HANDBOOK OF
Cosmetic Science and Technology

FOURTH EDITION

Edited by André O. Barel • Marc Paye • Howard I. Maibach

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1 Introduction

Marc Paye, André O. Barel, and Howard I. Maibach

Although cosmetics for the purpose of beautifying, perfuming, cleansing, or rituals have existed since the origin of civilization, only in the twentieth century has great progress been made in the diversification of products and functions and in the safety and protection of the consumer.

Before 1938, cosmetics were not regulated as drugs, and cosmetology could often be considered as a way to sell dreams rather than objective efficacy; safety for consumers was also sometimes precarious. Subsequently, the Food and Drug Administration (FDA), through the Federal Food, Drug, and Cosmetic Act, regulated cosmetics that were required to be safe for the consumer. With industrialization, many new ingredients from several industries (oleochemical and petrochemical, food, etc.) were used in preparation of cosmetics, offering a list of new functions and forms. For better control of these ingredients, US laws required ingredient classification and product labeling since 1966.

In Europe, the Council Directive 76/768/EEC of July 27, 1976, on the approximation of the laws of the member states relating to cosmetic products ("Cosmetics Directive") was adopted in 1976 to ensure the free circulation of cosmetic products and improve the safety of cosmetic products by placing the responsibility of the product on the cosmetic manufacturer.

In 1991, the Cosmetics Directive was amended for the sixth time and prohibited the marketing of cosmetic products containing ingredients or combinations of ingredients tested on animals, as of 1998. With the seventh amendment of the European Cosmetic Directive in 2003, a testing ban on finished cosmetic products was applied after September 11, 2004, whereas the testing ban on ingredients or combinations of ingredients was applied, at the latest, in 2009, irrespective of the availability of alternative nonanimal tests. For some end points where no alternative to animal testing was feasible (repeated-dose toxicity, reproductive toxicity, and toxicokinetics), a maximum deadline of March 11, 2013 was set up when those tests were also banned. Today, all finished cosmetic products and ingredients included in cosmetic products are prohibited in the European Union if they were tested on animals for cosmetics purposes (marketing ban).

In a field where the chemistry is evolving more quickly than the biology, new active ingredients are regularly identified and developed to improve the benefits of cosmetic products. However, with the current heterogeneity in worldwide regulations for placing new ingredients or cosmetic products on the market, it is anticipated that big differences are going to appear between the different regions, some favoring the innovation and a continuous improvement of products and some instead favoring stagnation by making so difficult the introduction of innovative ingredients on the market and stimulating a reformulation of products around the same bulk of authorized ingredients.

However, the safety of their products is the main preoccupation of cosmetic companies, and the disappearance of some safety tools pushed the companies to modify their approach and to consider any type of available information to establish the safe use of the product and the absence of risk for the consumers. An approach based more on the risk and on the weight of evidence of all types of information is, more and more, replacing an approach also based on the hazard of individual ingredients. A stricter postmarketing surveillance also ensures an overview of the undesirable effects due to cosmetic products and identification of any emerging concern on a product or a specific ingredient.

With regard to product efficacy, and despite the limitation in some parts of the world in developing new active ingredients, the trend continues to move towards products providing stronger benefits to their target, whether it is the skin, hair, nail, mucous membrane, or tooth. The competitive environment still pushes manufacturers to promise more to the consumers and to develop cosmetic products of higher efficacy. For cosmetic products to support these activities, raw materials became more efficacious and bioavailable, and bioengineering methods to analyze and measure those benefits became more sensitive, diversified, and reliable.

However, the availability and use of new active ingredients are not always sufficient for them to be optimally delivered to their targets and to sustain their activity. The cosmetic vehicle is also crucial to obtain this effect, and the role of the formulator is to combine the right ingredient into the appropriate vehicle. Cosmetology has thus become a science in its own, and the cosmetologist is not only a formulator chemist anymore but also a real life-science scientist who needs to fully understand the interaction of his or her products and ingredients with their targets to deliver the promised benefits.

Another and new factor changing the environment of the cosmetic industry is the growing extension of Web-based information. Today, any uncontrolled information may be posted on the Web and generate unconfirmed thoughts about a product or an ingredient. In some instances, the Web may be a useful tool to inform the consumers, but most of the time, the source of the information that is posted is not available or not controlled to allow its scientific analysis.

In this fourth edition of the *Handbook of Cosmetic Science and Technology*, the priority has been given to explaining the mechanism of action of cosmetic ingredients and products with their target in order for the cosmetic manufacturer to be able to develop a better scientific and evidence-based assessment of the safety and efficacy of their products and to better react against nonscientific, unfounded, Web-posted communication.

Additional sciences continue to develop and to contribute to the rise of active cosmetology; this is the case for biometric techniques, which have been developing for more than three decades and allow a progressive and noninvasive investigation of many skin properties. Instruments and methods are available to objectively evaluate and measure cutaneous elasticity, topography, hydration, and turnover rate, or even to see directly in vivo inside the skin through microscope evolution. Any claimed effect of a cosmetic on the skin should, today, find appropriate techniques for a clear demonstration. Several other books describe in detail all these methods, so purposefully, we have been selective in this edition to cover only some new and promising bioengineering methodologies.

Currently, big changes in the regulatory context are taking place and will greatly impact the cosmetic market. A recast of the European Cosmetic Directive has been adopted and entered into effect in July 2013. The implementation of REACH (Registration, Evaluation, and Authorization of CHEMICALS) also had implications by limiting the number of ingredients available to the cosmetic industry and creating high pressure on small and middle-sized enterprises (SMEs). Another topic that is clearly of interest today is the replacement of animal testing by alternative methods for testing the safety of cosmetic ingredients. The cosmetic industry, through separate activities or via its association, Cosmetics Europe (formerly the COLIPA), has been extremely active in developing in vitro methods and strategies for checking the safety of their ingredients. An extensive status on method developments is described in this book, which will provide a clear understanding of the progresses made but also of the way still to go in this area.

Finally, cosmetology has become a science based on the combination of various expertise domains: chemistry, physics, biology, bioengineering, dermatology, microbiology, toxicology, statistics, and many others.

Because of such a complexity in cosmetic science, it was not possible to cover in a useful manner all the aspects in one book. Details on most of the above fields are covered in the different volumes of the *Cosmetic Science and Technology* series. In the first edition of the *Handbook of Cosmetic Sciences and Technologies*, we especially aimed at producing a useful formulation guide and a source of ideas for developing modern cosmetics. Four years later, with the second edition of the handbook, about twenty chapters were added, while the others were updated by trying to cover the most recent innovations in terms of ingredients and cosmetic vehicle forms that should orient the type of products of the future. The third edition kept a few chapters from the first two editions but mainly included a new approach with chapters based on a detailed description of the mode of action and of the science behind the products, ingredients, or methodology. The fourth edition is a continuation of the third one, with a strong focus on the scientific aspect of the products and ingredients, but also provides up-to-date information on the new regulatory context, which has more and more influence on the innovation in cosmetology.

The fourth edition of the handbook has been reorganized and subdivided into nine sections. The first part of the handbook provides the reader with an overview of the different kinds of skin types and their specificities. This is especially important at a time when cosmetic products become more and more diversified and targeted to ethnic skin, sensitive skin, elderlies, or others.

"Skin Hydration" (part II), "Skin Barrier," and "Skin pH" (part III) are then addressed from product or ingredient, mechanism, and assessment perspectives. Part IV ("Skin Aging and Sun Care Products") covers the latest developments in terms of skin aging and sun care products, which represent a large contribution to the current cosmetic business.

Today, consumers are not satisfied anymore with the claims made on cosmetic products; they also want to see or perceive any claimed property of their product. This is why part V, devoted to skin perception, has been introduced with recent developments in measuring what has long been considered as subjective and not measurable. Covering various aspects of skin tolerance is an important section of the handbook (part VI) and provides the reader with relevant information from experts in skin irritation or allergy.

Section VII is devoted to skin color and appearance, while section VIII describes different types of cosmetics addressed to specific targets of the human body or with a less usual mode of application. For more conventional cosmetic products, the reader is referred to some excellent chapters from the first two editions.

Finally, the last section, "Ethics and Regulations" (part IX), provides a clear overview of the quickly evolving worldwide regulatory context and ethical requirements that should always lead any development and testing of new products.

Given the number of contributions, it has been a challenge to edit this fourth edition, only four years after the third; it has been possible because of the dedication of the authors and great support of Mr. R. Peden and Miss E. Pither from CRC Press. We thank them for making this enormous task easy, enjoyable, and fascinating.

2 Biophysical Characteristics of the Skin
Relation to Race, Sex, Age, and Site

Virginie Couturaud

INTRODUCTION

In addition to a number of other functions, the skin is mainly intended to protect human beings against physical or chemical external aggression as well as internal organic losses.

It fills this "barrier" part through a complex structure whose external part is made up of the stratum corneum, a horny layer covered with a hydrolipidic protective film. This function is only ensured when this horny layer made up of the accumulation of dead cells is properly moisturized, as the water is the keratin plasticizer.

The underlying epidermis also enables the reinforcement of the skin's defense capacity by ensuring the continuous and functional regeneration of the surface state (keratogenesis) and skin pigmentation (melanogenesis).

The dermis also plays this part and appears as a nutritional structure whose function is also particularly important for the maintenance, coherence, elasticity, and thermoregulation of the whole skin.

Finally, the hypodermis has a protective and reserve function.

According to its state, activity, and defense capacity, skin can have different appearances directly related to the water and fatty content of the hydrolipidic film.

A deficiency in fat, indispensable for retaining water in the teguments, favors water evaporation and, therefore, skin drying, whereas an excess of lipidic components favors a state defined as oily.

Among the numerous skin classifications that are proposed, the most closely connected with cosmetological requirements distinguishes four different types: normal, oily, dry, and mixed.

However, in practice, such a classification must be used cautiously. In fact, the words used are ambiguous and lead to various interpretations; the criteria of selection to define each category are difficult to standardize since they vary from one case to another. Some observations can even correspond to opposite clinical profiles.

So, for example, severe changes in epidermal water content associated with superficial pH changes can modify the skin's appearance and lead to the establishment of a visual diagnosis of dry skin, whereas it is actually oily skin.

For a long time, the research undertaken to try to understand the mechanisms leading to skin structural modification has been limited, with the researchers focusing more on the practical consequences than on the causes.

From now on, more recent works enable significant progress, but presently, the different classifications taken as the authority are still based on clinical observation instead of being based on the measurement of the biological parameters involved.

Dry skin would correspond to structural and functional modifications of the components of the epidermis.

Oily skin would result from an excessive seborrheic production invading the skin surface and possibly hair.

Resulting from totally independent processes, oily skin and dry skin therefore correspond to two states that must not be opposed, as some skins can be "dry" or "oily" and dehydrated at the same time.

The biophysical characteristics of skin also vary according to sex and age and can differ for the same subject according to the anatomical site considered.

Finally, the distribution of these different types of skin varies widely according to the ethnical group we are referring to.

A standardization of skin typologies based only on visual criteria therefore appears difficult and would require, in more or less the long term, resorting to other quantitative means of identification, notably referring to biochemical and biophysical data.

After a quick reminder of the parameters on which the traditional classification is based, an overview of the incidence of race, age, sex, and anatomical site in the measurement of the various skin biophysical characteristics will be proposed in order to show the limits of any kind of classification.

STRUCTURE OF THE SKIN

With a weight of about 4 kg and a surface of about 1.8 m², skin is the widest organ of an organism: its constitution is approximately the same at a qualitative level and on the whole body. However, it undergoes notable variations, especially concerning its thickness, its components, and above all, the way and variety of implantation of skin appendices. These variations enable skin to have a perfect functional adaptation.

In addition to its mainly protective role, skin also ensures numerous other essential functions such as permeation, metabolism, and thermoregulation and actively contributes to the sensorial function. This structural and functional diversity is influenced by intrinsic factors related to subjects—their ethnic group, their age, and their physiological,

psychological, and pathological state—and by extrinsic factors related to the immediate environment such as the dryness level, sun exposure, temperature, wind, and so forth.

General Considerations

It is not the intention of this chapter to approach in detail the complex biophysical structure of the skin, but it may be of importance to review some general aspects, allowing a better understanding of its ability to constantly adapt according to internal and environmental needs.

Being the largest and the outermost organ of the human body, the skin is the unique organ interfacing with the environment and has, as such, direct and important regulatory functions allowing the body to ensure a permanent defense of the whole organism against external aggressions. To do so, its anatomical structure is adapted to assume such physiological and barrier functions.

At the more external level is a very thin protective epithelium, constituting *the epidermis*, whose more superficial layer constitutes the *stratum corneum*. The epidermis serves mainly as protection of the organism against external aggressions, notably ensured through the cohesion of epithelial cells, the keratinocytes, that undergo a specific process of differentiation as they migrate from the dermoepidermal junction to the skin surface. This cohesion results from intercellular ties, the desmosomes, mainly responsible for the very great mechanical resistance of the epidermis. However, the migration of the keratinocytes remains possible since these desmosomal ties are submitted to a continuous process of dissolution and reconstitution associated with a progressive decrease in their adherence strength.

Keratinization corresponds to the most important structural and biochemical change undergone by the epithelial cells. Through this process, they synthesize keratin, a fibrous complex protein whose structure evolves during cell differentiation. This process starts at a basal level and ends with the transition between keratinocytes and corneocytes, cells mainly full of fibrous material. Corneocytes in degradation and intercellular lipids form a horny cover that reinforces the solidity and mechanical resistance of the stratum corneum, which also depends on the corneocyte supply in water.

In addition to this mechanical protection, the epidermis also has, through its structure and the presence of specialized cells such as the melanocytes, Merkel cells, and Langerhans cells, other more complex, functions among which are the regeneration of tissue, exchanges with the medium, and active defense against external aggressions.

Variables originating from the individual as well as exogenous factors have an important influence on the epidermal parameters [1].

At intermediate level, *the dermis*, dense conjunctive tissue, much thicker than the epidermis to which it is connected by the dermoepidermal junction, is an area of cohesion but also of intense exchanges.

This conjunctive tissue is globally made up of an amorphous extracellular substance in which more or less mobile cells float, the whole being kept together by a frame of elastic and collagen fibers. Numerous vessels, nerve fibers, and appendices with main functions, notably, the sweat and sebaceous glands and the hair follicles, go through the fundamental substance.

Among the cells, it is worth noting the presence of fibrocytes with proliferative capacity, responsible for the synthesis and maintenance of the extracellular material, histiocytes, mast cells, and leucocytes, involved in nonspecific defense and in immune supervision.

Due to its structure and the distribution of its components, the dermis is generally divided into two areas: the papillary dermis and the reticular dermis. The papillary dermis, at the dermoepidermal junction, fairly loose, very vascularized, rich in nerve fibers and endings. It therefore has multiple functions: nutritional exchanges with the epidermis and the capacity for percutaneous absorption through its vascular and lymphatic networks, protection against aggression and mechanical deformations through its fibrillar texture, sensory perception by the presence of most of the nerve endings, defense against foreign bodies by participating in the immune inflammatory and phagocytic processes through the existence of specialized cells, and tissue reconstruction.

The reticular dermis, thick, mainly made up of an interlacing of collagen fibers, is where the lower parts of the appendices are located, ensuring the junction between dermis and hypodermis. It mainly has a mechanical function through its capacity for deformation (extensibility, compressibility).

Finally, at the most internal level is the *hypodermis*, loose conjunctive tissue, linked to the lower part of the dermis by expansions of collagen fibers and elastic fibers, of different thickness according to the anatomical area. This tissue mainly contains adipocytes full of triglycerides, histiocytes, and mast cells. Its vascularization and innervation also vary according to the anatomical locations.

The hypodermis mainly has a function of protection and reserve of fat. Its mechanical properties are very badly known, but by enabling the skin to move as a whole on the underlying levels, this skin layer plays a main part in the breaking of the external strengths of deformation. In fact, it has been observed that the cicatricial elimination of the hypodermis results in a significant increase in the constraints of skin extension or friction due to a loss of mobility [2].

Variations According to the Anatomic Site

This preamble is of main importance when studying effects acting on the barrier function. As a matter of fact, a number of studies have objectivated that distinct anatomic sites posses different morphological and functional characteristics. Among others, the relationship between the corneocyte size and organization and the barrier function [3], the variation of the transepidermal water loss (TEWL) according to site [4], the incidence of the site in the stratum corneum hydration [5], the variation of quantitative composition between body locations [6]—all these examples underline the need for multiparametric approaches in the evaluation of the skin barrier properties [1].

Variations According to Age

Despite the numerous publications provided in the two last decades, and even if there is evidence that there are age-related changes in skin structure and function, data available are often conflicting and difficult to interpret. As an example, whole skin thickness is seen by some as increasing in youth, remaining constant during adulthood, and decreasing in the elderly [7]; others maintain that photoexposed areas thicken with age [8] or that changes with age are more related to skin location [9]. Difficulties result from the overlap between the different parameters involved. A recent review covering these aspects was unable to arrive at a conclusion, inviting researchers to develop new studies to clarify the situation [10].

Variations According to Gender

Very few studies on gender-related differences in skin physiology have been published, and the existing ones again provide conflicting results. Apparently, just a few gender-related differences were observed. In one recent publication, it was demonstrated that there was no difference in the amount of stratum corneum proteins between women and men but that their composition and amount in amino acid was different [11]. All in all, there are indications that most of the reported differences in skin physiology between men and women might be related to hormonal effects [12]. Changes in TEWL and cutaneous blood flow during the menstrual cycle have been reported [13].

CLASSIFICATION BY SKIN TYPES

Numerous skin classifications have been proposed; they all privilege specific criteria.

So, from a cosmetic point of view, the reference criterion is the user's feeling and, therefore, the surface state of his/her skin, his/her capacity for seduction and even attraction. There is a connotation of wellbeing and pleasure. This selective criterion generally leads to the classification skin into four main types, which still remain to be clearly defined, that is, normal skin, dry skin, oily skin, and mixed skin.

These denominations are based more on the feeling than on the causes, are imprecise and even erroneous, and entertain in practice significant misunderstandings between biologists and consumers, which will have to be progressively raised.

The improvement of the knowledge of the mechanisms involved actually leads to progressively being better able to differentiate what corresponds to an evolutionary process from a particular and immutable skin typology. If it is true, for example, that dry skin often has a genetic component [14], most people experienced it at a given moment of their life (because of climatic conditions, etc.). In the same way, most people at a given stage of their hormonal and sexual development have to face the troubles related to oily or mixed skin.

Normal Skin

Contrary to all expectations, it is worth noting that there is no definition of normal skin, which is qualified in comparison with the other skin types: normal skin is not dry skin, is not oily skin, is not mixed skin, and is not pathological skin.

A brief analysis of its structure and of its functions enables us to draw a more positive definition of normal skin.

Based on its structure and its functions, normal skin should be smooth skin, pleasant to the touch because of the cohesion of the cells of its more superficial layers, firm and supple skin because of the existence of a dense supportive tissue and of the presence of numerous elastic fibers of good quality, matte skin through its balanced seborrheic production, and clear and pinkish skin because of the perfect functionality of its microcirculatory network.

In reality, skin complying with all these characteristics would only exist in a healthy child before his/her puberty [15].

At a cosmetological level, we must be content with a less strong definition and consider normal skin as young skin, structurally and functionally balanced and requiring no care apart from that necessary for its cleaning.

Dry Skin

The concept of dry skin has also never been clearly defined. The term conceals several complementary or opposite points of view [16]. It remains completely different according to the way it is approached. People connect this notion to the effects observed and to their sensorial dimension. Therefore, for them, it is first of all a feeling of drying, a loss of skin suppleness and elasticity characterized by a rough appearance often associated with an important desquamation leading to a certain discomfort they intend to correct by using moisturizing products.

For the biologist, the xerosis would be first the consequence of a change of the coherence and functionality of corneocytes, the water deficiency of the superficial layers of the stratum corneum, when it exists, only resulting from it.

As a matter of fact, the physiopathogeny of most xerosis is still badly known, and it remains difficult to distinguish the causes from the consequences of these skin abnormalities [17].

As it has been said before, in normal conditions, the corneal layer is made up of a regular assembly of corneocytes forming a structure of modulated thickness whose physical qualities are unique [17].

Each corneocyte contains dampening substances called natural moisturizing factor (NMF) resulting from the enzymatic degradation of the fillagrins, which fix a certain quantity of intercorneocytar water and therefore exert a decreasing osmotic pressure as they migrate to the surface [17].

Any decrease in the enzymatic function therefore plays an important part in the NMF content and, consequently, in the osmotic pressure and in the opening of corneosomes, consequently easing a disorganized desquamation as it is observed with xerosis [17].

This dysfunction actually depends on a qualitative and quantitative change of enzymes and/or on an inadequate change of the pH of the corneum [18]. The intercorneocytar cohesion also depends on a complex mixture of lipids that constitute the lamellar structure interposed between the corneocytes (made up of fatty acids, sterols, and ceramides coming from the keratinosomes) [17].

Whereas most of the research focused on the study of the change of the function of the horny layer and of its constitution and led to the theory of moisture balance [19–24], few works have been undertaken to better understand the components of the epidermal cells that are involved in skin drying, which will enable a better understanding of the mechanisms that lead to xerosis.

Previous studies have shown the importance of four factors predisposing to dry skin:

- The lack of water of corneocytes, directly depending on the presence of NMF
- The epidermal hyperproliferation, resulting from a deficiency in the renewal process of the keratinocytes
- The change of lipidic synthesis at a cell level
- The deterioration of the functionality of the skin barrier following a degradation of intercellular cohesion

All these factors are interdependent.

Consequently, dry skin should be characterized by its rough appearance without referring to its hydration level [25].

Recent research has actually questioned some established ideas, notably, the influence of the inflammatory process or of the content in calcium ions of the epithelial cells in skin drying. In fact, experimental results have shown that the supply of nonsteroidal anti-inflammatory agents [26] or of calcic regulators [27] did not significantly modify skin state. On the other hand, the use of specific inhibitors of tryptic proteases, and particularly of the "plasminogen activation system," showed a capacity for restoring the skin's normal state and for simultaneously suppressing all the changes related to skin drying, notably against the mechanisms of cell regulation and differentiation, of increase in TEWL of the horny layer, and of acceleration of its renewal and the epidermal thickness resulting from it [28].

From now on, these works enable us to confirm that skin drying does not correspond to an irreversible state but results from a dysfunction involving the traditional "balance moisture theory" [29] and the "protease regulation theory," resulting from these new pieces of research [28].

As already seen, dry skin depends on numerous biological factors [25]; its reparation implies the restoration of the epidermal barrier, actually damaged by the loss of fat and dehydration of the superficial layers of the stratum corneum.

Such changes are more easily objectivable in black subjects, whose skin takes a perfectly visible ashy appearance. It is also advisable not to systematically associate dry skin with old skin even if, in elder subjects [30], we globally note a decrease in the hygroscopic quality of the stratum corneum and in the desquamation of corneocytes and the retention of keratin, contributing to giving a drier and rougher appearance to skin [31].

Oily Skin

Whereas dry skin reflects a functional change of different skin components, oily skin results from an overactivity of the sebaceous glands leading to an overproduction of sebum overflowing on skin, giving it a characteristic oily and shiny appearance.

In fact, sebum results from the disintegration of specific cells, the sebocytes, a short time before they go out from the sebaceous gland. Once again, it results from cell differentiation. Originally, sebum contains squalene, waxes, triglycerides, and sterols. Under the effect of resident bacteria, one part of the triglycerides is immediately hydrolyzed, and the main part of the cholesterol is esterified, the sebum excreted containing a significant quantity of free fatty acids contributing to the acidity of the pH of the skin's surface.

Then this sebum blends with epidermal lipids coming from the destruction of the desquamated horny cells that also contain triglycerides and cholesterol to form the surface lipidic film covering the stratum corneum.

Human beings have the particularity of having at their disposal sebaceous glands on almost the entire body, but their activity is not the same on all the anatomical sites. The production of sebum is more important on the head, face, neck, shoulders, and thorax, areas where a hyperseborrhea can be the conjunction of a high production of the glands and of a greater number of glands [32].

Sebum is a natural skin detergent that gives it an amphiphilic wettability through the presence of free fatty acids and waxes [33]. It also plays a part in the maintenance of the functional qualities of hairs, a fungistatic activity while having a nutritional function for bacterial species useful for the organism, and finally, a protective function against excessive dehydration in a dry environment through its effect on the epidermal barrier function, even if the sebum is not known to have a dampening activity [34] and has no influence on skin hydration level [35].

The change of its rate of production depends on genetic, endocrinic, and environmental factors [36].

The opposite of oily skin would not be dry skin, since they can coexist, for example, on the face. Such a statement is currently supported by many researchers [37].

Actually, young children almost never have a seborrheic outbreak before 7 years old, when the first secretion of androgenic precursors starts to form. This production will progress to reach its maximum at adolescence and then decrease with age.

It is also worth noting racial differences related to sex, men globally having oilier skin than women [31]. Finally, at a cosmetological level, it must be retained that oily skin is sometimes erythrosic, easily irritable, and particularly fragile.

Mixed Skin

It corresponds to complex skin where the different types previously described coexist on different areas of the body or face. The characteristic example is the face, where solid and oily skin with well-dilated pores on the medio-facial area can coexist with a fragile skin with fine grains on the cheeks.

Such skin requires conjugating the particularities and sensitivities peculiar to normal, dry, and oily skin.

BIOPHYSICAL CHARACTERISTICS OF THE SKIN

As it constitutes the external cover of the whole human body, skin has been reduced for a long time to a protective part against external aggressions.

The intense multidisciplinary exploration of skin carried out during the past 30 years progressively enabled us to better determine the specific function of its components, the nature and importance of the exchanges with the surrounding organs, and finally, the vital function that skin exerts on the organism in addition to its main part in natural defense.

These progressive discoveries show that skin, functionality, and immunity must not be separated anymore and lead to the concept of a real neuro-immuno-cutaneous endocrine system, the NEICS [38].

As a living organism, skin is in constant renewal and undergoes, at the same time, a progressive aging with a parallel decrease in its functionality; moreover, today, it still remains difficult to distinguish what depends on natural evolution from what is under external control, especially concerning the actinic aging.

At an external level, it leads to a progressive change of the skin surface state, a perceptible sign of the changes in both physiological functions and biophysical properties.

In order to measure the effects of aging and possibly to prevent its happening, it is important to identify analytical parameters, as realistic as possible, which correspond to the population concerned. It is particularly true for the analysis of biophysical data.

Beyond the interindividual variations or those that can result from the methodological approach or from the material of measurement used, many authors have tried to identify the influence of the race, sex, and age of the populations observed, and even the anatomical site on which the observations are made, on the results obtained. The results of these investigations are sometimes contradictory, but from now on, they enable us to emphasize some tendencies to be taken into consideration when conducting studies in the human being.

Good previous knowledge of these differences is notably essential to knowing the efficacy, acceptability, and even tolerance of products applied topically, such as cosmetics or dermatological products.

Their impact shall completely differ according to the market they are intended for, not necessarily for being inefficient but only for not being directly suitable for the targeted population, not necessarily for questions of habit and mode, but mainly because they do not correspond to the potential consumers' ethnological specificities.

This part will give a brief reminder of the incidence of race, age, sex, and exposure site in the most commonly explored biophysical characteristics of skin.

Incidence of Race

It is useless to talk about the interracial morphological differences. They are obvious and never gave rise to confusion the risk of complicate the problem of ethnical integrations.

At a macroscopic level, Caucasian (White), Hispanic, Asian, and African (Black) skins are very different at first sight as their color is enough to give them a very distinct appearance.

This difference disappears at a microscopic level as all types of skin have the same qualitative structure. However, this similarity is lower at a quantitative level. So, for example, the size and cytoplasmic dispersion of melanosomes are completely different between black and Caucasian skin [39–41] because they correspond to different needs of photoprotection [42]. In the same way, the surface of the corneocytes, even if identical for all types of skin [43], is different according to gender and anatomical site [44]. Another example is that bigger pore size in the facial skin of Japanese persons increases its cutaneous sensitivity [45].

The important functional differences existing between races seem to correspond to a necessary adaptation to the environment in which such populations are meant to live.

So, whereas the mean thickness of the horny layer is similar between the different races [46,47], the number of cell layers in the stratum corneum of black skin is higher than that noted in Caucasian or Asian skin. Black skin, therefore, has a more compact stratum corneum with a greater cohesion between cells that makes them difficult to remove [48]. However, the surface of corneocytes is identical for all types of skin [43]. In apparent contradiction to this greater cell cohesion, it is advisable to emphasize that the spontaneous surface desquamation is significantly more important in blacks than in Caucasians or in Asians [43].

These differences have to be taken into account notably when the capacity of the products for acting on cell renewal or for reducing skin drying is studied.

Interracial differences also exist concerning the melanocytic system. Even if each type of skin basically has the same number of melanocytes per unit of surface, there is no similarity concerning their structure [39] and their functionality [48]. Whereas the melanosomes are small and concentrated in the keratinocytes to be then degraded in the superficial layers of the epidermis of Caucasian skin, they are much bigger and widely scattered in all the layers of the keratinocytes and are not degraded when they arrive in the horny layer of black skin, giving them a characteristic color [49].

Colorimetric and spectrophotometric studies have shown that the interindividual and intersexual differences in skin coloration in the different races are mainly related to the blood concentration in hemoglobin for the Caucasian subject,

to both the hemoglobin and melatonic pigment content in the Asian subject, and to only the concentration of melanin in the black subject [50].

Racial differences concerning the functionality of the epidermal appendices also exist.

Contrary to a firmly fixed notion, the number of sweat glands is not different between the racial types whatever the geographical site as the variations depend more on exogenous than on genetic factors [51,52]. Today, nothing explains the different interracial smells, probably depending on bacteria [48].

It even never has been possible to demonstrate a possible racial incidence in sebaceous secretion, as some authors report a more important activity for black skins [53,54], whereas others report no substantial difference in sebaceous production between races in their comparative studies [55]. A recent study showed a more pronounced sebaceous production on the back in white than in black skin [56].

Thorough studies have explained the interracial differences in hair shape [57,58] and in pilosity but did not manage to objectivate the differences between their chemical components [59].

The advancement of knowledge enables us today to retain the assumption that the genetic factors and the intrinsic differences between ethnical groups actually have less importance than their capacity for adaptation to the environment they live in. Many recent publications reinforce this concept [60–62].

This different adaptation according to race can have significant repercussions according to the field investigated.

- *Skin Relief*

  There is little information concerning the possible racial differences as the intraethnic variations according to age and, possibly, anatomic site seem to have a much more important impact on the variability of the measurements. However, for the same age, it has been shown that the number of wrinkles is highest in Caucasians, followed at the same level by the Hispanic and black people; the smallest number of wrinkles is observed in Asian subjects [63].

  A more recent comparative study between German, Chinese, and Japanese populations specifies that the wrinkling in the border of the eye is more important in women aged 50 to 60 years old [64]. This observation is confirmed by an iconographic analysis showing that the wrinkles in the crow's foot of the eye of Asian females are more numerous but less deep compared to the European ones [65]. This same analysis has shown that the wrinkles above the upper lip or below the eye are less important for African American women. As a matter of fact, it was established that the increase with aging in the wrinkles of the crow's foot of the eye is linear for European women, while it corresponds to two different steps for Chinese women: after low growth till the 50s, the process then speeds up, both populations getting a similar wrinkling from the 60s on [66].

  According to another comparative analysis, the number of wrinkles on 10 anatomical sites of Caucasian and black subjects of the same ages shows that actually, the difference concerns only the periauricular area [67].

- *Color*

  The interracial difference is obvious and mainly depends on the content, size, and distribution of the melanosomes [68]. As it has been said, the number of melanocytes per unit of surface is the same for all the races, but their structure is different [41,69,70]. The color of black skin is mainly related to the particular migration of the melanosomes that invade all the epidermal layers and reach the horny layer without undergoing degradation, a process completely different from what is happening in the skin of Caucasians [42,71].

  Pigmentation leads to better protection against sun radiation and, therefore, actinic aging. The racial differences in constitutive pigmentation are directly related to the incidence of pigmentation disorders [72], black skin being much more exposed to hyperchromatic spots that appear under the effect of external aggressors or to hypochromatic spots for lack of sun exposure [71,73,74]. An order of increasing sensitivity to these alterations of pigmentation has been established, classifying black skin as the most exposed, followed by the white skin sensitive to hyperpigmentation spots, and then to a lesser degree, Hispanic and Asian skin [63]. However, it is advisable to note that the spots appear sooner and in a higher scale in Asian and African American women [65].

  Concerning skin brightness measured from the parameter L* of the International Commission on Illumination (CIE) L*a*b* system, the best improvement of skin brightness after sun exposure is noted in Caucasians, followed in decreasing order by Asian skin, Hispanic skin, and black skin, which mainly remains dull. Except black skin, which has a lower index of brightness, all the other types of skin have a similar index in the absence of sun exposure [63].

- *pH*

  No interracial difference has been shown concerning skin pH [63]. Some authors report a slightly higher pH for the Caucasian race in comparison with the black race [75–77]; these variations, rather, depend on the age of the population examined as the interracial deviations are mainly noted between 30 and 50 years of age.

- *Electrical Conduction*

  The measurement of electrical conductance on the skin superficial layers enabled us to show that it is the highest for black skin, lesser for Hispanic and Asian skin, and the lowest for Caucasian skin

[56,75,76,78–80]. This electrical resistance is reported to be twice as high in black as in white skin [76].

Another study [67] seems to demonstrate that on the contrary, there is no difference between the electrical conduction of the skin of subjects of Caucasian and African American races. It enables us to conclude that the racial criterion is not the only parameter to be taken into account in the study of skin electrical conductivity. So, the measurement of capacitance on different skin sites enables us to show contradictory interracial differences in the same study [67].

Aging allows us to observe a difference between races regarding skin dryness, this being more important for African American and Caucasian females than for Chinese and Mexican females [68].

It is worth noting that black skin shows a higher epidermal water content, although no change in the TEWL is observed; this particularity is justified by the greater cell cohesion of the stratum corneum, previously evoked for this ethnic group [69].

- *TEWL*

  Many experimental results show no interracial difference concerning the basal level of TEWL [56,78,79]. More advanced studies enabled us to establish that these global results were giving only an apparent response as the TEWL of the subjects of black race is actually significantly higher than that of, notably, Caucasian subjects, this difference being made up for in vivo by a lesser vasodilatation of black skin under the effect of external aggressors.

  This demonstration initially carried out in vitro [81] has been confirmed in vivo later on [56] by using substances able to neutralize the microcirculation locally.

  The interracial variation could be related to the skin content in ceramides, the TEWL being inversely proportional to their concentration [82].

  Interracial differences in skin permeability and barrier effect have been demonstrated under the effect of vasodilative agents [83] that show, under the same experimental conditions, the lowest TEWL in subjects of Caucasian race compared to those of Asian and Black race, comparable with each other. When the aggression is a stripping, it has been shown that the return to normal depends more on the phototype of the skin than on the race, the darkest skins having quicker recovery [84].

- *Biomechanical Properties*

  Measurements of the immediate extensibility (Ue), viscoelastic deformation (Uv), and capacity for immediate recovery (Ur) of the skin of the forearms of subjects of Asian, Caucasian, and Black races from a deformation created by the twistometer have shown significant interracial variations, particularly between Caucasian and black skin, which go in one or the other direction depending on whether the measurements are performed on sites protected from the sun or not [78]. For the three races, the extensibility is lower when skin is used to sunshine in comparison with when it is on a nonexposed site, this difference being clearly more marked for Caucasian skin (arbitrary values respectively ranging from 34 ± 3 to 40 ± 3 for black skin and from 49 ± 2 to 28 ± 2 for Caucasian skin).

  The variations in viscoelastic responses are not significant between protected and exposed sites for black subjects but are significant for the Caucasians and Hispanics, even if no interracial difference is noted.

  Black skin has the same capacity for recovery on both sides of the forearm, whereas there are significant differences between the two sites to the detriment of exposed areas for Hispanic and Caucasian skins.

  The capacity for recovery of black skin is higher than that of the Caucasian skin.

  The calculation of the modulus of elasticity (1/extensibility × skin thickness) that takes into account the incidence of skin thickness in the site of measurement showed significant differences between the three races to the advantage of black skin, whereas the deviation between the exposed site and the protected site was significant only for the White race [78].

  The elasticity index, measured by the ratio of recovery to extensibility, enabled us to show no appreciable difference between races. These results were confirmed by other authors using other sites and other equipment [75]. The best elasticity of black skin would result from its greater content of elastic fibers per unit of surface in comparison with white skin [85].

- *Seborrheic Production*

  Sebaceous secretion would be globally more important on black skin, followed by white skin, Hispanic skin, and to a lesser extent, Asian skin [46,53]. This variation is partly questioned by other authors who have found no substantial difference in sebaceous production between Caucasian subjects and black subjects [54]. Here again, the anatomical site taken into account seems to be a deciding factor. Black skin has the highest lipidic content of all races [86]. Concerning this point, a seasonal variation is noted, black skin being more lipidic in the summer than in the winter, notably on the face, an apparent paradox of skin both dry and shiny, a result of the superposition of a constitutional xerosis on a protective film of the surface, made up of a mixing of sweat and sebum [87].

- *Actinic Aging*

  The analysis of the penetration of light into skin and of the effects it induces was reported by many authors [88–91], who particularly took into account the behavioral difference between Caucasian skin

and black skin. In spite of structural differences in the stratum corneum, the total reflectance of light at its level is located between 4% and 7% for Caucasian and Black people [88]. On the contrary, there is a significant difference in the light transmission through the epidermis of Caucasian skin especially at wavelengths corresponding to the ultraviolet (UV) radiations, which results in a considerable decrease in the natural capacity for actinic protection of this ethnic group. This transmission is less important in the subjects with Hispanic skin [91]. Similar differences were noted with UVA.

On the whole sun spectrum, it results in a natural capacity for photoprotection of Caucasian skin three to four times lower than black skin [92,93]. This difference is directly related to the distribution of melanosomes in all the epidermal layers of black skin [94].

The examination of the available data concerning racial variations enables us to conclude that these differences affect a reduced number of parameters, that the variations noted have a limited incidence, and that the results published are often contradictory. As a consequence, the interracial studies on the biophysical properties of skin have to be tackled cautiously as the deviations observed actually depend on several factors that can act in a synergic or antagonistic way. Therefore, each experimental result will have to be confirmed. In addition, the dispersion of the results obtained in this type of study must incite the experimenter to establish study protocols that involve an enlarged number of subjects correctly selected in order to avoid the variability of individual responses hiding the reality of intergroup differences.

Incidence of Sex

Although the influence of sex on the results of biophysical measurements is often quoted in bibliography, little precise information is supplied, maybe because this criterion actually has little real influence on the results.

However, there are morphological differences in skin according to sex. In fact, skin thickness is greater in men on most of the sites usually used for biophysical measurements [94–96], whereas for women, skin is thicker at the dermal level [97].

Other authors reported no significant differences for the forearms [98–100]. Observations made on male and female Asian subjects showed no difference between sexes concerning the number of layers of corneocytes [101]. Skin thickness is reduced more quickly in women than in men with aging [102].

- *Skin Relief*

  To our knowledge, no publication brings relevant data concerning the influence of sex on the state and evolution of skin relief.

  The friction coefficient is also independent of sex [103].

- *Color*

  As already said, colorimetric and spectrometric studies have shown that pigmentation is more important in men than in women [50]. A study carried out with a colorimeter on a Caucasian population showed that the parameter a* is generally the highest but that actually, there is an interaction between sex and age for each of the parameters L*, a*, and b* [104].

- *pH*

  Measurements performed on different skin sites confirmed the absence of influence of sex on skin pH [105,106].

- *Electrical Conduction*

  A very great number of investigators have dealt with electrical conduction to characterize the hydration level of the skin's superficial layers, as it is a deciding factor in the study of the xerosis or of the functionality of cosmetic products.

  Several research teams have tried to determine the influence of sex on the variability of the results observed. Different parameters have been explored, some directly representative of skin electrical conductivity, such as the capacitance and impedance, and others representative of the opposite effect, that is, the resistivity to conduction, such as the measurement of resistance.

  No difference between sexes was shown concerning the conductance [103] and impedance [67]. The more controversial publications are concerned with the capacitance as some experimenters report no difference between sexes [107], whereas others, on the contrary, report a more pronounced resistance to conduction in women than in men, on the basis of measurements performed on several anatomical sites [98].

- *TEWL*

  Studies conducted by different authors on the TEWL have shown no variation between sexes [103,108,109]. Other researchers have reported a more pronounced water loss in men than in women [98,110]; one of them, in a study performed on Asians, has related this difference to a lower basal metabolism in women [111].

- *Biomechanical Properties*

  The incidence of the sexes in the measurements of the biomechanical properties of skin depends on the parameters used. Skin distensibility is reported to be higher in women, independently of the sites chosen [112]. Noncomparative measurements between sites have shown, on the forehead of women, an initial skin tension higher than that of men [94]. This elastic retraction is also reported to be relatively more important in women on the leg. The nonelasticity index is relatively more important in women than in men, but the absolute values of this index are clearly different according to the sites observed [94].

Finally, these different authors report that there is no difference between sexes, whatever the site, concerning the Young's modulus [94] and the hysteresis curve [112] for values that, in absolute, considerably differ between sites.

- *Seborrheic Production*

  The literature reports little relevant information on the incidence of sexes in sebum production. The rare publications mention a significant difference as men generally have, on the various sites studied, a higher sebum rate than women [98]. On the other hand, the extent of this variation would be low compared with the incidence of race [53]. The production of sebum decreases with age, more particularly in women [70]. Such a decrease seems to be related to hormonal incidence [106].

Incidence of Age

Due to the continuous aging of skin and its incidence in structure and functionality, the age of the subjects included in a study is often a main element to obtain relevant results. As we will consider in this chapter, age has a direct impact on the evolution of most of the biophysical parameters of skin. Health of the skin can influence and reduce the evolution of the biophysical constants. As a matter of fact, the maintenance of good skin hydration delays the appearance of wrinkles [113].

- *Skin Relief*

  Many publications have shown the incidence of aging in the increase in its roughness, the evolution of the microdepressionary network of skin [114], and the development of wrinkles whatever the ethnic group considered [63].

  In order to simplify, roughness can be considered as subject to external and internal influences such as the climatic environment, the sunshine, the effect of cosmetic products, and also the water content of the skin's superficial layers and so forth [115–118]. The destructuring of skin microrelief as the appearance of lines and then of wrinkles results from a deeper change of the proper skin structure, a characteristic that progressively becomes irreversible even if its term can be reduced by palliative care [119].

  Many methods have been proposed to measure as accurately as possible the levels of skin roughness, of its microdepressionary network, or of its different wrinkles.

  These methods, most of the time instrumental, resort to the use of microsensors, image analyzers, or photometric or echographic analyzers able to supply a very great number of parameters, among which only a few have a real relevance.

  Independently of the methodologies used, some facts have been established: the length of the microdepressionary network decreases with the age [114], and the depth of the folds grows hollow as the first wrinkles develop [76]. A systematic echographic analysis of wrinkles enabled the establishment a scale of values per ethnic group, according to the age and to the site observed [120]; the best correlation has been established for the number of wrinkles of the periocular area [121].

  All the bibliographical data show that the evolution of the microdepressionnary network is particularly sensitive beyond 40 years old as the main lines start to grow hollow progressively [122]. The lines of secondary orientation progressively disappear between 50 and 80 years old, and we observe monodirectional lines oriented in the direction of skin deformation and the multiplication of great spaces whose folds are not visible microscopically [114,117].

- *Color*

  For all the races, there is a decrease in the hyperpigmentation spots related to the age of the subjects [63]. The colorimetric examination enables us to note a decrease in skin brightness measured by the parameter L* of the L*a*b* CIE system [104], in the Japanese and in the Caucasians [123]. Concurrently, there is no significant change in the colorimetric parameters a* and b* and of the parameter C, corresponding to skin saturation: $C = \sqrt{a^2 + b^2}$ [124].

  In practice, these variations can differ according to the site observed and to the level of sun exposure [63].

  In summary, we can deduce from the bibliographical data that there is a decrease in skin brightness with aging but that this variation depends on the site where the measurement is performed.

- *pH*

  There are few available data on the subject. Publications are contradictory, underlining the absence of any variation in skin pH measured on several sites according to the age of the subjects taking part in the study [63] or, in a more recent publication, the increase in the pH with aging [106].

- *Electrical Conduction*

  The conductance generally increases with age in all the ethnic groups [63]. The capacitance measured comparatively in young and old subjects appears significantly lower in old subjects [76]. In practice, this evolution is not linear, as the capacitance actually increases with age until 50 years old and decreases later on [124].

  However, these observations must be considered cautiously because a more detailed analysis that takes into account the measurements on several anatomical areas shows that actually, the value of conductance and capacitance is also related to the measurement site [98,103,107,125].

  The electrical impedance measured with the spectrometer also varies according to age as the values of the indexes of magnitude (MIX), real part (RIX), and imaginary part (IMIX) increase with

age, whereas the index of phase (PIX) evolves in the opposite direction [110]. The indexes MIX and IMIX are considered as the most representative of aging.

- *TEWL*

  The relation between TEWL and age is very much questioned as some authors conclude that there is no relation between these two parameters [126,127], whereas others found that this relation does exist but is very slight [122] or that this correlation varies according to the anatomical sites where the measurements are performed. An increase in the TEWL on the forehead is described [98,128]. On the whole, the authors instead report a decrease in the TEWL according to age on most of the other sites examined [98,103,125].

  These contradictory data incite us to act with maximum attention to measure this parameter, taking care to have an objective reference at disposal for each measurement.

  Any correlation to the measurements of capacitance is strongly questioned [129–131].

- *Biomechanical Properties*

  Globally, a decrease in skin elasticity with age has been reported [114,132]. This is the same for tonicity and extensibility.

Incidence of Site

As previously seen, the racial criteria, age, and sex are not enough to define skin response to an aggression or to a possible restructuring effect. In fact, important variations exist in the subject considered separately according to the sites on which the measurements are performed, with these variations being sufficiently important to invalidate the experimental results.

Without trying to be exhaustive, this last part of the analysis supplies many concrete examples meant to incite the experimenters to choose accurately the site of measurement, according to its specificity, to the exploration that must be undertaken, and to the reference that is taken into account for the appreciation of the significance of the effects observed. The spontaneous changes of the skin's state over time according to intercurrent factors that depend on physiological and hormonal variations and on its proper aging therefore imply that their incidence is systematically taken into account, and such an approach can only be performed case by case.

Skin thickness is not the same between anatomical sites, as established in the publications of many authors through numbered data and different instrumental measurements. So, for example, skin thickness measured in a subject of Caucasian race is thinner on the forearm than on the forehead, respectively, on the order of 0.9 and 1.7 mm [94]. These values are slightly higher than those described by other authors [95,133–135] but can be taken into account as the approach was performed through a more elaborated technique based on high-resolution scanning [94,102]. In addition to the differences that exist between anatomical sites, there are great variations for the same area. This is the case, for example, between different areas of the face [98], between the dorsal and volar area of the forearm [78], and between different locations of the forearm [136].

Measurements performed with a scanner on 22 anatomical sites of young male and female Caucasians enabled us to note that skin is all the more echogenic since it is thinner and that, at acoustic level, the response of the reticular dermis is denser than that of the papillary dermis. This acoustic density, also inversely proportional to skin thickness, is consequently variable according to the thickness of the anatomical sites measured [97]. It must be noted that the evolution of the lips according to aging is similar to that one of the skin [137].

- *Skin Relief*

  As it has been already said, at basal state, skin relief is directly representative of the state of anisotropy of the local tensions, and the structural deformations or changes it undergoes are directly dependent on the constraints undergone (mechanical constraints, aging, but also external aggressions) [138]. This relief is therefore necessarily specific according to the sites observed as it can be shown by a simple visual examination of the structure and topography of skin at different levels, for example, the face, neck, limbs, and hands [139]. Beyond the structural differences between anatomical sites, there are also differences in levels of roughness [76,140–142].

- *Color*

  There are important natural variations in skin color between anatomical sites in absence of the additional effects on melanogenesis induced by sun exposure. Colorimetric measurements performed according to the L*a*b* CIE system on 18 different sites enabled us to note in the subjects of Caucasian race of phototypes I and II a more important variation in the parameter a*, directly connected to the redness of skin [143].

  A comparative analysis between the cheeks, forehead, and volar side of the forearm, usually exposed to the sun, showed that the forearm is lighter than the sites on the face, the values of the parameters a* and b* being significantly highest for the forehead [123,140]. Important variations between the measurements performed on a different site of the same anatomical area are also reported, for example, the variation in values a* and b* between distal and proximal forearm [143], or high and low part of the back [104}. For a given race, the parameter L* seems to be slightly influenced by the anatomical site where the measurement is performed [123,140,143].

  The location of the site of measurement is therefore very important during a repeated colorimetric analysis of skin. The interference that results from the variation in pigmentation according to its

exposure to sun UV radiations is very important and can also induce higher deviations than those existing between anatomical locations.

All the experimental studies that resort to colorimetric measurements have to take the incidence of this interference into account on the results recorded.

- *pH*

  To our knowledge, few authors took an interest in the incidence of the site of measurement in the value of skin pH, maybe only because the buffer function of skin does not enable us to note, for the same race, great variations between anatomical sites. However, work conducted by Zlotogorski [105] on 574 male and female Caucasians of different ages showed through repeated measurements that the pH of the cheek (4.2–6.0) is significantly higher than that of the forehead (4.0–5.6), which confirms previous observations [144]. Another study reports no difference between repeated measurements of the pH on the cheek, arm, and calf [63].

- *Electrical Conduction*

  A great number of research studies undertaken to have a better knowledge of the state of skin hydration, notably through the study of its electrical conduction, quickly enabled us to establish that it is not homogeneous on the whole human body. Most of the data refer to the anatomical sites most sensitive to skin drying, which are also the most exposed to external aggressions and particularly to the sun [68].

  The stability of the experimental results obtained depends, in great part, on the choice of the methodology implemented. According to some experimenters, the equipment that measures the capacitance actually seems to supply the most stable data [76,98,107,140].

  All the authors report significant deviations between anatomical areas and generally consider the forehead as the site where capacitance [63,76,98,103,107] and impedance [110] are the highest; the different sites of the face seem to give fairly similar results [98,140].

  Here, again, some researchers have shown that the different sites of a same anatomical area, for example, the dorsal side and volar side of the forearm, which correspond to different morphologies, have unequal conduction. However, these differences also differ according to the race considered [78].

  Here, again, the location of the site of measurement is very important in order that the analysis in the variation of electrical conduction over time remains relevant.

- *TEWL*

  The variation in trans-epidermal water loss according to the anatomical sites explored has been broadly demonstrated. On the whole, the comparative studies have shown a maximal water perspiration on palms followed by the sole of the foot, the back of the hand and then by the different sites of face [98, 103,110,140,145–147]. However, there seems to be no significant deviation between proximal and distal sites of a same geographical area [78,136]. On the other hand, measurements performed comparatively on 5 sites taken symmetrically on the both forearms of 16 subjects of Caucasian race showed the existence of significant deviations between symmetrical sites that do not enable to consider the contralateral site as equivalent concerning its trans-epidermal water loss. This fact is able to question a traditional experimental concept and justifies the randomisation of sites to take this dominance into account, related to the laterality of the subjects that take part in a study [148].

- *Biomechanical Properties*

  The variability of skin thickness and of its structure according to the geographical locations considered clearly has an influence on the biomechanical properties. The value of Young's module is consequently significantly higher on forehead that on forearm [94]. Conversely, the initial tension of skin is higher on the forearm [94]. The extensibility measured on 22 skin sites is the most important on the forehead and the lowest on the foot. This is the same for hysteresis [112].

  Tonicity, plasticity, and elasticity decrease with age in different proportions between sites, the measurements performed over time on the forearms giving the most stable results whatever the dimension of the probes used in an experimental model by extensometry [114].

  The variations in extensibility, elastic recovery, elasticity and viscoelasticity between sites of a same geographical area do not systematically vary in the same way according to the race considered. This is the case concerning the variations noted after measurements performed on the dorsal and volar sides of the forearms of Caucasian, Hispanic, and Black subjects [78].

- *Seborrheic Production*

  The global sebum rate also varies according to the sites as they do not have the same concentration in active sebaceous glands. It is the most important on the forehead, chin area, and upper part of plexus and back [148].

  Actually, there is no divergence concerning the sebum content of the different anatomical sites according to the authors that took an interest in this subject [63,98,140,149].

  For many researchers, this inter-site differences would correspond to different quantities of lipids [151], which have, according to the authors, equivalent [99] or different [150] compositions. This apparent disagreement could actually be explained by the fact that the studies are carried out at different periods of the year as the seasonality influences the contents in lipidic components particularly in Caucasians [151].

CONCLUSION

The resorting to biophysical methods to quantify the instantaneous state of the skin or its evolution under the effect of the aggression of the environment or inversely under the effect of products able to prevent its evolution is justified only when the methodologies implemented enable us to take into account its extraordinary structural and functional diversity.

In fact, to ensure its protective, moisturizing, thermoregulatory, and nutritional roles as well as its keratogenic, melanogenic, and reserve functions that are specific to the different layers it is made up of, skin has, beyond the global specificities related to the race, age, and sex of the subjects, functional specificities that do not allow us to analyze it globally.

Being the main organ in charge of the relation of the whole organism with its external environment, skin has a permanent capacity for adaptation that makes it able to interfere on the experimental data. Its incidence, therefore, has to be systematically taken into account.

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3 Ethnic Differences in Skin Properties*
The Objective Data

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INTRODUCTION

Ethnic differences in skin properties may explain disparities seen in dermatologic disorders and provide insight into appropriate differences in the management of these disorders. However, ethnic differences in skin have been minimally investigated by objective methods, and the data are often contradictory. The current experimental human model for skin is largely based upon physical and biochemical properties known about Caucasian skin. Thus, anatomical or physiological properties in skin of different races that may alter a disease process or treatment of that disease are not being accounted for.

Early studies show similarities in Black and White skin. For example, Thomson[1] and Freeman et al.[2] conclude that the stratum corneum (SC) is of equal thickness in Blacks and Whites. However, in 1974, Weigand et al.[3] demonstrated a difference in Black and White skin with regard to a variable other than color. They demonstrated that the SC of Black skin contains more cell layers and that Black skin requires more cellophane tape strips to remove the SC than White skin. Greater variability in the number of tape strips used within the Black subject pool was also found, compared with the White subject pool, but this variability was not correlated with the degree of skin pigmentation. The mechanisms behind greater intercellular adhesion among Black individuals may involve lipids[59] because the lipid content of the SC ranges from 8.5% to 18.4%, with higher values in Blacks.[25] Since SC thickness is believed to be equal,[12] the data reflected greater intercellular adhesion among the Black individuals.[3] Recently developed quantitative techniques for determining SC mass are yet to be utilized for this purpose.[4]

While Weigand et al.[3] objectively demonstrated a different physical property in Black and White skin, some other studies demonstrating differences used more subjective methods. For example, erythema has been used as a measure of demonstrating skin irritation.[57] Since erythema is difficult to assess in a person with dark skin, such subjective methods are not sufficient in evaluating ethnic disparities. Thus, in order to accurately report ethnic differences in skin properties, objective methods should be utilized. Objective measurements that have been studied are transepidermal water loss (TEWL); water content (WC, via conductance, capacitance, resistance, and impedance); corneocyte variability; blood vessel reactivity; elastic recovery/extensibility; pH gradient; lipid content; surface microflora; electron microscopy and immunoelectron microscopy of mast cell granules; confocal microscopy of epidermal innervation; microtopography; sebaceous function; vellus hair follicle distribution; facial pore attributes; morphology and distribution of melanosomes; and resistance to photodamage.

Given the ethnic diversity in the United States, it is essential to clarify relationships between race, color, ethnicity, and disease process. Even though these objective methods have been used to compare skin of different races, the data that exist remain minimal. Additionally, the data are often confusing and difficult to interpret. We explore and attempt to clarify the objective data available in differentiating skin properties of different races. Objective definitions of skin color are yet to be established. We introduce certain objective differences that have been established to date. We searched MEDLINE; MD Consult; Science Citation Index; the Melvyl Catalogue in the CDL-hosted database of the University of California, San Francisco, California, United States; Yahoo; Google; standard dermatology textbooks; and surge building library files from the University of California, San Francisco, from 1967 to August 2006. From August 2006 to October 2012, an additional search of citations was conducted using MEDLINE, MD Consult, Web of Science, PubMed, Google, and standard dermatology textbooks for relevant literature. Key words in searches included words pertaining to race (i.e., race, ethnicity, Black, African, White, Caucasian, Asian, Hispanic) and dermatology (i.e., skin, skin physiology, skin function). Studies pertaining to ethnic differences in hair were excluded to keep the review focused on skin function/physiology. Words used to describe race/ethnicity of study individuals are the same as those used by the authors in the respective texts.

TEWL

One role of the skin is to maintain an effective barrier against loss of body fluids and absorption of externally applied substances.[8] The total amount of water vapor passing the skin can be divided into water vapor passing the SC by passive diffusion and water vapor loss as a result of sweating.[9] Baseline water diffusion (imperceptible or

* Adapted with permission from *American Journal of Clinical Dermatology* 2003; 4(12): 843–860.

unnoticed perspiration) amounts to 2.25 μL/m²/s and is distinct and separate from sweat gland secretion.[10] Originally, the term *TEWL* was used to indicate the amount of water vapor passing through the SC by passive diffusion.[9] Current literature, however, refers to TEWL as the total amount of water vapor loss through the skin and appendages, under nonsweating conditions.[9] Therefore, note that TEWL is a true reflection of SC barrier function only when there is no sweat gland activity. In addition to characterizing the water barrier function of skin, measurement of TEWL has been utilized widely in studies to perform predictive irritancy tests and to evaluate the efficacy of therapeutic treatments on diseased skin.[11] To date, TEWL is the most studied objective measure in defining differences between the skins of different ethnicities.

Wilson et al.[12] demonstrated higher in vitro TEWL values in Black compared with White skin. Water evaporation measured from skin taken from 10 African American and 12 Caucasian cadavers matched for age and gender was then converted to TEWL using an equation. In addition to finding differences in Black and White skin physiology, the investigators also found that the TEWL of both races increased with skin temperature. These results were explained on the basis of a prior in vivo study from 1941 showing that Blacks had a lower skin and rectal temperature during exercise.[13] Thus, in maintaining equal temperatures between Black and White skin, they concluded that it would be expected that Black skin would have a greater rise in temperature to achieve the same end point temperature and therefore a higher TEWL.[12] Although comparisons between in vitro and in vivo studies are frequently made in medicine, note that the in vitro study may not have accounted for some physiological functions, such as sweating. Also, accounting for physiologic temperature differences by race in skin may be difficult in an in vitro study. Since TEWL depends on passive water vapor loss, and is based on laws of physics regarding passive diffusion, the rate of water vapor diffusion across the SC is theoretically directly related to the ambient relative humidity and temperature;[14] thus, it is reasonable to assume that the increased TEWL in Black skin is associated with an increase in temperature if, in fact, a difference in Black and White skin temperature does exist.

A subsequent in vivo study by Berardesca and Maibach[20] supported the findings of the in vitro study. The investigators determined the difference in irritation between young Black and White skin. They applied 0.5% and 2.0% sodium lauryl sulfate (SLS), a water soluble irritant (surfactant), to untreated, preoccluded, and predelipidized skin and quantified the resulting level of irritation using WC, TEWL, and laser Doppler velocimetry (LDV) of the SC. No statistical difference was found in irritation between the two groups based on WC and LDV; however, a statistical difference in the TEWL results of 0.5% sodium lauryl sulfate applied to the preoccluded skin was found. In that test, Blacks had 2.7 times higher TEWL levels than Whites ($P < .04$), suggesting that Blacks in the preoccluded state are more susceptible to irritation than Whites. This theory opposes the traditional clinical view, based on observing erythema,[7] that Blacks are less reactive to irritants than Whites.

Berardesca and Maibach[21] used the same model to compare differences in irritation between Hispanic and White skin. Although there were no significant differences in TEWL, WC, or LDV between the groups at baseline, the data showed higher values of TEWL for Hispanics compared with Whites after sodium lauryl sulfate-induced irritation. However, these values were not statistically significant. The investigators noted that the reaction of Hispanic skin to sodium lauryl sulfate resembles that of Black skin when irritated with the same substance.[20] Since skin pigmentation varies greatly within the Hispanic and Black communities, the degree of skin pigmentation, according to Fitzpatrick's[24] model, could represent an important variable.

However, in a later study, Berardesca et al.[15] found no significant difference in vivo in TEWL between races or anatomic sites for baseline observation. The investigators examined 15 Blacks, 12 Whites, and 12 Hispanics to account for degree of skin pigmentation, matched for age and gender, and measured TEWL, WC (via skin conductance), skin thickness, and biomechanical properties, such as skin extensibility, at two sites, the dorsal and volar forearm. Skin sites that vary in sun exposure were used to highlight the protective effects of melanin from ultraviolet (UV)-induced damage. Ethnic differences in skin conductance (Blacks > Whites) and skin elasticity were found and are discussed in "TEWL" and "Blood Vessel Reactivity" sections. However, even though the investigators expected a higher TEWL in Blacks, based on previous studies[12,20] and based on a higher WC (skin conductance) in Blacks found in their current study, no significant difference in TEWL was found between races or anatomic sites. They accounted for the higher WC in Black skin with no ethnic differences in TEWL on the basis that Black skin might have increased intercellular cohesion[3] and increased lipid content,[25] keeping the water in. In a subsequent study by Fotoh et al.[89] a photoexposed site (forehead) and a nonphotoexposed site (volar forearm) were also analyzed for differences among 25 sub-Saharan African Blacks or Caribbean Blacks, 25 African or Caribbean mixed races from intermarriage between Black African or Black Caribbean and White European Caucasian, and 25 European Caucasians. As was found by Berardesca et al.[15] there was no significant difference in TEWL found between races ($P > .05$) at either site. Fotoh et al. did find the forehead TEWL to be greater than the volar forearm TEWL for each group ($P < .001$), implying decreased barrier function on photoexposed sites.

In contrast, Kompaore et al.[17] found significantly higher TEWL values in Blacks and Asians compared with Whites. After application of methyl nicotinate (a vasodilator), the investigators evaluated TEWL and lag time to vasodilatation by LDV, before and after removal of the SC by tape stripping. The participants were seven Black men, eight White subjects (six male and two female), and six Asian men all living in France, aged 23–32 years, without skin disease. Before tape stripping, TEWL was 1.3 times greater in Blacks and Asians compared with Whites ($P < .01$); no difference

was found between Blacks and Asians. After eight and 12 tape strips, TEWL values were highest in Asians overall (Asians 1.7 times greater than Whites, $P < .05$). The investigators concluded that, similar to previous studies,[12,20] skin permeability measured by TEWL was higher in Blacks than in Caucasians. However, they also concluded that Asian skin had the highest permeability among the groups studied. Although the methods of this study were impressive and well documented, this finding has not yet been duplicated with statistical significance, despite similar patterns having been found. In 2012, Luther et al.[90] examined TEWL values of the volar forearm in six Caucasian (phototypes II–III), six African (phototypes V–VI), and six Asian (phototype IV) participants. The levels of respective TEWL values followed a similar pattern to those found by Kompaore et al.[17] with Asian TEWL values greater than African TEWL values, which were, in turn, greater than Caucasian TEWL values. These results, however, were not found to be statistically significant ($P > .05$).

Sugino et al.[19] (abstract only) also included Asians in their study but found that baseline TEWL was, in decreasing order, Blacks > Caucasians > Hispanics > Asians. Aramaki et al.[18] compared TEWL, SC hydration, sebum secretion, laser Doppler flowmetry, content of melanin, and erythema on the forearm at baseline and after sodium lauryl sulfate-induced irritation in 22 Japanese women (mean age 25.84 years) and 22 German women (mean age 26.94 years). There were no significant differences in TEWL between Japanese and German women before or after sodium lauryl sulfate stress. Another study (unpublished data) referenced in a review article[26] about Asian skin compared TEWL in Asians and Caucasians and also found no statistically significant differences at baseline or after tape stripping; however, no vasoactive substance was applied.

In an attempt to compare the degree of skin pigmentation as opposed to race, Reed et al.[22] compared seven subjects with skin types V and VI (four African Americans, two Filipinos, and one Hispanic) to 14 subjects with skin types II and III (six Asians and eight Caucasians). The investigators used TEWL to assess the ability of the SC to withstand or recover from insults to the epidermal permeability barrier (i.e., tape stripping). Subjects with skin type V/VI required more tape strippings (66.7 ± 6.9) compared to skin type II/III (29.6 ± 2.4) to achieve the same TEWL, that is, skin type V/VI had increased barrier strength (integrity). These findings correlate with those of Weigand et al.[3] that Black skin has more cell layers and increased intercellular adhesion. Furthermore, it was also found that water barrier function (measured by TEWL) in skin type V/VI recovered more quickly. This study demonstrated differences in SC barrier function as measured by TEWL between different skin types possibly independent of race. Since the sample size with skin types V and VI was small, further studies with larger sample sizes were needed to support these findings. In 2009, Gunathilake et al.[91] found that 51.7 ± 5.4 versus 20.1 ± 0.5 tape strippings were required to increase TEWL threefold at the volar forearm in 20 Fitzpatrick type IV–V versus 20 type I–II subjects, respectively ($P < .0001$). Muizzuddin et al.[92] further corroborated these findings in their study comparing African American, Caucasian, and East Asian females. Muizzuddin et al.[92] found that the skin of African Americans required the most strippings to reach the threshold TEWL value (10.3 ± 2.2, $P < .001$). In addition, Caucasian skin (6.1 ± 2.4 strippings) was demonstrated to have a significantly stronger barrier than East Asian skin (3.7 ± 1.8 strippings, $P < .001$).

Warrier et al.[16] recognized the discrepancies in data comparing skin of Blacks and Whites. Thus, in an attempt to clarify the data, the investigators studied TEWL, electrical capacitance, skin pH, elasticity, dryness/scaling, and skin microflora in 30 Black and 30 White women aged 18–45 years. In contrast to previous studies that found an increase in TEWL in Blacks compared with Whites,[12,17,20] Warrier et al.[16] found TEWL to be significantly lower on the cheeks (20% less) and legs (17% less) of Blacks compared with Whites ($P < .05$). TEWL was also lower on the forearms in Blacks, but this was not statistically significant. Prior studies examined the forearm, inner thigh, and back. Does the anatomic site act as a confounding variable in obtaining TEWL values? In a study on Caucasian subjects, TEWL values of the posterior auricular and forehead SC were higher than of the SC of the arm, forearm, or abdomen.[27] Thus, perhaps there are also differences in TEWL when comparing the sites examined (cheeks and lower legs) by Warrier et al.[16] to those of prior studies (forearm, inner thigh, and back).[12,15,17,20,21] Although this study used a larger sample size, the discrepancy in data warrants further studies with large sample sizes and comparisons of various anatomic sites.

Further evidence for differences in TEWL values at the facial cheek arose through a study performed by Muizzuddin et al.[92] which examined TEWL values of the right and left facial cheeks in 73 African American, 77 Caucasian, and 67 East Asian females. As demonstrated by Warrier et al.[16] TEWL values for the facial cheek were found to be lower in African American skin when compared to Caucasian skin ($P < .001$), indicating a relatively weaker barrier in Caucasian skin. East Asian TEWL values were also found to be significantly higher than those of African Americans ($P < .001$). Finally, Caucasian females exhibited slightly higher, although still significant, TEWL values than East Asian females ($P < .001$).

Berardesca et al.[23] examined differences in TEWL as well as pH in 10 Caucasian (skin types I and II) and eight African American (skin type VI) women at baseline and after tape strippings. TEWL increased for both races with each tape stripping. Interestingly, even though Black women had a higher TEWL at baseline and after each tape stripping compared with Caucasian women, the differences were only statistically significant (1.2 times greater) after three ($P < .05$) and six ($P < .03$) tape strips. Similar to the study by Reed et al.[22] it was also found that recovery of water barrier function, as measured by TEWL 48 h after stripping, was greater in Blacks as compared with Caucasians, but the difference was not statistically significant. Mohammed et al.[93] examined differences in TEWL at various depths via tape strippings in 12 Caucasian and 10 Black subjects as well.

TEWL values became greater with increasing number of tape strips removed ($P < .05$). As observed in the experiment performed by Berardesca et al.[23] there were no statistical differences in TEWL measurements between Black and Caucasian subjects at all depths measured at the mid-ventral forearm ($P > .1$).

Tagami[60] provided additional information on Asian skin by comparing TEWL between 120 Japanese and 322 French women, aged 20–70 years. The skin type of the French women was not specified. His research team measured TEWL on the cheeks and mid-flexor surface of forearms of all subjects, under the same environmental conditions. Although, TEWL was lower in Japanese women, the data were not statistically significant. These findings further supported those of Aramaki et al.[18] Yamashita et al.[94] however, presented statistically significant differences in TEWL values between 92 Japanese and 104 French men and women. Researchers found that Japanese subjects had lower TEWL values when compared to those of French subjects at both the inner upper arm and dorsal hand ($P < .01$).

More information about TEWL values in Asian skin was elucidated when Jung et al.[95] examined the effects of skin pH on wrinkle formation in Korean, Vietnamese, and Singaporean subjects. Upon measuring TEWL values of the front cheek in 110 Korean, 100 Vietnamese, and 100 Singaporean females, aged 20–35 years, Jung et al.[95] determined that Vietnamese skin had the lowest observed TEWL values. While TEWL values in Korean skin were significantly greater than those of both Vietnamese and Singaporean skin ($P = .000$), Singaporean TEWL levels were not significantly greater than those found in Vietnamese skin ($P = .395$).

Hicks et al.[61] grouped patients on the basis of skin color (Fitzpatrick skin type), as in Reed et al.[22] in studying the difference between susceptibility of Black and White skin to irritant contact dermatitis (ICD). The 14 participants were grouped as eight Whites (skin types II/III) and six Blacks (skin types V/VI), between the ages of 18 and 40 years. The skin on the volar forearm was exposed to 4% and 1% sodium lauryl sulfate and evaluated by reflectance confocal microscopy, TEWL, LDV, and routine histology at 6, 24, and 48 h after initial application. Changes in TEWL and SC thickness after exposure to 4% SLS at 48 h were negatively correlated in both groups. White participants showed a trend toward greater mean increases in TEWL after SLS exposure than Black participants, supporting the possibility that the barrier function in Black skin is more durable than in White skin, but the differences were not statistically significant. Overall, results from all methods of evaluation suggested reduced susceptibility of Black skin to ICD. However, while there was no significant difference between SC thickness of control sites in both groups (consistent with Weigand et al.[3]), the SC thickness was significantly less in Blacks as compared to Whites after exposure to 4% SLS at 48 h ($P < .05$). This pattern of SC thinning seems to contradict the findings of reduced susceptibility of Black skin to ICD. A larger sample size may be necessary to clarify this discrepancy and achieve a statistically significant trend in TEWL changes.

In another evaluation of differences between African American and White skin, Grimes et al.[62] did not find significant differences in TEWL in vivo. The subjects were comprised of 18 African American and 19 White adults between the ages of 35 and 65 years, with a subset of eight (three Black, five White) participating in chemical challenge of 5% SLS. Methods of evaluation included clinical evaluation and instrumental measurements of sebum level, pH, moisture content, and TEWL. Although there were differences in visual assessment of photoaging and hyperpigmentation, the baseline instrumental findings from all methods indicated no significant differences between African American and White skin. Within 6 h of irritation, there was a significant change in TEWL in White participants; however, after 24 h, TEWL measurements in both groups were similar. Due to the small sample size of the chemical challenge subset, statistical analysis on this data was not performed. The overall findings of this study support the postulation that, objectively, there is little difference between African American and White skin. However, again, based on small sample size, it is difficult to make definitive conclusions based on the data.

Pershing et al.[75] found a significant difference in TEWL between Caucasians and Asians with topical application of capsaicinoids. The study measured TEWL, skin surface temperature, and erythema after application of various capsaicinoid analogs at various concentrations on volar forearms of six Caucasians and six Asians aged 19–63 years. The results were baseline adjusted and control site corrected over time for each parameter to normalize data for inherent differences among skin sites. Increasing concentrations of total capsaicinoid were not associated with a proportional change in TEWL in either Caucasians or Asians. However, a capsaicinoid concentration of 16 mg/mL produced statistically less TEWL in Asians than Caucasians ($P < .05$); specifically, there was an increase in the mean TEWL in Caucasians but a decrease in Asians. The investigators concluded that changes in TEWL between Caucasians and Asians with capsaicinoids, but not irritants (such as SLS in Aramaki et al.[18]), may reflect the effect of vehicle composition (isopropyl alcohol for capsaicin vs. water for irritants) or other physiologic skin functions (such as cutaneous blood flow) in determining TEWL.

Astner et al.[76] evaluated ethnic variability in skin response to a household irritant (Ivory dishwashing liquid) by applying the irritant to the anterior forearms of 15 Caucasian subjects and 15 African American subjects. The investigators observed significantly higher mean values for TEWL in Caucasians compared to African Americans ($P \leq .005$) like Warrier et al.[16] had found previously. The 30 participants were patch tested to graded concentrations of Ivory soap and evaluated with clinical scoring, reflectance confocal microscopy, TEWL, and fluorescence excitation spectroscopy. There was a positive, dose-dependent correlation between TEWL values and irritant concentration in all groups. However, not only was the mean TEWL higher in Caucasians, but the relative increment of increase in response to the graded irritant concentrations was also higher

in Caucasians when compared to African Americans ($P \leq .005$). The researchers suggested that the lower values of TEWL in African Americans in this study may reflect the greater intercellular cohesiveness in African American skin.[3]

The notion that TEWL values are higher in Caucasians than in African Americans was further corroborated by studies performed by Gupta et al.[96] Chu and Kollias,[97] and Pappas et al.[98] Gupta et al.[96] sought to measure TEWL values in 138 White and 112 African American children in order to determine whether children with atopic dermatitis have altered skin barrier function. Their study found that TEWL values were elevated in children with atopic dermatitis compared to control groups ($P < .05$), suggesting compromised skin barrier function in children with atopic dermatitis. Through their procedures, Gupta et al.[96] also noted upon measuring TEWL values at the dorsal arm, volar forearm, lower leg, and cheek that White children exhibited consistently higher TEWL levels than African American children at the volar forearm. These results were statistically significant. Chu and Kollias[97] measured TEWL values of the dorsal forearm and upper inner arm in 84 Caucasian and 67 African American females, representative of a normal Central New Jersey population. Caucasian females displayed significantly higher TEWL levels than African American participants ($P < .05$). Chu and Kollias[97] discerned that lower TEWL levels in African Americans could possibly be a consequence of a more compact SC and greater lipid content than in Caucasians. Pappas et al.[98] (abstract only), noted a similar relationship with TEWL measurements of the face, with White participants exhibiting significant greater TEWL levels than African Americans ($P < .05$). Like Chu and Kollias,[97] Pappas et al.[98] suggested that differences in lipid composition may contribute to differences in barrier functions between ethnicities.

While the data regarding TEWL (summarized in Table 3.1) are conflicting, the overall evidence, except for the 1991 study by Berardesca et al.[15] and, later, the studies by Hicks et al.[61] Grimes et al.[62] Fotoh et al.[89] Luther et al.[90] and Mohammed et al.[93] supports some difference between Black and Caucasian skin. Most older studies using the forearm, back, and inner thigh[12,17,19,20,22,23] showed a greater TEWL in Blacks compared with Whites; however, more recent studies by Warrier et al.[16] Gupta et al.[96] Muizzuddin et al.[92] Chu and Kollias,[97] and Pappas et al.[98] which mostly possessed larger sample sizes than older studies, found TEWL to be less in Blacks than Whites at multiple anatomic sites. In addition, like Warrier et al.[16] a smaller study by Astner et al.[76] found the mean TEWL on forearms of Whites to be greater than those on Blacks after irritant stress. Perhaps the anatomic site examined causes discrepancies in TEWL values. Also, TEWL measurements with regard to Asian skin may be deemed inconclusive, as baseline measurements have found Asian skin to have TEWL values that are equal to Black skin and greater than Caucasian skin,[17] less than Caucasian skin and greater than African American skin,[92] less than all other ethnic groups,[19] and no different than other ethnic groups.[18,26,60,90] Additionally, Pershing et al.[75] found an increase in TEWL of Caucasians but a decrease in TEWL of Asians in response to high-potency capsaicinoids, the results of which are difficult to categorize.

If water barrier function truly depends on the degree of pigmentation, this has implications as to whether the SC gains or loses barrier integrity in cases of acquired hyperpigmentation or hypopigmentation. Further, differences in barrier integrity/function, as measured by TEWL, also have implications in the ability of people with different skin types and colors to withstand and recover from environmental insults as well as the ability to absorb topical therapeutic agents. Furthermore, TEWL may vary under different pathologic and physiologic conditions. Thus, the health and physiologic state of the subjects should be noted in future studies.

WC

WC or hydration of the skin can be measured by several methods including skin capacitance, conductance, impedance, and resistance. Using capacitance to measure WC is based on the high dielectric constant of water compared with other substances.[28] Conductance is also based on the changes in the electrical properties of the SC when the skin is hydrated.[29] Dry SC is a medium of weak electrical conduction, while hydrated SC is more sensitive to the electrical field.[28] Resistance is the reciprocal of conductance. In general, skin capacitance and conductance show similar behavior with regard to measuring WC of the skin, while resistance and impedance are opposite. Possible sources of error or variation in measurement include sweat production, filling of the sweat gland ducts, the number of hair follicles, the electrolyte content of the SC, and artifacts from applied topical agents.[29]

In 1962, Johnson and Corah[30] found that Blacks had higher levels of skin resistance at baseline than Whites ($P < .01$) at two different laboratories in St. Louis, Missouri, United States, and San Diego, California, United States. The St. Louis study examined 174 children (22 Black boys, 32 Black girls, 65 White boys, and 55 White girls), aged 83–92 months, while the San Diego study examined 42 subjects (16 Black men, five Black women, 16 White men, and five White women), with a mean age of 23 years. While the investigators in this study did not correlate their measurements of skin resistance to WC in the skin, knowing the relationship of skin resistance to WC, we can deduce that a higher resistance in Blacks may be correlated with a lower WC.

In 2010, El-Sheikh et al.[99] examined skin conductance levels (SCLs) in 128 girls and 123 boys (mean age 8.23 ± 0.73 years), of whom 64% were European American (EA) and 36% were African American (AA). From their procedures, El-Sheikh et al.[99] observed consistently higher SCLs in EA children than in AA children. The higher SCLs found in European American children were presumed to be a result of the inverse relation between the number of sweat glands and darker skin pigmentation.[99] An additional novel component

TABLE 3.1
Transepidermal Water Loss[a]

Study	Technique	Subjects	Site	Results
Wilson et al.[12] (1988)	In vitro	Blacks: 10 (mean age 38.6 years) Caucasians: 12 (mean age 41.1 years)	Inner thigh	TEWL: Blacks 1.1× > Caucasians (mean corrected log TEWL 2.79 and 2.61 μg/cm²/h, respectively; $P < .01$ for both values)
Berardesca and Maibach[20] (1988)	In vivo—topical application of SLS (irritant)	Black men: 10 (age 29.9 ± 7.2 years) White men: 9 (age 30.6 ± 8.8 years)	Back	No significant difference in TEWL between Blacks and Whites at baseline After SLS stress: TEWL: Blacks (untreated, preoccluded, and predelipidized) > Whites but only statistically significant (2.7× greater) for 0.5% SLS applied in the preoccluded area ($P < .04$)
Berardesca and Maibacj[21] (1988)	In vivo—topical application of SLS (irritant)	Hispanic men: 7 (age 27.8 ± 4.5 years) White men: 9 (age 30.6 ± 8.8 years)	Upper back	No significant differences in TEWL between Hispanics and Whites at baseline After SLS stress: TEWL Hispanics (untreated, preoccluded, and predelipidized) > Whites but not statistically significant
Berardesca et al.[15] (1991)	In vivo	Blacks: 15 (mean age 46.7 ± 2.4 years) Whites: 12 (mean age 49.8 ± 2 years) Hispanics: 12 (mean age 48.8 ± 2 years)	Volar and dorsal forearm	No significant difference in TEWL between site or ethnicity at baseline
Kompaore et al.[17] (1993)	In vivo—topical application of MN (vasodilator)	Blacks: 7 Caucasians: 8 Asians: 6 (ages 23–32 years, all)	Volar forearm	MN given before tape stripping: TEWL: Blacks and Asians 1.3× > Caucasians ($P < .01$); no difference between Blacks and Asians After 8 and 12 tape strips: TEWL: Asians > Blacks > Caucasians ($P < .05$, Asians 1.7× greater than Caucasians)
Sugino et al.[19] (1993)	In vivo	Blacks, Caucasians, Hispanics, Asians (no. of subjects, ages not specified)	Not documented	Baseline TEWL: Blacks > Caucasians ≥ Hispanics ≥ Asians
Reed et al.[22] (1995)	In vivo	Skin type V/VI: African American: 4 Filipino: 2 Hispanic: 1 Skin type II/III: Asian: 6 Caucasian: 8 (ages 22–38 years, all)	Volar forearm	Skin type V/VI required more tape strippings (66.7 ± 6.9) compared to skin type II/III (29.6 ± 2.4) to achieve the same TEWL, i.e., skin type V/VI had increased water barrier strength (integrity) Barrier function in skin type V/VI recovered more quickly
Warrier et al.[16] (1996)	In vivo	Black women: 30 White women: 30 (ages 18–45 years, all)	Left and right medial cheeks, mid-volar forearms, lateral mid-lower legs	TEWL: Blacks < Whites on cheeks (20% less) and legs (17% less) at baseline ($P < .05$); also lower on forearm but not statistically significant
Berardesca et al.[23] (1998)	In vivo	Black women: 8 Caucasian women: 10 (mean age 42.3 ± 5 years, both)	Mid-volar forearm	After tape stripping: TEWL: Blacks 1.2× > Caucasians after 3 ($P < .05$) and 6 tape strips ($P < .03$)

Aramaki et al.[18] (2002)	In vivo—topical application of SLS (irritant)	Japanese women: 22 (mean age 25.84 years) German women: 22 (mean age 26.94 years)	Forearm	No significant difference at baseline or after SLS stress
Tagami[60] (2002)	In vivo	Japanese women: 120 French women: 322 (ages 20–70 years, all)	Cheeks and mid-flexor forearm	TEWL: Japanese < Whites but not statistically significant
Hicks et al.[61] (2003)	In vivo—topical application of 1% and 4% SLS (irritant)	White: Skin type II: 6 Skin type III: 2 Black: Skin type V: 5 Skin type VI: 1 (ages 18–40 years, all)	Volar forearm	TEWL: Whites > Blacks but not statistically significant
Grimes et al.[62] (2004)	In vivo—topical application of 5% SLS (irritant)	African American: 18 White: 19 (ages 35–65 years, female, all) SLS stress: African American: 3 White: 5	Inner forearm	Baseline: No significant difference After SLS stress: immediate increase in TEWL of White subjects, but increase no longer evident after 24 h and found to be similar to African Americans (not statistically significant)
Pershing et al.[75] (2006)	In vivo—topical application of capsaicinoid analogs	Caucasians: Male: 3 Female: 3 Asians: Male: 3 Female: 3 (ages 19–63 years, all)	Volar forearm	Increasing concentrations of total capsaicinoid not associated with proportional change in TEWL, in all subjects Capsaicinoid concentration of 16 mg/mL produced ↑ mean TEWL in Caucasians, ↓ mean TEWL in Asians ($P < .05$)
Astner et al.[76] (2006)	In vivo—topical application of Ivory soap (irritant)	Caucasians: 15 (skin type II/III) African Americans: 15 (skin type V/VI) (ages 18–49 years, all)	Anterior forearm	Positive dose-dependent correlation between TEWL and irritant concentration: mean TEWL: Caucasians > African Americans ($P \leq .005$) Relative increment of increase in TEWL after irritant: Caucasians > African Americans ($P \leq .005$)
Gupta et al.[96] (2008)	In vivo	White children: 138 African American children: 112 (ages >3 years, all)	Volar forearm, dorsal arm, lower leg, cheek	TEWL: White children > African American children, statistically significant at volar forearm
Fotoh et al.[89] (2008)	In vivo	Black African or Caribbean women: 25 African or Caribbean mixed-race women: 25 European Caucasian women: 25 (ages 20–32 years, all)	Forehead and volar forearm	TEWL: No significant difference between Black, African, or Caribbean mixed-race and Caucasian healthy women ($P > .05$)

(*continued*)

TABLE 3.1 (Continued)
Transepidermal Water Loss[a]

Study	Technique	Subjects	Site	Results
Gunathilake et al.[91] (2009)	In vivo—tape strippings	Epidermal integrity was assessed in two groups of healthy volunteers, each having 20 subjects with type I–II (age 31.8 ± SD 6.9 years) and type IV–V (age 32.4 ± SD 9.7 years) skin	Volar forearm	51.7 ± 5.4 vs. 20.1 ± 0.5 tape strippings required to increase TEWL threefold in type IV–V vs. I–II subjects, respectively; $P < .0001$
Muizzuddin et al.[92] (2010)	In vivo—sticky tape (irritant)	African Americans: 73 Caucasians: 77 East Asians: 67 (ages 18–45 years, female, all)	Right and left facial cheek	TEWL: Caucasians > East Asians > African Americans ($P < .001$) Tape stripping: skin of African Americans required most strippings to reach threshold TEWL (10.3 ± 2.2, $P < .001$); Caucasian skin significantly stronger barrier than East Asian skin
Chu and Kollias[97] (2011)	In vivo	Caucasian females: 84 African American females: 67 (ages 14–75 years, all)	Dorsal forearm and upper inner arm	TEWL: Caucasians > African Americans ($P < .05$)
Pappas et al.[98] (2011)	In vivo	White, African American, and East Asian females (no. of participants not indicated) (ages 18–25 or 35–35 years, all)	Face	TEWL: White > East Asian > African American, statistically significant difference between Whites and African Americans ($P < .05$)
Mohammed et al.[93] (2011)	In vivo—tape strippings	Black subjects: 10 Caucasian subjects: 12 (ages 20–58 years, all)	Mid-ventral forearm	No statistical differences in TEWL between Black and Caucasian subjects at all depths (t-test, $P > .1$)
Yamashita et al.[94] (2012)	In vivo	Japanese (mean age 41.1 ± 12.8 years): 43 males, 49 females French (mean age 40.4 ± 14.4 years): 52 males, 52 females	Cheek, dorsal aspect of hand, inner upper arm	TEWL: Japanese < French, statistically significant difference at inner upper arm and dorsal hand ($P < .01$)
Luther et al.[90] (2012)	In vivo	Caucasian: 6 (phototypes II–III) (mean age 28.1 ± 4.3 years) African: 6 (phototypes V–VI) (mean age 27.3 ± 3.2 years) Asian: 6 (phototype IV) (mean age 23.5 ± 1.6 years)	Volar forearm	TEWL Asian > African > Caucasian but not statistically significant ($P > .05$)
Jung et al.[95] (2012)	In vivo	Korea (K): 110 Vietnam (V): 100 Singapore (S): 100 (ages 20–35 years, female, all)	Front cheek	TEWL ($g/m^2/h$): K (18.45 ± 5.44), V (14.65 ± 2.61), S (15.4 ± 3.47)

Note: MN = methyl nicotinate; SLS = sodium lauryl sulfate.

[a] Berardesca et al.[15] Hicks et al.[61] Grimes et al.[62] Fotoh et al.[89] Luther et al.[90] and Mohammed et al.[93] all found no significant difference between Blacks and Whites. Warrier et al.[16] Astner et al.[76] Gupta et al.[96] Muizzuddin et al.[92] Chu and Kollias,[97] and Pappas et al.[98] found Blacks < Whites. All other evidence supports TEWL: Blacks > Whites. TEWL measurements of Asian skin are inconclusive as they have been found to be equal to Black skin and greater than Caucasian skin,[17] less than Caucasian skin and greater than African American skin,[92] equal to Caucasian skin,[18,60] and less than all other ethnic groups.[19] Pershing et al.[75] found an increase in TEWL of Caucasians but a decrease in TEWL of Asians in response to high concentrations of topical capsaicinoids.

of this study included comparisons of ethnic SCL trajectories as children of different ethnicities developed. Graphing analyses showed that EA boys possessed SCL levels at age eight years higher than AA boys and had significant negative slopes as they aged, while trajectories of SCL for AA boys were shallow and did not display considerable growth over time. Neither AA nor EA girls exhibited significant SCL changes over development. Implications of these various developmental changes in SCL patterns for different ethnicities require further study.

In addition to comparing TEWL, Berardesca and Maibach also compared WC by capacitance before and after topical administration of sodium lauryl sulfate in Blacks and Whites and, in another study, in Hispanics and Whites.[20,21] There were no significant differences in WC between Blacks and Whites at baseline or after sodium lauryl sulfate stress.[20] In comparing Hispanics and Whites, there was an increase in WC in Hispanics at baseline, but the difference was not significant; however, after sodium lauryl sulfate application, they found a significant increase in WC in Hispanics compared with Whites when a negative visual score (i.e., no erythema) was given for irritation ($P < .01$).[21] In reviewing the data, however, we found that the although the mean values for WC in Hispanics were greater than in Whites, the standard deviations (SDs) were also large. When an irritant reaction was visually detectable, the WC was proportionally increased in both races, eradicating a difference between them.

Berardesca et al.[15] examined WC by conductance on the volar and dorsal forearm of 15 Blacks, 12 Whites, and 12 Hispanics in addition to examining TEWL, skin thickness, and extensibility. Within each race studied, significant differences existed in WC between the volar and dorsal forearms (Table 3.2). Whites and Hispanics demonstrated decreased WC on the dorsal aspect of each arm compared with the volar side (22% less and 11% less, respectively), whereas Blacks demonstrated a 13% decrease in WC on the volar aspect compared with the dorsal side. The differences, however, were statistically more relevant for White skin ($P < .001$) and less for Blacks ($P < .02$) and Hispanics ($P < .05$). In comparing the races with each other, Blacks and Hispanics had increased WC compared with Whites on the dorsal forearm. On the volar forearm, however, Hispanics demonstrated greater WC than Blacks and Whites. Their findings do not correlate with those of the prior studies; however, this study measured WC at baseline using conductance, whereas the prior studies measured WC at baseline using resistance,[30] and at baseline and after sodium lauryl sulfate stress using capacitance.[20,21] The variability in WC observed between site and race is difficult to interpret. The investigators noted, however, that the White subjects had an increased amount of hair on the forearms compared with the other two groups, possibly accounting for some differences in the results.

Sugino et al.[19] measured WC with an impedance meter in Blacks, Whites, Hispanics, and Asians. They found that WC was highest in Asians compared with Caucasians, Blacks, and Hispanics. The exact values and study size were not documented. The investigators correlated high WC with high ceramide and low TEWL values also measured in their study. In a later study, Diridollou et al.[100] measured WC by capacitance in younger (mean age 36 ± 9 years) and older (mean age 61 ± 8 years) African American, Chinese, Caucasian, and Mexican American women, for a total of 311 participants, at the dorsal and ventral forearms. Skin dryness was not significantly different among ethnicities for the younger group of participants for both anatomic sites tested. Among the older participants, the dryness index at the dorsal forearm was greater in African American ($P < .05$) and Caucasian ($P < .05$) women than Chinese women. No other significant differences between ethnic groups were observed. Among older participants being tested at the ventral forearm, the dryness index was determined to be higher in African American women than Mexican ($P < .01$) and Chinese ($P < .001$) women, with no other statistically significant differences noted. Only African American and Caucasian women exhibited significantly higher dryness indices with age. Of note, dryness was significantly higher on the sun-exposed dorsal site in comparison to the less exposed ventral site in lighter skin (Chinese and Caucasian skin), but no difference in skin dryness was found in darker African American and Mexican skin. Diridollou et al.[100] suggest that this may be due to melanin having a protective effect.

Warrier et al.[16] examined WC by capacitance at baseline in 30 Black and 30 White women aged 18–45 years. Black women had a significantly higher WC on the cheeks ($P < .05$), but there were no significant differences at baseline between Blacks and Whites on the forearms and the legs. They proposed that the difference found on the cheeks might be related to evidence of more elaborate superficial vasculature and more apocrine and mixed eccrine–apocrine glands in facial skin of Blacks,[32] as well as to differences in melanin content, the packaging of melanocytes, and their ability to prevent epidermal photodamage.[33,35]

Manuskiatti et al.[31] studied seven Black and five White women with a mean age of 25.8 ± 4.2 years and five Black women and five White women with a mean age of 64.7 ± 3.8 years and measured WC (by capacitance) as well as desquamation index, as a measure of skin scaling, on the preauricle, posterior neck, dorsal upper arm, dorsal forearm, volar forearm, lower back, abdomen, thigh, and lower leg. The results of the desquamation index are discussed in "WC" section. They found no ethnic differences in WC but did find significant differences between the younger and older women (younger women had higher WC than older women).

Sivamani et al.[65] compared differences in friction coefficient, impedance, and amplitude/mean calculation of friction coefficient curves between Caucasian, African American, Hispanic, and Asian subjects. Participants included 22 Caucasians, 14 African Americans, 14 Hispanics, and nine Asian volunteers, aged 18 to 60 years. In addition to measuring baseline differences, the researchers assessed differences in response to polyvinylidene chloride occlusion, topical petrolatum, and topical glycerin applied to the volar forearm, based on gender, age, and ethnicity. Baseline measurements showed no significant differences in impedance

TABLE 3.2
Water Content[a]

Study	Technique	Subjects	Site	Results
Johnson and Corah[30] (1962)	In vivo—resistance	St. Louis study: Black boys, 22; Black girls, 32; White boys, 65; White girls, 55 (ages 83–92 months, all) San Diego study: Black men, 16; Black women, 5; White men, 16; White women, 5 (mean age 23 years, all)	First and third fingers of right hand	Skin resistance: Blacks > Whites at baseline ($P < .01$), i.e., Blacks have lower water content
Berardesca and Maibach[20] (1988)	In vivo—topical application of SLS (irritant)—capacitance	Black men, 10 (age 29.9 ± 7.2 years) White men, 9 (age 30.6 ± 8.8 years)	Back	No significant differences between Blacks and Whites at baseline or after SLS stress
Berardesca and Maibach[21] (1988)	In vivo—topical application of SLS (irritant)—capacitance	Hispanic men, 7 (age 27.8 ± 4.5 years) White men, 9 (age 30.6 ± 8.8 years)	Upper back	No significant differences between Hispanics and Whites at baseline After SLS stress: Hispanics > Whites when negative visual score was given for irritation ($P < .01$) (large standard deviations)
Berardesca et al.[15] (1991)	In vivo—conductance	Blacks, 15 (mean age 46.7 ± 2.4 years) Whites, 12 (mean age 49.8 ± 2 years) Hispanics, 12 (mean age 48.8 ± 2 years)	Volar and dorsal forearm	Blacks (13% less) volar < dorsal forearm ($P < .02$) Whites (22% less): dorsal < volar forearm ($P < .001$) Hispanics (11% less): dorsal < volar forearm ($P < .05$) Black and Hispanics > Whites on dorsal forearm at baseline Hispanics > Blacks and Whites on volar forearm at baseline
Sugino et al.[19] (1993)	In vivo—impedance	Blacks, Caucasians, Hispanics, Asians (no. of subjects, ages not specified)	Not documented	Asians > Caucasians, Blacks, and Hispanics
Warrier et al.[16] (1996)	In vivo—capacitance	Black women, 30 White women, 30 (ages 18–45 years, both)	Left and right medial cheeks, mid-volar forearms, lateral mid-lower legs	Blacks > Whites on cheeks at baseline ($P < .05$) No significant difference between two groups on forearms and legs
Manuskiatti et al.[31] (1998)	In vivo—capacitance	Black women, 7 White women, 5 (mean age 25.8 ± 4.2 years, both) Black women, 5 White women, 5 (mean age 64.7 ± 3.8 years, both)	Preauricle, posterior neck, dorsal upper arm, dorsal forearm, volar forearm, lower back, abdomen, thigh, lower leg	No significant differences between Blacks and Whites at baseline

Sivamani et al.[65] (2003)	In vivo—impedance, topical application of petrolatum and glycerin	White, 22 African American, 14 Hispanic, 14 Asian, 9 (ages 18–60 years, all)	Volar forearm	Baseline: no significant differences in electrical impedance between age, gender, or ethnicity; impedance of proximal < distal forearm ($P < .001$) After topical interventions: all interventions produced decrease in impedance; degree of decrease varied by intervention; no significant differences between age, gender, or ethnicity
Grimes et al.[62] (2004)	In vivo—capacitance	African American, 18 White, 19 (ages 35–65 years, female, all)	Inner forearm	Baseline: African Americans < Whites but not statistically significant
Diridollou et al.[100] (2007)	In vivo—capacitance	African American (AA), Chinese (CH), Caucasian (CA), and Mexican (ME) American women 56 AA, 44 CH, 41 CA, 30 ME (mean age 36 ± 9 years) 58 AA, 45 CH, 22 CA, 15 ME (mean age 61 ± 8 years)	Dorsal and ventral forearm	Ventral forearm: Younger group, no significant difference in dryness index between ethnic groups Older group, dryness index significantly higher for AA than ME ($P < .01$) and CH ($P < .001$) No significant difference was seen between CH and ME, or between CA and other three ethnic groups Dorsal forearm: Younger group, no significant difference Older group, dryness index significantly higher for AA ($P < .05$) and CA ($P < .05$) than CH No significant difference between CA and AA, or between ME and three other ethnic groups
Fotoh et al.[89] (2008)	In vivo—capacitance	Black African or Caribbean women, 25 African or Caribbean mixed-race women, 25 European Caucasian women, 25 (ages 20–32 years, all)	Forehead and volar forearm	Mean hydration index of Black women is less than other groups but no significant difference between Black, African, or Caribbean mixed-race and Caucasian women ($P > .05$)
El-Sheikh et al.[99] (2010)	In vivo—conductance	128 girls and 123 boys (mean age 8.23 ± 0.73 years) European American (EA), 64% African American (AA), 36%	Volar surface on distal phalanges of second and third digits of nondominant hand	Higher levels of skin conductance levels were observed across the session for EA rather than AA children
Doberenz et al.[102] (2011)	In vivo—conductance	Caucasian, 23 Asian, 21 Other, 4 (age 37.3 ± 11.8 years, all, 67% female)	Nondominant hand	Asians had lower skin conductance levels ($P < .05$) and fewer nonspecific skin conductance fluctuations ($P < .05$) than Caucasians but higher coefficients of skin conductance variation ($P < .01$)
Chu and Kollias[97] (2011)	In vivo—conductance	Caucasian females, 84 African American females, 67 (ages 14–75 years, all)	Dorsal forearm and upper inner arm	Conductance of the upper inner arm is similar for both ethnicities Conductance of dorsal forearm: African Americans > Caucasians ($P < .05$)
Pappas et al.[98] (2011)	In vivo	White, African American, and East Asian females (no. of participants not indicated) (ages 18–25 or 35–35 years, all)	Face	Skin hydration: African American > East Asian > White

(*continued*)

TABLE 3.2 (Continued)
Water Content[a]

Study	Technique	Subjects	Site	Results
Yamashita et al.[94] (2012)	In vivo—Japanese, hygrometer; French, corneometer	Japanese (mean age 41.1 ± 12.8 years): 43 males, 49 females French (mean age 40.4 ± 14.4 years): 52 males, 52 females	Cheek, dorsal aspect of hand, inner upper arm	Japanese > French, statistically significant at all sites ($P < .01$)
Bailey et al.[101] (2012)	In vivo—capacitance	African American, 27 Asian, 7 Caucasian, 36 Hispanic, 16 (ages 18–61 years, all)	Forehead, mid-cheek, jowl, neck, abdomen	Moisture content did not vary significantly when stratified by ethnicity
Luther et al.[90] (2012)	In vivo	Caucasian, 6 (phototypes II–III, mean age 28.1 ± 4.3 years) African: 6 (phototypes V–VI, mean age 27.3 ± 3.2 years) Asian: 6 (phototype IV, mean age 23.5 ± 1.6 years)	Volar forearm	Caucasian > African > Asian but not statistically significant ($P > .05$)
Jung et al.[95] (2012)	In vivo—capacitance	Korea (K), 110 Vietnam (V), 100 Singapore (S), 100 (ages 20–35 years, female, all)	Front cheek	Skin hydration (AU): 53.23 ± 12.8 (K), 63.18 ± 11.23 (V), 56.26 ± 12.65 (S)

Note: AU = arbitrary units; SLS = sodium lauryl sulfate.

[a] Ethnic differences in water content, as measured by resistance, capacitance, conductance, and impedance, are inconclusive.

between age, gender, or ethnicity. Notably, although there were no significant differences between right and left forearms, significant baseline variation was found between the distal and proximal volar forearms; the proximal forearms showed lower impedance than the distal forearms ($P < .001$). As impedance is a measure of WC, we can infer baseline differences in WC among anatomic sites from this study. Additionally, all interventions produced decreases in impedance from baseline (degree of decrease varied by intervention) but no significant differences between age, gender, or ethnicity. The authors concluded that there is little variation in volar forearm skin across gender, age, and ethnicity, providing an adequate site for testing of skin and cosmetic products.

Grimes et al.[62] measured baseline moisture content on the inner forearms of 18 African American and 19 White women aged 35–65 years based on capacitance. The study found no significant variation in baseline moisture content between African American and White subjects' inner forearms. Fotoh et al.[89] in addition to their TEWL measurements, also analyzed WC differences between various ethnicities via capacitance measurements of the forehead and volar forearm. Overall, the mean hydration index of Black women was less than the other groups, but there was no significant difference present between the 25 Black, 25 African or Caribbean mixed-race, and 25 Caucasian women ($P > .05$).

Subsequent studies performed by Bailey et al.[101] and Luther et al.[90] also failed to show significant differences in WC between ethnicities. Bailey et al.[101] measured capacitance levels in 27 African American, seven Asian, 36 Caucasian, and 16 Hispanic participants, aged 18–61 years old. Upon examining the forehead, mid-cheek, jowl, neck, and abdomen, it was determined that moisture content did not vary significantly when stratified by ethnicity. Luther et al.[90] showed a general trend of SEC WC decreasing from Caucasians to Africans to Asians, but none of these differences in skin moisture were statistically significant ($P > .05$).

Chu and Kollias[97] measured conductance values in Caucasian ($n = 84$) and African American ($n = 67$) females at the dorsal forearm and upper inner arm. While conductance of the upper inner arm was similar for both ethnicities, conductance levels in the dorsal forearm were found to be significantly higher in African Americans than in Caucasians ($P < .05$), indicating higher SC hydration in African Americans at the dorsal forearm. These results at the dorsal forearm support earlier findings made by Berardesca et al.[15] In addition, although there were no age or body site differences in WC detected among Caucasian participants, conductance levels were found to differ with body site and increase with age in African American females. In regard to a different anatomic site, Pappas et al.[98] (abstract only) observed significantly higher skin hydration in the faces of African American panelists than in White panelists ($P < .05$).

Doberenz et al.[102] provided additional information regarding WC differences between Asian and Caucasian skin through their study examining SCLs of the nondominant hand in 23 Caucasian and 21 Asian subjects. Asian participants exhibited lower SCLs ($P < .05$) and fewer nonspecific skin conductance fluctuations ($P < .05$) than Caucasians but higher coefficients of skin conductance variation ($P < .01$). This contrast between number of fluctuations and variability implies that after the effect of mean levels is decreased, Asians display greater variability in SCLs.[102]

Yamashita et al.[94] examined WC differences in Japanese ($n = 92$) and French ($n = 104$) subjects at the cheek, dorsal aspect of the hand, and inner upper arm. It was observed that Japanese skin WC was significantly greater than WC in French skin at all sites tested ($P < .01$). Yamashita et al.[94] referencing the study performed by Gunathilake et al.[91] suggested that the higher SC integrity and skin barrier function in Japanese subjects may be a factor of darker skin types in Japanese subjects. Darker skin types have been found to be associated with an increase in melanosomes and a more acidic pH of the SC.[91]

Jung et al.[95] provided further information regarding WC in Asian skin. In this study, Vietnamese skin possessed significantly greater skin hydration, as measured by capacitance, than both Korean skin and Singaporean skin ($P = .000$). Differences in skin hydration between Korean and Singaporean skin were not statistically significant ($P = .176$).

The WC results of each study are summarized in Table 3.2. While Johnson and Corah[30] did not correlate resistance to WC in their study, it can be inferred from their data that ethnic variance was found in WC. However, the sodium lauryl sulfate-induced irritation studies by Berardesca and Maibach[20,21] revealed no significant differences in WC between the races at baseline or after sodium lauryl sulfate stress, except for a questionable difference (high SDs) of Hispanics having greater WC than Whites after sodium lauryl sulfate stress. Since it is believed that artifacts from topically applied substances may alter values measured by capacitance or conductance,[28] this may play a factor in some of the values obtained in the study on sodium lauryl sulfate irritant-induced stress. Additionally, Manuskiatti et al.[31] found no difference in WC between Blacks and Whites but did find differences based on age. Berardesca et al.[15] and Warrier et al.[16] however, did demonstrate ethnic variability in WC, but the values varied by anatomic site. Diridollou et al.[100] detected higher dryness indices in African Americans compared to Chinese and Mexican American participants at the ventral forearm, but no significant differences were observed between Caucasians and African Americans. Older African American and Caucasian women exhibited significantly higher dryness indices than their younger counterparts, indicating the importance of age, as Manuskiatti et al.[31] previously described. In further studies of Asian populations, Doberenz et al.[102] noted lower SCLs in Asians compared to Caucasians, while Yamashita et al.[94] observed higher WC in Japanese subjects than in French subjects at all anatomic sites tested. Sugino et al.[19] also demonstrated ethnic variability, with Asians having a higher WC than other ethnic groups based on impedance. In contrast, Sivamani et al.[65] recently reported no ethnic differences in WC, both baseline and after various topical

interventions, based on impedance; they did find variation of WC between different anatomic sites and with specific interventions. Of note, impedance, as used in the latter two studies, is less widely used than capacitance, and conductance has been shown to be more sensitive to environmental and technical factors that affect the SC;[28] this makes it difficult to compare the results presented by these latter two studies. In more recent studies using capacitance, Grimes et al.[62] Fotoh et al.[89] and Bailey et al.[101] showed no significant variation in baseline moisture content between African American and White subjects' skin, further supporting studies by Berardesca and Maibach[20,21] and Manuskiatti et al.[31] Luther et al.[90] also failed to find significant differences in WC among African American and White subjects' skin but did not indicate the method by which skin moisture was measured. Utilizing conductance measures, El-Sheikh et al.[99] observed higher skin conductance in European American children than African American children, but Chu and Kollias[97] failed to show a significant difference in WC between Caucasians and African Americans at the upper inner arm. Chu and Kollias,[97] however, did find African American females to have a significantly higher conductance of the dorsal forearm, supporting earlier findings made by Berardesca et al.[15] Pappas et al.[98] identified higher skin hydration in the faces of African Americans in comparison to Whites as well.

These findings, by measuring skin capacitance, conductance, impedance, and resistance, are difficult to interpret in terms of SC WC because other physical factors, such as skin microrelief, sweat production, and the presence of hair on the measuring site, may modify the quality of skin electrode contact.[28] Thus, it seems there may be factors other than race in the determination of WC, and no conclusions with regard to race and WC can be made at this time. Studies with more subjects and with the use of more than one method of measuring WC for accuracy should be considered in the future. In addition, since variation has been shown by anatomic sites, care should be taken to use consistent anatomical sites when comparing measurements of WC.

CORNEOCYTE VARIABILITY

Corneocytes differ in shape from the keratinocytes that produce them. The disk-like shape of corneocytes allows them to present with a large surface area in the horizontal position.[27] In Caucasians, the surface area of corneocytes differs by body site[27,36] and age.[27,37] It has also been demonstrated in Caucasians that corneocyte surface area is an important factor in the permeability of the skin to water loss and to percutaneous absorption of topically applied substances.[27]

Corcuff et al.[38] compared corneocyte surface area and spontaneous desquamation (via corneocyte count) on the upper outer arm in Black African Americans, White Americans of European origin, and Asian Americans of Chinese extraction. There were 18–25 age-matched subjects per group who were free from dermatological disorders. No difference in corneocyte surface area was found between the groups. However, spontaneous desquamation (corneocyte count) was increased in Blacks by factor of 2.5 compared with White and Asian skin ($P < .001$). The investigators felt that their findings were not consistent with earlier studies that showed increased intercellular adhesion or increased TEWL[12,20] in Black skin. This enhanced desquamation may (partially) account for "ashing" frequently seen clinically in Black people.

In contrast, Warrier et al.[16] conducted a study that included corneocyte desquamation and had different results. The investigators studied 30 Black and 30 White subjects, matched for age, and found that the desquamation index was greater on the cheeks and forehead of White subjects compared with Black subjects. No difference was found on the legs. The investigators attributed the lower corneocyte desquamation on the cheeks and foreheads of Blacks compared with Whites to possible differences in moisturizing properties of sebum. These findings did not correlate with dry skin frequently seen clinically in Black people. Since it is believed that corneocyte surface area varies by anatomic site in Caucasians,[27] perhaps corneocyte desquamation also varies by site. Corcuff et al.[38] studied the upper outer arm, whereas Warrier et al.[16] examined the cheeks, forearms, and lower legs. More studies of corneocytes desquamation should be conducted on the anatomic areas where dry skin is more frequently experienced. Additionally, the climate of the area where the study is done should be considered as it may influence desquamation. Warrier et al.[16] conducted their study over a 6-week period in winter, from December through February in Cincinnati, Ohio, United States, when temperatures and relative humidity are low and frequency of dry skin (winter xerosis) is high. In contrast, the city and climate are not documented in the study by Corcuff et al.[38]

In addition to measuring WC, Manuskiatti et al.[31] also examined the desquamation index in seven Black and five White women (mean age 25.8 ± 4.2 years) and five Black women and five White women (mean age 64.7 ± 3.8 years) on the preauricle, posterior neck, dorsal upper arm, dorsal forearm, volar forearm, lower back, abdomen, thigh, and lower leg. There were no differences in desquamation index between Blacks and Whites at all areas measured, except at the preauricular area ($P = .02$). However, whether Blacks or Whites had a higher desquamation index at this area was not specified. The investigators discounted the difference found at the preauricular area and attributed it to the small sample size used. Like the results found for WC, they also found significant differences in desquamation index based on age (older individuals had higher desquamation index than younger individuals at the preauricle). Overall, they concluded that age and anatomic site, but not race, demonstrate a significant influence on skin roughness and scaliness.

More recently, Fotoh et al.[89] measured the desquamation index in 25 sub-Saharan African Blacks or Caribbean Blacks, 25 African or Caribbean mixed races, and 25

European Caucasians on the volar forearm and forehead by assessing the thickness of the spontaneous desquamation of the corneocytes on these anatomic sites. No significant differences in the desquamation index among the ethnicities being studied were found ($P > .05$). Mohammed et al.[93] also failed to find significant differences in corneocyte characteristics, specifically corneocyte surface area and maturity, between 10 Black and 12 Caucasian subjects at the midventral forearm.

In 2009, Gunathilake et al.[91] studied various characteristics of corneocytes in two pigment groups, type I–II and type IV–V. At least 30 measurements were taken from at least five subjects in each pigment group. Anatomic sites were not indicated. Investigators determined that while there was no difference detected in corneocyte cytosol between the pigment groups, darkly pigmented subjects displayed significantly thicker cornified envelopes than their lightly pigmented counterparts ($P < .0006$). The authors of the study, however, were unable to identify a basis for this significant difference.

Muizzuddin et al.[92] developed an assay to measure the amount of cross-linking in corneocytes taken from the skin by tape stripping, called the index of maturation. This index of maturation is positively associated with barrier integrity. When looking at corneocytes derived from 40 African Americans, 97 Caucasians, and 40 East Asians at the ventral forearm, the found the highest maturation index in African Americans ($P < .001$). These results, as suggested by the authors, signifies African Americans possessing a greater amount of covalently bound proteins in the corneocytes and an improved rigidity of the cornified envelope, in comparison to Caucasians and East Asians.[92] The Caucasian cohort was found to possess a higher maturation index than the East Asian group ($P < .001$). In addition to the above findings, stratum corneum chymotryptic enzyme (SCCE) levels were also obtained for each ethnicity. SCCE is a serine protease of the kallikrein family, which contributes to the proteolysis of intercellular corneodesmosomes. As such, SCCE levels act as a valuable marker for the skin's ability to eliminate corneocytes naturally through the desquamation process.[92] Differences in SCCE between Caucasian and East Asian groups were insignificant. Caucasian subjects, however, possessed significantly higher SCCE levels than those found in the African American group ($P < .001$). Muizzuddin et al.[92] suggest that the low SCCE levels found in African Americans may partially contribute to their displaying a higher amount of cohesion in the uppermost layers of the SC compared to East Asians ($P < .001$).

Chu and Kollias[97] measured differences in the amount of corneocyte detachment from the dorsal forearms and upper inner arms of 84 Caucasian and 67 African American females, using adhesive tape. Dermatoscopy revealed differences in Caucasians and African Americans to be most significant in older age groups. In the age groups aged 55–60 and 65–75 years, the dorsal forearms of Caucasians displayed more corneocyte detachment than those of African Americans. Similar relationships were seen at the upper inner arm in the oldest age groups for both ethnicities. Dermatoscopy revealed minimal differences between the younger participants of each ethnicity. The authors suggest that as African Americans become older, they are better able to sustain the strength of their intercellular bonds of superficial corneocytes, and cell turnover may be greater. While both ethnicities had increased corneocyte removal from the dorsal forearm with age ($P < .05$), only Caucasians displayed a direct relationship between corneocyte detachment and age at the upper inner arm ($P < .01$).

Finally, Yamashita et al.[94] compared corneocyte characteristics at the cheek, dorsal aspect of the hand, and inner upper arm between Japanese and French subjects. Among the various anatomic sites, there was only a significant difference in the size of corneocytes at the cheek, with corneocytes in French subjects significantly greater in size than those in Japanese subjects ($P < .01$). Further, French participants were found to possess greater SC thick abrasion than Japanese participants at all anatomic sites ($P < .01$), supporting the notion that barrier functions of Japanese skin are superior to French skin.[94]

Overall, Corcuff et al.[38] Warrier et al.[16] Manuskiatti et al.[31] Gunathilake et al.[91] Muizzuddin et al.[92] and Chu and Kollias[97] reveal statistically significant results, but the findings are at times contradictory (Table 3.3). Corcuff et al.[38] demonstrated greater corneocyte desquamation in Blacks compared with Whites on the upper outer arm. In contrast, Warrier et al.[16] found a greater desquamation index on the cheeks and foreheads of Whites compared with Blacks. Additionally, Manuskiatti et al.[31] found a difference on the preauricular area only out of the numerous areas examined, but whether Blacks or Whites have a higher desquamation index is not specified. While Fotoh et al.[89] and Mohammed et al.[93] failed to find significant differences between Blacks and Whites, more recent results consistently support African Americans possessing stronger barrier functions in the skin compared to Whites.[91,92,97] While Gunathilake et al.[91] noted significantly thicker cornified envelopes in darkly pigmented subjects, Muizzuddin et al.[92] demonstrated greater cross-linking of corneocytes in African Americans compared to Caucasians and East Asians. Further, Chu and Kollias[97] noted significantly higher corneocyte detachment with adhesive tape in older Caucasians compared to similarly aged African Americans. In a separate analysis between Japanese and French subjects, Yamashita et al.[94] determined Japanese skin to have greater barrier integrity than French skin, as noted by French subjects experiencing more SC thick abrasion at all anatomic sites tested.

Do the site of measurement of corneocyte desquamation, the WC and TEWL at that site, and the climate of the area where the study was done act as confounding variables for these results? In light of what is now known about WC and TEWL, the issue of corneocyte desquamation should be revisited as these may be contributing variables. Corneocyte desquamation may have clinical implications in issues regarding the diagnosis and treatment of xerosis frequently seen in African Americans.

TABLE 3.3
Corneocyte Variability[a]

Study	Subjects	Site	Results
Corcuff et al.[38] (1991)	Black (mean age 33.5 ± 7.5 years) Caucasian (mean age 31 ± 7 years) Asian (mean age 26.5 ± 7.5 years) (18–25 subjects per group)	Upper outer arm	No difference in corneocyte surface area Spontaneous desquamation (corneocyte count) Blacks 2.5× > Caucasians and Asians ($P < .001$)
Warrier et al.[16] (1996)	Black women, 30 White women, 30 (ages 18–45 years, both)	Left and right medial cheeks, mid-volar forearms, lateral mid-lower legs	Desquamation index: Blacks < Whites on cheeks (18% less) and forearms (20% less, $P < .05$) but no significant differences on the legs
Manuskiatti et al.[31] (1998)	Black women, 7 White women, 5 (mean age 25.8 ± 4.2 years, both) Black women, 5 White women, 5 (mean age 64.7 ± 3.8 years, both)	Preauricle, posterior neck, dorsal upper arm, dorsal forearm, volar forearm, lower back, abdomen, thigh, lower leg	No difference in desquamation index between Blacks and Whites except at preauricular area ($P = .02$, which ethnicity greater not specified)
Fotoh et al.[89] (2008)	Black African or Caribbean women, 25 African or Caribbean mixed-race women, 25 European Caucasian women:,25 (ages 20–32 years, all)	Forehead and volar forearm	No significant differences in desquamation index ($P > .05$)
Gunathilake et al.[91] (2009)	At least 30 measurements were taken from at least 5 subjects in each pigment group (type I–II and type IV–V)	Not indicated	No differences detected in corneocyte cytosol Subjects with types IV–V skin significantly thicker cornified envelopes (CEs) in comparison with types I–II (19.7 ± 0.6 vs. 15.5 ± 0.4 nm, respectively, $P < .0006$) No visible differences in immunostaining for several constituent CE peptides, including loricrin, filaggrin, and involucrin
Muizzuddin et al.[92] (2010)	African Americans, 40 Caucasians, 97 East Asians, 40 (ages 18–45 years, female, all)	Ventral forearm	Index of maturation (cross-linking of corneocytes): African Americans > Caucasians ($P < .001$) Caucasians > East Asians ($P < .001$)
Chu and Kollias[97] (2011)	Caucasian females, 84 African American females, 67 (ages 14–75 years, all)	Dorsal forearm and upper inner arm	Corneocyte removal with adhesive tape: Dorsal forearms of Caucasians (55–60 and 65–75 years) > African Americans of the same age In younger groups, minimal differences were observed between ethnicities
Mohammed et al.[93] (2011)	Black subjects, 10 Caucasian subjects, 12 (ages 20–58 years, all)	Mid-ventral forearm	No significant differences in corneocyte surface area between groups (t-test, $P > .05$) No significant differences in overall corneocyte maturity between groups at any depth (t-test, $P > .1$)
Yamashita et al.[94] (2012)	Japanese (mean age: 41.1 ± 12.8 years): 43 males, 49 females French (mean age: 40.4 ± 14.4 years): 52 males, 52 females	Cheek, dorsal aspect of hand, inner upper arm	Significant difference in size of corneocytes only at cheek: French > Japanese ($P < .01$) Stratum corneum thick abrasion: French > Japanese, significant at all sites ($P < .01$)

[a] Ethnic differences in corneocyte desquamation are inconclusive. The most clinically provocative observation is that of Corcuff et al.[38]—a 2.5 times greater spontaneous desquamation rate in Blacks compared to Caucasians and Asians.

BLOOD VESSEL REACTIVITY

Cutaneous blood flow has been examined on numerous occasions to assess skin physiology, irritation, evaluation of dermatologic pathology/treatments, effects/delivery of drugs, and wound healing among other areas of interest.[39] The visual assessment of cutaneous microcirculation has been measured for centuries by the degree of erythema or pallor/blanching (visual scoring). However, the introduction of objective techniques for the evaluation of blood flow has shown that the human eye is rather unreliable. Two techniques utilized by the papers to be discussed are LDV and photoplethysmography (PPG).

LDV is a noninvasive method that continuously follows the flow of red blood cells. It is based on measurement of the Doppler frequency shift in monochromatic laser light backscattered from moving red blood cells. It detects the frequency-shifted signal and derives an output proportional to the number of erythrocytes multiplied by their velocity in the cutaneous microcirculation.[39,40] LDV has been applied to skin physiology; diagnostics (especially scleroderma, Raynaud disease, and patch test reactions); predictive testing of irritancy (topical drugs, cosmetics, detergents, cleansing agents, products used in industry); and effects of drugs (vasodilators, minoxidil, sunscreens and UV light, topical corticosteroids [blanching]).[39]

PPG can be defined as the continuous recording of the light intensity scattered from a given source by the tissues and collected by a suitable photodetector.[41] Specific to the skin, it allows the registration of pulsative changes in the dermal vasculature and is synchronized with heartbeat. Infrared light from a transducer is absorbed by hemoglobin, and the backscattered radiation is detected and recorded. The backscattered light depends on the amount of hemoglobin in the skin, and the result obtained will therefore reflect the cutaneous blood flow. PPG has been used for studies of skin physiology, dermatological disorders, as well as systemic diseases.[39]

Guy et al.[42] enrolled six Black subjects aged 20–30 years, six White subjects aged 20–30 years, and six White subjects aged 63–80 years, with good general health and no recent skin disease, and taking no prescription medications, and studied their response to topically applied vasodilator methyl nicotinate. The substance was applied to the volar forearm, and blood vessel reactivity was measured by LDV and PPG. There was no significant difference between the ethnic groups in time to peak response, area under the response–time curve, or time for response to decay to 75% of its maximum value. However, the PPG maximum response was 40% less in the young Black group than in the young White group ($P < .05$). The authors made note of the fact that the sensitivities of the two methods of study (LDV and PPG) were not equivalent. They concluded that, overall, the data suggested a similarity in response among races and ages. The investigators did not discuss the significance or implications of the lower maximum PPG seen in the Black subjects.

Berardesca and Maibach[20] performed a study to determine the difference in irritation between young Black and Caucasian skin. They applied 0.5% and 2.0% sodium lauryl sulfate to untreated, preoccluded, and predelipidized skin and then quantified the resulting level of irritation using LDV, TEWL and WC of the SC. There were no significant differences between Black and White skin for LDV at baseline or after application of sodium lauryl sulfate. The authors did note, however, that in Blacks, application of the 0.5% sodium lauryl sulfate to untreated skin revealed minimal changes in cutaneous blood flow (as measured by LDV) compared with baseline. They used this finding to explain the decreased irritant-induced perceptible erythema in Blacks.[6] However, after reexamining the data, we might consider that there was about the same degree of minimal change from baseline to application of 0.5% sodium lauryl sulfate in untreated skin in the Caucasian group.

Berardesca and Maibach[21] used the same model to compare differences in irritation between Hispanic and Caucasian skin. Like the sodium lauryl sulfate-induced irritation study comparing Blacks and Whites, the same study comparing Hispanics and Whites revealed equivalent blood vessel responses between the two groups.

Berardesca and Maibach[43] performed a subsequent study using LDV but this time examined ethnic differences induced by corticosteroid application (a vasoconstrictive stimulus). They examined six Black and eight Caucasian men, matched for age, and measured cutaneous hyperemia using LDV, before and after the application of 0.05% clobetasol ointment to the forearm. The following parameters were analyzed: (1) the area-under-the-curve response from the starting point of the hyperemic response until the return of blood flow to basal values; (2) the magnitude of the maximum peak response; (3) the slope of the rise from immediate postocclusion to peak reactive hyperemic flow; and (4) the slope of the decay from peak reactive hyperemic flow to resting levels. After the vasoconstrictive stimulus was given, the Black subjects showed a 40% decreased area-under-the-curve response ($P < .04$), a 50% decreased peak response ($P < .01$), and a decreased decay slope after peak blood flow ($P < .04$) compared with the Whites. Overall, their data were consistent with a decrease in blood vessel reactivity of Blacks compared with Whites.

Gean et al.[44] also found differences in blood vessel reactivity between different ethnic groups; however, their data conflict with the findings of Berardesca and Maibach.[43] Gean et al.[44] examined five Black subjects (skin types V or VI), five Asian subjects (skin type IV), and five Caucasian subjects (skin type II), aged 20–35 years, with no history of skin disease, who were nonsmokers and were not taking prescription medications, and applied three different concentrations of methyl nicotinate to the upper third of the ventral forearm. Methyl nicotinate-induced vasodilatation was assessed visually and by LDV. At three different dose levels, the following parameters were compared: (1) the diameter of the maximum visually perceptible erythematous area; (2) the area under the erythematous diameter

versus time curve; (3) the maximum LDV response; and (4) the area under the LDV response versus time curve. Since we are reviewing only the objective data, the first two parameters measuring erythema revealed no significant differences and will not be discussed. The investigators observed that the area under the curve for LDV response versus time was greater in Blacks than Caucasians for all methyl nicotinate concentrations ($P < .05$). This contrasts with prior studies, which found either no difference[20,42] or a decrease[43] in the area-under-the-curve response in Blacks. Note, however, that in this study, a vasodilator (methyl nicotinate) was given, whereas in the prior study by Berardesca and Maibach[43] a vasoconstrictor was given. They also found that the area-under-the-curve response versus time was greater in Asians compared with Caucasians for higher dose levels of methyl nicotinate ($P < .05$).

Kompaore et al.[17] evaluated TEWL and lag time to vasodilatation by LDV, before and after removal of the SC by tape stripping in seven Black men, eight Caucasian subjects (six male and two female), and six Asian men. After application of methyl nicotinate, but before tape stripping, there was no difference between the groups in basal perfusion flow (by LDV), but lag time before vasodilatation was greater in Blacks and less in Asians compared with Caucasians ($P < .05$). After eight and 12 tape strips, lag time before vasodilatation decreased in all three groups but decreased significantly more in Asians compared with Caucasians and Blacks ($P < .05$). The order of sensitivity to methyl nicotinate was Asian > Caucasian > Black. After topical application of methyl nicotinate, TEWL measurements indicated that Black and Asian skin was more permeable to water than Caucasian skin (Table 3.1); however, LDV-recorded lag time to vasodilatation results revealed that Asian skin had a higher permeability to methyl nicotinate than Caucasian and Black skin. This study confirmed the importance of the SC in barrier function, but could not explain the reason behind the ethnic differences in TEWL and lag time to vasodilatation.

Aramaki et al.[18] evaluated LDV at baseline and after sodium lauryl sulfate-induced irritation in 22 Japanese and 22 German women. There was no difference in LDV at baseline and after sodium lauryl sulfate-induced irritation. Few studies measuring LDV have examined persons of Asian descent. Although it is difficult to compare a study that used tape stripping[17] with one that used a vasoactive substance,[8] note that Aramaki et al.[18] had a larger sample size than Kompaore et al.[17] and found no baseline difference in LDV.

An investigation done by Hicks et al.[61] demonstrated no significant difference in blood vessel reactivity between Black and White participants. SLS was applied to the volar forearm, and response was recorded using LDV. The results obtained are in conflict with several previous studies that have suggested differences between Black and White skin.[17,42–44] However, the investigators expressed doubt in the validity of the LDV measurements due to technical difficulties in using the flowmeter while conducting the study.

Wijayanto et al.[103] sought to examine the differences in thermoregulatory responses to heat between 10 Japanese and 10 Malaysian men, via, among other characteristics, skin blood flow measurements from Laser Doppler flowmetry. Upon examining multiple anatomic sites, investigators discovered no significant differences in changes in skin blood flow upon heating in the scapula and thigh between groups. After the passive heating of legs, however, the change in skin blood flow was significantly greater in Japanese participants compared to Malaysian subjects ($P < .05$). The authors suggest that this difference in change in blood flow reflects lower cutaneous vasodilation in tropical natives.

Yim et al.[104] further studied skin blood flow in Asian skin with their comparison of 10 Caucasian and 10 Korean participants for differences in endothelial function in response to local heating and vascular occlusion at the forearm. The study found that Caucasians possessed higher skin blood flows than Koreans for all temperatures being tested ($P = .001$) and in response to occlusion ($P = .016$). These results, as indicated by the authors of the study, imply that Koreans have lower vascular endothelial function in comparison to Caucasians, which may predispose Koreans to cardiovascular disease.[104]

Park et al.[105] investigated skin blood flow differences at the ventral aspect of the left forearm among 148 Indian Asians and 147 Europeans. After adjusting for diabetes and cardiovascular disease risk factors, one of the most significant findings was postocclusive reactive hyperemia (PORH) being greater in Europeans than in Indian Asians ($P = .008$). Essentially, ischemia-induced microvascular flow is reduced in Indian Asians.[105] These findings were later corroborated by a study performed by Petrofsky et al.[106] in which 10 Southeast Asian Indians were compared with 10 Caucasians for differences in endothelial function at the forearm in response to local heat and vascular occlusion. When occlusion was applied at various skin temperatures (31°C and 42°C), Caucasians displayed a significantly higher skin blood flow response to the occlusion than did Southeast Asian Indians ($P < .01$). Investigators in this latter study also examined the circulatory response of each ethnicity to heat. Petrofsky et al.[106] determined that Caucasians possessed a higher peak skin blood flow compared to Southeast Asian Indians at each temperature examined ($P < .05$).

The results of the studies on blood vessel reactivity are summarized in Table 3.4. Overall, recent studies support Caucasians possessing superior blood flow responses to occlusion when compared to Asian Indians.[105,106] However, since each study administered different vasoactive substances that may act on different receptors in blood vessels, they could not be accurately compared.[45] As was noted by Hicks et al.[61] it has been previously reported that small changes in position of the measuring probe can produce significant changes in measurements and may result in decreased reliability of results. Additionally, measurements may differ according to anatomic sites.

TABLE 3.4
Blood Vessel Reactivity[a]

Study	Technique	Subjects	Site	Results
Guy et al.[42] (1985)	Topically administered MN (vasodilator), LDV and PPG	Blacks, 6 (age 20–30 years) Whites, 6 (age 20–30 years) Whites, 6 (age 63–80 years)	Volar forearm	MN given: No significant difference in time to peak response, area under response–time curve, or time for response to decay to 75% of its max value PPG max response: young Black (40% less) < young White ($P < .05$)
Berardesca and Maibach[20] (1988)	Topically administered SLS (irritant), LDV	Black men, 10 (age 29.9 ± 7.2 years) White men, 9 (age 30.6 ± 8.8 years)	Back	SLS stress: No significant difference between Blacks and Whites Blood vessel reactivity minimal in Blacks from baseline to application of 0.5% SLS on untreated skin
Berardesca and Maibach[21] (1988)	Topically administered SLS (irritant), LDV	Hispanic men, 7 (age 27.8 ± 4.5 years) White men, 9 (age 30.6 ± 8.8 years)	Upper back	SLS stress: Similar LDV response in Hispanics and Whites
Berardesca and Maibach[43] (1989)	Topically administered corticoid (vasoconstrictor), LDV	Black men, 6 Caucasian men, 8 (mean age 27 ± 3 years, both)	Forearm	After vasoconstrictor given: 40% decreased area-under-the-curve response in Blacks compared with Whites ($P < .04$) 50% decreased peak response in Blacks compared with Whites ($P < .01$) Decreased decay slope after peak blood flow in Blacks compared to Caucasians; in Blacks, $y = 3.3672 - 0.0737x$ before treatment compared to $y = 2.5347 - 0.0367x$ after treatment ($P < .04$), i.e., less blood vessel reactivity in Blacks
Gean et al.[44] (1989)	Topically administered MN (vasodilator), LDV	Blacks, 5 Caucasians, 5 Asians, 5 (ages 20–35 years, all)	Upper 1/3 volar forearm	MN given: Area under the curve for LDV response vs. time: Blacks > Caucasians for all MN concentrations ($P < .05$) Area under the curve for LDV response vs. time: Asians > Caucasians for higher dose levels of MN ($P < .05$)
Kompaore et al.[17] (1993)	Topically administered MN (vasodilator), LDV	Blacks, 7 Caucasians, 8 Asians, 6 (ages 23–32 years, all)	Volar forearm	MN given: Before tape stripping: no difference between the groups in basal perfusion flow, but lag time before vasodilatation was Blacks > Caucasians > Asians ($P < .05$) After 8 and 12 tape strips: lag time before vasodilatation decreased in all three groups but significantly decreased in Asians > Caucasians > Blacks ($P < .05$)
Aramaki et al.[18] (2002)	Topically administered SLS (irritant), LDV	Japanese women, 22 (mean age 25.84 years) German women, 22 (mean age 26.94 years)	Forearm	No significant difference at baseline or after SLS stress

(*continued*)

TABLE 3.4 (Continued)
Blood Vessel Reactivity[a]

Study	Technique	Subjects	Site	Results
Hicks et al.[61] (2003)	Topically administered SLS (irritant), LDV	White, 7 Black, 6 (ages 18–40 years, all)	Volar forearm	SLS stress: no significant difference in LDV response between groups
Wijayanto et al.[105] (2011)	Passive heating, LDF	Japanese men (JP), 10 (mean age 20.8 ± 0.9 years) Malaysian men (MY), 10 (mean age 22.3 ± 1.6 years)	Forehead, left scapula, left forearm, and thigh	After passive heating of legs, change in skin blood flow greater in JP than in MY for forehead and forearm ($P < .05$) No significant difference in change in skin blood flow in scapula and thigh between groups
Park et al.[105] (2012)	Heating and arterial occlusion, laser Doppler fluximetry	Indian Asian, 148 European, 147 (ages stratified by 5—year age bands, 35–75 years, all)	Ventral aspect of left forearm	Skin temperature under resting conditions did not differ by ethnicity Postocclusive reactive hyperemia (PORH): Europeans > Indian Asians ($P = .008$) Maximum hyperemia and minimum microvascular resistance showed minimal differences between groups
Yim et al.[104] (2012)	Local heat and vascular occlusion, LDI	Caucasian, 10 (mean age 27.8 years) Korean, 10 (mean age 25.4 years)	Forearm	Skin blood flows for all temperatures: Caucasians > Koreans ($P = .001$) Skin blood flow response to occlusion: Caucasians > Koreans ($P = .016$)
Petrofsky et al.[106] (2012)	Local heat and vascular occlusion, LDI	Caucasian (C), 10 (mean age 26.78 ± 2.64 years) Southeast Asian Indian (SAI),10 (mean age 25.2 ± 2.6 years)	Forearm	Occlusion at skin temp. 31°C: Blood flow: C > SAI ($P < .01$) The same effect was seen at skin temperatures of 42°C Circulatory response to heat: C > SAI at each temperature examined ($P < .05$)

Note: Corticoid = clobetasol propionate 0.05% ointment; LDF = laser Doppler flowmetry; LDI = laser Doppler imaging; LDV = laser Doppler velocimetry; MN = methyl nicotinate; PPG = photoplethysmography; SLS = sodium lauryl sulfate.

[a] Studies cannot be compared to each other because each uses different vasoactive substances. However, each study, except for those of Berardesca and Maibach[21] comparing Hispanics and Whites, Aramaki et al.[18] comparing Japanese and German women, and Hicks et al.[61] comparing Blacks and Whites, reveals some degree of ethnic variation in blood vessel reactivity. Studies support Caucasians possessing increased blood flow responses to occlusion when compared to Asian Indians.[107,108]

ELASTIC RECOVERY/EXTENSIBILITY

In addition to examining TEWL and skin conductance, Berardesca et al.[15] also examined biomechanical properties, such as elastic recovery and skin extensibility, on the dorsal and volar forearm in 15 Blacks, 12 Whites, and 12 Hispanics. These biomechanical properties were determined by applying a specific torque parallel to the skin's surface and then measuring how stretchable the skin was (skin extensibility) and recording the time required for the skin to return to its original state after release of the torque (elastic recovery). For skin elastic recovery, they found no significant difference between the races on the dorsal forearm (Blacks > Whites, but not significant). However, elastic recovery was 26% less in Blacks compared with Whites on the volar forearm ($P <$ .001). There was no significant difference in elastic recovery between Whites and Hispanics. The authors explained the significantly decreased elastic recovery in Blacks compared with Whites on the volar forearm, with a higher recovery in Blacks on the dorsal side (although not significant), on the basis of greater actinic damage on the dorsal side of Whites, with melanin as a photoprotective factor in Blacks.

For skin extensibility, within each race, Berardesca et al.[15] found significant differences between dorsal and volar forearms in Hispanics and Whites (dorsal < volar, $P < .0002$ and $P < .0001$, respectively], but extensibility was the same on both sides of the forearm in Blacks. When comparing the races to each other, Blacks had greater extensibility than Whites on the dorsal forearm but decreased extensibility compared to Whites on the volar forearm ($P < .01$ for both). Skin elasticity overall is defined as elastic recovery divided by extensibility. When looking at this ratio, the investigators found no significant differences between the races. They explained the variability in these biomechanical properties of skin based on the protective role of melanin from UV rays. They believed that Blacks did not show differences in skin extensibility between the dorsal and volar forearm because they were more photoprotected. Furthermore, they believed that Blacks had greater extensibility on the dorsal forearm compared with Whites for the same reason. However, if Blacks are presumed to also be more photoprotected on the volar forearm compared with Whites, this reasoning does not explain why Whites were found to have a greater extensibility than Blacks on the volar side.

Warrier et al.[16] examined elastic recovery in 30 Black and 30 White women but did not record skin extensibility. There was no significant difference between Blacks and Whites on the legs, but elastic recovery on the cheeks was 1.5 times greater in Blacks than in Whites ($P < .05$). These findings contradicted those of Berardesca et al.[15] who found a 26% decrease in elastic recovery on the volar forearm of Blacks. Warrier et al.[16] explained their findings of higher elastic recovery on the cheeks of Blacks based on the higher WC that they found on the same anatomic area, thus presumably resulting in a higher elastic deformation.

In 2011, Wolff et al.[107] investigated the skin rigidity and occurrence of wrinkles at the forehead and face of 21 Black and 65 White recently menopausal women. Durometer measurements of skin rigidity were not different between races/ethnicities, nor was age associated with skin rigidity. Skin wrinkles, however, were found to vary in prevalence based on ethnicity. Wrinkle scores in Black women were significantly lower than in White women at all facial locations except the neck ($P < .05$). Investigators suggest that photoaging is at least partly due to the protective features of melanin.[107]

The data on skin biomechanics, specifically elastic recovery and extensibility, vary by anatomic site and by race. However, the conclusions drawn by Berardesca et al.[15] contradict those of Warrier et al.[16] The data not only vary by race and by site, but age may also be a contributing factor. In the study by Berardesca et al.[15] the subjects were all within the same age range (mean age 46.7–49.8 years). However, even though Warrier et al.[16] had a larger number of study subjects, the age range was 18–45 years. Wolff et al.[107] attempted to measure variations in skin rigidity between Black and White women but did not observe significant differences between ethnicities. Further, Wolff et al.[107] indicated that age did not have an association with skin rigidity in their study. Overall, the ethnic differences in skin biomechanics are inconclusive and warrant further study (Table 3.5).

MICROTOPOGRAPHY

Skin microrelief reflects the three-dimensional organization of the deeper layers and functional status of the skin.[77] Research has been performed relating changes in skin microtopography to age and, more recently, relating changes to ethnic origin (Table 3.5). Guehenneux et al.[77] (abstract only) studied changes in microrelief with age in 356 Caucasian and 120 Japanese women, aged 20–80 years, whose volar forearms were examined via skin replicas and analyzed by interferometry, simultaneously during winter in Paris and Sendai. Of the 12 "global parameters" and 13 "local parameters," the abstract reported the analysis of three local parameters: orientation of lines, depth of lines, and anisotropy index. Both Caucasian and Japanese women showed an increase in the density of lines measuring >60 μm in depth and a decrease in the density of lines measuring <60 μm with increasing age. However, this change was found to be more pronounced and occur at a younger age in Caucasian women. In addition, although no changes in orientation of lines with age were found in Japanese women, changes correlating with an increase in skin anisotropy with age were found in Caucasian women. Note that it is difficult to assess the reliability of comparing these results as the subjects were studied in two distinct geographical locations, where environmental exposures may differ.

A study by Diridollou et al.[78] (abstract only) compared skin topography among 310 women, aged 18–61 years, including subjects of African American, Caucasian, Asian, and Hispanic descent; the ethnic distribution was not delineated in the abstract. Skin microrelief of the dorsal and ventral forearms was investigated according to age and ethnicity in terms of the density of line intersections, in which a higher

TABLE 3.5
Additional Objective Differences in Skin Properties

Study	Technique	Subjects	Site	Results
		(a) Skin Elastic Recovery[a]		
Berardesca et al.[15] (1991)	In vivo	Blacks, 15 (mean age 46.7 ± 2.4 years) Whites, 12 (mean age 49.8 ± 2 years) Hispanics, 12 (mean age 48.8 ± 2 years)	Volar and dorsal forearm	No significant difference between groups on dorsal forearm Elastic recovery: Blacks (26% less) < Whites on volar forearm ($P < .001$)
Warrier et al.[16] (1996)	In vivo	Black women, 30 White women, 30 (ages 18–45 years, both)	Left and right medial cheeks, mid-volar forearms, lateral mid-lower legs	No significant difference between groups on the legs Elastic recovery: Blacks 1.5× > Whites on cheeks ($P < .05$)
		(b) Skin Extensibility[a]		
Berardesca et al.[15] (1991)	In vivo	Blacks, 15 (mean age 46.7 ± 2.4 years) Whites, 12 (mean age 49.8 ± 2 years) Hispanics, 12 (mean age 48.8 ± 2 years)	Volar and dorsal forearm	Significant dorsal < volar extensibility within Whites and Hispanics ($P < .001$ and $P < .002$, respectively) Black > White extensibility on dorsal forearm ($P < .01$) Black < White extensibility on volar forearm ($P < .01$)
Wolff et al.[107] (2011)	In vivo	Black women, 21 White women, 65 (mean age 53.3 ± 2.7 years, all)	Forehead and cheek	Durometer measurements of skin rigidity were not different among races/ethnicities, nor was age associated with skin rigidity
		(c) Microtopography[b]		
Guehenneux et al.[77] (2003)	In vivo—skin replicas and interferometry	Caucasian, 356 Japanese, 120 (ages 20–80 years, female, all)	Volar forearm	↑ in the density of lines >60 μm and ↓ in the density of lines <60 μm in depth with increasing age in both; change in Caucasians > Japanese and at earlier age in Caucasians Anisotropy: ↑ with age in Caucasians, no change in Japanese
Diridollou et al.[78] (2005)	In vivo—SkinChip	310 women (ages 18–61 years, all; African American, Caucasian, Asian, Hispanic)	Dorsal and ventral forearms	Roughness and anisotropy ↑ with age on both dorsal and ventral forearms in all groups; Caucasians > Hispanic, Asians, and African Americans Density of the line intersections: Caucasians and Hispanics < Asians and African Americans
Fujimura et al.[108] (2009)	In vivo—skin replicas and Primos system	Japanese, (JA) 105 Chinese, (CH) 96 German, (GE) 90 (ages 18–76 years, age-matched females, all)	Corner of eye and lower eyelid	Corner of eye: CH and GE > JA for both R_a and R_{max} roughness parameters in most age groups ($P < .05$) CH > GE and JA in the age group of 50s and 60s ($P < .05$) Lower eyelid: Results are not consistent with results from corner of eye GE > JA and CH in some ages for roughness values No significant difference between JA and CH Similar results were obtained for R_{max} ↑ roughness from below eye with age was much lower than from corner of the eye

(*continued*)

TABLE 3.5 (Continued)
Additional Objective Differences in Skin Properties

Study	Technique	Subjects	Site	Results
Luther et al.[90] (2012)	In vivo—Primos system	Caucasian, 6 (phototypes II–III) (mean age 28.1 ± 4.3 years) African, 6 (phototypes V–VI) (mean age 27.3 ± 3.2 years) Asian, 6 (phototype IV) (mean age: 23.5 ± 1.6 years) (males, all)	Volar forearm	Measurement of skin roughness revealed no statistically significant differences ($P > .05$)
		(d) pH Gradient[c]		
Berardesca et al.[23] (1998)		Black women, 8 Caucasian women, 10 (mean age 42.3 ± 5 years, both)	Mid-volar forearm	No significant difference in pH at baseline After tape stripping: pH significantly decreased in Blacks after three tape strips, i.e., superficial SC layers No differences between ethnicities after 9, 12, and 15 tape strips, i.e., deeper SC layers
Warrier et al.[16] (1996)		Black women, 30 White women, 30 (ages 18–45 years, both)	Left and right medial cheeks, mid-volar forearms, lateral mid-lower legs	pH: Blacks (pH = 5.15) < Whites (pH = 5.52) on cheeks at baseline ($P < .05$) No significant difference in pH on the legs at baseline
Grimes et al.[62] (2004)		African American, 18 White, 19 (ages 35–65 years, females, all)	Above left eyebrow	Baseline: African Americans < Whites but not statistically significant
Fotoh et al.[89] (2008)		Black African or Caribbean women, 25 African or Caribbean mixed-race women, 25 European Caucasian women, 25 (ages 20–32 years, all)	Forehead and volar forearm	Black women have a cutaneous pH significantly higher than mixed-race and Caucasian women ($P < .01$)
Gunathilake et al.[91] (2009)		German (n = 110, 72 females, age 29 ± 6.6 years) and Sri Lankan (n = 129, 117 females, age 25 ± 2.1 years) nurses with type I–II and IV–V skin, respectively	Volar forearm and dorsum of hand	Type IV–V subjects had significantly more acidic surface pH ($P = .0001$ for both forearm and hand)
Luther et al.[90] (2012)		Caucasian, 6 (phototypes II–III, mean age 28.1 ± 4.3 years) African, 6 (phototypes V–VI, mean age 27.3 ± 3.2 years) Asian, 6 (phototype IV, mean age 23.5 ± 1.6 years)	Volar forearm	No significant difference in pH ($P > .05$)
Jung et al.[95] (2012)		Korea (K), 110 Vietnam (V), 100 Singapore (S), 100 (ages 20–35 years, females, all)	Front cheek	Skin pH: 5.741 ± 0.451 (K), 5.069 ± 0.614 (V), 5.696 ± 0.573 (S) P value: K–V, .000; K–S, .000; V–S, .000
		(e) Lipid Content[d]		
Reinerston and Wheatley[25] (1959)		Cadavers: Black man, 1 White man, 3 Living: Black man, 1 White man, 1 (ages 49–68 years, all)	Cadavers: Abdomen Living: Back and thigh	Lipid and sterol content in total epidermis: Blacks > Whites
Sugino et al.[19] (1993)		Black, White, Hispanic, and Asian (no. of subjects, age not specified)	Not documented	Ceramide levels: Blacks (50% less) < Whites and Hispanics ($P < .05$)

(*continued*)

TABLE 3.5 (Continued)
Additional Objective Differences in Skin Properties

Study	Technique	Subjects	Site	Results
Harding et al.[46] (2002)		UK, 41 Thai (dry season), 31 Thai (humid season), 31 (ages 20–40 years, all)	Scalp	UK and Thai subjects demonstrated similar levels of total lipids
Gunathilake et al.[91] (2009)		5 subjects in each pigment group (type I–II and type IV–V)	LB density in two SG layers, immediately beneath the SC–SG junction	The darkly pigmented skin has higher LB density as illustrated by random electron micrographs ($P = .01$) Increased LB production correlated with a readily apparent increase in epidermal lipid content, shown in frozen sections stained with Nile Red
Muizzuddin et al.[92] (2010)		African Americans, 60 Caucasians, 115 East Asians, 156 (ages 18–45 years, female, all)	Ventral forearm	Caucasians and East Asians had similar ceramide levels in the SC (1.18 ± 0.46 and 1.14 ± 0.51, respectively); African Americans had significantly fewer ceramides (0.74 ± 0.25) than other groups ($P < .001$)
Jungersted et al.[109] (2010)		Asian, 25 Danish (White-skinned), 28 African, 18 (ages 20–39 years, all)	Volar forearm	Ceramide/cholesterol ratio: Asians > Whites > Africans
		(f) Sebaceous Function[e]		
Aramaki et al.[18] (2002)	In vivo—sebumeter; topical application of SLS (irritant)	Japanese women, 22 (mean age 25.84 years) German women, 22 (mean age 26.94 years)	Forearm	Baseline sebum levels: Japanese < Whites ($P < .05$) After SLS stress: Japanese > Whites ($P < .05$)
Grimes et al.[62] (2004)	In vivo—sebumeter	African American, 18 White, 19 (ages 35–65 years, females, all)	Forehead	Baseline sebum levels: African Americans < Whites but not statistically significant
de Rigal et al.[80] (2005)	In vivo—sebumeter; sebutape	387 women (ages 18–70 years, all; African American, Hispanic, Caucasian, Chinese)	Forehead and cheeks	Mean sebum excretion rate: same across all ethnic groups Number of sebaceous glands: Chinese and Hispanics < Caucasians and African Americans Sebum level decrease with age: linear in Chinese; sudden ↓ around age 50 years for other 3 groups
Fotoh et al.[89] (2008)	In vivo—sebumeter	Black African or Caribbean women, 25 African or Caribbean mixed-race women, 25 European Caucasian women, 25 (ages 20–32 years, all)	Forehead	No significant difference was found in the sebum quantity between different ethnic groups
Pappas et al.[98] (2011)	In vivo—sebutapes	White (W), African American (AA), and East Asian (EA) females (no. of participants not indicated) (ages 18–25 or 35–35 years, all)	Face	Production of facial lipids: AA > EA > W Analyzing lipid classes (free fatty acids, triglycerides, and wax esters): Significant difference ($P < .05$) in wax ester fraction comparing AA to W

(*continued*)

TABLE 3.5 (Continued)
Additional Objective Differences in Skin Properties

Study	Technique	Subjects	Site	Results
Luther et al.[90] (2012)	In vivo	Caucasian, 6 (phototypes II–III, mean age 28.1 ± 4.3 years) African, 6 (phototypes V–VI, mean age 27.3 ± 3.2 years) Asian, 6 (phototype IV, mean age 23.5 ± 1.6 years)	Volar forearm	No statistically significant differences observed
Jung et al.[95] (2012)	In vivo—sebumeter	Korea (K), 110 Vietnam (V), 100 Singapore (S), 100 (ages 20–35 years, females, all)	Front cheek	Sebum excretion rate (μg/cm²/h): 29.76 ± 44.88 (K), 54.43 ± 24.83 (V), 49.8 ± 74.09 (S) *P* value: K–V, .002; K–S, .015; V–S, .803
		(g) Mast Cell Granules[f]		
Sueki et al.[48] (2001)	EM of biopsy specimen	Black men, 4 (mean age 29.2 ± 3 years) Caucasian men, 4 (mean age 29.4 ± 1.2 years)	Medial–lateral buttock	Mast cells contain 1.5× larger granules in Black skin compared to White skin ($P < .0001$) Mast cells contain 15% more PLS in Blacks compared to Whites ($P < .05$) Mast cells contain 30% less curved lamellae in Blacks compared to Whites ($P < .05$) Tryptase immunoreactivity localized to PLS regions in Black skin, compared to curved lamellae regions in White skin ($P < .0001$) Cathepsin G localized to electron-dense amorphous subregions in both Black and White skin
		(h) Vellus Hair Follicles[g]		
Mangelsdorf et al.[81] (2006)	In vivo—skin surface biopsies	Asian, 10 African American, 10 (ages 25–50 years, males, all) (results compared to Caucasians studied in Otberg et al.[82])	Forehead, back, thorax, upper arm, forearm, thigh, calf	Distribution of follicle density for different body sites same in all groups: highest on forehead, lowest on calf Follicle density on forehead: Caucasians > African Americans > Asians ($P < .01$); no significant differences on other sites Calf and thigh: Asians and African Americans—smaller values for volume ($P < .01$, both), potential penetration surface ($P < .01$, both), follicular orifice ($P < .01$ and $P < .05$, respectively), and hair shaft diameter ($P < .01$, both)
Luther et al.[90] (2012)	In vivo—skin surface biopsies	Caucasian, 6 (phototypes II–III, mean age 28.1 ± 4.3 years) African, 6 (phototypes V–VI, mean age 27.3 ± 3.2 years) Asian, 6 (phototype IV, mean age 23.5 ± 1.6 years)	Scalp, calf	Vellus hair shaft diameter on scalp and calf showed similar diameters in all ethnic groups; Africans had significantly larger diameter in the calf vs. Caucasians ($P < .05$) Surface of terminal follicular infundibulum on scalp, representing penetration surface, largest in Caucasians ($P < .05$) Vellus hair follicle density significantly higher in Asians than Africans and Caucasians ($P < .05$)

(*continued*)

TABLE 3.5 (Continued)
Additional Objective Differences in Skin Properties

Study	Technique	Subjects	Site	Results
		(i) Facial Pores[h]		
Sugiyama-Nakagiri et al.[110] (2009)	Surface replicas, in vivo CLSM	Caucasian, 20 Asian, 20 (10 Chinese and 10 Japanese) Hispanic, 20 African American, 20 (ages 30–39 years, female, Dallas, Texas, USA, all)	Facial cheek	Asians showed significantly smaller total pore areas than Caucasians and Hispanics ($P < .05$) and significantly lower pore numbers than Caucasians ($P < .05$) No significant differences in average pore size African Americans showed substantially more severe impairment of architecture around facial pores than any other racial group ($P < .05$) African Americans had significantly more dermal papillae per area of facial skin than the other ethnic groups ($P < .05$) Hispanics had significantly more dermal papillae per area of facial skin than Caucasians ($P < .05$)

Note: R_a = mean roughness; R_{max} = maximum roughness; LB = lamellar body; SC = stratum corneum; SG = stratum granulosum; EM = electron microscopy; PLS = parallel-linear striations; SC = stratum corneum; CLSM = confocal laser scanning microscopy.

[a] Unable to draw conclusions regarding ethnic differences in skin biomechanics (skin elastic recovery and extensibility) due to insufficient and conflicting evidence.

[b] Difficult to compare studies due to different techniques and/or ethnicities being studied. However, earlier studies demonstrate an increase in anisotropy with age in Caucasians.[77,78]

[c] Four studies demonstrate pH of dark skin less than light skin.[16,23,62,91] However, Berardesca et al.[23] demonstrate this difference after superficial tape stripping of the volar forearm but not at baseline, while Warrier et al.[16] demonstrate the difference at baseline on the cheeks but not on the legs, and the results from Grimes et al.[62] did not reach statistical significance. A study performed by Fotoh et al.[89] opposed the results found in these previous studies.

[d] Ethnic differences in lipid content are inconclusive.

[e] Ethnic differences in sebaceous function are inconclusive.

[f] Larger mast cell granules, increased PLS, and increased tryptase localized to PLS in Black compared to White skin.

[g] Unable to draw conclusions regarding ethnic differences in vellus hair follicle distribution and morphology due to insufficient evidence. Want to delete this and instead write: "Insufficient evidence due to only two studies present, but both studies support increased follicular reservoir function in Caucasians versus Africans and Asians."

[h] Significant ethnic differences between structural properties of facial skin are evident.

density of the intersection indicated smoother skin, and line orientation, in which a smaller angle difference between the two main directions of the lines indicated higher anisotropy. The results were gathered using SkinChip, a device based on active capacitance imaging technology and image analysis software. On the ventral forearms, the data supported that the roughness and anisotropy of the skin increases with age in all four ethnic groups; the density of intersection decreased, and the angle between lines of different orientation became smaller. The same results were produced on the dorsal forearm, a sun-exposed site, but changes were significantly less pronounced for the African American subjects, indicating a possible resistance to photoaging in this group. Overall, the density of the intersections was less for Caucasians and Hispanics than for Asians and African Americans. In addition, the anisotropy was higher for Caucasians than for Hispanics or Asians, and significantly higher than for African Americans.

Fujimura et al.[108] analyzed differences in skin roughness among 105 Japanese, 96 Chinese, and 90 German age-matched women, using skin replicas and the Primos system. Skin roughness was measured using the Primos system, which creates three-dimensional images of skin surface structure.[90] At the corner of the eye, Chinese and German subjects possessed greater mean and maximum roughness values than Japanese subjects for most age groups ($P < .05$). Further, among participants aged between 50 and 69 years, the Chinese subgroup possessed greater skin roughness values ($P < .05$). Skin roughness results at the lower eyelid were not consistent with results from the corner of the eye, finding Germans to have greater roughness values than Japanese and Chinese subjects in some age groups. Skin roughness was primarily used in this study in order to quantitatively characterize skin wrinkles in different ethnicities.

Luther et al.[90] attempted to further elucidate differences in skin roughness among six Caucasian (phototypes II–III), six African (phototypes V–VI), and six Asian (phototype IV) subjects at the volar forearm, using the Primos system. Although no statistically significant differences were found among any of the ethnicities being studied, the investigators noted several potential confounding variables. These included, but were not limited to, the young age of the

volunteers (Caucasian, African, and Asian volunteers possessed mean ages of 28.1, 27.3, and 23.5 years, respectively); gender (volunteers were all male) and the anatomic skin sites being measured.[90]

Diridollou et al.[78] concluded that roughness and anisotropy are more pronounced in Caucasian skin than in Hispanic, Asian, and African American skin. Guehenneux et al.[77] also found more pronounced changes of topography and higher anisotropy in Caucasian skin as compared to Asian skin, and at an earlier age. However, the results of both studies cannot be compared or integrated as they used different tools of investigation and different evaluation parameters. Fujimura et al.[108] observed different relationships between skin roughness and ethnicities depending on the anatomic site being tested. Luther et al.[90] failed to observe significant differences in skin roughness among Caucasians, Africans, and Asians but noted that this may be due to a variety of confounding variables. As such, the results are inconclusive and warrant further study.

pH GRADIENT

Ethnic differences in pH of the skin have also been explored. In addition to examining TEWL, Berardesca et al.[23] also examined differences in pH in 10 Caucasian (skin types I and II) and 8 Black African American (skin type VI) women at baseline and after tape strippings. They found no significant differences between the two races in pH at baseline. After tape stripping, however, they found a significantly lower pH in Blacks compared with Whites after three tape strippings but no significant differences after nine, 12, and 15 strippings. Thus, there was a lower pH in Black skin compared with White skin in the superficial layers of the SC but not in the deeper layers. The investigators stated that the data were difficult to explain. It was hypothesized that since the TEWL was also found to be increased after three and six tape strippings, the increased TEWL might allow for an increase in the hydrogen ion concentration in a normally hydrophobic SC. Of note, although the difference between the races in pH was not significant at deeper layers of the SC, the pH in both races did decrease with more tape strippings, but TEWL did not follow the same trend. Thus, an increase in TEWL does not fully explain the findings in pH.

Warrier et al.[16] also included pH in their study of 30 Black and 30 White women; however, they only examined pH at baseline, not after tape stripping. There was a decreased pH on the cheeks of Blacks compared with Whites, pH = 5.15 versus pH = 5.52, respectively ($P < .05$). There was also a decreased pH in Blacks on the legs, but the difference was not significant. The authors attributed the decreased pH in Blacks to lactic acid and dicarboxylic amino acids in sweat secretions mixed with sebum and evaporation of sweat causing acidity to increase,[49] with the idea that there might be a greater number of sweat glands in Blacks.[32]

Similar results were also produced in the study by Grimes et al.[62] The skin pH, measured above the left eyebrow in 18 African American and 19 White women aged 35–65 years, was found to be lower in African Americans than Whites, but the results did not reach statistical significance.

In contrast to previous literature, Fotoh et al.[89] determined that 25 Black women had a cutaneous pH significantly superior to 25 mixed-race and 25 Caucasian women at the forehead and volar forearm ($P < .01$). Investigators in the study recognized that their results conflicted with those of Berardesca et al.[23] and Warrier et al.[16] They also hypothesized that sweat and/or sebaceous secretion is likely varied among ethnicities due to the statistically significant differences present in pH. For the Black subgroup, which was found to have the highest cutaneous pH, adaptations to a temperate climate give rise to less sweat secretion (decreased amino acids and lactate secretion) and/or a change in sebaceous secretion (decrease or change of free fatty acids).[89]

Gunathilake et al.[91] further explored differences in surface pH in 110 German and 129 Sri Lankan nurses, with type I–II and IV–V skin, respectively. Researchers determined that type IV–V subjects displayed significantly more acidic surface pH for both the forearm and hand in comparison to the lighter-skinned (type I–II) subjects. To account for differences in geographical locations, investigators repeated the study with a smaller group of volunteers in San Francisco and found similar results. Gunathilake et al.[91] noted that a possible mechanism for this pH difference may be explained by the fact that darkly pigmented melanocytes distribute more melanosomes, which are acidic organelles, to the outer epidermis of neighboring keratinocytes.

Luther et al.[90] attempted to distinguish differences in pH at the volar forearm among Caucasian, African, and Asian subjects, but no statistically significant differences were observed ($P > .05$). Jung et al.[95] explored pH differences of the front cheek in 110 Korean, 100 Vietnamese, and 100 Singaporean females. Korean subjects presented the highest pH values, followed by Singaporean volunteers. Statistically significant differences were present for every ethnicity comparison ($P = .000$). Jung et al.[95] also noted a negative correlation between skin pH and skin hydration, sebum excretion rate, and skin temperature.

The skin pH has been found to be lower in Blacks compared with Whites in three different studies but under different circumstances.[16,23,62] Berardesca et al.[23] only demonstrate this difference in the superficial layers of the SC on the volar forearm but not at baseline; while Warrier et al.[16] demonstrate the significant difference at baseline on the cheeks but not on the legs. Grimes et al.[62] demonstrated a difference on the forehead, but it lacked statistical significance. Similarly, Gunathilake et al.[91] showed more acidic pH values in darkly pigmented individuals at the volar forearm and dorsum of the hand. In contrast, Fotoh et al.[89] demonstrated higher cutaneous pH levels in African women than in mixed-race and Caucasian women at the forehead and volar forearm. Luther et al.[90] noted no significant differences in skin pH between Caucasians, Africans, and Asians at the volar forearm. Thus, it can be implied that there may be some differences between Whites and Blacks in SC pH, but the etiology of this finding and its confounders remain to be explored (Table 3.5).

LIPID CONTENT

Skin lipids may play a role in modulating the relation between SC WC and TEWL, resulting in higher conductance values in Blacks and Hispanics; greater intercellular cohesion with a normal TEWL could produce a higher WC.[59] Sugino et al.[19] correlated high WC values with high ceramide (lipid) levels and low TEWL. They studied WC (by impedance), TEWL, and ceramide levels in Black, White, Hispanic, and Asian subjects. The number of subjects, age, and sample site were not documented in the published abstract. Ceramide levels were 50% lower in Blacks compared with Whites and Hispanics (total ceramides: 10.7 ± 4.7, 20.4 ± 8.1, and 20.0 ± 4.3 μg/mg, respectively; $P < .05$). Though they noted that WC levels were highest in Asians, they did not document the ceramide levels of Asians (according to their hypothesis, Asians should have the highest ceramide levels). Thus, the correlation that was made between WC, TEWL, and ceramide levels was not fully exemplified.

The finding of low ceramide levels in Blacks by Sugino et al.[19] is important because several studies base their findings of increased WC in Blacks[15,16] upon a 1959 study by Reinerston and Wheatley,[25] which, in contrast to Sugino et al. showed higher total epidermis lipid and sterol content in Blacks compared with Whites. They took abdominal skin from four cadavers (one Black man and three White men), and back and thigh skin from one Black and one White man, all aged 49–68 years, and examined lipid and sterol content. Although they found that lipid and sterol levels were higher in Blacks, they had a small sample size and compared skin from both deceased and living subjects at different anatomic sites.

Harding et al.[46] analyzed scalp SC lipid content in 41 UK, 31 Thai (dry season), and 31 Thai (humid season) subjects, aged 20–40 years, in an attempt to evaluate ethnic differences in dandruff. They observed that decreased levels of scalp SEC free fatty acids, cholesterol, and ceramides were found in subjects with dandruff. However, the overall levels of scalp lipids were similar in the UK and Thai subjects.

In 2009, Gunathilake et al.[91] measured lamellar body density in the two stratum granulosum layers immediately beneath the SC–stratum granulosum junction. After evaluating five subjects in each pigment group (type I–II and type IV–V), investigators concluded that darkly pigmented skin had higher lamellar body density than lightly pigmented skin ($P = .01$). Increased lamellar body production correlates with an increase in epidermal lipid content, which, the authors suggest, may possibly explain the enhanced barrier function of darkly pigmented skin.[91]

A subsequent study by Muizzuddin et al.[92] demonstrated that Caucasians and Asians possess significantly higher ceramide levels than African Americans. Muizzuddin et al.[92] examined 73 African American, 119 Caucasian, and 149 East Asian females for ceramide levels at the ventral forearm. While Caucasians and East Asians had similar ceramide levels in the SC, both ethnic groups had significantly more ceramides than African Americans ($P < 0.001$). Low ceramide levels have been found to be associated with dry skin, which may explain the increased frequency of xerosis in patients with darker skin.[92] In spite of the relationship between ceramide levels and increased barrier function, African Americans possessed the strongest barrier function compared to the other ethnicities in the study, reaffirming that there are many factors that contribute to barrier integrity.

Jungersted et al.[109] measured ceramide/cholesterol ratios in 25 Asian, 28 Danish (White-skinned), and 18 African volunteers at the volar forearm. Results showed that Asians possessed the highest ceramide/cholesterol ratios, followed by White-skinned volunteers, with Africans exhibiting the lowest ceramide/cholesterol ratios. These results were all statistically significant. One limitation of this study is that the differences in ratios cannot be analyzed in regard to whether ceramides or cholesterol leads to higher and lower ratios for each ethnic group.

Overall, it seems as though ethnic differences in skin lipid content are inconclusive because while some studies find decreased lipids in Blacks compared with Whites,[19,92,109] others find increased lipids in Blacks,[25,91] and still another finds no difference between people from the UK and Thailand (Table 3.5).[46]

SEBACEOUS FUNCTION

Sebum is a semisolid secreted onto the skin surface by glands attached to the hair follicle by a duct.[79] The functions of sebum include protection from friction, reduction of water loss, and protection from infection. Sebum levels have been confirmed to decline with age; however, there are few studies on the effect of race on baseline sebum secretion. Grimes et al.[62] used a sebumeter to measure sebum levels on the forehead as a component of baseline parameters in their study of 18 African American and 19 White women aged 35–65 years. The results showed lower levels of sebum on African American skin than on White skin, but differences were not statistically significant. Similar findings were found by Fotoh et al.[89] whose results showed a pattern of lower levels of sebum on African American skin at the forehead compared to the mixed-race and Caucasian groups, but these differences were not statistically significant. In a subsequent study, Luther et al.[90] did not note any statistically significant differences in sebum excretion among Caucasians, Africans, and Asians at the volar forearm.

A study by de Rigal et al.[80] (abstract only) investigated the skin sebaceous function of 387 women of African American, Hispanic, Caucasian, or Chinese descents, aged 18 to 70 years. Measurements were performed using a sebumeter and sebutape on the forehead and cheeks to compare sebum excretion rate and number of sebaceous glands according to ethnicity and age. The mean gland excretion was the same across ethnic groups. However, the number of sebaceous glands was lower in Chinese and Hispanic groups as compared to Caucasian and African American groups. In addition, the normal sebum decrease with age was different in each population; the decrease was linear in the Chinese

group, but the other three groups exhibited a sudden decrease around 50 years of age.

Aramaki et al.[18] assessed sebum secretion as a part of their study investigating skin reaction to sodium lauryl sulfate at concentrations of 0.25% and 0.5%. Before and after application of SLS to the forearms of each subject, sebum levels were determined by a sebumeter. The baseline sebum levels were lower in Japanese women than in White women. However, after SLS 0.25% and 0.5% application, sebum levels were higher in the Japanese women ($P < .05$).

Pappas et al.[98] (abstract only) examined sebum content using sebutapes on the faces of women from White, African American, and East Asian populations. In this study, African Americans produced the greatest amount of facial lipids, followed by East Asians and then Whites. Upon a closer analysis of actual lipid classes, differences between African Americans and Whites became significant when comparing the wax ester fraction ($P < .05$). Seven lipids in the wax ester fraction also significantly differed in quantity between African Americans and Whites ($P < .05$). The authors suggest that these differences may account for the differences in barrier function, which was strongest in African Americans.[98]

Jung et al.[95] analyzed differences in sebum content at the front cheek for Korean, Vietnamese, and Singaporean subjects. In all, the sebum excretion rates for Koreans were significantly lower than those found in Vietnamese and Singaporean subjects ($P = .002$ and $P = .015$, respectively). Differences between Singaporean and Vietnamese individuals were minimal.

Aramaki et al.[18] de Rigal et al.[80] Pappas et al.[98] and Jung et al.[95] all suggest that significant differences exist in sebum levels according to ethnicity. The de Rigal et al.[80] study found that, although the mean sebum excretion was the same across ethnic groups, the number of sebaceous glands and the normal sebum decrease with age varied between groups. This may indicate a difference in distribution of sebum independent of sebum levels among ethnic groups. Aramaki et al.[18] determined sebum levels to be lower in Japanese women as compared to White women at baseline, but Japanese women expressed an increase in sebum levels in response to irritant stress. This irritant response may represent a physiologic attempt to increase barrier defense. Pappas et al.[98] correlated the higher sebum levels found in African Americans with their increased barrier integrity. Jung et al.[95] noted significantly lower sebum excretion rates in Koreans compared to Singaporean and Vietnamese subjects. Further studies will be useful to elucidate whether differences in barrier defense between ethnic groups are based on varying baseline sebum levels or varying sebaceous response to physical stress (Table 3.5).

MAST CELL GRANULES

Based on frequent clinical observations of pruritus and scratching in African Americans, Sueki et al.[48] evaluated differences in mast cells between Black and White skin (Table 3.5). They took 4 mm punch biopsies of normal buttock skin from four African American males (mean age 29.2 ± 3.0 years) and four White males (mean age 29.4 ± 1.2 years) with no prior history of skin disease or atopy and processed the biopsies routinely for electron microscopy. Mast cells in Black skin contained 1.5 times larger granules ($P < .0001$), 15% more parallel-linear striations (PLSs, $P < .05$), and 30% less curved lamellae ($P < .05$) compared with White skin. In addition, the investigators also examined the subgranular distribution of mast cell proteases, tryptase, and cathepsin G by immunoelectron microscopy. They found that tryptase immunoreactivity localized to PLS regions in Black skin, compared with curved lamellae regions in White skin ($P < .0001$). In contrast, cathepsin G localized to electron-dense amorphous subregions in both Black and White skin.

The investigators attributed the larger mast cell granules in Black skin to possible increased fusion or division of the granules in Blacks. On the other hand, they noted that the larger percentage of PLS and smaller percentage of curved lamellae in Blacks was more difficult to explain. They hypothesized that it might be influenced by the mediator content, especially the amount of tryptase. From other studies suggesting the participation of mast cells in aberrant fibrosis in skin disorders such as keloid scars[51] and hypertrophic scars,[52] the investigators suggested the involvement of tryptase in these disorders. Keloid scarring is frequently observed in Black individuals, and Blacks were found to have increased amounts of tryptase in this study compared with Whites. Even though the study had a small sample size and only examined skin from one anatomic region, the researchers still found significant structural differences in mast cells between Black and White skin. Further investigation of proinflammatory mediators should be done to corroborate these findings. This discovery should also prompt further electron microscopic evaluation of other cells involved in dermatologic disorders.

VELLUS HAIR FOLLICLES

As follicular morphology and distribution may affect penetration of topical medications and consequent treatment response, Mangelsdorf et al.[81] investigated vellus hair follicle size and distribution in Asians and African Americans as compared to Whites. Skin surface biopsies were taken from seven body sites of 10 Asians and 10 African Americans, ages 25 to 50 years. The body sites were matched to locations described by Otberg et al.[82] in their study on Caucasians. In comparing the results of the three ethnic groups, the distribution of follicle density at different body sites was the same; the highest average density was on the forehead and the lowest on the calf for all groups. However, follicular density on the forehead was significantly lower in Asians and African Americans ($P < .001$). The Asians and African Americans also exhibited smaller values for volume ($P < .01$, both groups), potential penetration surface ($P < .01$, both groups), follicular orifice ($P < .01$ and $P < .05$, respectively), and hair shaft diameter ($P < .01$, both groups) on the thigh and calf regions. In addition, the follicular reservoir, as described by

follicular volume, was generally higher in Caucasians. The authors concluded that the significant ethnic differences in follicle structure and pattern of distribution, especially in calf and forehead regions, emphasize the need for skin absorption experiments on different skin types to develop effective medications for prevention and treatment of skin disorders.

Luther et al.[90] also investigated vellus hair follicle characteristics among Caucasian, African, and Asian subjects. As was performed by Mangelsdorf et al.[81] skin surface biopsies were obtained, and hair follicle morphology was assessed according to guidelines set forth by Otberg et al.[82] The skin surface biopsies were taken from the scalp and calf of each volunteer. Luther et al.[90] demonstrated that while vellus hair shaft diameter on the scalp and calf showed similar diameters in all ethnic groups, Africans displayed significantly larger diameters in the calf in comparison to Caucasians ($P < .05$). The surface of terminal infundibulum on the scalp, representing the penetration surface, was largest in Caucasians ($P < .05$).[90] Finally, vellus hair follicle density was significantly higher in Asians than in Africans and Caucasians ($P < .05$). In addition to these findings, Luther et al.[90] also measured follicular penetration of sodium fluorescein via differential stripping after topical application. After 96 h, concentrations of sodium fluorescein were significantly higher in the SC of Asians than in Caucasians and Africans ($P < .05$). Researchers acknowledge that this may be due to differences in cultural habits, with Asians wearing shorts more often than other ethnicities, leading to lower amounts of sweating and therefore less removal of the topically applied substance in comparison to Caucasians and Africans.

As discerned by the authors of this more recent study, Mangelsdorf et al.[81] demonstrated an increased follicular reservoir in the majority of anatomic sites in Caucasians versus Africans and Asians.[90] Similar findings and patterns were obtained by Luther et al.[90] but these observations reached statistical significance only in the scalp regions, not the calf. Differences in absorption among different ethnicities have important clinical implications in the dosing of certain therapeutic regimens and deserve further study.

EPIDERMAL INNERVATION

While TEWL, WC, and blood vessel reactivity have been used as measures of irritancy, Reilly et al.[53] sought to explain ethnic differences in irritancy in terms of differences in skin innervation and nociceptor activity. They utilized confocal microscopy to examine epidermal innervation of the volar forearm pretreated with capsaicin in 20 European Caucasian, eight Japanese American, and eight Chinese American volunteers. However, no differences in innervation, including the biochemical properties of the nerve fibers, were found.

FACIAL PORES

In 2009, a study performed by Sugiyama-Nakagiri et al.[110] examined differences in structural properties of facial skin between 20 Caucasian, 20 Asian (10 Chinese and 10 Japanese), 20 Hispanic, and 20 African American women from the same geographic location (Dallas, Texas, United States). Specifically, researchers examined facial pore attributes and analyzed the epidermal architecture around facial pores. In doing so, Sugiyama-Nakagiri et al.[110] determined no significant differences in average pore size between ethnicities but found African Americans to have more severe impairment of architecture around facial pores than any other group tested ($P < .05$). The African American cohort also possessed more dermal papillae per area of facial skin than any other ethnicity ($P < .05$). In regard to dermal papillae, the Hispanic group displayed significantly greater dermal papillae per area of facial skin than the Caucasian participants ($P < .05$). Asian participants, as a whole, exhibited substantially smaller total pore areas than Caucasians and Hispanics ($P < .05$), along with significantly lower pore numbers than Caucasians ($P < .05$). In all, results support significant differences in structural properties of facial skin between different ethnicities, which may impact the appearance of facial pores.[110]

MELANOSOMES

Ethnic differences in number of melanocytes, number of melanosomes, and morphology of melanosomes have been of great interest in working toward the development of objective definitions of skin color (Table 3.6). The biosynthesis of melanin, a cutaneous pigment, occurs in a melanosome, a metabolic unit within the melanocyte; melanosomes are then transported via melanocyte dendrites to adjacent keratinocytes.[66]

In 1969, Szabo et al.[83] examined five Caucasoids, six American Indians, three Mongoloids (from Japan and China), and seven Negroids to observe melanosome groupings using electron micrographs. The melanosomes in keratinocytes of Caucasoids and Mongoloids were found to be grouped together with a surrounding membrane. In contrast, the Negroid keratinocytes showed numerous melanosomes, longer and wider than in other racial groups and mostly individually dispersed. Additionally, they observed an increase in melanosomes of keratinocytes of all races after irradiation, with grouping of melanosomes maintained in Caucasoids and Mongoloids. The authors concluded that individually dispersed melanosomes give a more uniform and dense color than the grouping found in fair skin.

In 1973, Konrad et al.[84] studied melanosome distribution patterns in hyperpigmented White skin alone and found that when comparing hyperpigmented lesions to control areas, there were no uniform differences in the distribution patterns of melanosomes. In addition, the degree of clinical hyperpigmentation was not associated with specific distribution patterns. However, they did note an important relationship between melanosome size and distribution: the percentage of melanosomes dispersed singly increased with increasing melanosome size. The authors also reported findings with experimental pigment donation showing that large melanosomes are taken up individually by keratinocytes and dispersed singly within their cytoplasm, while small

TABLE 3.6
Melanosomes and Skin Surface Microflora

Study	Technique	Subjects	Site	Results
		(a) Melanosomes[a]		
Szabo et al.[83] (1969)	In vivo—EM	Caucasoid, 5 American Indian, 6 Mongoloid, 3 Negroid, 7 (age not reported)	Not reported	Caucasoids and Mongoloids: grouped melanosomes Negroids: longer and wider melanosomes, predominantly individually dispersed
Alaluf et al.[87] (2002)	In vivo—EM; alkali solubility of melanin	European, 10 Chinese, 8 Mexican, 10 Indian, 10 African, 10	Dorsal forearm and volar upper arm	Average melanosome size: dorsal forearm > volar upper arm, in all ethnic groups ($P < .001$); African > Indian > Mexican > Chinese > European Melanosome size correlated with total melanin content (Pearson product moment: r = 0.62, $P < .0001$) Light melanin fraction: African < Mexican and Chinese < Indian < European Dark melanin fraction: African and Indian > Mexican and Chinese > European Total amount of melanin: African and Indian > Mexican, Chinese, and European ($P < .001$)
Thong et al.[86] (2005)	In vivo—EM	Chinese, 15 (skin type IV/V, ages 10–73 years) Caucasian, 3 (skin type II, ages 22–49 years) African American, 3 (skin type VI, ages 18–52 years)	Volar forearm	Proportion of individually distributed to clustered melanosomes: African Americans > Asians > Caucasians ($P < .05$) Mean ± SD size of melanosomes distributed individually > clustered, in all ethnic groups Mean ± SD size of random melanosomes: African Americans > Asians > Caucasians ($P < .05$)
Wakamatsu et al.[111] (2006)	In vitro	60 primary cultures of human melanocytes isolated: Very light, 28 (L) Light, 13 (L+) Fairly dark, 7 (D) Dark, 12 (D+)	51 neonatal and 9 adult skins	Large differences in total melanin (TM) and eumelanin (EM); L < L+ < D < D+ Lighter melanocytes tend to contain more pheomelanin In adult melanocyte cultures, EM correlates with ethnic background of donors (African American > Indian > Caucasian)
Yoshida et al.[112] (2007)	In vitro—HSS on mice	Caucasian (L) and African (D) descent melanocytes (M), keratinocytes (K), fibroblasts (F) K_DM_D; K_LM_D; K_DM_L; K_LM_L	Melanocytes, keratinocytes, and fibroblasts from neonatal foreskin	Melanin content in epidermis and maturation stage of melanosomes in basal keratinocytes significantly increased in HSS composed of dark compared to light keratinocytes Ratio of individual/clustered melanosomes higher in HSS composed of dark keratinocytes
		(b) Skin Surface Microflora[b]		
Rebora and Guarrera[47] (1988)		Black men, 10 White men, 10 (ages 21–59 years, both)	Forearm	*Candida albicans*: Blacks (150% greater) > Whites ($P < .025$) Aerobes: Blacks (650% greater) > Whites ($P < .025$)
Warrier et al.[16] (1996)		Black women, 30 White women, 30 (ages 18–45 years, both)	Left and right medial cheeks, mid-volar forearms, lateral mid-lower legs	Density of *Propionibacterium acnes*: Blacks > Whites but not statistically significant No significant difference in aerobes

Note: HSS = human skin substitute; EM = electron micrograph; SD = standard deviation.

[a] Darker skin has more individually dispersed melanosomes in comparison to lighter skin; individually dispersed melanosomes tend to be larger in size than clustered melanosomes. Darkly pigmented melanocytes are more acidic than lightly pigmented melanocytes.

[b] Insufficient and conflicting evidence to draw conclusions regarding ethnic differences in skin microflora.

melanosomes are incorporated and maintained as aggregates. These data suggested melanosome size differences as the basis for skin color differences between Black skin and White skin.

More recently, Thong et al.[86] quantified variation in melanosome size and distribution pattern in Asian, Caucasian, and African American skin. The volar forearms of 15 Chinese (phototypes IV–V, ages 10–73 years), 3 Caucasians (phototype II, ages 22–49 years), and 3 African Americans (phototype VI, ages 18–52 years) were examined by electron microscopy of 4 mm punch biopsies. The proportions of individual and clustered melanosomes were compared for each ethnic group and showed statistically significant differences ($P < .05$). Melanosomes in Caucasian skin were distributed as 15.5% individual versus 84.5% clustered. Meanwhile, in African Americans, the melanosomes were distributed as 88.9% individual versus 11.1% clustered. The Asian melanosome distribution was intermediate between the latter two groups, at 62.6% individual versus 37.4% clustered. The investigators also determined the mean ± SD size of melanosomes distributed individually to be larger in comparison to those distributed in clusters for each ethnic group. The mean ± SD of random melanosomes in each group differed as African American skin showed significantly larger melanosome size than Caucasian skin, and Asian skin showed melanosome size as intermediate between the other two groups. Thus, there was a trend of progressive increase in melanosome size when moving from Caucasian to African American skin that corresponded with the progression from predominantly clustered to predominantly individual melanosome distribution. In addition, degradation patterns of melanosomes in the upper levels of epidermis varied by ethnic group. As keratinocytes became terminally differentiated and migrated to the SC, melanosomes were completely degraded and absent in the SC of light skin, while intact melanosomes could be seen in the SC of dark skin. Asian skin showed an intermediate pattern where few melanosomes remained in the corneocytes; interestingly, the remaining melanosomes were predominantly individual, indicating that clustered melanosomes may be degraded more efficiently during this process.

Alaluf et al.[87] examined the morphology, size, and melanin content of melanosomes on the volar upper arms and dorsal forearms of 10 European, eight Chinese, 10 Mexican, 10 Indian, and 10 African subjects living in South Africa. Four-millimeter punch biopsies were analyzed based on electron micrographs of melanosomes and on alkali solubility of extracted melanin. The melanosome size of dorsal forearm (photoexposed) skin was observed as approximately 1.1 times larger than melanosome size of volar upper arm (photoprotected) skin ($P < .001$) when data were pooled from all ethnic groups; each ethnic group separately showed a similar trend, but this lacked statistical significance. In addition, a progressive and statistically significant increase in average melanosome size was observed when moving from European (light) to African (dark) skin types. The melanosome size was directly correlated with total melanin content in the epidermis of all subjects ($P < .0001$). When comparing the epidermal melanin content among ethnic groups, the investigators found a downward trend in the amount of alkali-soluble melanin (light-colored pheomelanin and dihydroxyindole-2-carboxylic acid (DHICA)-enriched eumelanin) in the epidermis as the skin type became progressively darker; African skin contained the smallest amount ($P < .02$). Indian skin presented an exception to this trend with higher concentrations of light melanin fractions than both Mexican and Chinese skin ($P < .05$). However, both African and Indian skin showed about two times more of the alkali insoluble melanin (dark-colored dihydroxyindole (DHI)-enriched eumelanins) than the Mexican, Chinese, and European skin types ($P < .001$). Overall, the melanin composition showed a trend toward higher fractions of alkali-soluble melanins while moving from darker (African) skin to lighter (European) skin. In addition, African and Indian skin revealed the highest total amount of melanin ($P < .001$) and did not differ significantly from each other. There was no significant difference in total epidermal melanin between the remaining groups.

Wakamatsu et al.[111] further examined differences in eumelanin and pheomelanin content through their study of 60 primary cultures of human melanocytes, isolated from 51 neonatal and nine adult skins. These cultures consisted of 28 very light (L), 13 light (L+), 7 fairly dark (D), and 12 dark (D+) melanocyte groups. Like Alaluf et al.[87] Wakamatsu et al.[111] noted lighter melanocytes to be generally more pheomelanic in composition than darker melanocytes. As a whole, total melanin (eumelanin plus pheomelanin) and eumelanin increased progressively from very light to dark melanocyte cultures. Eumelanin, but not pheomelanin, was found to correlate well with the ethnic background of donors, with African Americans possessing greater eumelanin content than Indians, who in turn possessed more eumelanin than Caucasians.[111] Finally, investigators discerned greatly varying eumelanin-to-pheomelanin ratios regardless of the pigmentation of the cultured melanocytes. Wakamatsu et al.[111] suggest that this may be due to the influence of keratinocytes and the regulation of cysteine levels, a pheomelanin precursor.

Yoshida et al.[112] implemented an interesting approach to study relationships between melanocytes and keratinocytes, along with their impact on skin color variation. Upon acquiring primary cultures of melanocytes, keratinocytes, and fibroblasts from Caucasian and African neonatal foreskin, combinations of cells were grafted onto the back skin of severe combined immunodeficient (SCID) mice, creating human skin substitutes (HSSs). From this procedure, investigators determined that the epidermal melanin content and maturation stage of melanosomes, measured by the ratio of stage IV melanosomes from electron microscopy, were significantly increased in HSSs composed of African skin-derived keratinocytes compared to Caucasian skin-derived keratinocytes, indicating an influence of keratinocytes on melanogenesis. At the same time, this in vivo HSS model found that melanocyte numbers within the basal layer did not change with light or dark keratinocytes.[112] As supported

by previous studies, the actual total number of melanosomes did not vary based on the type of keratinocyte producing the HSS.[112] Yoshida et al.[112] further corroborated findings made by Szabo et al.[83] and Thong et al.[86] through their observation of higher ratios of individual to clustered melanosomes in HSSs composed of dark keratinocytes.

In 2009, Gunathilake et al.[91] observed differences in pH of the cell bodies and dendrites between 27 lightly pigmented melanocytes and 33 darkly pigmented cells. In all, darkly pigmented melanocytes possessed significantly more acidic cell bodies ($P < .05$) and more acidic dendritic processes ($P < .001$) than lightly pigmented melanocytes being studied. Investigators were able to localize the acidity of the dendritic processes, which concentrate and secrete melanosomes, to vesicular organelles.[91] Gunathilake et al.[91] suggest that these vesicular organelles most likely represent melanosomes.

Despite the data showing differences in number and distribution of melanosomes, recent studies find no evidence of differences in numbers of melanocytes among ethnic groups.[66] For example, Alalluf et al.[85] found no significant difference in melanocyte number between African ($n = 10$), Indian ($n = 10$), Mexican ($n = 10$), and Chinese ($n = 8$) skin types using immunohistochemical methods. They did consistently find 60%–80% more melanocytes in European ($n = 10$) skin than all other skin types ($P < .01$), but the authors felt that a larger sample size would be necessary to confirm this observation. Tadokoro et al.[88] also found approximately equal densities of melanocytes in unirradiated skin of Asian, Black, and White subjects ranging from 12.2–12.8 melanocytes per mm.

Thus, it is generally accepted that differences in skin color are supported more by differences in melanosome distribution, size, and content rather than melanocyte number. Szabo et al.[83] observed larger and more individually dispersed melanosomes in Negroid keratinocytes and concluded that individually dispersed melanosomes may contribute to a more dense skin color. Konrad et al.[84] further noted that the number of singly dispersed melanosomes increased as melanosome size increased. Thong et al.[86] quantified the ethnic differences in melanosome size and distribution, finding a gradient in relative proportion of individual versus clustered melanosomes that corresponded with size of melanosomses. At one extreme, African American skin showed larger melanosomes that were predominantly individually dispersed; with Asian skin displaying intermediate results, Caucasian skin was at the other extreme, showing smaller melanosomes that were predominantly clustered. Alaluf et al.[87] also revealed a progressive increase in melanosome size as ethnic skin went from lighter to darker. Furthermore, dark skin contained more total melanin and a larger fraction of DHI-enriched (dark-colored) eumelanin than light skin. Both Wakamatsu et al.[111] and Yoshida et al.[112] corroborated many of the above findings, in addition to noting eumelanin's correlation with ethnic background and the influence of keratinocyte type on melanogenesis. Finally, Gunathilake et al.[91] showed darkly pigmented melanocytes to be significantly more acidic than lightly pigmented melanocytes.

SURFACE MICROFLORA

Ethnic differences in skin microflora have also been examined. Rebora and Guarrera[47] inoculated the forearm skin of 10 Black men and 10 White men (age 21–59 years) with *Candida albicans* and examined the severity of ensuing dermatitis as well as the population of *Candida* and other aerobes at the inoculum site. The severity of dermatitis was scored subjectively by observation of pustules. However, population of microflora was assessed objectively by colony counts after aerobic incubation at 95°F (35°C) for 2 days. Black skin harbored 150% more yeast after inoculation with *C. albicans* and 650% more aerobes both at baseline and after inoculation than White skin ($P < .025$).

In addition to investigating TEWL, capacitance, desquamation index, elastic recovery, and skin pH, Warrier et al.[16] also examined facial skin microflora in 30 Black and 30 White women aged 18–45 years. They found no significant differences in the density of aerobes (mostly *Staphylococcus* spp.) between Blacks and Whites. In contrast, although not statistically significant, there was a higher density of Propionibacterium acnes in Blacks compared with Whites. They felt that this might be due in part to a believed increase in sebum output in Blacks.[50]

Both studies demonstrated increased skin microflora in Blacks in that Rebora and Guarrera[47] found that Blacks harbor significantly more *C. albicans* after inoculation and Warrier et al.[16] found higher density of *P. acnes*, but the values were not statistically significant. However, Rebora and Guarrera[47] found Blacks to have significantly higher levels of aerobes both at baseline and after inoculation with *C. albicans,* while Warrier et al.[16] found no significant ethnic differences in the density of aerobes. Since the minimal data that exist are conflicting, no conclusions regarding skin microflora can be made until investigators examine the issue further (Table 3.6). Perhaps the age of subjects, anatomic site, and humidity of the geographic environment where the study was conducted cause variation in skin microflora and should be accounted for in future studies.

ANTIMICROBIAL PROPERTIES

In 2001, Mackintosh[68] reviewed evidence discussing the role of melanization of skin in the innate immune defense system. He reported that a major function of melanocytes, melanosomes, and melanin in skin is to inhibit the proliferation of bacterial, fungal, and other parasitic infections in the dermis and epidermis. Numerous studies are cited showing evidence that melanocytes and melanosomes exhibit antimicrobial activity and are regulated by known mediators of inflammatory response. The review aims to support the hypothesis that immunity and melanization are genetically and functionally linked. The author notes that previous reports have implied a reduced susceptibility of dark-skinned individuals to skin disease. In addition, it is postulated that the evolution of Black skin could represent high pressures from infection, especially in tropical regions. In five out of six recent

investigations, people of African descent have been shown to be less susceptible than Whites to scabies, fungal dermatophytosis, cutaneous *C. albicans* infections, and bacterial pyodermas. Additionally, although Rebora and Guarrera[47] demonstrated increased skin microflora in Blacks, they found that the severity of dermatitis in Black subjects was significantly less ($P < .01$), suggesting the possibility of increased barrier defense. This evidence may explain why the existence of melanocytes and melanization among different parts of the body is independent of sun exposure, as in the genitalia, as well as the latitudinal gradient in skin melanization. The evolutionary data presented in this review article are compelling and indicate a necessity for controlled studies to clarify whether the number of melanocytes, size of melanosomes, or type of melanin can affect the antimicrobial properties of skin.

PHOTODAMAGE

Although there is evidence for objective differences in skin color, it remains unclear what role these differences in melanin and melanosomes play in dermatologic disorders. "Surface Microflora" section of this chapter introduced the potential role of melanosomes in antimicrobial defense. The most extensively studied function of darker skin color, however, has been resistance to photodamage from UV radiation. End effects of photodamage include skin cancer, which are well documented as affecting lighter-skinned individuals more than those with darker skin.

In determining a relationship between melanosome groupings and sun exposure, studies have observed that dark-skinned Whites, when exposed to sunlight, have nonaggregated melanosomes, in contrast to light-skinned, unexposed Whites, who have aggregated melanosomes. Similarly, there are predominantly nonaggregated melanosomes in sunlight-exposed Asian skin and primarily aggregated melanosomes in unexposed Asian skin.[66,67]

Alaluf et al.[87] noted an increase in melanosome size in photoexposed skin versus photoprotected skin in all ethnic groups; the melanosome size was directly correlated with epidermal melanin content, suggesting increased melanogenesis in photoexposed areas. Van Nieuwpoort et al.[70] demonstrated that with increased melanogenesis, light-skin melanosomes showed elongation and reduction in width with no significant change in surface area, while dark-skin melanosomes enlarged in both length and width, with an increase in volume. Based on these data, although all skin types show an increase in epidermal melanin with sun exposure, both distribution and morphology may influence unequal filtering between light and dark skin types.

In another study, Rijken et al.[74] investigated response to solar-simulating radiation (SSR) among White and Black skin. Six healthy Dutch White subjects, with skin phototype I–III and mean age of 24.5 years, were exposed to 12,000–18,000 mJ/cm2 of SSR. Six healthy West African or African (South) American Black subjects, with skin phototype VI and mean age of 25.3 years, were exposed to 18,000 mJ.cm^2 of SSR. Six other White subjects were also added to study the effects of erythema-effective doses of SSR. Skin pigment, DNA photodamage, infiltrating neutrophils, photoaging-associated proteolytic enzymes, keratinoctye activation, and the source of interleukin 10 (IL-10) in skin biopsies were taken before and after radiation. The significance of IL-10 lies in the fact that IL-10–producing cells may be involved in skin carcinogenesis. In each White volunteer, SSR caused DNA damage in epidermal and dermal cells, an influx of neutrophils, active proteolytic enzymes, and keratinocyte activation. Also, three White volunteers showed IL-10–producing neutrophils in the epidermis. In Black-skinned individuals, aside from DNA damage in the suprabasal epidermis, there were no other changes found; basal keratinocytes and dermal cells were not damaged. The authors concluded that these results were best explained by the difference in skin pigmentation and that melanin functions as a barrier to protect basal keratinocytes and the dermis from photodamage.

Other studies have suggested that filter properties of melanin alone, do not provide sufficient protection against DNA damage in underlying cells. Tadokoro et al.[69] investigated the relationship between melanin and DNA damage after UV exposure in 37 subjects of five ethnic origins (Black, White, Asian, others not specified) and Fitzpatrick phototypes I through VI. They found measurable damage to DNA in all groups, and DNA damage was maximal immediately after irradiation, gradually returning to baseline over time. The immediate DNA damage levels were higher in Whites and Asians in comparison to Blacks and Hispanics. In addition, the Whites and Asians showed lower constitutive levels of melanin content. However, the kinetics of DNA damage differed among subjects. Upon monitoring the percentage of removal of damage toward baseline 7 days after UV exposure, no correlation was found between melanin content or ethnic group and the efficiency of DNA damage removal. There were variable rates of DNA repair within individual groups, indicating that DNA repair rates were not associated with skin type. The authors noted that other properties of melanin, such as antioxidant properties and radical scavenging properties, may play roles in minimizing UV damage. Ethnic differences in expression of receptors involved in melanosome uptake and melanocyte-specific proteins, both before and after UV exposure, are also being investigated.

CONCLUSION

The US census bureau estimates that the population is composed of 13.1% Black or African American, 16.7% Hispanic or Latino, and 8.7% other non-Whites.[71] It has been predicted that people with skin of color will constitute a majority of the United States and international populations in the twenty-first century.[72] These statistics highlight the importance of objective investigation of differences in structure and function of skin of different colors; relationships between race, color, and ethnicity; and process and presentation of disease in these groups. It is imperative that we have a deeper understanding of these characteristics, as they play an important

role in learning how to appropriately modify patient treatment. Differences do exist in structure and physiology of skin among different races and may differentially affect disease. However, data on ethnic differences in skin, physiology, and function are few; the studies that do exist consist of typically small patient populations. Consequently, few definitive conclusions can be made. The Food and Drug Administration (FDA) currently recommends inclusion of more ethnic groups in dermatologic trials, citing evidence that physiologic differences in skin structure between races can result in varying efficacies of dermatologic and topical treatments.[63]

There exists reasonable evidence (Table 3.7) to support variable blood vessel reactivity, lower skin surface pH, larger mast cell granules, and larger melanosomes with more individual distribution in colored skin when compared with White skin by means of objective measurements. Although some deductions have been made about Asian and Hispanic skin, the results are contradictory, and further evaluation of Asian and Hispanic skin needs to be done. A review by Robinson[54] also supported the notion that the evidence comparing Asian and Caucasians is insufficient and less than compelling, but there have been an increasing number of studies comparing these ethnicities in recent years. Perhaps more specificity about the origin of their heritage, as has been found in more recent studies cited, should also be included since "Asian" and "Hispanic" encompasses a broad spectrum of people. Ethnic differences in skin WC, corneocyte desquamation, skin elastic recovery/extensibility, microtopography, lipid content, sebaceous function, follicular morphology and distribution, and skin microflora, although statistically significant, are minimal and contradictory. Thus, no conclusions regarding these objective data can be made.

One issue that must be raised when interpreting these studies is the definition of race or ethnicity. Race seems to encompass genetic variations based on natural selection, which include, but are not limited to pigmentation.[63] Pigmentation appears to be based mainly on erythema, melanin, and the skin's response to physiologic insult. Anthropologists divide racial groups into Caucasoid (e.g., Europeans, Arabs, Indians), Mongoloid (e.g., Asians), Australoid (e.g., Australian aborigines), Congoid or Negroid (e.g., most African tribes and descendants), and Capoid (e.g., the Kung San African tribe), with the idea that racial variations were selected to facilitate adaptations to a particular environment.[56,57] Some reject the relevance of any genetic basis for race, stating that 90%–95% of genetic variation occurs within geographic populations rather than across racial groups.[63] Furthermore, the concept of race has been dismissed by some as being an artificial, nongenetically defined construct, lacking scientific basis, and a hindrance for research, diagnosis, and treatment of skin disease.[63,73]

Ethnicity, on the other hand, is a more general term, encompassing biologic and cultural factors. Ethnicity has been defined as how one sees oneself and how one is seen by others as part of a group on the basis of presumed ancestry and sharing a common destiny, often with commonalities in skin color, religion, language, customs, ancestry, and/or occupation or region.[58] Thus, ethnicity encompasses a set of categories that overlaps with race but also depends on more subjective and cultural factors, while race seems to encompass genetic variations based on natural selection. With these obscure definitions based on both biology and the subjective manner in which one labels oneself, the basis of objective research on racial or ethnic differences is already somewhat subjective and, therefore, problematic. However, studies show that differences, whether based on genetic variations or on subjective labels, do exist. Perhaps future studies in dermatology should also address how one defines oneself as part of a particular race or ethnic group in addition to examining the degree of skin pigmentation. This will help determine whether the differences are truly the result of genetic variations that were selected for by race or of biologic variations in melanin content that vary between and within each race. Further research in both genetics and dermatology are warranted to draw any final conclusions with regard to race/ethnicity as the etiology for differences in skin physiology.

TABLE 3.7
Summary

Evidence Supports	Insufficient Evidence for	Inconclusive
• Variable ethnic blood vessel reactivity • pH, Black < White skin • Larger mast cell granules, increased PLS, and increased tryptase localized to PLS in Black compared to White skin • Darker skin has more individually dispersed melanosomes; individually dispersed melanosomes larger than clustered melanosomes • Darkly pigmented melanocytes are more acidic than lightly pigmented melanocytes	Ethnic differences in[a] • Skin microflora • Epidermal innervation • Vellus hair follicle morphology and distribution, although both studies support increased follicular reservoir function in Caucasians vs. Africans and Asians • Facial pore structure	Ethnic differences in • TEWL • Water content • Corneocyte desquamation • Lipid content • Sebaceous function

Note: PLS = parallel-linear striations; TEWL = transepidermal water loss.

[a] Skin microflora, epidermal innervation, vellus hair follicle morphology/distribution, and facial pore structure were labeled as "insufficient evidence for" ethnic differences rather than "inconclusive" because only two studies or less examined these variables.

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4 Sensitive Skin
Sensory, Clinical, and Physiological Factors

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INTRODUCTION

The sensory reactions of consumers to common products such as health and beauty products strongly influence purchasing decisions[1]; in fact, of consumers who claimed unusually sensitive skin, 78% avoided specific products due to prior experiences of unpleasant sensory effects with their use.[2] Manufacturers of consumer products conduct intensive premarket testing intended to ensure that marketed products are free of irritant potential. It is not uncommon, however, for postmarketing surveillance efforts to receive reports of sensory perceptions not predicted by even the most robust development methodology.[1] These sensory perceptions, though often transient and unaccompanied by a visual dermatological response, define an equivocal and still evolving dermatological condition known as *sensitive skin*.

Sensitive consumers report an impressive variety of sensory reactions (prickling, burning, tingling, stinging, itching, dryness, and/or tightness[3,4]) in response to contact exposures to certain products (Table 4.1).[3,5] Investigations spurred by these unpredicted reactions to contact exposures added environmental insults such as wind, pollution, and cold to the list of possible triggers, as well as intrinsic insults like stress and hormonal fluctuations (Table 4.2).[3] Reports of some sensory distress occur primarily in the absence of obvious physical signs of irritation such as erythema.[3]

Sensitive skin, a term whose definition continues to be refined, is now generally agreed to describe unpleasant subjective sensory reactions to common contact and environmental exposures, which occur without classically demonstrable irritation or immunological response.[6] The term "reactive skin" occurs occasionally in the literature as well.

Consumer products have generated a menagerie of professed symptoms over a wide range of intensity in response to a multitude of both contact and environmental exposures. Diagnostic criteria for a condition based on largely subjective and often ephemeral symptoms therefore have been, not surprisingly, rather elusive.

A lack of correlation between sensory perceptions and response to known irritants has hampered the initial research. Some who describe themselves as nonsensitive develop clear signs of objective irritation, while many who claim to have sensitive skin report intense sensation without any accompanying physical sequelae.[7] Extreme intrapersonal variability is also observed in reaction to potential exposures: an irritant dose completely tolerable in 99 subjects can cause pronounced irritation in the hundredth.[7] The intensity of individual responses to specific irritants also varies tremendously.[2]

In addition, multiple intrinsic and extrinsic factors appear to influence sensory perceptions, including but probably not limited to age, genetics, hormonal factors, race, skin type, anatomic region, and concurrent skin diseases. Sensory response, for example, can vary substantially within the same individual at different[8] and even at the same anatomical site on symmetric limbs.[9]

Factors that differ between geographical locations and individual cultures must also be considered. As many common consumer products are now being marketed worldwide, an understanding of the causes and mechanisms of sensitive skin becomes even more important.[10]

WORLDWIDE DISTRIBUTION OF SENSITIVE SKIN

The phenomenon of sensitive skin, particularly its global prevalence, has been largely explored throughout much of the world using epidemiological surveys. Although sensitive skin was initially believed to be an abnormal reaction to common products that occurred in only small subsets of consumers, these surveys consistently find a high prevalence of sensitive skin across the industrialized world (Figure 4.1). The majority of women in the United States, Europe, and Japan (most of the patients queried to this date) now believe they have sensitive skin.[11] Self-assessed claims to skin sensitivity have also increased steadily over time,[12] with the rate of increase being particularly noteworthy in men.[13]

The fact that self-diagnoses solicited through the use of consumer surveys reveal a majority of consumers who believe they have unusual, unpleasant, and unpredicted (through premarket testing) sensory responses to products well characterized as nonirritant in premarket testing, combined with the lack of any measurable physical component using a variety of methodologies, led some to doubt that sensitive skin might be of physiological origin.[12,14]

PHYSIOLOGICAL BASES OF SENSITIVE SKIN

The phenomenon is recorded in all nations studied,[2] however, and the finding of equivalent prevalence in two separate

TABLE 4.1
Reported Signs and Symptoms of Sensitive Skin

Symptoms	Signs
Burning[24]	Excoriation[89]
Itching[24]	Hyperkeratosis[89]
Pain[81]	Lichenification[89]
Prickling[24]	Papules[89]
Smarting[69]	Redness/erythema[81]
Stinging[81]	Scaling[24]
Tickling[89]	Vesicles[89]
Tightening[24]	Xerosis/dryness[24]

TABLE 4.2
Reported Factors Contributing to Sensitive Skin

Specific Contact Exposures	Environmental and Internal
Deodorants/antiperspirants powder/talcs[15,91]	Cold[3]
Fabrics[15,19,91,92]	Dry air[19]
Facial cosmetics[15]	Heat[29]
Facial moisturizers, cleansers, astringents[15]	Hormonal fluctuations[3]
Hair products (e.g., shampoo, conditioners, colorants)[15]	Humidity[19]
Household cleaners and dishwashing liquids[15,91]	Pollution[3]
Laundry products: detergent and fabric softeners[15,91]	Spicy food[2]
Pantiliners and menstrual pads[91]	Stress[3]
Perfumes/fragrances[91]	Sun[15]
Soaps[3,47,91]	Temperature changes[2]
Sunscreen[15,91]	Wind[47]

continents (North America [United States], 68%,[13] and Europe [Greece], 64%[15]) lends physiological credibility to consumer complaints. Although initial studies could find no predictable correlation between reported sensory perception and frank signs of irritation, and documented sensory responses to tested irritants were not predictive of generalized sensitivity, a subgroup of sensitive subjects termed "stingers" were shown to experience stronger sensory irritation to chemical probes for stinging and burning.[16]

A few studies also successfully demonstrated that sensitive-skin patients are capable of distinguishing products based on blinded sensory end points. Of two studies that evaluated the relationship between neurosensory responses and objective clinical irritation, and that included only subjects with demonstrated sensory sensitivity, both showed a correlation between sensory and objective signs.[6] In a study regarding sensitivity to facial tissue that did not exclude nonsensitive individuals, sensory effects, in fact, were demonstrated to be the most reliable measure of product differences.[6]

Research since has steadily identified a set of intrinsic factors that contribute to sensitive skin, a clear indication of a physiological basis for this as-yet not-fully-understood dermatological condition.

HOST FACTORS AFFECTING SKIN SENSITIVITY

Numerous potential host factors are known, through epidemiological studies, to influence the perception of sensitive skin.

Gender

Sensitive skin is historically claimed far more often by women than by men, an observation with biological plausibility, since men have thicker skin then women[17] and hormonal differences produce inflammatory sensitivity in the skin of females.[9,18] However, a recent study in 1039 subjects found a 68.4% prevalence of self-reported sensitive skin, with no difference between men and women.[19] Products targeted at sensitive skin, particularly with regard to men's personal hygiene products, are one of the fastest-growing segments of the health and beauty industry. It may be that with increased marketing of products for sensitive skin in men, it has become more culturally acceptable for males to define themselves as having sensitive skin.

Ethnicity

Racial differences in skin structure and associated susceptibility to disease are among the fundamental questions in dermatotoxicology.[20] Two large epidemiological studies have reported no observed racial differences in self-reported sensitivity to consumer products.[2,21] Most survey respondents, however, have been Caucasian females. Less subjective methodologies suggest genuine racial differences. Skin structure is known to vary significantly by skin type, and structural differences with the potential to influence permeability have been observed. Epidermal thickness was found to correlate with pigmentation ($P = .0008$)[1]; fair skin, in addition to being thinner, has a much higher tendency to flush or blush, both associated with barrier impairment and increased vascular reactivity.[21]

Although no significant differences in barrier function (Asian versus Caucasian) were observed,[22] Blacks and Asians were shown to have higher baseline transepidermal water loss (TEWL) values than Caucasians.[23] Racial differences in the ceramide content of the stratum corneum (SC) have also been observed,[22] as has a difference in the buoyant density of the SC.[24] Skin hydration has been observed to be higher in Black, Asian, and Hispanic subjects than in Caucasians.[25]

The number of sweat glands in the skin has been proposed as an influencing factor in permeability[22]; substantial variation in the distribution and size of apocrine glands among races has also been observed.[22] There has been some association observed in Blacks between sweat gland activity and conductance,[22] which could be related to differences in the chemical composition of sweat.[20] The increased electrical resistance observed in Blacks implies an increased cohesion or thickness of the SC.[26] Although studies of racial

FIGURE 4.1 Prevalence (highest reported percentage of sensitivity, 1992–2012) of sensitive skin around the world. (a) Europe.[47,87,93] (b) Asia.[91,94] (c) United States.[2,13] (Maps courtesy of www.begraphic.com.)

differences with regard to irritant response have yielded conflicting evidence, methyl nicotinate assessment of vasoactive response suggests that there may be genuine racial differences in permeability.[6] Increased percutaneous absorption of benzoic acid, caffeine, and acetylsalicylic acid was demonstrated in Asians when compared with Caucasians, and decreased percutaneous absorption was observed in Blacks.[22]

Sensory testing, however, has not correlated well with permeability. Despite no observed difference in SC function (measured through multiple methodologies), Japanese women living in Marburg, Germany, were significantly more likely to report stinging sensations in response to 10% lactic acid application than the German women tested.[22]

Another investigation used capsaicin to evaluate sensory response in 114 women by assessing threshold detection of capsaicin. Although individual variability was substantial (some volunteers detected the lowest concentration [0.0000316%], while almost one-third did not detect the highest concentration [100 times higher]), and distribution differed only slightly between groups, Caucasians evidenced higher sensitivity to capsaicin than did Asians, who had higher sensitivity than African women; 56.8% of African women did not detect even the highest concentration, as compared to 42% of Asian subjects and only 34% of Caucasians.[10]

Survey-based epidemiologic studies have observed that, while overall prevalence of skin sensitivity is similar across skin types and ethnic groups, there were differences with regard to what triggers discomfort and how it is experienced. Euro-Americans were found to have higher susceptibility to wind relative to other ethnic groups.[2] Asians had higher sensitivity to spicy food, and Hispanics had relatively less reactivity to alcohol.[2] Caucasians reported visual effects like erythema more than African Americans, while African Americans were more likely to report sensory effects.[27] In addition, African Americans of both genders were more likely to report sensitivity in the genital area than other groups ($P = .0008$).[13]

Age

The physiological changes that occur as skin ages would predict an increased susceptibility to irritants. SC integrity in the elderly is more fragile.[6] While tactile sensitivity has been shown to decrease with age, however, pain sensation is preserved.[6] Susceptibility to known irritants, however, is decreased in older people,[28] and when sensory response to a lactic acid challenge was evaluated in more than 100 elderly Italian subjects, the intensity of the stinging response was inversely proportional to age.[29]

A survey of sensitive-skin perception in 1029 individuals in Ohio (United States), however, found that those over 50 years of age were more likely to claim sensitive skin than younger adults, adding that skin had become more sensitive over time (46%).[28] Those over 50 years of age were also more likely to perceive genital skin to be more sensitive.[28] Sensitivity of the skin of the scalp was also found to increase with age.[30]

Sensitivity to mechanical stimuli, stinging, and skin wetness were shown to decline after the menopause, in contrast to sensations of burning and itching, which did not.[31] Interestingly, sensitivity to mechanical stimuli was restored by estrogen supplementation.[31]

Anatomic Site

Sensory response to various stimuli has been widely demonstrated to depend on the anatomical region studied, biologically reasonable as skin is known to differ by location with regard to skin structure and function.[32] Analysis of structural differences found that SC density varies tremendously by anatomical site: Palms and soles are the thickest, while the genital area is very thin (six layers).[6] The SC on the face is thinner than on the limbs and torso,[33] with faster turnover [6] but relatively poorer barrier function.[33] Permeability also varies by anatomical site[34] in correlation with numbers of layers in the SC.[33] TEWL, a reflection of the structural integrity of the SC, also varies in parallel with its thickness and maturity.

The face is the most common site of skin sensitivity.[13,35] In a study of 1039 men and women, 77.3% reported facial sensitivity, compared to 60.7% for the body and 56.2% in genital skin.[13] Saint-Martory et al.[3] also found the face to be the most commonly reported site of sensitivity, with hands, scalp, feet, neck, torso, and back also reported, in order of frequency.

The nasolabial fold has been reported to be the most sensitive region of the facial area, followed by the malar eminence, chin, forehead, and upper lip.[3,5] Misery et al.[36] found that 44.22% of sensitive-skin subjects questioned also experienced sensitivity of the scalp, with itching and prickling being the most frequent symptoms of scalp sensitivity.[30] Women, in particular, experience facial sensitivity, most likely related to the number of products used on the face, a thinner barrier in facial skin,[35] and twice the density of nerve fibers in facial skin.[37]

Most existing studies have been conducted in facial skin because of its sensitivity (stinging sensations, particularly, are readily elicited on facial skin[38]) and the fact that it is easily accessible for both visual[39] and biophysical assessments.[40] The hands have also been shown to be particularly sensitive, specifically with regard to household cleaners.[41]

Vulva

The vulva is an area of particular interest, as it differs from skin at exposed body sites.[42] Skin is thinnest in the genital area,[43] and nonkeratinized vulvar skin exhibits clearly increased permeability related to the absence of keratin and a loosely packed, less structured lipid barrier.[42] Vulvar skin is also characterized by a frictional component, occlusion, increased hydration, increased hair follicles and sweat glands, and increased blood flow.[6]

This potential for heightened vulvar susceptibility to topical agents is not widely reported in the literature, despite the fact that 29% of patients with chronic vulvar irritation were demonstrated to have contact hypersensitivity. Of those patients, an impressive 94% were determined to have developed secondary sensitization to topical medications, symptoms that could readily be interpreted by consumers as sensitive skin.[6]

Irritant effects on the vulva are seemingly dependent on the relative permeability of the specific irritant in vulvar skin.[42,44] Irritant reactions to feminine-care products have been reported with a few feminine products that contain chemicals known to be irritants in certain doses.[45]

Recent studies have evaluated skin sensitivity in the vulvar area with regard to sensory responses to consumer products meant for the vulvar area. Although patients with existing genital erythema related to a concurrent infection did not evidence an increased sensitivity to feminine hygiene pads,[38] women who perceived themselves as particularly susceptible to facial erythema were significantly more likely to have medically diagnosed vulvar erythema, a potential indicator of an underlying systemic source.[38]

Women with urinary incontinence, surprisingly, did not evidence an increased sensitivity of genital skin but were significantly more likely to assess themselves as having overall skin sensitivity than continent subjects (86.2% versus 68.3%, respectively).[46] A recent study evaluated vulvar sensitivity to a variety of stimuli by measuring sensory thresholds and found that the vulva was less sensitive to punctate touch and vibration than other body sites.[31]

Environmental Factors

The lower temperatures and humidity characteristic of winter are known to cause lower water content in the SC.[9] Correspondingly, a majority of sensitive-skin sufferers report unpleasant sensory responses to cold temperatures and wind but also to sun, pollution, and heat.[2,24] Air conditioning, which lowers temperature and humidity, is also reported to be a trigger for sensitive skin.[47] The frequency of sensitive skin in women was observed to be significantly higher in summer than in winter (71.2% in July versus 59.39% in March).[48] Unusual occupational or leisure exposures to chemicals may also contribute to sensitive skin.[14]

Allergy and Atopy

Data continue to accumulate that implicate atopy and allergy in sensitive skin, a hypothesis with biological plausibility, as contact allergy and skin sensitivity share similar cytokine induction[49] and the density of cutaneous nerves has been demonstrated to be higher in atopic skin than in normal skin.[50] Stingers are also more likely to have atopic dermatitis (AD).[50]

One very early epidemiological study in the United Kingdom with 2368 respondents observed the incidence of atopy to be higher in subjects with sensitive skin.[21] In another survey-based assessment of 1039 individuals (83.6% female), subjects who claimed sensitive skin were five times more likely to report medically diagnosed skin allergies ($P < .0000$) than those without sensitive skin and three and a half times more likely to have relatives with sensitive skin.[49] Löffler et al.[14] also observed a link between sensitive skin and self-reported nickel allergy.

One study compared Greek women with medically diagnosed AD to women with unrelated dermatological problems and found a significant association between the clinical diagnosis of AD and a self-report of skin sensitivity ($P < .001$). All patients (100%) in the AD group claimed at least some degree of skin sensitivity, as compared to 64% of individuals with other skin conditions. The claim to moderate or severe sensitivity was 80% in the AD group, as compared to 16% in the controls.[15] Patients with AD were also significantly more likely to indicate a family history (68% to 24%, $P = .004$) of sensitive skin; 76% of the sensitive family members were parents.[15] The frequency, severity, and history of skin sensitivity in patients with AD were also far more pronounced than in controls.[15]

Based on available data linking sensitive skin to atopic and allergic disorders, a three-question algorithm was developed for identifying atopic individuals out of a population of sensitive-skin patients. Able to identify 88% of known atopic individuals in testing, the algorithm provides a rapid and useful tool for screening in industry, postmarket surveillance, and epidemiological testing.[51] Such a strong correlation of sensitive skin with other, more well-described skin conditions with reliable diagnostic criteria underscores the physiological origins of the sensitive-skin phenomenon.

POSSIBLE PHYSIOLOGIC BASES OF SENSITIVE SKIN

Subclinical Response to Classic Irritants

It is possible that skin sensitivity simply represents a subclinical indication of classical skin irritation. Although most sensitive-skin reports define only sensory manifestations of irritation, and no consistent correlation of objective signs with subjective perceptions has been defined,[6] a few did find some association between subjective and objective signs. Some self-reported sensitive-skin sufferers describe physical signs of irritation like erythema.[15] Specific biophysical parameters were assessed in 32 subjects previously diagnosed with sensitive skin (without any accompanying clinical manifestations) as compared to a nonsensitive-skin control group. Patch testing found that patients with sensitive skin were 10 times more likely to respond to traditional irritants ($P < .01$) and three times more likely to respond to cosmetic allergens ($P < .01$) than those without sensitive skin.[52] Sensitive subjects also had significantly less sebum production ($P < .01$) and dryer skin ($P < .05$).[52]

Vascular reactions to methyl nicotinate and acetyl-b-methyl chloride in sensitive-skin patients were linked to a significant hyperreactivity of skin blood vessels and increased erythema[52]; the risk of intense vascular reaction to methyl nicotinate was 75 times higher in sensitive patients than nonsensitive controls. A strong association of sensitivity with fair skin was also observed.[52]

Part of the reason for the observed breakdown between sensory effects and objective signs is the fact that an objective sign like erythema is the end result of a complex, multistep physiological process. Numerous underlying processes (e.g., changes in blood flow, moisture content, pH) would be expected to occur before the appearance of visible external changes.[1] Advances in testing protocols reveal subclinical changes, refuting the hypothesis that sensitive skin is without physical sequelae.

Exaggeration of Test Conditions

Simion et al.,[53] by exaggerated arm washing with synthetic detergent bars, observed physiological skin effects like dryness that correlated statistically with sensory perceptions (dryness, tightness, and itching). In addition, consumers were able to reproducibly distinguish between test products purely on the basis of sensory effects.

One study evaluated four versions of facial tissues with tape stripping prior to repeated wiping in order to accentuate irritation, daily evaluating erythema, dryness, and product preference. Panelists' subjective product preferences distinguished among the four test products more reliably than either erythema or dryness.[54]

A second method of accentuating test conditions, developed specifically for testing paper products such as catamenial products, has proven very effective at accentuating irritant response to inherently mild products. The behind-the-knee (BTK) protocol, developed specifically for testing inherently mild paper products like feminine pads, uses product secured by an elastic band at the popliteal fossa as a test site, which adds a critical mechanical friction component to traditional testing.[55] Levels of irritation produced in BTK testing are consistently higher than those achieved with standard patch testing, consistently reproducible.[55] Used with the advances in quantifying sensory responses described below, BTK has proven useful in the development of valuable protocols for sensitive-skin testing.

Quantifying Sensory Responses

Feminine hygiene products (inherently nonirritant) were evaluated using the BTK protocol[56] according to four combinations of test conditions (wet/dry, intact/compromised skin)

in parallel with a traditional arm patch.[57] Although no differences were observed between any combinations tested with regard to erythema, skin sites where patients experienced burning, itching, or sticking had consistently higher mean irritant scores.[57] Altogether, eight similar studies positively associated perceived sensory effects with an increase in irritant scores.[55]

Increasing Sensitivity of Assessment of Physical Response

Visual grading of erythema has been relied on for a number of years as trained graders achieve a high degree of reproducibility with no specialized equipment. A new approach, however, utilized cross-polarized light, which allows visualization of the skin at a depth of 1 mm below the surface. Testing was performed using sodium lauryl sulfate (SLS) in a standard patch test, as well as two different feminine hygiene products behind the knee. Subsurface visualization provided no advantage over visual scoring with the minor irritation produced by low-level SLS. In BTK subjects, however, enhanced visual scoring through subsurface visualization allowed the observation of significant differences in the irritation produced by the two different products, as early as the first day.[58]

In further research, subclinical changes were observable after initial exposure; enhanced visualization was able to correlate subclinical effects on skin to previously established consumer preferences between two products,[58] a correlation that had not been verifiable in prior testing.[59] Enhanced visual scoring, used successfully with both traditional patch testing and BTK (with SLS and catamenial pads), provides a first link between sensory and physiological effects.

Enhanced visualization was also employed in assessing the genital area of symptomatic patients, with the conclusion that enhanced visualization through cross-polarized light may assist in diagnosing subclinical inflammation in vulvar conditions heretofore characterized as sensory syndromes.[60]

A second approach demonstrated a correlation between surface-temperature measurements and inflammatory response.[61] A high-precision handheld infrared thermographic scanner facilitates rapid convenient measurement of changes in skin temperature in situ.[62]

Two catamenial products were compared in a BTK protocol, where skin surface temperature was measured using the thermographic scanner. Skin temperature changes observed were closely associated with visual scores, both correlated also with subject reports of sensory discomfort such as rubbing, chafing, burning, sticking, itching, and pain. Furthermore, the diaries of the subjects' sensory experiences made a clear distinction between the two test products, a distinction that was consistent with both visual scoring and skin temperatures; six of eight sensory effects were associated with higher visual scores.[62]

Collection of skin-derived cytokines can be achieved via a commercially available product called Sebutape (CuDerm Corporation, Dallas, Texas), applied to skin for 60 s and then removed. Application of the tape to both healthy skin and compromised skin was followed by extraction of different cytokines from the tape, which were then quantified. Compromised skin was associated significantly with interleukins (ILs) and receptor antagonists (RAs), such as increased IL-1α levels, increased IL-8 levels, and increased IL-1RA/IL-1α ratio. This technique has potential usefulness in further characterizing sensitive skin.[63]

Abrogation of the Stratum Corneum

The sensitive-skin phenomenon emerged from reports of consumer intolerance to common health and beauty products; certain ingredients of some of these products are known to be irritants at certain concentrations.[64] Recent work has demonstrated that the pH of skin care products can impair SC integrity and increase permeability. Products of pH 8 (as compared to pH 3 or 5) were associated with a decrease in the pH of the skin surface and swelling of the SC. Skin exposed to that pH also evidenced a significant increase in TEWL as well as desquamation, signs of disruption of SC integrity.[65] Sensitive-skin patients often describe "tightness" as an unpleasant sensory response to contact with common products, a sensation produced by rapid evaporation of water from the skin surface (TEWL) induced by loss of SC integrity.[66]

The SC acts as barrier against both water loss and penetration by potentially harmful irritants and is therefore integral to maintaining a normal skin surface pH of 5.5 as well as an internal pH of 7.4, both of which help to preserve a functional barrier.[65] When compromised, penetration of foreign substances may stimulate keratinocytes and Langerhans cells to produce mediators that produce an inflammatory response as well as the onset of symptoms of sensitive skin.[67] Alteration of skin-barrier function may allow irritants to penetrate more easily, causing a more rapid and intense reaction than in the "normal" population.[68]

Although highly sensitive to environmental conditions, TEWL is often used as a measure of SC integrity. Most studies have looked for baseline differences in TEWL between those who claim sensitive skin and controls, with equivocal results. Most studies found a meager difference between baseline biophysical parameters.[32,40,67,69]

A recent study, however, compared sensitive skin to nonsensitive by calculating dynamic water mass and evaporation half-life based on mathematical modeling of TEWL desorption curves obtained after occlusion. This dynamic approach found significant differences between groups and unequivocal evidence of barrier impairment in those with sensitive skin, successfully correlating subjective sensory experience with impairment in the SC barrier.[67]

The role of the stratum in sensitive skin is also supported by research showing that daily supplementation with flaxseed oil leads to significant improvement in skin sensitivity (after irritant exposure) as well as decreases in TEWL.[70] Skin hydration may also ameliorate skin sensitivity[11]; in postmenopausal women, use of an emollient relieved skin sensitivity in parallel with improvements in SC function.[71]

Evaluation of the potential role of the SC in sensitive skin using corneosurfametry confirmed that subjects with a self-reported sensitivity to detergents had an increased reactivity to tested products as compared to the control group.[72] Corneocytes in SC in sensitive skin may react in exaggerated fashion to contact with cosmetics or other products, producing a release of cytokines, prostaglandins, and leukotrienes, which stimulate release of neuromediators may act directly to stimulate nerve endings.[66]

Abnormalities in intercorneocyte lipids have been observed in some skin diseases, and in patients with a compromised SC, such as AD, psoriasis, and ichthyosis, decreased SC ceramides are reported. The ceramide composition of the SC (in vitro) in sensitive-skin individuals combined with lactic acid testing found that although the persons in the sensitive-skin group were more likely to be stingers, and although no differences in either TEWL or erythema were observed, sensitive-skin sufferers had fewer SC ceramides than controls. Six sites were tested, but the difference reached significance only in the face.[73]

Reduction of the Neurosensory Threshold

Sensitive skin is predominantly sensory in nature and thus includes a neural component. Sensory differences may be related to innervation; viable epidermis is replete with free nerve endings, but this network is still poorly characterized.[74]

Studies with lactic acid sting tests support a physiologic etiology for sensitive skin. Local anesthetics block response in lactic acid sting tests, and those who perceive a pronounced burning sensation in response to lactic acid ("stingers") also respond more vigorously to vasodilators.[35]

An Italian study compared self-reports of sensitivity to response in the lactic acid test and found that the prevalence of stingers was very similar to the prevalence of self-reported skin sensitivity (54.3% and 56.9%, respectively). In addition, those who believed their skin to be sensitive were more likely to be stingers (59%) than nonstingers (48.9%).[29]

Functional magnetic resonance imaging (MRI), which measured cerebral activation associated with skin discomfort, found that lactic acid-induced skin discomfort resulted in increased activity in multiple areas of the brain, with significantly greater increases in neural activity in sensitive-skin patients than in controls.[75]

Another study measured calibrated electrical stimulation of the skin by stimulating nerve fibers with three different current strengths (5 and 250 Hz, 2 kHz) in addition to applying capsaicin (0.075%) to the zygomatic arch. With stimulation at 5 Hz (a current known to selectively stimulate the c-fibers of sensory nerves), subjects with sensitive skin (confirmed by sensory testing) displayed a significantly lower perception threshold. Capsaicin application, in nonsensitive subjects, had no effect on perception of the 5 Hz current, while the sensory perception threshold increased in sensitive subjects, a dulling of perception that lasted for 60 min. These findings suggest that sensory perception in sensitive subjects is easily disturbed by weak stimulation, inducing a wide variability of response compared to nonsensitive subjects, an effect which appears to be c-fiber modulated.[76]

Sensory thresholds may involve free nerve endings and nerve corpuscles, which receive both excitatory stimuli and suppressive signals. Suppression of an antagonist acts to amplify the efferent neurosensory input, which is interpreted by consumers as the unpleasant sensory manifestations of sensitive skin.[66] It has been long believed that only C-terminals in the epidermis affect skin surface perception; it is now recognized, however, that neurotransmitters and their receptors, as well as receptors that regulate the neuroendocrine system of the skin, are present in keratinocytes, which recognize environmental stimulants and react. Neurogenic inflammation probably results from the release of neurotransmitters such as substance P, calcitonin gene-related peptide (CGRP), and vasoactive intestinal peptide, which induce vasodilation and mast cell degranulation. Nonspecific inflammation may also be associated with the release of ILs (IL-1, IL-8, prostaglandin E2, prostaglandin F2, and tumor necrosis factor-α).[36]

Mast cells may also mediate sensory perception. Both the numerical density and size of the lymphatics in the upper levels of the dermis were increased in sensitive-skin subjects (as defined by electric current perception) as compared to controls.[77]

Another important neural modulator is the heat receptor transient receptor potential vanilloid 1 (TRPV1), involved in nociception and which mediates not only warmth but also burning.[78] A study of capsaicin detection thresholds between ethnic groups observed a 2log unit range of observed values,[10] confirming the high level of variability consistently reported in the literature. Variability could be explained by existing genetic differences in the expression level of TRPV1 or by varying functionality in keratinocyte cell membranes in the surface layers of the skin.[79]

Interestingly, support for both SC abrogation and a decreased neural threshold was observed through the use of a lysate of probiotic lactic acid bacteria. Use of the lysate in ex vivo human skin implants resulted in statistically significant improvement versus placebo in parameters of inflammation; when nerve cell cultures were used in vitro, the probiotic lysate also significantly inhibited capsaicin-induced CGRP release by neurons. Initial investigations were followed by a randomized, double-blind placebo-controlled trial in which 66 volunteers used either lysate cream or placebo for 2 months. Subjects who used the lysate cream showed a significant decrease in lactic acid sensitivity and decreased TEWL, as well as resistance to further barrier function disruption by stripping. The authors suggest that skin sensitivity decreases because neurons become less accessible in an intact SC, as well as less reactive.[80]

A PHYSIOLOGICAL CONDITION WITH A PSYCHOSOCIAL COMPONENT

Although skin sensitivity to normal household products was initially believed to be an abnormal reaction to common products, a reaction that, by definition, would be expected to

occur in only a small subset of consumers, epidemiological surveys consistently find a high prevalence of sensitive skin across the industrialized world (Figure 4.1). This, combined with the difficulty of correlating objective signs to sensory perceptions in order to arrive at reliable diagnostic criteria, led some experts to question sensitive skin as an actual physiological condition.[14]

It was proposed in both the popular media and the medical literature[11,21] that the emergence of sensitive skin in industrialized countries represents a "Princess and the Pea" effect, wherein it is now culturally fashionable to claim sensitive skin. Its widespread prevalence—in temporal association with the burgeoning of consumer products marketed for those who believe they suffer from sensitive skin—tends to support a psychosocial component.

Cultural practices may produce widely different exposures to potential irritants.[81] For example, hygiene practices (use of douches, perfumes, medications, antifungal medications, and contraceptives) are the most common cause of vulvar irritation.[18] Older women were observed to be more likely to report irritation due to incontinence products than younger women, who were more likely to report irritation due to tampons.[28] Asians reported being more sensitive than Caucasians to spicy food.[2] These findings are almost certainly based on culturally driven levels of exposure than on actual physiological differences.

The expression and report of pain is influenced by social environment and culture as well. Cultures tolerate expressions of pain differently: some prize stoicism,[82] while some prize freedom of expression, a value system passed from one generation to another.[10,83]

Although multiple studies have failed to document consistent ethnic differences in pain threshold, pain studies do document a significant influence of culture on pain expression.[83] African American cancer patients, for example, reported more pain and as well as more pain-related dysfunction than white patients[84]; Asians were observed to be more stoic than whites.[85] Cultural values regarding pain expression and their influence on sensitive-skin prevalence are virtually unknown.

Cosmetics and skin products marketed specifically for sensitive skin are one of the fastest-growing segments of the cosmetic market,[86] and advertising plays an extremely significant role in consumer perceptions. A 2009 investigation surveyed 4506 individuals across Europe and found overall prevalence rates for sensitive skin ranging from 59% in Germany to 91% in Italy. Since the European population is considered essentially to be a genetically homogenous population, the authors attributed the significant variability largely to differences in advertising.[87] Regional differences observed within the United States may also reflect cultural distinctions (Miranda Farage, unpublished data).[88]

CONCLUSION AND RECOMMENDATIONS

Dermatology, as a medical science, requires diagnostic tests that can distinguish sensitive skin from healthy skin on a physiologic basis.[73]

The challenge in the field of sensitive skin has been to integrate a nearly dizzying array of variables as follows: a multitude of possible irritants, the variety of possible sensory and/or physical responses, the differences between anatomical areas, the cultural influences, the hormonal components, and a substantial number of potential mechanisms. The magnitude of this task reveals that diagnostic criteria are still, so far, elusive.[68]

Sensitive skin, however, for so long exclusively a self-defined condition, is beginning to reveal its physiological underpinnings, through advances in methodology and the gradual elucidation of the various mechanisms that play a part.

Evidence suggests that sensitive skin may not, in fact, be a single entity but instead a heterogeneous syndrome,[4] which encompasses different categories of subjects and sensitivities based on different mechanisms. Neural function, indeed, does not act in isolation but is interdependent with both the immune system and the skin, sharing numerous

TABLE 4.3
Suggested Subdivisions of the "Umbrella Term" *Sensitive Skin*

Author	Group 1	Group 2	Group 3
Muizzuddin[90]	**Delicate** Easily disrupted barrier function not accompanied by rapid or intense inflammatory response	**Reactive** Strong inflammatory response without a significant increase in permeability	**Stingers** Heightened neurosensory perception to minor cutaneous stimulation
Pons-Guiraud[24]	**Very Sensitive** Reactive to a wide variety of both endogenous and exogenous factors with both acute and chronic symptoms and a strong psychological component	**Environmentally Sensitive** Clear, dry, thin skin with a tendency to blush or flush and primarily reactive to environmental factors	**Cosmetically Sensitive** Transiently reactive to specific and definable cosmetics products
Löffler[89]	**Very sensitive** Subjects who claim heightened sensitivity of skin	**Irritable** Subjects who develop a stronger objective skin reaction to irritant or allergen	**Stingers** Individuals who consistently perceive pain response to sensory inducing chemicals

cellular contact as well as the same language of cytokines and neurotransmitters. All three interact to affect cutaneous responses.[52]

Three different authors, in fact, have proposed three different sets of three different subgroups (Table 4.3).[24,89,90] None of these, however, consider a psychosocial component. It seems certain that as the phenomenon of sensitive skin continues to be unraveled and the cultural interplay with physiological factors better understood, this multifactorial condition will be revealed to be comprised of subgroups based on mechanism of origin. As the experience of sensitive skin for consumers can significantly impact quality of life,[12] a better understanding of sensitive skin and the development of effective therapies for those who suffer from it are needs in dermatological research.

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5 Neurophysiology of Self-Perceived Sensitive-Skin Subjects by Functional Magnetic Resonance Imaging

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INTRODUCTION

The diagnosis of sensitive skin is defined by neurosensory hyperreactivity of the skin and is essentially based on self-perceived sensations of people who report facial skin discomfort as stinging, burning, and itching when their skin is exposed to some environmental factors (wind, sun, or pollution) or after application of topical products (hard water, soap, or cosmetics) [1–3]. Epidemiological studies performed on large populations have shown that about 50% of women declare that they have self-perceived sensitive skin (SPSS), and 10% fall into the category "very sensitive" [4]. Similar percentages have been obtained in different populations: African Americans, Asians, Caucasians, or Hispanics [5]. SPSS is lower in the male population (30%) and tends to decrease with age [4,6].

Even if reported adverse reactions could be the very first symptoms of an irritant contact dermatitis [7], sensitive skin is not a pathological disorder [8].

This chapter will first present a short review of the different approaches for assessing sensitive skin. Then we will present in detail a new approach based on the analysis of the pattern of brain activation in self-assessed sensitive-skin subjects compared with nonsensitive-skin subjects using functional magnetic resonance imaging (fMRI).

TESTS AVAILABLE: A REVIEW

Psychophysical tests were proposed to measure the chemosensory response of the skin after application of lactic acid or capsaicin, for instance [9–11]. With constant stimulation (for instance, a 10% lactic acid product as the stimulus), it has been shown that there was a statistically significant difference in the global degree of discomfort combining the sensations of stinging, burning, and itching, allowing two populations of subjects to be defined. A first group, characterized by low scores, can be classified as subjects with nonsensitive skin, while a second group, characterized by high scores, can be classified as subjects with sensitive skin. However, these psychophysical tests are still based on the subject's self-perceived response.

A slightly modified procedure to the lactic acid stinging test proposed in 1977 [8] is, nowadays, the most widely used. However, it has been reported that it does not fully render the complexity of self-assessed sensitive skin, as illustrated by the discrepancy between lactic acid response and self-perception of sensitive skin [12–14]. In 2000, this difference was taken into account for the recommendation to include "stingers" with a concomitant self-declared sensitive skin as panelist for safety testing [13].

Owing to the great similarity of symptoms induced by topically applied capsaicin to those associated with sensitive skin [10], a new elicitation test using a 0.075% emulsion of a pungent component extracted from chili peppers was proposed [11,15]. Topical application of capsaicin leads to a short release of neuropeptides (substance P, calcitonin gene-related peptide [CGRP]) from peripheral nerve endings and causes the appearance of uncomfortable sensations. Authors reported that unpleasant reactions are more intense and also more frequent in SPSS subjects.

All these provocative tests are based on the quantification of the degree of discomfort in response to a defined stimulation (10% lactic acid or 0.075 capsaicin). In psychophysics, an alternative method is based on detection threshold. This procedure has been tested recently [16] and consisted in attaining the detection threshold of topically applied capsaicin. Five capsaicin concentrations were used in 10% ethanol aqueous solution (3.16×10^{-5}%, 1.0×10^{-5}%, 3.16×10^{-4}%, 1.0×10^{-4}%, and 3.16×10^{-3}%). This new test of skin neurosensitivity which is easy, quick, and painless, appears to be promising for the diagnosis of sensitive skin and could also provide a basis for the assessment of modulators of skin neurosensitivity.

In 1998, another psychophysiological test based on the assessment of peripheral sensitivity to thermal stimuli was suggested as a possible diagnosis of sensitive skin [17]. Two recent studies reported contradictory results, which could indicate that differences in thermal sensitivity were too weak to consider this thermal indicator as an accurate predictive indicator of sensitive skin [16,18].

As both epidemiological surveys and psychological tests are partly subjective, these approaches being based on the verbal response of the volunteers, some authors have used noninvasive methods to analyze skin properties such as transepidermal water loss, skin hydration, or skin color. Instrumental measurements do not show large differences

between subjects with sensitive skin and those with nonsensitive skin, even if some alteration of the barrier function in people with sensitive skin has been reported by some authors [14,19,20].

BRAIN PATTERN ANALYSIS OF SENSITIVE-SKIN SUBJECTS BY FMRI

Rationale

Our knowledge on sensitive skin shows us that it is not easy to assess because it mainly lacks visible, physical, or histological measurable signs, and such a phenomenon has even led some authors to question the reality of this skin condition [21]. However, when people report the subjective perception of discomfort or low-intensity painful sensations, it should be informative to study the responses of those with sensitive skin and those with nonsensitive skin during the final step of integration of the information, which takes place in the central nervous system. Regarding this topic, most studies have analyzed the processes in the central nervous system of nociceptive information such as pain perception and intensity. More recently, some studies have analyzed a more subjective aspect of pain perception, including feelings of unpleasantness and emotions associated with future implications, termed "secondary affect" [22,23]. Some authors have studied less severe sensations than pain such as itch and reported activation of some similar structures as described for pain [24–26].

The aim of the study, detailed in the next paragraphs, was to assess brain activation during a provocation test involving very slightly painful stimulation and a feeling of discomfort, in two groups of subjects classified as sensitive skin or nonsensitive skin.

Materials and Methods

Subjects

After informed consent, 18 healthy young women (mean age: 33 ± 9 years) participated in this study, which was approved by the hospital ethics committee. The main inclusion criteria were absence of any dermatological, neurological, or vascular condition affecting the face; nonuse of topical or systemic treatments that might interfere with the results of the test; and no contraindications to magnetic resonance imaging (MRI).

Nine subjects were classified as having sensitive skin and nine as having nonsensitive skin, based on their responses to the questionnaire described in the following section.

Questionnaire

To maximize differences between the two groups, subjects were required to have a response profile highly characteristic of sensitive skin on the questionnaire (Table 5.1). Sensitive skin was characterized by the cutaneous reaction to topical applications and to environmental factors.

Answers to the 13 questions were actually used to allocate groups. The following subjects were considered as having sensitive skin: those answering "yes" to two of the first three questions (sensitive skin, reactive skin, and irritable skin), "yes" to three of the four questions on skin reaction to cosmetics (questions 4–7), and "yes" to three of the six questions on the environment (questions 8–13). In contrast, subjects who answered "no" to the 13 questions were classed as having nonsensitive skin. Table 5.1 shows the frequency of "yes" answers to the 13 questions in both groups. The table shows that the two groups were very different with regard to the auto-evaluation of skin sensitivity.

TABLE 5.1
Sensitive-Skin Questionnaire with Frequencies of Positive Responses for Both Groups

Questionnaire	Sensitive Skin (n = 9)	Nonsensitive Skin (n = 9)
1. Do you regard yourself as having a sensitive facial skin?	100%	0%
2. Do you consider yourself as having a facial skin prone to irritation?	89%	0%
3. Do you consider yourself as having a reactive[a] facial skin?	100%	0%
4. Do you avoid certain cosmetics, which you feel may cause your facial skin to react[a]?	100%	0%
5. Do you consider that your facial skin reacts[a] readily to cosmetics or toiletries?	89%	0%
6. Do some cosmetics or toiletry products make your facial skin itch, sting, or burn?	100%	0%
7. Have you ever experienced an adverse reaction on your face to a cosmetic or toiletry product?	100%	0%
8. Does the expression "does not tolerate cold weather or a cold environment" apply to your facial skin?	89%	0%
9. Does the expression "does not tolerate hot weather or a hot environment" apply to your facial skin?	78%	0%
10. Does the expression "does not tolerate fast changes in temperature" (e.g., going into a warm shop from a cold street) apply to your facial skin?	100%	0%
11. Does going out in the wind cause your facial skin to itch, burn, or sting?	56%	0%
12. Does going out in the sun cause your facial skin to itch, burn, or sting?	67%	0%
13. Does your facial skin react[a] to air pollution?	56%	0%

[a] Stinging, burning, and/or itching sensations with or without redness.

Task

Before the magnetic resonance (MR) examination, it was clearly explained to the volunteers what would happen in the scanner and what they would be asked to do. It consisted of simultaneous application to the face of two products described as "likely to induce discomfort." Volunteers did not know that the lactic acid product was applied on the right side of their face (single-blind protocol).

During the MR acquisition, whenever they saw an arrow on the screen, subjects were asked to press the four-position keyboard to report the level of discomfort perceived on the left side of the face when the arrow was pointing to the left and on the right side of the face when the arrow was pointing to the right. Particular attention was taken to check that all the subjects had the same understanding of the global degree of discomfort corresponding to the cumulative effect of stinging, burning, and itching.

A four-level rating system was used:

- 0: no or very slight discomfort
- 1: slight discomfort
- 2: moderate discomfort
- 3: severe discomfort

fMRI Protocol

Three-dimensional MR images were first acquired to have the exact brain anatomy for each subject. Then products A and B were applied simultaneously on the nasolabial folds for 10 s (Figure 5.1), and fMRI acquisition (echo-planar imaging sequence) started immediately and consisted of following brain activation every 3 s during 10 min.

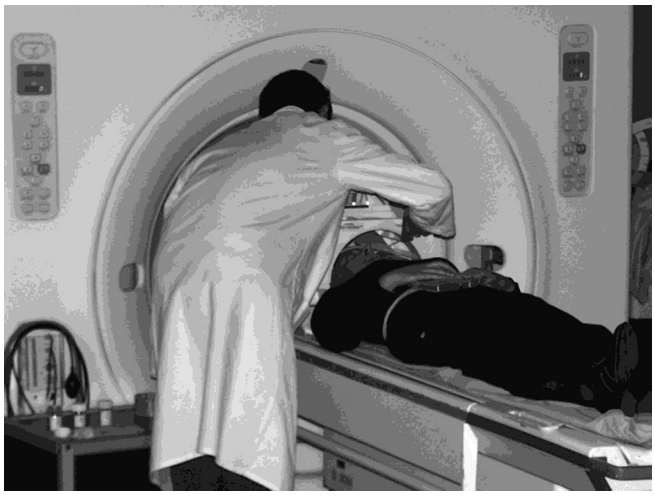

FIGURE 5.1 Lactic acid and saline solution as control were simultaneously applied to the nasolabial areas with a cotton wool bud. The subject's hand was on the four-position keyboard to quantify the degree of discomfort induced by the products during the magnetic resonance acquisitions.

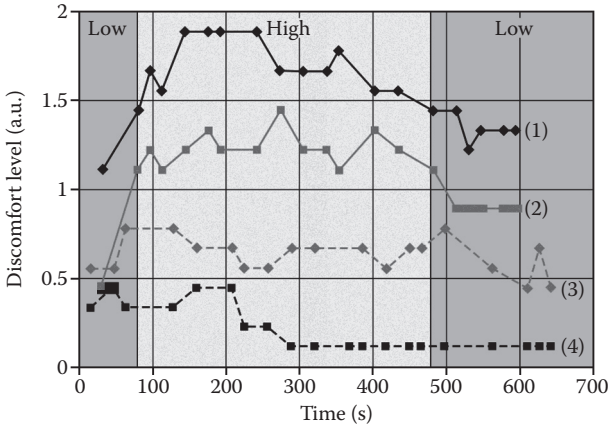

FIGURE 5.2 Kinetics of discomfort for both groups and for the two products. These curves were used to construct the functional magnetic resonance imaging contrast by differentiating a phase between 80 and 480 s corresponding to a high degree of discomfort from a phase between 0 and 80 s and a phase between 480 and 640 s corresponding to a low degree of discomfort. (1) Lactic acid (10%) on sensitive-skin subjects. (2) Lactic acid (10%) on nonsensitive-skin subjects. (3) Saline solution on sensitive-skin subjects. (4) Saline solution on nonsensitive-skin subjects.

Results

Self-Assessment Results

A mean cumulative degree of discomfort was calculated for each group and each product and confirmed a statistically significant increase of discomfort on the side where the lactic acid was applied compared with the saline-solution side. The difference was greater in the sensitive-skin group.

We report (Figure 5.2) the mean kinetic curve of discomfort for each condition.

The time intervals between 0 and 80 s and between 480 and 640 s were classified as low- or null-discomfort periods, while the phase between 80 and 480 s was classified as a medium- or high-discomfort period.

We used these results to construct the fMRI time contrast, as fMRI can only analyze brain activation by varying only one condition, which, in this protocol, is the degree of discomfort.

fMRI Results

Brain Activation when the Arrow Was Pointing to the Control Side (Saline Solution)

Figure 5.3a and b presents mean activation maps for both groups corresponding to periods of time when subjects responded looking at the arrow pointing to the left (saline solution). It can be seen on the 3-D images that no activation was detected in any part of the brain. However, at least the visual cortex should have been activated as subjects received visual stimuli (the arrow projected on the screen), and the motor cortex should have been activated as subjects pressed the keyboard to rate the degree of discomfort. As the central

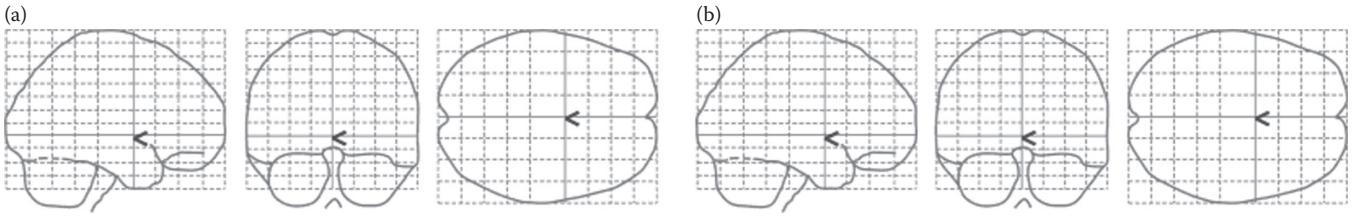

FIGURE 5.3 Brain activation maps obtained by functional magnetic resonance imaging. Saline solution as control. (a) Subjects with nonsensitive skin. (b) Subjects with sensitive skin. No changes in brain activation were observed as a function of time. Visual and motor stimuli were stable during the acquisition time.

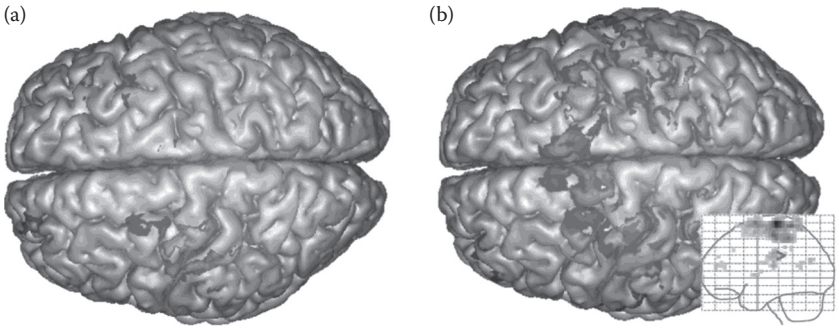

FIGURE 5.4 Brain activation maps obtained by functional magnetic resonance imaging. Lactic acid as a provocation test. (a) Subjects with nonsensitive skin. (b) Subjects with sensitive skin. Nonspecific activation was recorded in both groups in the primary contralateral sensory cortex, which can be considered as the first cortical pathway of this type of sensory perception. Bilateral extensions in the sensory cortex and the prefrontal cortex. Inset activation in internal structures, such as in the cingulate cortex, was specific to the sensitive-skin group.

phase was compared with the beginning and end phases of the time period, activation was stable over time, so that no difference was detected related to time for the visual and motor tasks, which were constant during the acquisition time.

Brain Activation when the Arrow Was Pointing to the Stimulated Side (Lactic Acid Solution)

Figure 5.4a and b present the mean activation maps for both groups during periods pointing to the right (the lactic acid solution). In the nonsensitive-skin group, most of the activated pixels were located in the left primary area of the sensory cortex (first step of the cortical pathway). Other small areas of activation can be seen in associated areas.

In the sensitive-skin group, the mean activated maps were very different. There was considerable activation in the left primary sensory area and considerable bilateral activation in the sensory cortex and in the prefrontal cortex, as well as some activation in deeper structures located in the limbic system (Figure 5.4b inset).

Discussion

The results of subjective data (self-perceived clinical signs) from the lactic acid test in a limited number of subjects were consistent with the results in the literature obtained in a greater number of subjects [27,28]. In both groups, the discomfort rating was higher in subjects with sensitive skin, and the kinetics were comparable over about 10 min, with rapid onset of discomfort and a perceptible decrease after 7 to 8 min. It is also important to relate this to the capacity to lateralize the discomfort perceived in the two facial zones, which were separated only by a few centimeters.

During responses concerning the control saline solution applied to the left side of the face (Figure 5.3), no cerebral activation change with time was observed in either group. However, throughout the acquisition, subjects saw the luminous arrow, which activated areas of the visual cortex, and had to press the keyboard to give their responses, which activated areas of the motor cortex. It can clearly be seen that there was no difference during the two phases chosen, since these stimuli were constant during the recording. The control recording demonstrates that the activation maps corresponding to perception of discomfort with lactic acid can be interpreted with confidence, based on the only stimulation changing over time in the protocol: the degree of discomfort.

In the group with nonsensitive skin, cerebral activation was essentially located in the left primary somatosensory area of the cortex. Since the afferent nerve fibers cross in the spinal cord, contralateral activation corresponds to the first step in neural treatment of the stimulation. Other activations, in very small areas, are more difficult to interpret. In the group of subjects with sensitive skin, cerebral activation maps present a very different pattern. As the first step of cortical integration, there was considerable activation of the primary area of the left sensory cortex, as in the group with nonsensitive skin. Bilateral extensions in the sensory cortex and the prefrontal cortex, together with activation of the subcortical areas (the cingulate cortex), showed multidimensional perception of the

sensation. These activations may be interpreted as the consequence of attention, emotion, and possibly planning the action in response to the unpleasant sensation induced by the stimulation particularly felt by subjects with sensitive skin.

As a consequence, these fMRI results contribute to reinforcing the confidence in self-assessment results, since groups differentiated on the basis of the questionnaire present different cerebral activation maps, and the contrast needed for the fMRI to compare two situations (presence/absence of discomfort) was based on the subjects' feelings in the MRI scanner and measured using a keyboard.

CONCLUSION

Although fMRI could not be considered as a tool to evaluate efficiency in routine products on SPSS subjects, the results we have reported here are of great interest in this field. The different brain activation observed with fMRI between subjects with high SPSS and no SPSS clearly reinforces the neural pattern for this disorder.

In addition, it is of importance to observe that with the questionnaire we have developed, we can select subjects with different neurophysiologic patterns as demonstrated by fRMI. Consequently, with this very simple means, we could get pertinent phenotypes regarding sensitive skin.

Finally, we also have to underline that the activated brain areas are those that are usually involved in the pain process. Everything occurs in SPSS subjects as if the threshold to feel discomfort of the skin is lower than the one for SPSS subjects. The origin of this low threshold could be linked to specific central nervous system patterns, peripheral neural patterns, or both. New studies are still needed to answer these questions.

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6 Tests for Sensitive Skin

Enzo Berardesca

It is difficult to find accurate parameters for categorizing skin as sensitive or nonsensitive; this condition often lacks visible, physical, or histologically measurable signs. Subjects with sensory irritation tend to have a less hydrated, less supple, more erythematous, and more teleangiectatic skin compared to the normal population. In particular, significant differences were found for erythema and hydration/dryness [1]. Tests for sensitive skin are generally based on the report of sensation induced by topically applied chemicals. Consequently, the use of self-assessment questionnaires is a valuable method to identify "hyperreactors" [2] and a useful tool for irritancy assessment of cosmetics [3].

SENSORY TESTING METHODS

Psychophysical tests based on the report of sensation induced by topically applied chemical probes have been increasingly utilized to provide definite information on sensitive skin. These methods of sensory testing can be validated by the use of functional magnetic resonance imaging (fMRI), which represents one of the most developed forms of neuroimaging. This technique measures changes in blood flow and blood oxygenation in the brain, closely related to neural activity manifested as sensory reaction [4]. Querleux et al. [4] evaluated two groups according to their self-perceived characterization by using a dedicated questionnaire about their skin reactivity. Event-related fMRI was used to measure cerebral activation associated with skin discomfort induced by a simultaneous split-face application of lactic acid and of its vehicle.

In both groups, skin discomfort due to lactic acid increased activity in the primary sensorimotor cortex contralateral to application site and in a bilateral frontoparietal network including parietal cortex, prefrontal areas around the superior frontal sulcus, and the supplementary motor area. However, activity was significantly larger in the sensitive skin group. Most remarkably, in the sensitive skin group only, activity spread into the ipsilateral primary sensorimotor cortex and the bilateral peri-insular secondary somatosensory area [5].

Quantification of Cutaneous Thermal Sensation

In dermatology, thermal sensation testing analysis is the most utilized quantitative sensory testing (QST) technique [6]. It assesses function in free nerve endings and their associated small myelinated and nonmyelinated fibers. This method is able to measure quantitatively the threshold for warm and cold sensation as well as hot and cold pain. Yosipovitch et al. [7] used this technique to evaluate ethnic differences in sensorial irritation and perception of pain (with and without stratum corneum stripping), finding no major differences among Asian skin [7].

Stinging Test

Stinging test has been used as a method for the assessment of skin neurosensitivity. Stinging seems to be a variant of pain that develops rapidly and fades quickly any time the appropriate sensory nerve is stimulated. The test relies on the intensity of stinging sensation induced by chemicals applied on the nasolabial fold [8]. The procedure differs depending on the chemical utilized. It is still not sure how it relates to identifying sensitive skin as high variation in the results is observed with these tests.

Lactic Acid

After a 5- to 10-min facial sauna, an aqueous lactic acid solution (5% or 10% according to different methods) is rubbed with a cotton swab on the test site while an inert control substance, such as saline solution, is applied to the contralateral test site. After application, within a few minutes, a moderate to severe stinging sensation occurs for the "stingers group." Subjects are then asked to describe the intensity of the sensation using a point scale. Hyperreactors, particularly those with a positive dermatologic history, have higher scores. Using this screening procedure, 20% of the subjects exposed to 5% lactic acid in a hot, humid environment were found to develop a stinging response [8]. Lammintausta et al. [9] confirmed these observations identifying in their study 18% of subjects as stingers. In addition, stingers were found to develop stronger reactions to materials causing nonimmunologic contact urticaria and to have increased transepidermal water loss and blood flow velocimetry values after application of an irritant under patch test.

Capsaicin

An alternative test involves the application of capsaicin. Recently, a new procedure assessed by Jourdain et al. [10] appears to be accurate and reliable for the diagnosis of sensitive skin. After facial cleansing, five increasing capsaicin concentrations in 10% ethanol aqueous solution are applied on the nasolabial folds. The formulation of capsaicin in hydroalcoholic solution accelerates the action of capsaicin on the face in comparison with the previously used 0.075%

capsaicin emulsion, without being associated with painful sensation.

The capsaicin detection thresholds are more strongly linked to self-declared sensitive skin than the lactic acid stinging test [10].

Dimethylsulfoxide

The alternative application of 90% aqueous dimethylsulfoxide (DMSO) does not have the same efficacy of lactic acid or capsaicin stinging test, and, after application, intense burning, tender wheal, and persistent erythema often occur in stingers.

Nicotinate and Sodium Lauryl Sulfate Occlusion Test

A different approach to identify sensitive skin relies on vasodilation of the skin as opposed to cutaneous stinging. Methyl nicotinate, a strong vasodilator, is applied to the upper third of the ventral forearm in concentrations ranging from 1.4% to 13.7% for a 15-s period. The vasodilatory effect is assessed by observing the erythema and the use of laser Doppler velocimetry (LDV). Increased vascular reaction to methyl nicotinate was reported in subjects with sensitive skin [11]. Similar analysis can be performed following application of various concentrations of sodium lauryl sulfate (SLS).

Evaluation of Itching Response

Itchy sensation seems to be mediated by a class of C fibers with an exceptionally lower conduction velocity and insensitivity to mechanical stimuli [12].

Indeed, no explanation has been found of the individual susceptibility to the itching sensation without any sign of coexisting dermatitis. Laboratory investigations have also been limited.

An itch response can be experimentally induced by topical or intradermal injections of various substances such as proteolytic enzymes, mast cell degranulators, and vasoactive agents.

Histamine injection is one of the more common procedures: histamine dihydrochloride (100 μg in 1 ml of normal saline) is injected intradermally in one forearm. Then, after different time intervals, the subject is asked to indicate the intensity of the sensation using a predetermined scale and the duration of itch is recorded. Information is always gained by the subject's self-assessment.

A correlation between whealing and itching response produced by applying a topical 4% histamine base in a group of healthy young females has been investigated [13].

The cumulative lactic acid sting scores were compared with the histamine itch scores in 32 young subjects: all the subjects who were stingers were also moderate to intense itchers, while 50% of the moderate itchers showed little or no stinging response [13].

Furthermore, the histamine-induced itch sensation decreases after topically applied aspirin [14]. This result can be attributed to the role that prostaglandins play in pain and itch sensation [15].

Washing and Exaggerated Immersion Tests

The aim of these tests is to identify a subpopulation with an increased tendency to produce a skin response.

In the washing test [16], subjects are asked to wash their face with a specific soap or detergent. After washing, individual sensation for tightness, burning, itching, and stinging is evaluated using a point scale previously determined.

The exaggerated immersion test is based on soaking the hands and forearms of the subjects in a solution of anionic surfactants (SLS) at 40°C for 20 min. After soaking, hands and forearms are rinsed under tap water and patted dry with a paper towel. This procedure is repeated two more times, with a 2-h period between each soaking, for two consecutive days. Prior to the procedure, baseline skin parameters are evaluated. The other evaluations are taken 2 h after the third and sixth soaking and 18 h after the last soaking (recovery assessment). All of the skin parameters are performed after the subjects have rested at least 30 min at 21°C ± 1°C.

Behind the Knee Test

A second method of exaggerating conditions in the testing of consumer products is the behind-the-knee (BTK) protocol, which employs the popliteal fossa as a test site. BTK testing consists of the test product placed behind the knee and held securely by an elastic knee band, which in the course of daily activities adds a crucial mechanical friction component to the traditional testing [17].

Levels of irritation produced in BTK testing are consistently higher than those achieved with standard patch testing due to the frictional irritation screening in addition to just chemical irritation. BTK testing, in conjunction with the other two approaches below, has proven useful in the development of potentially valuable protocols for sensitive-skin testing, especially when self-declared vs. normal populations are tested side by side with the same products. Visual grading of erythema, the method of choice for many years, achieves a high degree of reproducibility with trained graders. A novel approach, however, utilized cross-polarized light, which allows visualization of the skin at a depth of 1 mm below the surface. Following minor irritation produced by low-level SLS, subsurface visualization provided no improvement over visual scoring. In BTK, however, enhanced visual scoring through subsurface visualization detected significant differences in irritation produced by two different test products, differences that were visible on the first day [18]. Enhanced visual scoring was used successfully with both traditional patch testing, forearm controlled application test (FCAT), and BTK, providing a first link between sensory and physiological effects. Subclinical changes were apparent after initial exposure, and enhanced visualization was able to correlate subclinical effects with consumer preferences between products [19], a correlation that had not been verifiable in prior testing.

Enhanced visualization was also evaluated in the genital area of symptomatic patients, demonstrating that

cross-polarized light may be useful in diagnosing subclinical inflammation in ostensibly sensory vulvar conditions [20].

A study of facial tissues with and without lotion evaluated four versions of facial tissues by employing repeated wiping in order to accentuate irritation. Affected skin had been compromised by tape stripping prior to the initiation of wiping. Erythema, as well as dryness, was evaluated daily. Statistical analysis revealed that the panelists' subjective product preferences were more consistent in distinguishing between the test product than were the visuals signs (erythema and dryness) [21].

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7 Mechanisms of Skin Hydration

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INTRODUCTION

One of the main functions of the skin is to maintain a competent barrier to water loss (Table 7.1). Water is continuously lost from the outermost skin layers to the atmosphere (evaporative water loss); and in order to control the rate of water loss, the barrier integrity must be preserved. Maintaining the barrier to water loss is important since hydration affects the skin's appearance, mechanical properties, and cell signaling processes [1–9]. The barrier integrity can be compromised by chemical insult (e.g., the use of surfactant-containing cleansing products or harsh chemicals), mechanical insult, dry relative humidity conditions, and sun exposure [10–16].

There are several excellent review articles discussing stratum corneum structure, biochemical processes, and the importance of maintaining well-hydrated skin [17–25]. In this chapter, we will build on these reviews with data pertaining to the importance of cleansing with mild products, the adaptability of the skin to changing environments, the effect of excess water exposure, and the influence of diet to skin hydration. This chapter begins by examining the skin's intrinsic mechanisms for maintaining adequate hydration and concludes by discussing the external influences that affect the skin water content (i.e., the environment, cleansing products, moisturizing systems, and dietary practices).

STRATUM CORNEUM

The skin is divided into three main components: the epidermis, dermis, and subcutaneous fat tissue. The stratum corneum is the uppermost layer of the epidermis. It is, in most body sites, 10–20 μm in depth and is composed of intercellular lipids and dead cells known as corneocytes [26]. Corneocytes are flat, hexagonal-shaped keratin containing structures surrounded by a protein-strengthened envelope. The protein envelope is made up of a variety of proteins including involucrin, loricrin, filaggrin, proline-rich proteins, and keratolinin [27,28]. Corneocytes originate from proliferative epidermal cells known as keratinocytes. As the keratinocytes divide and migrate up toward the outermost skin layers, a process known as differentiation, they change their morphology and cell content. By the time they reach the stratum corneum, they have become flattened, protein-rich sacs. The corneocytes have no nucleus or any other cell organelles. Although the stratum corneum is sometimes referred to as the nonviable epidermis, perturbation of this tissue initiates a cascade of events occurring in the stratum corneum as well as the viable epidermis (e.g., changes in protease activity, lipid biosynthesis, aquaporin, and filaggrin expression) [29].

The epidermis is divided into four main continuous layers: the stratum corneum, stratum granulosum, stratum spinosum, and stratum basale. As Figure 7.1 illustrates, the character of the cells within each of these layers is quite distinct. The basal, keratinocyte cells are columnar in shape, are found in the deepest layer of the viable epidermis, and divide and migrate upward to eventually replace the corneocyte cells. The entire process from cell birth to the "desquamation" of the corneocyte cells is 3–4 weeks. The cells in the stratum spinosum are more polygonal shaped and have spine-like projections that cross intercellular spaces and form desmosomes and tight junctions. It is within these cells that keratin synthesis is initiated. In the stratum granulosum, the cells begin to flatten and the major organelles (including the mitochondria and nucleus) begin to degenerate. The stratum corneum represents the uppermost skin "horny layer" that consists of dead, keratin-filled corneocytes.

Elias et al. [30–34] have proposed a model for the stratum corneum, known as the "brick and mortar" model. The rigid, keratin-filled corneocytes are the bricks and the intercellular lipids are the mortar. The intercellular lipids, along with lectins, desmosomes, and corneodesmosomes, bind to corneocytes to help to hold them in place [35]. It is the physical arrangement of corneocytes and lipids that enables the skin to resist high transepidermal water loss (TEWL) and prevent foreign microbial and chemical entities from gaining entry into the body.

Natural Moisturizing Factor

In addition to keratin, which can bind a substantial amount of water, the stratum corneum contains a number of other hydrophilic agents listed in Table 7.2. These materials are called natural moisturizing factors (NMFs) [36–38]. The NMFs constitute about 20%–30% dry weight of the stratum corneum [39] and are found intracellularly as well as extracellularly (e.g., sugars, hyaluronic acid, urea, and lactate) [40]. The major contributors to the intracellular NMFs are basic amino acids and their derivatives, such as pyrrolidone carboxylic acid and urocanic acid, comprising up to about 50% weight of the total NMF. The NMF concentration varies as a function of age and skin depth [20]. Harding et al. [20] report that for healthy skin not exposed to surfactant damage, the NMF content is independent of depth until one approaches the filaggrin containing levels of the skin. In

TABLE 7.1
Main Functions of the Skin

Functions of the Skin	Activity
Protective shield	Protects body from mechanical insult, chemical penetration, germ invasion, and UV radiation.
Barrier to water loss and foreign body penetration	Prevents the evaporation of excess water and thwarts the penetration of chemicals and pathogens.
Temperature regulator	Contains sweat ducts that modulate body temperature.
Detoxification system	Because skin continuously desquamates, provides an avenue for the body to eliminate toxins.
Early defense system	Langerhans cells capture and transfer foreign material (e.g., viruses and bacteria) to the lymph nodes for their safe removal from the body.
Sensory organ	The presence of nerve endings and Merkel cells enables the sense of touch.
Appearance	The skin defines a person's physical appearance.
Wound repair	Natural restorative response to repairing tissue damage.

TABLE 7.2
Composition of NMF

Components	Mole Percent (%)
Amino acids	40
Sodium pyrrolidone carboxylic acid	12
Lactate	12
Urea	7
Ions (e.g., Cl^-, Na^+, K^+, Ca^{2+}, Mg^{2+}, PO_4^{3-})	18.5
Sugars	8.5
Ammonia, uric acid, glucosamine, creatine	1.5
Citrate and formate	0.5

Sources: Adapted from Rawlings AV, Harding CR, *Dermatol Ther*, 17 Suppl 1:43–48, 2004; Verdier-Sevrain S, Bonte F, *J Cosmet Dermatol*, 6(2):75–82, 2007.

older individuals (50–65 years), the concentration of NMF is lower, particularly in the deeper stratum corneum layers, a reflection of the skin's diminished ability to degrade filaggrin.

Because NMFs are effective humectants, they have a positive impact on the biochemical and mechanical properties of the stratum corneum. Blank [12,14] communicated the importance of maintaining effective concentrations of water in the stratum corneum to prevent or reduce skin tightness, cracking, scaling, and flaking. In addition to enhancing skin water content, the NMFs improve skin plasticity due to specific interactions with keratin. NMFs reduce the water's mobility as well as the intermolecular forces between the keratin fibers [41]. Neutral and basic amino acids appear to be the major contributors to the plasticization process. Removal of soluble NMFs can occur during water rinsing and cleansing [42]. Mild cleansing systems should thus be used to minimize NMF removal.

Most amino acid-based NMF (and their derivatives pyrrolidone carboxylic acid and urocanic acid) are derived by the enzymatic hydrolysis (proteolysis) of the protein, filaggrin, and to a lesser extent by the hydrolysis of corneodesmosomes [17,43–45]. Filaggrin is a protein found in the stratum granulosum layer. It is derived from the 500-kDa, highly basic pro-filaggrin protein found in the keratohyalin granules of the epidermis. Pro-filaggrin is degraded to filaggrin (via a dephosphorylation process) in the uppermost layers of the viable epidermis. Because pro-filaggrin is osmotically inactive, the skin has engineered a process to protect the water-rich epidermal cells from osmotic pressure-induced lysis [17]. Conversely, the ability of filaggrin to degrade into the NMF components in the stratum corneum makes it possible

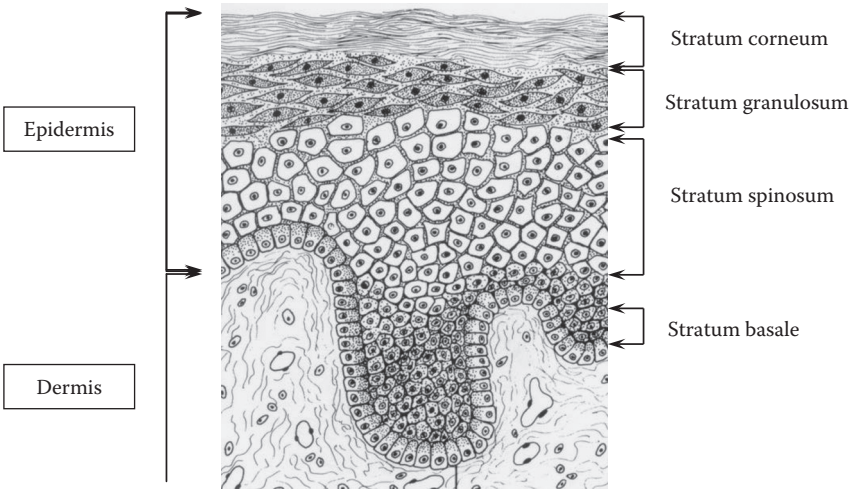

FIGURE 7.1 Schematic diagram of the skin's major epidermal layers. (Reprinted from Polefka T, Surfactant interactions with skin, In: Broze G, editor, *Handbook of Detergents*, New York: Marcel Dekker, 1999, 433–468; Parker F, Structure and function of the skin, In: Orkin M, Maibach H, Dahl MV, editors, *Dermatology*, Norwalk, CT: Appleton & Lange, 1991, 1–7.)

for the outermost skin layers to maintain an adequate water supply when exposed to dry environments. The breakdown of filaggrin is strictly controlled by the water activity [1,18,46]. Based on in vitro experiments, the degradation of filaggrin only occurs when the water activities are between 0.7 and 0.95. At higher activities, no breakdown occurs [47]. At lower activities, the proteolytic enzymes are inactivated and the desquamation process ceases. Consequently, when the skin is occluded (or when the relative humidity is high), there is minimal breakdown of filaggrin. Drier conditions lead to an increase in proteolytic activity resulting in the production of more NMFs. A mechanism is thus present that ensures adequate skin water content in the skin layer most influenced by changes in environmental conditions or chemical insult.

Using tape stripping methods [48,49] and confocal Raman spectroscopy [42], investigators have shown that the concentration of NMF decline substantially as one approaches the stratum granulosum. This is consistent with the fact that filaggrin degradation begins in the stratum compactum. Given the higher water content, one expects that low amounts of NMF would be formed near the stratum granulosum/stratum corneum border. As the concentration of water decreases in the upper stratum corneum, an enhanced degradation of filaggrin occurs. Surprisingly, Egawa and Tagami [50] reported no changes in the concentration of NMF (other than lactic acid and urea, which could have been produced via sweating) as a function of season. The only correlation was the panelist's subjective feeling of "not feeling dry" and higher amounts of NMF. In this same report, younger Japanese individuals (mean age 32 years) had a lower amount of NMF versus older individuals (mean age 67 years). This result was attributed to the faster stratum corneum turnover of the younger age group. Unlike what was reported previously [20], these authors showed a high amount of NMF at the skin surface that decreased as a function of depth. Typically, the uppermost layer of the stratum corneum has a lower NMF content than the mid corneum presumably because cleansers remove the surface material.

Some NMFs behave as simple humectants and have other functions. Lactate and potassium, for example, affect the pH and stiffness of the stratum corneum [51]. The L isomer of lactic acid stimulates ceramide biosynthesis and improves barrier function [52].

Two additional NMFs, hyaluronic acid (HA) [53] and glycerol, have also been found in the stratum corneum. HA, a nonsulfated glycosaminoglycan, is a hygroscopic polymer of repeating disaccharide units of N-acetylglucosamine and glucuronic acid. It is a well-known component of the dermis, maintaining its hydrated state and providing structural integrity. In the stratum corneum, it functions as a humectant but also interacts with the intercellular lipids and regulates the mechanical properties of the stratum corneum.

Glycerol may be derived from the breakdown of sebaceous triglycerides or originate from the conversion of phospholipids to free fatty acids. The importance of glycerol was revealed in a study completed by Fluhr et al. [54]. These authors employed mice models where sebaceous glands (which produce triglycerides that degrade to glycerol) were largely absent and showed that although the permeability barrier responded to mechanical abrasion similar to the control, skin hydration was only enhanced by the addition of glycerol. Like HA, glycerol also influences the skin's pliability by interacting with skin lipids. Froebe et al. [55] and Mattai et al. [56] showed how glycerol could modulate the phase behavior of intercellular lipids favoring a more pliable, liquid crystalline structure at low relative humidities.

Stratum Corneum Lipids

Stratum corneum lipids play a major role in maintaining skin hydration. These intercellular lipids are composed of approximately 40%–50% ceramides, 20%–25% cholesterol, 15%–25% fatty acids (that have chain lengths of between 16 and 30 carbons, C24:0–C28:0 being the most abundant), and 5%–10% cholesterol sulfate; the approximate molar ratio of these lipids is 1:1:1 (ceramide/fatty acid/cholesterol) [57–59]. They represent about 15% of the dry weight of the stratum corneum [60]. These intercellular lipids are arranged in a highly organized lamellar arrangement (or bilayer) with only very small amounts of water present, presumably interacting with the lipid polar head groups [61]. This compact lamellar structure is a very effective barrier to TEWL. When skin is exposed to solvents such as toluene, n-hexane, or carbon tetrachloride, which remove barrier lipids, the TEWL is increased [62]. The ceramides are major components of the intercellular lipids, and this is reflected in their contribution to the structural organization of the lamellar bilayer. There are about 11 major ceramide classes that are synthesized from glucosylceramides, epidermosides (acylglucosylceramides), and sphingomyelin [63,64]. These ceramides have complex structures varying in both their polar head groups and their dual hydrophobic chains [64,65]. Each ceramide contributes in specific ways to stratum corneum organization and cohesion and thus to the integrity of the barrier. In particular, the ω-hydroxyacyl portion of ceramide EOS completely spans a lamellar bilayer, and the linoleate tail is believed to intercalate between a closely apposed bilayer essentially linking two bilayers together [59,66]. In fact, when any of the acylceramides are extracted, the periodicity of the lamellar bilayer structure is eliminated [67].

The ceramide naming nomenclature was proposed by Motta et al. [68] and later extended by Masukawa et al. [69] to include dihydrosphingosines. Ceramides are designated CER FB, where F is the type of fatty acid and B is the type of base. N represents nonhydroxy fatty acids; A stands for alpha-hydroxy fatty acids; and O represents esterified omega-hydroxy fatty acids. S, P, H, and DS represent sphingosines, phytosphingosines, 6-hydroxysphingosine, and dihydrosphingosine, respectively. Masukawa et al. [64] identified over 300 different ceramide species within the human stratum corneum.

Lipid Organization and Structural Models

Electron diffraction studies [70] have shown that as corneocytes migrate from the lower regions of the stratum corneum to the outer layers, there is a corresponding change in lipid packing from a more ordered, orthorhombic packing to a more fluid hexagonal phase. This observation is consistent with the known weakening of the barrier and complete loss of lamellar ordering in the topmost layers of the stratum corneum [71–73]. Changes to the composition of the stratum corneum lipids in the upper stratum corneum (i.e., increased concentration of cholesterol sulfate, hydrolysis of CER EOS, increased concentration of short chain length fatty acids, crystallization of cholesterol, and decreased levels of ceramides) presumably influence the loss of lamellar order [72]. Indeed, factors that can affect lipid composition, such as washing with harsh cleansers, perturb the lamellar structure and adversely change the condition of the skin [73].

There are several models that have been proposed to describe the structural phases of the lipid bilayer (Table 7.3). The domain-mosaic model suggests that lipids coexist within a two-phase system consisting of discontinuous crystalline domains embedded within liquid crystalline or gel phases [74]. The more ordered gel phase allows for greater packing of the lipids and hence a more effective barrier. Molecular transport across the bilayer occurs through the disordered crystalline domains.

X-ray diffraction studies of hydrated stratum corneum have shown two types of lamellar structures, having repeat distances of 13.2–13.4 nm (long periodicity phase) and 6.0–6.4 nm (short periodicity phase) [61,75]. Bouwstra et al. [76] have proposed a molecular arrangement of the long periodicity phase, called the sandwich model, consisting of two broad lipid layers of about 5 nm each with a crystalline structure separated by a narrow central lipid layer of about 3 nm with fluid domains. Cholesterol and ceramides are important for the formation of the lamellar phase, while fatty acids mostly impact the lateral packing of the lipids. This model is an illustration of a multiphase system. The upper and lower domains contain more ordered lipid phases (i.e., orthorhombic or gel) while the central zone is more fluid in nature. Two other sandwich models have also been proposed, but there is no clear evidence of affirming the molecular structural features of one model over the other [77–80].

Norlen [81] has proposed yet a third skin barrier model. This model suggests that the lipid matrix has a homogeneous lamellar gel phase with a low degree of lipid fluidity. Stratum corneum epidermal lipid heterogeneity, the long length of the fatty acid chains, and the presence of cholesterol are used to support this model since these factors have been shown to stabilize gel phases [82,83]. This model does not require the presence of water or any bilayer conformation. The presence and location of the cholesterol define the variation in permeability within this model system. However, the absence of orthorhombic phases detected in human stratum corneum lipid lamellae calls into question the validity of this model.

There are also different models describing the mechanism of skin barrier formation. The Landmann model suggests that "lamellar bodies" or stacked monobilayer vesicles separate from the trans-Golgi network, are extruded into the intercellular space at the stratum granulosum/stratum corneum border, fuse with the cell plasma membrane of the stratum granulosum, and discharge the lipids into continuous multilamellar membrane sheets in the intercellular space [84]. The membrane folding model [85–87] argues against abrupt changes in lipid phase transitions that would result from the disruption, diffusion, and fusion of the lamellar bodies. Based on this model, the skin barrier formation takes place as a direct, continuous unfolding of a three-dimensional membrane into a flat, multilayered two-dimensional lipid structure (having only hexagonal hydrocarbon chain packing and no abrupt phase transitions) [85,86]. By evaluating vitreous sections of nonpretreated, nonstained, full-thickness, hydrated skin using cryo-transmission electron microscopy, evidence of cubic-like membrane structures were observed. This organizational pathway was proposed to be more thermodynamically preferred to that previously described by Landmann [84]. The cryo-transmission electron microscopy preparation was also reported to be improved over conventional electron microscopy methods because it does not require dehydration and chemical fixation of the sample. With additional innovations in instruments and instrumental techniques, active research will be sure to continue in this area.

TABLE 7.3
Proposed Models Describing How Barrier Lipids Structure within Stratum Corneum

Skin Barrier Model	Description
Domain mosaic	Stratum corneum barrier lipids coexist in liquid crystalline (water permeable) and highly ordered gel phase (water impermeable) domains. Water is expected to be most permeable at the phase boundaries. The more fluid crystalline phase allows for the permeation of water.
Sandwich model	Proposes a more structured arrangement of liquid crystalline and gel domains. A narrow central lipid layer with fluid domains (3 nm wide) lies between two broad, crystalline lipid layers (6.4 nm wide).
Single gel phase model	Skin barrier lipids exist as a single lamellar gel phase with no phase boundaries.

Lamellar Lipid Arrangement and Water Permeability

The lamellar or bilayer arrangement, independent of the nature of the lipids from which it is derived, is a natural barrier to water permeability [88]. In skin, there is a relatively large gradient in water chemical potential between the viable epidermis, where the water content is about 70% by weight, and the stratum granulosum/stratum corneum junction, where the water content drops to 15%–30% [89]. Under this large water gradient, the stacked bilayer arrangement

of lipids, which is a continuous region in the stratum corneum, provides an optimal way to reduce water loss through the skin. Water escaping from the stratum corneum would have to traverse the tortuous pathway of the bilayer [74,90]. In addition, fully matured corneocytes would also increase the tortuosity and hence the diffusional path length of water [19]. The combination of a lamellar arrangement of lipids and increased diffusional path length due to corneocytes reduces water diffusion to the atmosphere.

AQUAPORINS AND TIGHT JUNCTIONS

Another mechanism by which the skin maintains its hydrated state is the use of aquaporins (AQPs). These transmembrane proteins form water channels across cell membranes, facilitating the transport of small polar molecules across the cell membrane. Specific AQPs also have the ability to facilitate the transport of glycerol and urea. AQP3 is most relevant to skin hydration [19]. It is localized in the basal and suprabasal layers of the epidermis and is not expressed in the stratum corneum. In AQP3-deficient mouse skin, the skin is less hydrated and less elastic, the permeability of water and glycerol within the skin is reduced, and there is a delayed barrier recovery [91–93]. Only by adding glycerol is the skin condition improved [94]. Skin diseases associated with impaired barriers and low skin hydration also tend to have reduced expression of AQP3. Boury-Jamot et al. [95] found that AQP3 expression was inversely correlated to the severity of patients with eczema and spongiosis.

Tight junctions consist of more than 40 transmembrane (i.e., claudins, occludin, and junctional adhesion molecules [JAMs]) and plaque proteins (zonula occludens) [96]. This protein combination forms a semipermeable barrier between aligning cell membranes making it very difficult for water to pass through the space between the epidermal cells. Ions or fluids must actually diffuse or be actively transported through the cell in order to pass through the tissue. Claudins, occludins, and JAMs are principally responsible for controlling water permeability. Claudin 1-deficient mice die within 1 day of birth because of excessive TEWL [97]. The presence of organized tight junctions and an intact stratum corneum barrier ensures low values of TEWL. For those diseases where patients experience dry skin and a compromised barrier (e.g., psoriasis vulgaris and ichthyosis vulgaris), the location of tight junction proteins may also be altered. Proteins that may be expressed homogeneously throughout the epidermis may be preferentially expressed in the upper or lower layers.

DESQUAMATION

So far, the above discussion has centered on natural ways human skin has evolved to retain water. In addition to hydrating the skin, water also plays a crucial role in the exfoliation or desquamation of corneocytes. Corneocytes are linked in the lower stratum corneum by corneodesmosomes, which are macromolecular glycoprotein complexes. As the corneocytes move from the lower to the outer region of the stratum corneum, the corneodesmosomes are progressively degraded by hydrolytic enzymes. This leads to desquamation in the outer stratum corneum. These enzymes include serine proteases such as stratum corneum chymotryptic enzyme (SCCE) and stratum corneum tryptic-like enzyme (SCTE), which are more effective at neutral pHs and are most active on the outermost layers of the stratum corneum [19,46,98–100]. The cathepsin family of proteases is more active under lower pH conditions and is present throughout the stratum corneum. Other proteases include cysteine proteases, sulfatases, and glycosidases. Many of these enzymes are localized in the intercellular space, and their activity is affected by both the lipid organization and water content [20,101]. Clearly, low water content within the stratum corneum affects the activities of stratum corneum proteases, which leads to dry, flaky skin. A review has recently been published describing how skin proteases influence the desquamation process [102]. These changes have also been studied as a function of season, anatomical site, and skin depth [103]. An optimal skin water content to maintain these processes based on in vitro assessments is 10%–20% [13].

ENVIRONMENTAL IMPACT ON SKIN HYDRATION

Changes in lipid biosynthesis [72,104], epidermal DNA synthesis [9], barrier function [105], and skin thickness [106] are all influenced by the skin water content. There are many studies showing that biochemical processes are also altered as a function of changes in the environmental relative humidity [107,108]. Rawlings et al. [109] demonstrated that dry conditions inhibit corneodesmosomal degradation, while increasing humidity increases corneodesmosomal degradation. Moreover, when human skin was exposed to low humidity conditions (10%) even for short exposure periods (3 and 6 h), a significant decrease in stratum corneum water content and increase in skin roughness were observed [3].

Even in humid conditions, the skin is still subject to a number of environmental insults, which can negatively affect skin hydration. Excess UV radiation, for example, causes UV-induced erythema leading to a compromised barrier [110]. Several animal studies have demonstrated that abrupt changes in the environment (going from humid [80% RH] to dry [<10% RH] conditions) increase the time required for barrier function to return to normal [105]. In this situation, the skin does not have enough time to adapt to the new climatic conditions. DeClercq et al. [5] have further demonstrated that skin can adapt to dry climatic conditions. They found that panelists living in a hot, dry climate such as Arizona had a better barrier function and less dry skin compared to panelists living in New York, which had a more humid climate [5].

While prolonged exposure to conditions of low relative humidity (<20%) enhance barrier function, sustained exposure to high humidity conditions leads to a gradual deterioration in the barrier [1]. A relative humidity greater than 80% is associated with a decrease in NMFs and corneocyte hydration in the epidermis of hairless mice [1]. It has also been shown that when normal skin is exposed to a moist

environment, the kinetics of barrier recovery is delayed due to a reduction in the number of epidermal lamellar bodies and lipid content, in direct contrast to what is observed at low humidities [108]. Therefore, when skin adapts to a high humidity environment, its capacity to respond to external changes is decreased, partially due to a reduction in the reservoir of stratum corneum lipids.

It is remarkable that a human fetus has a mechanism to protect the outermost skin barrier to the damaging effects of amniotic fluid, an environment that would result in a loss of barrier function in adults [111]. During the third trimester of gestation, a biofilm known as vernix caseosa forms and coats the prenatal skin. This film acts as a barrier and facilitates the formation of the acid mantle, which provides an optimal environment for inhibiting bacterial colonization [112,113]. Vernix caseosa consists of ~80% water, 10% protein (corneocytes with no desmosomal attachments), and 10% lipids by weight (consisting of barrier and sebaceous lipids not arranged in any lamellar structure). This material has been shown to have multiple functions including being an efficient moisturizer and osmoregulator [114]. Based on transmission electron microscope images, the limited structure of vernix caseosa is very similar to that of the topmost layers of the stratum corneum. The body appears to have retained this structural feature of vernix caseosa during the course of stratum corneum maturation.

PERSONAL CARE PRODUCTS AND SKIN HYDRATION

Effect of Cleansing Systems

Cleansers are designed to remove unwanted materials from the skin such as dirt, oils, and sebum. However, the use of harsh surfactants damages the skin barrier, increases the skin's susceptibility to environmental sources of irritation and sensitization, and reduces skin moisture and smoothness [115]. Charged surfactants, such as anionic and cationic, are the most aggressive. Sodium lauryl sulfate (SLS) is a harsh surfactant that, given its small hydrodynamic radius, is the only surfactant that can extract the intercellular lipids and disrupt the lipid bilayer [116]. It, along with most charged surfactants, adsorbs to skin proteins causing them to denature and swell. Rhein et al. [117] reported that the extent of protein denaturation is dependent on the surfactant monomer concentration and exposure time. Because surfactants denature skin proteins, enzymatic reactions that control desquamation, inflammation, and oxidation processes are negatively impacted [118,119]. The resulting enhanced barrier permeability leads to skin dryness, roughness, cracking, and inflammation [10,46,120].

Fortunately, there are a number of surfactants used commercially that are mild to the skin. These include mostly nonionic and amphoteric variants, and for the anionic variants, highly ethoxylated (at least 5-EO) alkyl sulfates, sulfosuccinates, isethionates, sarcosinates, taurates, alkyl phosphates, and alkyl glutamates. The aggressiveness of charged surfactants can be mitigated by reducing the concentration of the surfactant's monomer species, reducing the charge by incorporating various counterions and/or cosurfactants to form mixed micelles, and introducing ethoxylation [10]. The improved mildness reduces the incidence of barrier damage, which aids in the maintenance of hydrated skin (i.e., nondrying cleansers).

Surfactants also negatively impact the skin hydration properties by removing NMF. Blank and Shappirio [14] showed that when isolated human stratum corneum was exposed to 1% solutions of soap, alkyl sulfate, or alkyl benzylsulfonate, all surfactants reduced the ability of the tissue to absorb water from the atmosphere, relative to water. This water-holding capacity correlated with the loss of NMF. A similar correlation has been found between natural saponified soaps and mild synthetic surfactants using confocal Raman spectroscopy [121] (Figure 7.2).

There has been a great deal of research focused on delivering enhanced skin moisturization using cleansers [115]. Emollient-containing cleansers have been found to alleviate the dry skin condition of people having rosecea, sensitive skin, and/or atopic dermatitis [122,123]. Emulsion-based liquid body washes are commonly employed to mildly cleanse and moisturize the skin. Although delivering a moisturization benefit using lipophilic agents is difficult to achieve in a rinse-off system, clinical studies have confirmed that enhanced moisturization can be achieved in formulas containing a large quantity of oils and/or humectants [124]. The patent literature is replete (but will not be further discussed in this chapter) with examples of approaches to improve the delivery of actives from cleansing systems. Invariably, it has been demonstrated that cleansing systems are able to remove dirt and bacteria while simultaneously depositing oils on the skin to improve skin feel, smooth desquamating corneocytes, and improve barrier function.

Research has demonstrated that oatmeal is a good choice for gentle cleansing and moisturizing of dry, sensitive skin [125]. Oatmeal has been used for centuries as a soothing agent to relieve itch and irritation associated with various xerotic dermatoses. Many clinical properties of colloidal oatmeal are derived from its chemical polymorphism. Its high concentration of starches and beta-glucan is responsible for the protective and water-holding functions of oat. The presence of different types of phenols confers antioxidant and anti-inflammatory activity. Some of the oat phenols are also strong ultraviolet absorbers. The cleansing activity of oat is mostly due to saponins. Its many functional properties make colloidal oatmeal a good cleanser, moisturizer, buffer, as well as a soothing and protective anti-inflammatory agent [126].

Although cleansers have been formulated to successfully deliver oils to the skin, delivering humectants has been more challenging. Humectants are highly water soluble and, consequently, harder to deposit onto the skin during the washing process. Special delivery systems have yet to be developed to improve the competency in this area.

Moisturizing the stratum corneum using lotions and creams is typically the best way to hydrate the skin. This is typically accomplished by using emulsion formulas, which

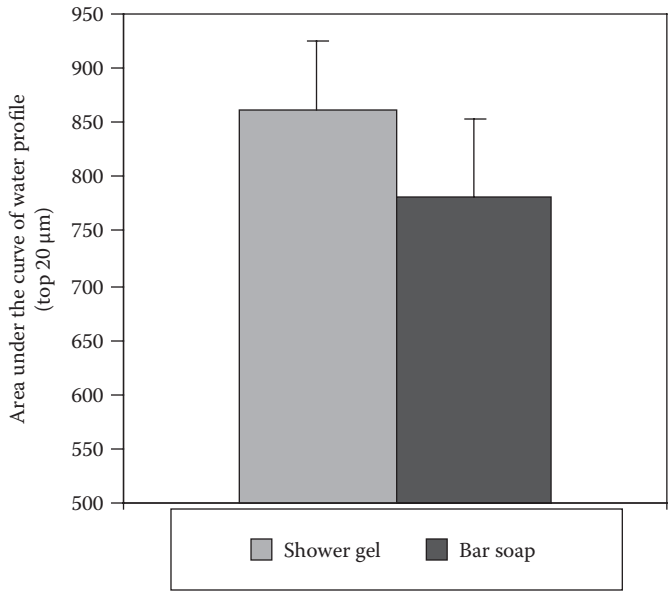

FIGURE 7.2 Water content as a function of cleanser type determined using confocal Raman spectroscopy. (From Wu J, Polefka T, *Int J Cosmet Sci*, 30:47–56, 2008.)

contain humectants, emollients, and/or occlusive agents [127]. Humectants attract and hold on to water. Occlusive agents form a barrier across the skin, reducing the TEWL. "Emollient" comes from the Latin derivation meaning a material designed to soften and soothe the skin [128]. Emollients can be occlusive or semiocclusive, meaning they may not be very effective at preventing evaporative water loss but are effective in smoothing skin.

Glycerol and urea are well-known humectants [129–131]. Glycerol also prevents the crystallization of stratum corneum lipids at low relative humidity, which leads to less TEWL and a higher skin water content. Previous studies evaluated the influence of glycerol on the recovery of damaged stratum corneum induced by repeated washings with SLS. The authors found that glycerol created a stimulus for barrier repair and improved stratum corneum hydration [132].

Petrolatum and mineral oil are common occlusive agents. Application of hydrophobic materials such as petrolatum to prevent skin dryness may be as old as mankind itself. In recent times, however, manufacturers are incorporating lipids that can form lamellar bilayers in their formulations to enhance the barrier properties of the skin [133,134]. They typically use ceramides or ceramide-like molecules to accomplish this goal and have found an even greater benefit when they combine the lipid technology with glycerol [135]. Niacinamide has also been shown to enhance lipid biosynthesis, which again improves barrier function [136]. As in the above situation, the addition of glycerol further improves the clinical dry skin condition. Using Fourier transform infrared spectroscopy, emollients such as isostearyl isostearate and isopropyl isostearate have been shown to increase the thermotropic stability of the orthorhombic phase of a stratum corneum lipid model system containing ceramides, cholesterol, and free fatty acid [137]. Further analysis demonstrated that there was actually an increase in orthorhombic packing following the addition of either of these lipid moisturizers [138]. Like glycerin, these emollients are able to alter the phase behavior of the lipid domain within the stratum corneum to reduce water loss. This is the mechanism ascribed to improving the hydration conditions in the skin when applied in vivo.

Water in Excess

Skin exposure to extrinsic water is usually considered to be harmless. Oftentimes, it is used as the "control" site in experiments that investigate the way compounds interact with the skin. However, there is evidence that prolonged contact with water can negatively affect SC barrier function, similar to surfactants [139]. In addition to eliciting erythema, inflammation, and intense dermatitis, excess water exposure can increase SC swelling and suppleness, weakens SC corneocyte cohesion, and increases the permeability of all substances, especially water. Warner et al. [120,139] showed that overexposure of skin to water causes a disruption of the SC intercellular lamellar bilayer ultrastructure in vitro as well as in vivo. Similar to surfactant exposure, the swelling response was time-dependent and wide intracellular clefts between corneocytes were observed. These studies, as well as others, show that prolonged hydration of the SC can directly disrupt the barrier lipids leading to compromised skin [120,139,140].

DIETARY IMPACT ON SKIN CONDITION

It is generally stated that topically applied cosmetic products can be helpful in restoring normal hydration to dry skin. However, less recognized is the positive influence that

drinking plenty of water can have on the skin's appearance. Approximately 45%–70% of human body weight consists of water. One-third of the total body water is extracellular and two-thirds are within the intracellular compartment [141]. Water is free to move between the cell membranes with any net movement controlled by the effective osmotic and hydrostatic pressures. This balance of body fluid is dependent on the intake of water through drinking, food, and metabolism, and the loss of water through natural processes. The three components of the skin, the epidermis, dermis, and subcutaneous fat tissue, play a major role in water regulation, with the SC water content helping to maintain many of the skin's biophysical properties [142]. Soft, smooth skin has an optimally hydrated SC with a water content of approximately 20%–30% and a water content of less than 10%–20% resulting in abnormally dry skin [141,142]. While the environment can play a role in TEWL, a good balance between water intake and loss is vastly important in helping to maintain healthy water content in the SC, which has a positive influence on skin hydration.

An increased intake of pure, healthy water helps to enhance nutrient absorption, skin hydration, detoxification, and virtually every aspect of better health. However, studies have also shown that drinking dietary natural mineral water or taking a food supplement containing pro-hydrating actives maintains adequate skin hydration as well. Mac-Mary et al. [143] showed that the magnitude of change in a corneometer measurement on the forearm of healthy subjects increased by 14% when 1 L of mineral water was consumed per day for 42 days, which was clinically significant and similar to observed modifications with moisturizing cosmetic products (10%–30%). Primavera and Berardesca [141] investigated how a capsule containing an active product based on vegetable ceramides, amino acids, sea fish cartilage, antioxidants, and essential fatty acids improved skin hydration after oral use. Significant improvement in corneometer readings was seen in the active-treated groups (+30%), in addition to a decrease in skin roughness and improved skin smoothness after 40 days, as measured using a Visioscan. Self-assessment and clinical assessment data confirmed the results of the biophysical measurements. These studies demonstrate that a proper diet with adequate water and mineral intake is just as important in the management of skin hydration as a complementary cosmetic approach. Puch et al. [144] further showed that ingesting a probiotic-containing dairy product enriched in gamma linolenic acid (an omega 6-polyunsaturated fatty acid that has been shown to enhance the rate of barrier recovery when applied topically and when taken orally), vitamin E, and catechins improved barrier function after 6 weeks of taking a twice/day dosage. The average improvement was 13% [144]. The reduction in TEWL was observed throughout the 6-month study despite the changes in season. More recently, topical supplementation of cholecalciferol (vitamin D3) was shown to significantly improve skin moisturization, and vitamin D3 serum levels were also found to correlate with a more hydrated stratum corneum [145].

SUMMARY

Maintaining hydration of the stratum corneum can be accomplished using a number of different mechanisms. From using mild surfactants that minimally compromise the skin barrier, to delivering moisturizers (humectants, occlusive oils, lipid-modulating agents), these materials offer a means of adding moisture back to the skin or, alternatively, reducing water loss [146,147]. The skin itself, in fact, has a natural process to minimize excess water loss. Through the water-dependent production of intercellular skin lipids and NMF, an intricate mechanism is in place to function optimally in an often arid, external environment. The skin is a remarkable organ, producing vernix caseosa to protect (as a barrier, anti-infective, and antioxidant) the fetus while it is immersed in amniotic fluid, a potential damaging environment, and following birth enhancing the acid mantle development, which facilitates skin maturation during the postnatal period. The production of urocanic acid and free fatty acids in the stratum corneum further contributes to the regulation of stratum corneum pH [148,149]. For those living in dry climates, the skin can adapt and generate an improved barrier function and increased water content. The development of the confocal Raman spectrometer has allowed researchers to noninvasively monitor skin water content and skin composition changes as a function of the environment and product use [42,150–152]. The identification of aquaporins and tight junctions provides increasing evidence for internal mechanisms that the skin is using to improve the opportunities for corneocyte hydration. More importantly, there are increasing data confirming the importance of maintaining optimal skin water content to ensure the activity of processes that occur in the epidermis. Protecting and maintaining an adequate skin water content and barrier function are proving to be essential to achieving healthy, youthful looking skin.

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8 Hydrating Substances

Marie Lodén

INTRODUCTION

Hydrating substances are used in cosmetic products to retard moisture loss from the product and to increase the moisture content in material that is in contact with the product. This function is generally performed by hygroscopic substances, or humectants, which are able to absorb water from the surroundings. In the European Commission database with information on cosmetic substances and ingredients (CosIng), more than 900 substances are listed as humectants.

Target areas in the body for treatment with humectants are dry skin, but also hair and mucous membranes may benefit from application of humectants. Dry hair is brittle, is rough, has a tendency to tangle, and has hardly any luster. Humidity of the atmosphere is the only source of moisture to hair, except shampooing, and the addition of humectants to the hair will, therefore, facilitate its retention of water. The same is true for the skin, although it is constantly supplied with water from inside of the body. The skin forms a critical structural boundary for the organism and is frequently compromised as a result of underhydration. The water held by the hygroscopic substances in the stratum corneum is a controlling factor in maintaining skin flexibility and desquamation [1,2]. Hydration plays an important role in maintaining the metabolism, enzyme activity, mechanical properties, appearance, and finally, barrier function of the skin.

The special blend of humectants found in the stratum corneum is called natural moisturizing factor (NMF) [3]. NMF can make up about 10% of the dry weight of the stratum corneum cells [3]. Substances belonging to this group are amino acids, pyrrolidone carboxylic acid (PCA), lactates, urea, and inorganic ions (Tables 8.1 and 8.2) [3]. Furthermore, glycerol is found naturally in the stratum corneum, and the mean amounts are found to be about 0.7 μg cm^{-2} on the cheek and 0.2 μg cm^{-2} on the forearm and sole of the foot [4]. The proportion of the inorganic ions and lactate in the stratum corneum differs from that in sweat and does also change between winter and summer [5]. The levels of lactate and potassium in the stratum corneum appear to correlate with each other as well as with the physical properties of the stratum corneum [5]. The levels of lactate are found to be approximately 100 times higher than that of glycerol [4,5].

NMF is formed from the protein filaggrin, whose formation is regulated by the moisture content in the stratum corneum [1]. Mutations in the filaggrin gene have been identified as the major predisposing factor for atopic eczema [6,7]. In skin diseases such as ichthyosis vulgaris [8,9] and psoriasis [10], there is a virtual absence of NMF. In ichthyosis vulgaris, the stratum granulosum is thin or missing due to a defect in the processing of profilaggrin, which also is noticed as tiny and crumbly keratohyalin granules [11].

Glycerin is another humectant suggested to be important for the stratum corneum hydration (Tables 8.1 and 8.2). Skin dryness in sebaceous gland-deficient mice has been found to be linked to reduced levels of glycerin because of the absence of triglycerides, which are the primary source for glycerin [12]. This type of dryness may also be applicable to clinical situations where sebaceous glands are absent or involuted, such as in prepubertal children showing eczematous patches, which disappear with the onset of sebaceous gland activity. Moreover, xerosis in the distal extremities of aged skin and in patients receiving systemic isotretinoin for treatment of acne may be linked to glycerin depletion because of the lower sebaceous gland activity [12].

Physiologically occurring and synthetic substances are used as humectants in cosmetic products (Tables 8.1 and 8.2). The water-binding capacity of the sodium salts of lactic acid and PCA appears to be higher than that of glycerin and sorbitol (Table 8.3) [13,14]. Treatment of solvent-damaged guinea pig footpad corneum with humectant solutions shows that the water held by the corneum decreases in the following order: sodium PCA > sodium lactate > glycerin > sorbitol [15]. Urea also has strong osmotic activity [16,17]. However, which of these substances most efficiently reduces xerosis or other dry skin conditions is not known. Besides differences in water-binding capacity, their absorption into the skin is important for the effect. Hence, the in vitro humectancy should be distinguished from the in vivo moisturizing effect [18]. Some factors to consider during product development are highlighted in Table 8.4.

This chapter will provide basic information about some commonly used humectants, which are primarily used for treatment of the skin. Moreover, safety information is provided.

BUTYLENE GLYCOL

Description

Butylene glycol usually means 1,3-butanediol, but the term can also be used for 2,3-butanediol (Tables 8.1 and 8.2). The alcohol is a viscous, colorless liquid with sweet flavor and bitter aftertaste [41]. It is soluble in water, acetone, and castor oil but practically insoluble in aliphatic hydrocarbon [41].

TABLE 8.1
Chemistry of Hygroscopic Substances

Name	CAS No.	MW g/mol	Other Names	Natural Source
Butylene glycol	107-88-0	90.1	1,3-butanediol, 1,3-butylene glycol	
Glycerin	56-81-5	92.1	Glycerol, 1,2,3-propanetriol	Hydrolysis of oils and fats
Lactic acid	50-21-5	90.1	2-hydroxypropanoic acid	Sour milk and tomato juice
Panthenol	81-13-0	205.3	Dexpanthenol, pantothenol, provitamin B_5	Plants, animals, bacteria
PCA	98-79-3	129.11	L-pyroglutamic acid, DL-pyrrolidonecarboxylic acid, 2-pyrrolidone-5-carboxylic acid	Vegetables, molasses
Propylene glycol	57-55-6	76.1	1,2-propanediol	
Hyaluronic acid	9004-61-9	5×10^4– 8×10^6	Hyaluronan	Cockscomb, biofermentation
Sorbitol	50-70-4	182.17	D-glucitol	Berries, fruits
Urea	57-13-6	60.08	Carbamide, carbonyl diamide	Urine

Sources: Rawlings, A.V., and Matts, P.J., *J. Invest. Dermatol.*, 124, 2005. Sweetman, S.C., ed., *Martindale: The Complete Drug Reference*, Pharmaceutical Press, London, 2005. Budavari, S., *The Merck Index*., Merck & Co., Rahway 1989. Rowe, R.C. et al., *Handbook of Pharmaceutical Excipients*, 4th ed., Pharmaceutical Press, London, 2003.

Note: CAS, chemical abstract service; MW, molecular weight; PCA, pyrrolidone carboxylic acid.

General Use

Butylene glycol is used as a humectant for cellophane and tobacco [41]. It is also used in topical products and as a solvent for injectable products. Butylene glycol is claimed to be most resistant to high humidity and is often used in hair sprays and setting lotions [19]. The alcohol also retards loss of aromas and preserves cosmetics against spoilage by microorganisms [19].

Safety

Butylene glycol is considered safe by the Cosmetic Ingredient Review (CIR) Expert Panel [20]. A human skin patch test on undiluted butylene glycol produced a very low order of primary skin irritation, and a repeated patch test produced no evidence of skin sensitization [20]. The substance is reported to be less irritating than propylene glycol [21,22]. Few reports of contact allergy exist, but the substance does not seem to cross-react with propylene glycol [21].

GLYCERIN

Description

In 1779, the Swedish scientist C.W. Scheele discovered that glycerin could be made from a hydrolyzate of olive oil. The alcohol is a clear, colorless, odorless, syrupy, and hygroscopic liquid (Tables 8.1 and 8.2) approximately 0.6 times as sweet as cane sugar. Glycerol has three hydrophilic hydroxyl groups that are responsible for its hygroscopicity. It is miscible with water and alcohol, slightly soluble in acetone, and practically insoluble in chloroform and ether.

General Use

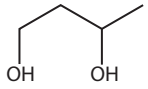
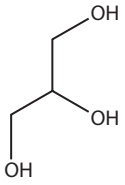
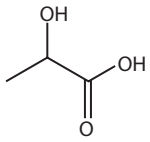
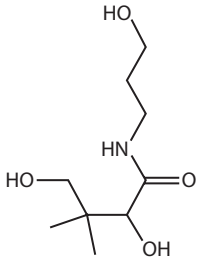
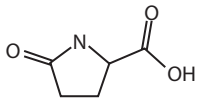
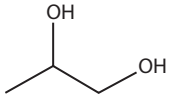
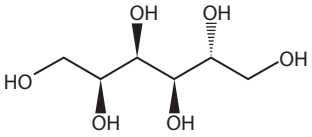
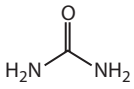
Glycerin is used as a solvent, plasticizer, sweetener, lubricant, and antimicrobial agent. The substance has also been given intravenously or by mouth in a variety of clinical conditions in order to benefit from its osmotic dehydrating properties [23]. This effect can also be used topically for the short-term reduction of vitreous volume and intraocular pressure of the eye [23]. Moreover, concentrated solutions of glycerin are used to soften earwax [23], and suppositories with glycerin (dose of 1–3 g) promote fecal evacuation [23].

Effects on Skin

The importance of glycerin in skin care products is well established. To explain its benefits, studies have focused on its humectant and protecting properties. Levels ranging between a few percent and 20% to 25% are used in moisturizers for treatment of dry skin conditions [24]. Glycerin not only attracts water but also has been suggested to modulate the phase behavior of stratum corneum lipids and to prevent crystallization of their lamellar structures in vitro at low relative humidity [25]. Incorporation of glycerin into a stratum corneum model lipid mixture enables the lipids to maintain the liquid crystal state at low humidity [25]. The biochemical consequences of these properties may be due to the influence of the activity of hydrolytic enzymes crucial to the desquamatory process in vivo [26]. Thereby, the rate of corneocyte loss from the superficial surface of human skin increases, probably due to enhanced desmosome degradation [1,26].

The mode of action of glycerol on both stratum corneum hydration and epidermal barrier function seems to be related to the aquaporin 3 channel. The aquaporins are a family of small, integral membrane proteins that function as plasma membrane transporters of water and, in some cases, small polar solutes (reviewed in ref. [27]). Glycerol is transported very slowly into the epidermis, and thus, its transport rate is sensitive to the intrinsic glycerol permeability of the basal keratinocyte layer. Repeated tape stripping taken from skin treated with 15% glycerin cream indicates that glycerin

TABLE 8.2
Chemical Formulas of Humectants

Humectant	Formula
Butylene glycol	
Glycerin	
Lactic acid	
Panthenol	
PCA	
Propylene glycol	
Sorbitol	
Urea	

diffuses into the stratum corneum to form a reservoir [28]. During some hours after application, a decrease in transepidermal water loss (TEWL) has been noted [28–31] followed by increased values after some hours in animal skin [31]. No evidence of deterioration of the skin barrier function has been noted after long-term treatment of normal and atopic skin with 20% glycerin [32,33]. Instead, glycerin has been found to accelerate barrier recovery after acute external perturbations [34]. Glycerol leads to a more rapid reconstitution of the protective skin barrier following mechanical (tape stripping) or chemical (repeated sodium lauryl sulfate [SLS] application, acetone) damage. It can absorb water, thus creating water flux in the stratum corneum, which may lead to a stimulus for barrier repair [35]. In the studies by Andersen et al. [36–38], only glycerol treatment improved skin barrier recovery after acute and cumulative irritations induced by SLS or nonanoic acid applications in hairless guinea pig model and in human volunteers. The high hygroscopicity of glycerol can be involved in this action, supporting TEWL and ion movement (especially calcium) [39].

Moreover, in human skin, its surface profile, electrical impedance, and increase in the coefficient of friction were found to accompany an improvement in the skin condition, as assessed by an expert [29]. Glycerin is also suggested to induce a shrinking of superficial corneocytes, which was independent from osmotic effects [40]. This contraction might give a more compact stratum corneum and reduce the risk for irritant contact dermatitis [40].

Safety

Very large oral or parenteral doses can exert systemic effects because of the increase in the plasma osmolality, resulting in the movement of water by osmosis from the extravascular spaces into the plasma [23]. Glycerin dropped on the human eye causes a strong stinging and burning sensation, with tearing and dilatation of the conjunctival vessels [41]. There is no obvious injury, but studies have indicated that glycerin can damage the endothelial cells of the cornea [23,41]. Glycerin has been shown to have excellent skin tolerability and treatment, with 20% not showing any signs of adverse effects in atopic dry skin [24].

HYALURONAN (HYALURONIC ACID)

Description

The earliest work on skin was devoted predominantly to the cells that make up the layers of skin: epidermis, dermis, and underlying subcutis. Now it is beginning to be appreciated that the materials that lie between cells, the matrix components, have major instructive roles for cellular activities. This extracellular matrix endows skin with its hydration properties. The components of the extracellular matrix appear amorphous by light microscopy but form a highly organized structure of glycosaminoglycans (GAGs), proteoglycans, glycoproteins, peptide growth factors, and structural proteins such as collagen and, to a lesser extent, elastin. The predominant component of the extracellular matrix, however, is hyaluronan; one of the first extracellular matrix components to be elaborated in the developing embryo (reviewed in ref. [42]). The term *hyaluronan* is used to cover both hyaluronic acid and sodium hyaluronate. Hyaluronan is a member of the class of amino sugars containing polysaccharides known as the GAGs widely distributed in body tissues. The polymer provides the turgor for the vitreous humor of the eye, and the name *hyaluronic* acid derives from the Greek *hyalos* (glossy, vitreous) and uronic acid. Molecular weight is within the

TABLE 8.3
Moisture-Binding Ability of Humectants at Various Humidities

Humectant	31%	50%	52%	58%–60%	76%	81%
Butylene glycol						38 [43]
Glycerin	13 [68], 11 [14]	25 [13]	26 [14]	35–38 [68,109]	67 [14]	
Na PCA	20 [68], 17 [14]	44 [13]	45 [14]	61–63 [68,109]	210 [14]	
Na lactate	19 [14]	56 [13]	40 [14]	66 [109]	104 [14]	
Panthenol	3 [63]		11 [63]		33 [63]	
PCA	<1 [68]				<1 [68]	
Propylene glycol					32 [109]	
Sorbitol			1 [13]		10 [109]	

Note: PCA, pyrrolidone carboxylic acid.

TABLE 8.4
Parameters to Consider During Product Development to Obtain the Desired Effect

Formulation Related	Effect on the Target Area
Price and purity?	Product claim?
Chemical stability during production and shelf life?	Substantivity in rinse-off products?
Sensitive to heat? UV light? pH?	Penetration characteristics?
Incompatibilities with other ingredients?	Hygroscopicity?
Adsorption to the packaging material?	Adverse effects?
Effects on the preservation system?	Changes of the microbial flora?

range of 50,000 to 8×10^6 depending on source, methods of preparation, and determination [43]. Hyaluronic acid is a regulator of cell behavior and influences cellular metabolism. Moreover, the molecule binds water and functions as a lubricant between the collagen and the elastic fiber networks in the dermis during skin movement. A 2% aqueous solution of pure hyaluronic acid holds the remaining 98% water so tightly that it can be picked up as though it were a gel [44].

The skin is the largest reservoir of body hyaluronic acid, containing more than 50% of the total. The papillary dermis has more prominent levels of hyaluronic acid than the reticular dermis. Hyaluronic acid is extracted from cockscomb or obtained from streptococci (Lancefield groups A and C) [23]. During manufacturing, the large, unbranched, noncross-linked, water-containing molecule is easily broken by shear forces [44]. The carbohydrate chain is also very sensitive to breakdown by free radicals, ultraviolet (UV) radiation, and oxidative agents [44].

General Use

A viscous solution of sodium hyaluronate is used during surgical procedures on the eye and is also given by intra-articular injection in the treatment of osteoarthritis of the knee [23]. Hyaluronic acid is also applied topically to promote wound healing. Topical application of 0.1% solution in patients with dry eye has been suggested to alleviate symptoms of irritation and grittiness [23].

Effects on Skin

High-molecular-weight hyaluronic acid solutions form hydrated viscoelastic films on the skin [44]. The larger the molecular size, the greater the aggregation and entanglement of the molecules, and hence, the more substantial and functional the viscoelastic film associated with the skin surface [44]. Owing to the high molecular weight, hyaluronic acid will not penetrate deeper than the crevices between the desquamating cells. The polymer may also be injected to obtain a smoother surface and reduce the depth of wrinkles.

Safety

Sodium hyaluronate is essentially nontoxic. When the substance is used as an ophthalmic surgical aid, transient inflammatory ocular response has been described [23].

LACTIC ACID

Description

Lactic acid is colorless to yellowish crystal or syrupy liquid, miscible with water, alcohol, and glycerol but insoluble in chloroform [23]. Lactic acid is an α-hydroxy acid (AHA), that is, an organic carboxylic acid in which there is a hydroxy group at the two, or alpha (α), position of the carbon chain (Table 8.2). Lactic acid can exist in a DL, D, or L form. The L and the D forms are enantiomorphic isomers (mirror images). Lactate is also a component of the natural hygroscopic material of the stratum corneum and constitutes about 12% of this material (Table 8.1) [3].

Formulations containing lactic acid have an acidic pH in the absence of any inorganic alkali or organic base. The pH is increased in several formulations by partial neutralization.

General Use

Lactic acid has been used in topical preparations for several decades because of its buffering properties and water-binding capacity [15,23]. Lactic acid and its salts have been used for douching and to help maintain the normal, acidic atmosphere of the vagina. Lactic acid has also been used for correction of disorders associated with hyperplasia and/or retention of the stratum corneum, such as dandruff, callus, keratosis, and verrucae (viral warts) [23]. Moreover, lactic acid has been suggested to be effective for adjuvant therapy of mild acne [45]. Also, ethyl lactate has been proposed to be effective in the treatment of acne, due to its penetration into the sebaceous follicle ducts with subsequent lowering of pH and decrease in the formation of fatty acids [46].

Investigators have also reported increases in the thickness of viable epidermis [47,48] as well as improvement in photoaging changes [47,49]. Lactic acid in combination with other peeling agents is used to produce a controlled partial-thickness injury to the skin, which is believed to improve the clinical appearance of the skin [50].

Effects on Skin

In guinea pig footpad corneum, it has been shown that both lactic acid and sodium lactate increase the water-holding capacity and skin extensibility [15]. Potassium lactate has been suggested to restore stratum corneum hydration more effectively than sodium lactate, suggesting that potassium ion itself may play certain roles in maintaining the physical properties of the stratum corneum [5] With increasing pH, the adsorption of lactic acid decreases, due to the ionization of the acid [15]. In another study on strips of stratum corneum from human abdominal skin, the uptake of water by sodium lactate was greater than that by lactic acid, but the stratum corneum was plasticized by lactic acid and not by sodium lactate [13]. Lactic acid also reduces the cohesion between the corneocytes and interferes with the bonding between the cells, which causes increased cell turnover, especially at a pH of around 3 [51–53].

The concentrations used for treatment of ichthyosis and dry skin have ranged up to 12% [54]. After treatment with 5% lactic acid combined with 20% propylene, glycol increased TEWL has been noted in patients with lamellar ichthyosis [55]. However, lactic acid has been suggested to stimulate ceramide synthesis and improve skin barrier function [56,57].

Safety

Lactic acid is caustic to the skin, eyes, and mucous membranes in a concentrated form [41]. Compared to other acids, lactic acid has no unusual capacity to penetrate the cornea, so its injurious effect is presumably attributable to its acidity [41].

Immediately after the application of an AHA, stinging and smarting may be noticed; this is closely related to the pH of the preparations and the substances themselves [52,53,58]. The emulsion type has been reported to influence the degree of stinging, where water-in-oil emulsions induced less stinging than ordinary oil-in-water [59]. In normal skin, irritation and scaling may be induced when the acids are applied in high concentrations and at low pH [60]. At a fixed lactic acid concentration, the desquamative effect is highly pH dependent, while at fixed pH, the turnover rate of skin is concentration dependent [53]. Increased sensitivity to UV light has also been detected, which raises concerns over long-term use [61]. Due to insufficient safety data, the Food and Drug Administration (FDA) recommend that lactic acid should be used up to a maximum level of 2.5% and a pH ≥5 [61].

PANTHENOL

Description

D-panthenol is a clear, almost colorless, odorless, and viscous hygroscopic liquid, which may crystallize on prolonged storage (Tables 8.1 and 8.2) [23]. Panthenol is an alcohol, which is converted in tissues to pantothenic acid (vitamin B5), a component of coenzyme A in the body. The substance can be isolated from various living creatures, which gave the reason for its name (Table 8.1) (*panthoten* is Greek for *everywhere*) [62]. Panthenol is very soluble in water and freely soluble in alcohol and glycerol but insoluble in fats and oils [63]. The substance is fairly stable to air and light if protected from humidity, but it is sensitive to acids and bases and also to heat [63]. The rate of hydrolysis is lowest at a pH of 4 to 6 [63].

General Use

Panthenol is widely used in the pharmaceutical and cosmetic industry for its moisturizing, soothing, and sedative properties [62,64]. It is also found in topical treatments for rhinitis, conjunctivitis, sunburn, and wound healing (ulcers, burns, bed sores, and excoriations); usually, 2% is used [23,62]. The mechanisms of action are only partly known.

The hygroscopic alcohol can further be used to prevent crystallization at the spray nozzles of aerosols [63].

Effects on Skin and Hair

Topically applied panthenol is reported to penetrate the skin and hairs and to be transformed into pantothenic acid [62,65]. Treatment of SLS-induced irritated skin with panthenol accelerates skin barrier repair and stratum corneum hydration [64]. Moreover, skin redness decreased more rapidly by panthenol treatment [64].

Pantothenic acid can be found in normal hair [63]. Soaking of hair in 2% aqueous solution of panthenol has been reported to increase the hair diameter up to 10% [66].

Safety

Panthenol has very low toxicity and is considered safe to be used in cosmetics [65]. Panthenol and products containing

panthenol (0.5%–2%) administered to rabbits caused reactions ranging from no skin irritation to moderate-to-severe erythema and well-defined edema [65]. Low concentrations have also been tested, on humans and those formulations did not induce sensitization or significant skin irritation [65]. Contact sensitization to panthenol present in cosmetics, sunscreens, and hair lotion has been reported, although allergy to panthenol among patients attending dermatological clinics for patch testing is uncommon [62,67].

PCA AND SALTS OF PCA

Description

PCA is the cosmetic ingredient term used for the cyclic organic compound known as 2-pyrrolidone-5-carboxylic acid (Tables 8.1 and 8.2). The ''L'' form of the sodium salt is a naturally occurring humectant in the stratum corneum at levels about 12% of the NMF [3], corresponding to about 2% by weight in the stratum corneum [68]. The sodium salts of PCA are among the most powerful humectants (Table 8.3). PCA is also combined with a variety of other substances, like arginine, lysine, chitosan, and triethanolamine.

Effects on Skin

A significant relationship has been found between the moisture-binding ability and the PCA content of samples of stratum corneum [68]. Treatment with a cream containing 5% sodium PCA also increased the water-holding capacity of isolated corneum compared to the cream base [69]. The same cream was also more effective than a control product containing no humectant, and equally effective as a similar established product with urea as humectant, in reducing skin dryness and flakiness [69].

Safety

In animal studies, no irritation in the eye and the skin was noted at concentrations up to 50%, and no evidence of phototoxicity, sensitization, or comedogenicity was found [70]. Minimal, transient ocular irritation has been produced by 50% PCA [70]. Immediate visible contact reactions in back skin have also been noted after application of 6.25% to 50% aqueous solutions of sodium PCA [71]. The response appeared within 5 min and disappeared 30 min after application. PCA should not be used in cosmetic products in which *N*-nitroso compounds could be formed [70].

PROPYLENE GLYCOL

Description

Propylene glycol is a clear, colorless, viscous, and practically odorless liquid having a sweet, slightly acrid taste resembling glycerol (Tables 8.1 and 8.2) [72]. Under ordinary conditions, it is stable in well-closed containers, and it is also chemically stable when mixed with glycerin, water, or alcohol [72].

General Use

Propylene glycol is widely used in cosmetic and pharmaceutical manufacturing as a solvent and vehicle, especially for substances unstable or insoluble in water [72]. It is also often used in foods as antifreeze and emulsifier [43,72]. Propylene glycol is also used as an inhibitor of fermentation and mold growth [43].

Effects on Skin

Propylene glycol has been used in the treatment of a number of skin disorders including ichthyosis [55,73,74], tinea versicolor [75], and seborrheic dermatitis [76], due to its humectant, keratolytic, antibacterial, and antifungal properties [72,77].

Safety

Propylene glycol has been given an acceptable daily intake (ADI) value of 25 mg/kg by the Joint FAO/WHO Expert Committee of Food [72,78]. Poisoning has been found after oral doses of around 100 to 200 mg/kg to children [79–81] and after topical treatment with high concentrations in burn patients [82], but the alcohol is considered safe for use in cosmetic products [83].

Clinical data have shown skin irritation and sensitization reactions to propylene glycol in normal subjects at concentrations as low as 10% under occlusive conditions and in dermatitis patients as low as 2% [22,83]. The nature of the cutaneous response remains obscure, and therefore, the skin reactions have been classified into four mechanisms: (1) irritant contact dermatitis, (2) allergic contact dermatitis, (3) nonimmunologic contact urticaria, and (4) subjective or sensory irritation [84]. This concept allows a partial explanation of effects observed by different authors [84].

PROTEINS

Description

Proteins and amino acids for cosmetics are based on a variety of natural sources. Collagen is the traditional protein used in cosmetics. Collagen has a complex triple-helical structure, which is responsible for its high moisture retention properties. Vegetable-based proteins have, during recent years, grown in importance as an alternative to using animal by-products. Suitable sources include wheat, rice, soybean, and oat.

In cosmetics, native proteins can be used, but perhaps the most widely used protein types are hydrolyzed proteins of intermediate molecular weight with higher solubility. An increased substantivity is obtained by binding fatty alkyl quaternary groups to the protein. Improved film-forming properties can be obtained by combining the protein and polyvinylpyrrolidone into a copolymer. Such modifications may increase the moisture absorption compared with the parent compound. Potential problems with proteins are their odor and change in color with time. Furthermore, as they are nutrients, their inclusion in cosmetics may require stronger preservatives.

Efficacy and Safety

Amino acids belong to the NMF and account for 40% of its dry weight [4]. Because of their relatively low molecular weight, they are capable of penetrating the skin and cuticle of the hair more effectively than the higher-molecular-weight protein hydrolyzates.

Salts of the condensation product of coconut acid and hydrolyzed animal protein [85], and wheat flour and wheat starch [86], are considered safe as cosmetic ingredients by the CIR. The most frequent clinical presentation of protein contact dermatitis is a chronic or recurrent dermatitis [87]. Sometimes, an urticarial or vesicular exacerbation has been noted a few minutes after contact with the causative substance [87,88]. Hair conditioners containing quaternary hydrolyzed protein or hydrolyzed bovine collagen have induced contact urticaria and respiratory symptoms [88]. Atopic constitution seems to be a predisposing factor in the development of protein contact dermatitis [88].

SORBITOL

Description

Sorbitol is a hexahydric alcohol appearing as a white crystalline powder, odorless, and having a fresh and sweet taste (Tables 8.1 and 8.2) [23]. It occurs naturally in fruit and vegetables and is prepared commercially by the reduction of glucose. Sorbitol is most commonly available as 70% aqueous solution, which is clear, colorless, and viscous. It is easily dissolved in water but not so well in alcohol. It is practically insoluble in organic solvents.

Sorbitol is relatively chemically inert and compatible with most excipients, but it may react with iron oxide and become discolored [72].

General Use

Sorbitol is used in pharmaceutical tablets and in candies when noncariogenic properties are desired. It is also used as a sweetener in diabetic foods and in toothpastes. Sorbitol is also used as a laxative intrarectally and believed to produce less troublesome side effects than glycerin [23]. Its hygroscopic properties are reported to be inferior to those of glycerin (Table 8.3) [13,89].

Safety

When ingested in large amounts (>20 g/day), it often produces a laxative effect [23,72].

UREA

Description

Urea is another physiological substance occurring in human tissues, blood, and urine (Tables 8.1 and 8.2). The amount is on the order of 2% in urine. The extraction of pure urea from urine was first accomplished by Proust in 1821, and pure urea was first synthesized by Wöhler in 1828 [90].

Urea is a colorless, transparent, slightly hygroscopic, odorless or almost odorless, prismatic crystal, or white crystalline powder or pellet. Urea is freely soluble in water, slightly soluble in alcohol, and practically insoluble in ether [23]. Urea in solution hydrolyzes slowly to ammonia and carbon dioxide, which may cause swelling of the packaging [23].

General Use

Urea is used as a 10% cream for the treatment of ichthyosis and hyperkeratotic skin disorders [90,91] and, in lower concentrations, for the treatment of dry skin. In the treatment of onychomycosis, urea is added to a medicinal formulation at 40% as a keratoplastic agent to increase the bioavailability of the drug [92].

Effects on Skin

An increased water-holding capacity of scales from psoriatic and ichthyotic patients has been observed after treatment with urea-containing creams [91,93]. The lipid matrix of the skin seems not to be affected by urea, since the transition temperatures of mouse skin lipids was not changed by exposure to 12% urea [94]. Urea could also protect against osmotic stress by replacing water and retaining the liquid crystalline phase at lower humidity [95].

Concern has been expressed about the use of urea in moisturizers, with reference to the risk of reducing the chemical barrier function of the skin to toxic substances [16]. The increase in skin permeability by urea has been shown in several studies, where it has been found to be an efficient accelerant for the penetration of different substances [96–98]. Not all studies, however, support the belief that urea is an effective penetration promoter [99,100], and treatment of normal skin with moisturizers containing 5% to 10% urea has been found to reduce TEWL and also to diminish the irritative response to the surfactant SLS [101,102]. One moisturizer with urea also reduced TEWL in atopic patients [32,103] and made skin less susceptible to irritation to SLS [104]. Improvement in skin barrier function has also been shown in dry skin [105] and in ichthyotic patients [91].

Safety

Urea is a naturally occurring substance in the body, as the main nitrogen-containing degradation product of protein metabolism. Urea is an osmotic diuretic and has been used in the past for treatment of acute increase in intracranial pressure due to cerebral edema [23]. No evidence of acute or cumulative irritation has been noted in previous studies on urea-containing moisturizers, but skin stinging and burning are reported after treatment with 4% to 10% urea creams in dry and lesioned skin [105–107]. A recent study suggests that urea affects cutaneous arterial sympathetic nerve activity and elevates blood flow via histaminergic H3 receptor [108].

CONCLUSIONS

A number of interesting humectants are available as cosmetic ingredients. Most of them have a long and safe history of use, and several are also naturally occurring in the body or accepted as food additives. The low-molecular-weight substances are easily absorbed into the skin, providing a potential drawback of stinging sensations from some of them. The high-molecular-weight substances usually do not penetrate the skin, but instead, they are suggested to reduce the irritation potential of surfactants. However, case reports of urticarial reactions have been reported after exposure to modified proteins [88].

The advantage with the larger and chemically modified materials are that they have an increased substantivity to target areas, whereas it is apparent that small amounts of several low-molecular-weight hygroscopic substances have a questionable contribution to the water content of hair and stratum corneum in rinse-off products (Table 8.4).

Another issue worth considering is whether the obtained humectancy is the only mode of action. Some humectants may modify the surface properties and increase the extensibility of stratum corneum without influencing the water content. Furthermore, humectants may also modify skin barrier function and influence specific metabolic processes in the skin. One should also keep in mind that humectants can improve the cosmetic properties of the formulation, and some of them also facilitate marketing of the product just because of their names.

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9 Skin Care Products

Howard Epstein

AN OVERVIEW OF EMULSION-BASED SKIN CARE PRODUCTS

A variety of skin care products exist in today's marketplace. They fulfill a variety of functions by either acting directly on the skin (e.g., moisturizers) or being a cosmetically elegant vehicle for the delivery of specific active ingredients (e.g., sunscreens or antipyretic or antiacne medicaments). In general, these products are categorized in the United States into three functional groups:

- Drugs: to prevent or ameliorate diseases by altering the structure and/or function of the body.
- Cosmetics: to beautify and improve the feeling or sensory aspects of normal and/or nondiseased skin. Dry skin would be included in this category.
- Cosmeceuticals: an intermediate classification for cosmetic products that may enhance the function of the skin. Currently, the Food and Drug Administration (FDA) does not recognize this category [1].

The three product groups can also be classified by their physical properties. Most common forms of skin care products are emulsions. Emulsions are mixtures of two insoluble materials that are stabilized against separation. An example is a mixture of oil and water, which will not mix unless an intermediate emulsifier is incorporated into the mixture.

Different Types of Emulsions

Emulsifiers can act as solubilizers and spreading or dispersing agents. Correct use of emulsifiers permits one to formulate homogeneous mixtures, dispersions, or emulsions of oily, waxy substances with water. Solids may be dispersed in liquids or insoluble liquids within other liquids. Greasy anhydrous ointments can be designed to be more washable. These types of properties may be achieved by appropriate selection of emulsifiers, active ingredients, and other compatible ingredients in the vehicle.

Emulsions may be formulated of water in oil (w/o), oil in water (o/w), aqueous gel, and silicone in water. Other products may be formulated as semisolids containing oleaginous ingredients, absorption bases, and water-soluble types containing polyethylene glycol (PEG). Recently, there has been a growing interest in water-in-oil-in-water (w/o/w) emulsions, also referred to as multiple emulsions.

O/w emulsions are the most commonly formulated. These types of emulsions tend to feel less greasy and have a lower cost of formulation because of a higher water content. W/o emulsions have historically been less popular because of a characteristic greasy, oily feel on application to skin. However, the development of newer emulsifiers has enabled a skilled formulator to develop w/o emulsions of a lighter texture. Silicone formulation aids may also be used to form stable water-in-silicone (w/Si) or w/o emulsions. These are polymeric surface-active agents with long bond lengths and wide bond angles. This provides for free rotation of functional groups permitting formulations of w/o and w/Si emulsions with exceptional elegance and good coverage when applied to skin [2]. This enables formulation of stable emulsions with medium-to-low viscosity. These different chemical-type emulsions are commonly referred to as vehicles when "cosmetic"-active or drug-active ingredients are incorporated into them (Table 9.1).

Not all emulsifiers behave in the same way. Properties of the emulsifier will determine the emulsion type. Their compatibility with oils having different polarities is also a critical concern. Emulsifiers will impact the desired sensory properties of the product such as color, odor, and desired viscosity (e.g., lotion or cream consistency).

Different Types of Emulsifiers

Emulsifying agents, which are surface-active agents (surfactants), are available in a wide range of chemical types. These include nonionic, hydrophobic, lipophilic, ethoxylated, and nonethoxylated types. A recent trend is to lower the amount of or even eliminate surfactants in an effort to minimize the already low irritation potential of the formulation. It is possible to formulate emulsifier-free emulsions with cross-linked acrylic polymer derivatives. These materials are hydrophilic polymers that are hydrophobically modified by adding an alkylic chain. These molecules, known as polymeric emulsifiers, provide additional formulation options for new product development [4].

FORMULATING HYDRATING CREAMS AND LOTIONS

The continuing development of biophysical instrumentation and test techniques has enabled formulation of highly effective skin care formulations. Formulators now have several options with respect to formulating new products. When initiating formulation development, it is important to understand project/product requirements, type of product(s),

TABLE 9.1
Examples of Vehicle Types

Type of Emulsion	Examples
W/o	Cold creams, cleansing or evening creams (overnight creams)
O/w	Common moisturizers, hand and body lotions
Oleaginous	Petrolatum
Water soluble	Polyethylene glycol-based ointments
Aqueous gels	Lubricating jelly; gelling agents such as carbomers, hydroxyethylcellulose, and magnesium aluminum silicate may be used in the formulation.
Absorption bases	Hydrophilic petrolatum; these vehicles may contain raw materials able to function as w/o emulsifiers permitting large quantities of water to be incorporated as emulsified droplets.

Source: Block, H., Medicated Applications, in: Gennaro, A.R., ed., *Remington's Pharmaceutical Sciences*, 18th ed. Mack Publishing Company, Pennsylvania, 1980.

Note: w/o, water in oil; o/w, oil in water.

performance and aesthetics needs, formulation cost constraints, packaging needs, product claims, and formulation safety. To what part of the body will the formulation be applied, and at what time of the day, morning or overnight? Will makeup be applied over the product; will clothing come into contact with the product? Will the targeted consumer apply a fragrance to the body after application of the product, and if so, will the fragrances conflict? Once these requirements are defined, the formulator can consider active ingredients, emulsion systems, preservative systems, color, and fragrance.

Emulsions allow the formulating chemist to combine otherwise incompatible ingredients into an effective commercially desirable cosmetic product. The key to product development is the technique employed to select appropriate raw materials. Commonly used emulsifying agents are ionic (anionic or cationic) or nonionic. The function of the emulsifying agent is dependent upon the unique chemical structure of the emulsifier. Each emulsifier has a hydrophilic (water-loving) and lipophilic (oil-loving) part. Examples of hydrophilic moieties are polyhydric alcohols and polyethylene chains. Lipophilic parts may be a long hydrocarbon chain such as fatty acids, cyclic hydrocarbons, or a combination of both. Nonionic agents may have hydrophilic action generated by hydroxyl groups and ether linkages, such as polyoxyethylene chains. Nonionic emulsifying agents can be neutral or acidic, giving formulators greater flexibility regarding pH requirements for cosmetic actives. Nonionics can be used in formulating w/o- or o/w-type emulsions and will help to mitigate the characteristic oily feel of w/o emulsions.

Thousands of emulsifying agents are available on the world market today. Choosing the best agent is the key responsibility of the formulator. Many agents used in the cosmetic and drug industry are classified by a system known as hydrophilic–lipophilic balance (HLB) number. This system, developed in the mid-1950s, is a useful starting point in emulsifier selection. In this system, each surfactant having a specific HLB number is used to emulsify an oil phase having an HLB required for a stable emulsion. Using an emulsifier or combination of emulsifiers matching the required HLB of the oil phase will form a stable emulsion. Limitations to this method include incomplete data for required HLBs of many cosmetic ingredients. Combinations of or single emulsifying agents giving the appropriate theoretical HLB may not be the optimal combination for emulsion stability or product performance. Other emulsifying agents may work better and provide a more elegant formulation with greater efficacy. In addition, theoretical HLB numbers of complex mixtures may not follow a linear additive rule specified in the calculation [2].

In this classification system, emulsifying agents with an HLB of 10 would indicate a more water-soluble agent compared with one having an HLB of 4.

For nonionic detergents of the ester type,

$$\mathrm{HLB} = 20\left(1 - \frac{s}{a}\right)$$

s = saponification number of the material
a = acid number of the fatty acid moiety of the product

For ethoxylated esters and ethers, when the saponification value is not known,

$$\mathrm{HLB} = \mathrm{E} + \frac{\mathrm{P}}{5}$$

E = percentage of ethylene oxide
P = percentage of polyalcohol in the molecule

When the hydrophobic portion contains phenols and monoalcohols without polyalcohols, the equation can be simplified to

$$\mathrm{HLB} = \frac{\mathrm{E}}{5}$$

Most nonionics fall into this category; manufacturers who provide HLB values in their product specifications most frequently use the latter formula (Table 9.2).

Mixtures of anionic and nonionic agents obtain the best emulsion; mixtures of cationic and nonionic emulsifiers may not be as elegant. Examples of nonionic emulsifiers are alcohol ethoxylates, alkylphenol ethoxylates, block polymers, ethoxylated fatty acids, sorbitan esters, ethoxylated sorbitan esters, and ethoxylated castor oil. The solubility of nonionic surfactants in water can often be used as a guide in approximating the HLB and usefulness.

TABLE 9.2
Relationship Between HLB Range and Water Solubility

Water Solubility	HLB Range
No dispersibility in water	1–4
Poor dispersion	3–6
Milky dispersion after agitation	6–8
Stable milky dispersion	8–10
Translucent to clear dispersion	10–13
Clear solution	13+
HLB	**Application**
4–6	W/o emulsifier
7–9	Wetting agent
8–18	O/w emulsifier
13–15	Detergent
15–18	Solubilizer

Source: *The HLB System*, ICI Americas, Inc., Wilmington, Delaware 1984.
Note: HLB, hydrophilic–lipophilic balance; w/o, water in oil; o/w, oil in water.

Oil-in-Water Emulsions

O/w emulsions typically contain 10% to 35% oil phase; a lower-viscosity emulsion may have an oil phase reduced to 5% to 15%. Water in the external phase of the emulsion helps hydrate the stratum corneum of the skin. This is desirable when one desires to incorporate water-soluble active ingredients in the vehicle. Oil droplets in emulsions have a lower density than the phase they are suspended in. To have a stable emulsion, it is important to adjust the specific gravity of the oil and water phases as closely as possible. Viscosity of the water phase (external phase) may be increased to impede the upward migration of the oil particles. Addition of waxes to the oil phase will increase specific gravity but may have a profound effect on the appearance, texture, and feel on application to skin of the product. Increasing water phase viscosity is one of the most common approaches. Natural thickeners (alginates, caragenates, xanthan) and cellulosic (carboxymethyl cellulose) gums are used for this purpose.

Carbopol[1] resin is perhaps the most popular gum thickener for contributing toward emulsion stability, especially at higher temperatures. The addition of a fatty amine to a Carbopol resin will further enhance stability by strengthening the interface of the water and oil phases through partial solubilization into the oil droplets. Electrolytes and cationic materials will have a destabilizing effect on anionic sodium carboxymethyl cellulose and should not be used together. Veegum, an inorganic aluminum silicate material, is also commonly used to thicken emulsions. Carbopol and Veegum may be used together to modify the characteristic draggy feel of Carbopol when used at the higher levels.

Emulsifier blends with HLBs ranging from 7 to 16 are used for forming o/w emulsions. In the blend, the hydrophilic emulsifier should be formulated as the predominant emulsifier to obtain the best emulsion. A popular emulsifier, a glycerol monostearate and polyoxyethylene stearate blend, is a self-emulsifying, acid-stable blend. Emulsifiers are called self-emulsifying when an auxiliary anionic or nonionic emulsifier is added for easier emulsification of the formulation. Formulating with self-emulsifying materials containing nonionic emulsifiers permits a wide range of ingredient choice for the formulator, especially with acid systems. In alkaline formulations, polyoxyethylene ether-type emulsifiers are preferred with respect to emulsion stability.

An alternative to glycerol monostearate self-emulsifying emulsifier is emulsifying wax NF (meeting the standards of the National Formulary). This emulsifier, when used with a fatty alcohol, will form viscous liquids into creams depending on the other oil-phase ingredients. Use levels may vary from 2% to 15%; at lower levels, a secondary emulsifier such as the oleths or PEG glycerides will give good stability. This system is good for stabilizing electrolyte emulsions or when other ionic materials are formulated into the vehicle. Polysorbates are o/w emulsifiers, wetting agents, and solubilizers that are often used with cetyl or stearyl alcohol at 0.5% to 5.0% to produce o/w emulsions [6].

Water-in-Oil Emulsions

Although less popular than o/w emulsions, these systems may be desirable when greater release of a medicating agent or the perception of greater emolliency is desired. Emulsifiers having an HLB range of 2.5 to 6 are frequently selected. When multiple emulsifiers are used, the predominant one is generally lipophilic, with a smaller quantity of a hydrophilic emulsifier. These emulsions typically have a total of 45% to 80% oil phase.

During the last few years, formulators have become interested in more elegant w/o emulsions by formulating with new emulsifying agents, for example, emollients such as esters, Guerbet alcohols, and silicones. Selection of a suitable emollient depends on the ability of the material to spread on skin with low tack, dermal compatibility, and perceived elegance by the user. In achieving this elegance, some researchers suggest a correlation of emollient and molecular weight of the emollients. In these studies, viscosity of w/o creams has correlated with molecular weight of the emollients used in test formulations. High-molecular-weight coemulsifiers formulated with high-molecular-weight emollients gave more stable w/o emulsions. The polarity of the emollients used was found to be important as well. Emollients or mixtures of emollients with medium polarity gave test lotions the most desirable stability results [7]. Anionic emulsifiers are generally inefficient w/o emulsion stabilizers because more surface-active agents are often needed to stabilize these emulsions. Sorbitan stearates and oleates are effective emulsifiers when used at 0.5% to 5.0%; sorbitan isostearates, being branched-chain materials, give a very uniform particle size for w/o emulsions.

Multiple Emulsions

Multiple emulsions are of interest to the skin care formulator because of the elegant appearance and less greasy feel of these formulation types. Two types of multiple emulsions are encountered in skin care, w/o/w, where the internal and external water phases are separated by oil, and oil-in-water-in-oil (o/w/o), where the water phase separates the two oil phases. The method of preparation for each multiple emulsion type is similar.

Benefits of these types of formulations are the claimed sustained release of entrapped materials in the internal phase and separation of various incompatible ingredients in the same formulation.

A suggested technique for forming a w/o/w emulsion is to first create a w/o primary emulsion by combining water as one phase with oil and a lipophilic emulsifier as the second phase in the traditional method. Next, water and a hydrophilic emulsifier Are combined with the w/o primary emulsion at room or warm temperature (i.e., 40°C) with mixing, forming a w/o/w multiple emulsion. These emulsions typically contain about 18% to 23% oil and 3% to 8% lipophilic emulsifier. The continuous oily phase is stabilized with about 0.5% to 0.8% magnesium sulfate. W/o emulsifiers have an HLB less than 6 and are frequently nonionic or polymeric. O/w emulsifiers have an HLB greater than 15 and are ionic with high interfacial activity. For o/w/o multiple emulsions, w/o emulsifiers have an HLB less than 6 with similar properties as a w/o/w w/o emulsifier. O/w emulsifiers have an HLB greater than 15 and are nonionic, with lower interfacial activity.

Water-in-Silicone Emulsions

Silicone compounds have evolved into a class of specialty materials used for replacement, substitutes, or enhancers for a variety of organic surface-active agents, resulting in the ability to formulate products with unique properties. Previously, silicone compounds were available as water-insoluble oily materials almost exclusively. Newer silicone compounds such as polyethylene oxide bases grafted to polydimethylsiloxane hydrophobic polymers, known as dimethicone copolyol emulsifiers, have been developed. These types of emulsifiers permit formation of water-in-cyclomethicone emulsions. Further work in this field led to adding hydrocarbon chains to silicone polyether polymers. This resulted in improved aesthetics to oil in silicone emulsions as well. Silicone copolyols exhibit high surface activity and function similarly to traditional emulsifiers. Unlike hydrocarbon emulsifiers with higher molecular weights, high-molecular-weight silicone emulsifiers can remain fluid. This gives very stable viscoelastic films at the water/oil interface. The ability to make silicones more formulator-friendly has led to the development of several new silicone-based surfactants. Both a water-soluble and an oil-soluble portion are needed to make a surface-active molecule. Silicone surfactants substitute or add on silicone-based hydrophobicity, creating a distinctive skin feel and other attributes of typical silicones as well as attributes of fatty surfactants. These emulsions may be prepared in a traditional two-phase method, for example, 2% to 3% weight/weight (wt/wt) of laurylmethicone copolyol in 23% wt/wt oil phase can be mixed in a separate water phase with electrolyte to form a hydrating cream [8].

Water-Soluble Ointment Bases

PEG polymers are available in a variety of molecular weights. These materials are water soluble and do not hydrolyze or support mold growth. For these reasons, PEGs make good bases for washable ointments and can be formulated to have a soft-to-hard consistency. PEGs dissolve in water to form clear solutions; they are also soluble in organic solvents. PEG ointment USP (United States Pharmacopeia) is a mixture of PEG 3350 and PEG 400 heated to 65°C, cooled, and mixed until congealed. To formulate a water-soluble ointment base, water and stearyl alcohol may be incorporated into this base.

Absorption Bases and Petrolatum

Absorption bases can serve as concentrates for w/o emollients; water may be added to anhydrous absorption bases to form a cream-like consistency. Petrolatum, a component of some absorption bases, has been shown to be absorbed into delipidized skin and to accelerate barrier recovery. Bases can be made washable by addition of a hydrophilic emulsifier. For example, formulation with polysorbate-type emulsifiers with polyoxypropylene fatty ethers will improve washability. These surfactants will form o/w emulsions with rubbing on skin. W/o petrolatum creams can be formulated by mixing 50% to 55% petrolatum with a sorbitan sesquioleate at 5% to 10% having an HLB of about 3 to 7 in one phase and water in a second phase. Both phases are blended at 67°C to 70°C with mixing.

OTHER INGREDIENTS

Consumer-perceived benefits of a cream or lotion are often a result of ingredients remaining on the skin after water and other volatile materials have evaporated. Emollients and other skin conditioners are commonly used for this reason. The following are frequently used ingredients to modify the feel of the emulsion on skin (Table 9.3).

Preservative Systems

Most formulations require preservative systems to control microbial growth. Microbial contamination with pathogenic microorganisms can pose a health risk to the consumer, especially from *Pseudomonas* infection in the eyes or from an existing illness. Microbial contamination may cause an emulsion to separate and/or form "off" odors. Contaminated products are also subject to recall, which is undesirable from a commercial viewpoint.

Preservatives can be divided into two groups: formaldehyde donors and those that cannot produce formaldehyde. The

TABLE 9.3
Examples of Moisturizer Ingredients and Their Functions

Ingredient	Use Level (%)	Comments
Emollient esters	5–25	Modify the oily, greasy feel of mineral oil and petrolatum, light-to-moderate feel on skin.
Triglyceride oils	5–0	Light-to-heavy feel, often used as spreading agents.
Mineral oil/petrolatum	5–70	Heavy, oily feel, provides occlusion for appropriate vehicles.
Silicone oils	0.1–15.0	Helps to prevent soaping of formulations, improves spread on skin, is water repellent, and has skin-protective properties.
Humectants (glycerin, propylene glycol, sorbitol, polyethylene glycol)	0.5–15.0	Moisture-binding properties help retard evaporation of water from formulation, control viscosity, and impact body and feel of emulsion.
Thickeners (Carbopol[1], Veegum)	0.1–2.0	Help obtain viscosity, enhance stability, bodying agents.

former group includes 1,3-Dimethylol-5, dimethylhydantoin (DMDM hydantoin), diazolidinyl urea, imidazolidinyl urea, quaternium 15, and the parabens (esters of *p*-hydroxybenzoic acid), whereas preservatives such as Kathon GC, phenoxyethanol, and iodopropynyl butylcarbamate work by alternative mechanisms. The formulator is advised to consult appropriate preservative manufacturers to select the optimal preservative system for the emulsion (Table 9.4).

TABLE 9.4
Examples of Emulsifiers

Nonionic	
Polyoxyethylene fatty alcohol ethers	Very hydrophobic to slightly hydrophobic
Polyglycol fatty acid esters	Very hydrophobic to slightly hydrophobic
Polyoxyethylene modified fatty acid esters	Very hydrophilic to slightly hydrophilic
Cholesterol and fatty acid esters	Slightly lipophilic to strong lipophilic
Glyceryl dilaurate	Secondary emulsifier
Glycol stearate	Secondary emulsifier
Anionic	
Disodium laureth sulfosuccinate	
Sodium dioctyl sulfosuccinate	
Alcohol ether sulfate	
Sodium alkylaryl sulfonate	
Cationic	
PEG alkyl amines	
Quaternary ammonium salts	
Self-emulsifying bases (form o/w emulsions)	
PEG-20 stearate and cetearyl alcohol	
Cetearyl alcohol and polysorbate 20	
Glyceryl stearate SE	
Absorption bases	
Lanolin alcohol and mineral oil and octyldodecanol	
Petrolatum and ozokerite and mineral oil	

Note: PEG, polyethylene glycol; SE, self-emulsifying.

SKIN CARE EMULSIONS FOR THE AGING POPULATION

Consumers frequently refer to young skin as having a healthy glow, radiance, or vitality that tends to diminish over time. These changes in appearance in part are related to the diminished ability of older skin to retain moisture. Cosmetic and cosmeceutical products that address the needs of the aging population by enhancing appearance are predicted to grow in product sales at twice the rate of the overall cosmetic market in the near future [9].

Early moisturizers were formulated primarily with lipids on the basis of the assumption that fats and oils make the skin soft and supple. In reality, it is difficult to specify exactly how much water content of skin is required for adequate moisturization. The water content of keratinocytes in the basal layer is about 70%. This decreases to about 15% to 20% as mature stratum corneum reaches the desquamating layers [10]. The current moisturizing strategy is to

- Increase water-holding capacity of the stratum corneum by external application of hydroscopic ingredients, known as humectants. These ingredients act in the same way as natural moisturizing factor (NMF) in skin; some materials used in moisturizers such as lactic acid and urea are components of NMF.
- Hold water in the stratum corneum by deposition of a water-insoluble oily material on the skin surface; these materials are known as occlusive agents. Oily materials mimic the effect of the natural lipid bilayers of the skin to restrict evaporation from the surface, that is, petrolatum.

In general, required levels of occlusive agents are relatively high and will cause a formulation to become tacky when applied to skin. Emulsification of occlusive agents in combination with hydroscopic agents can reduce the ability of the agent to be effectively occlusive in the finished product. Humectants are used to improve moisturization of the skin, but there are conditions when humectants may actually deprive the skin of water. Once a humectant has absorbed water, the activity coefficient of water is lowered. "If the water

in skin tissue does not have a lower water activity compared to the surrounding humectant-water blend, water molecules will not be transferred to skin [10].” Consideration should be given in the selection of humectant to ensure that the formulation does not hamper the enzyme-controlled normal desquamatory process. Glycerin is frequently the humectant of choice for this reason. More recent formulations contain hydrophilic polymers (Table 9.5) that may function as humectants and help smooth skin as well [10] (Table 9.6).

Emulsion formulators are aware that the health of the epidermis may be affected by

- The intracorneal lipid layer, its formation, hydrolysis, and oxidation
- Enzymatic dependency of synthesis of NMF
- Climatic changes

A disadvantage of formulating with glycerin-based moisturizers is that they are poor solvents for cosmetic lipids [10]. When it is desirable to have a lipophilic “cosmetic active” in the formulation, the formulator must use skill and experience to optimize the formulation.

TABLE 9.5
Hydrophilic Polymers Used in Skin Care Moisturizers

Alginic acid
Chitosan (and salts)
Collagen
Hyaluronic acid

Source: Rieger, M.M., ed., *Harry’s Cosmeticology*, 8th ed., Chemical Publishing Comp., Inc, New York, 2000.

TABLE 9.6
Examples of Common Skin Care Moisturizing and Conditioning Agents

Emollients	Humectants	Occlusives
Acetylated lanolin	Acetamide MEA	Acetylated lanolin alcohol
C14–15 alcohols	Ammonium lactate	Caprylic/capric triglyceride
Dimethicone copolyol	Copper PCA	Cetyl ricinoleate
Hexyl laurate	Glucuronic acid	Dimethicone
Isopropyl myristate	Glycerin	Hydrogenated lanolin
Lanolin	PCA	Mineral oil
PPG-20 cetyl ether	Propylene glycol	Myristol myristate
Squalene	Sodium PCA	Petrolatum
Sucrose oleate	Sorbitol	Soybean lipid
Wheat germ glycerides	Urea	Squalane

Source: Rieger, M.M., ed., *Harry’s Cosmeticology*, 8th ed., Chemical Publishing Comp., Inc, New York, 2000.

Note: MEA, monoethanolamine; PCA, pyrolidone carboxylic acid; PPG, polypropylene glycol.

FORMULATING FOR IMMEDIATE IMPROVEMENT IN APPEARANCE AND TEXTURE OF SKIN

Various strategies are available to formulate emulsions that provide immediate cosmetic benefits to skin. Epidermis of young skin is translucent; it allows light to partially pass through it. Skin that appears translucent will exhibit a shine or glow. The layer between the epidermis and dermis has ridges known as rete pegs. In aging skin, this region becomes smaller and flatter, tending to reduce the translucent effect of skin. Further, keratinocytes at the surface of the skin do not slough off as quickly. This results in skin that has a dull and uneven appearance. Other contributing factors to loss of “skin glow” or “radiance” include the irregular pattern of melanocytes that tends to develop in aging skin.

In normal daylight, one observes light that is partially reflected from the surface of stratum corneum and light that is partly reflected back from the dermis. Younger-looking skin will reflect light from the lower epidermis and blood vessels in the dermis with color contributed from melanin and hemoglobin. Incident light reflecting off dry skin will not penetrate as deeply and reflect back with a dull appearance.

Interference Pigments

One approach to altering the way light is reflected back from skin is to formulate with interference pigments. This approach initially used in facial products has recently found popularity in body moisturizers. Effect-enhancing pigments are used to “add natural, transparent luster to skin”; they can improve the tactile qualities of the skin by giving the emulsion a silky feel. The same effect-enhancing pigments may be used to impart an elegant luster to the appearance of the product [11].

Effect pigments are composed of thin, translucent platelets that produce luster by partially reflecting and partially transmitting light. Pigments are available as natural pearl, mica, and bismuth oxychloride-based materials. Bismuth oxychloride crystals have a “brilliant” white pearlescense; some grades create metallic effects, while other grades provide a “subtle luster and smooth feel.” Natural pearls can provide a “satiny luster” to emulsions. Metal oxide-coated mica pigments with thin films of iron oxide or titanium dioxide are most commonly used. The colors in these materials will shift with the viewing angle to create complex iridescence on curved body surfaces. Smaller platelets provide a “satiny-smooth, silky luster, while larger ones provide sparkle, glitter, and a lively appearance” [11]. Use of appropriate particle size and color combinations can give the skin a “radiant glow.”

Interference pigments are formulated in skin care products at levels of 0.1% to 2.0% by weight, depending upon the qualities the formulator wishes to achieve. The selection of particle size can help diminish the appearance of age spots, fine lines, and uneven skin color. Interference effects are maximized when a variety of particle sizes are formulated.

Soft Focus Effects

Fine particles, such as microspheres, are used in emulsions and anhydrous formulations to enhance the feel and appearance of skin. The chemical compositions of microspheres are diverse. Examples are polymethyl methacrylate, polyethylene, ethylene/acrylate copolymer, nylon, polyurethane, silicone resins, and silica. Selection of the appropriate material can provide "optical blurring" effects to the formulation, minimizing the appearance of fine lines and uneven skin tone. Some skin care products can deposit a transparent layer on the skin, making fine lines more visible to the eye. Formulation with appropriate microspheres can help to minimize this effect and give the skin an enhanced appearance [12]. Formulating with varied particle size will further help minimize the appearance of uneven skin [13].

When formulating with interference pigments and soft focus materials, a critical consideration is the refractive index (RI) of the primary vehicle and the material(s) to be incorporated into the vehicle. When the vehicle is applied to skin, the portion of the vehicle remaining on the skin after evaporation is considered the "primary vehicle." For example, in an emulsion of oils and polymers applied to skin, the oil/polymer portion will be the primary vehicle after the water has evaporated from the skin's surface. In general, the RI of the light-diffusing particle must be greater than that of the skin and the vehicle to be effective (Table 9.7).

Emollient Esters

Chemically, esters are the covalent compounds formed between acids and alcohols. Esters can be formed from inorganic and carboxylic acids and any alcohol. Esters, when formulated in cosmetic emulsions, have diverse functions. They serve as emollients, skin conditioners, solvents, fragrance compounds, and preservatives [14].

More recently, emollient esters have been used in place of more expensive silicones to provide aesthetic benefits to cosmetic emulsions. Esters can be formulated with silicones to enhance stability and feel of the emulsion when applied to skin [15]. Esters that function as coemulsifiers provide improved skin adhesion of the reduced formulation tackiness and can improve hydration properties of humectants.

Esters display properties that reflect their chain length and structural arrangement of their two starting materials. For this reason, different esters will have differing emollience. A simple monoester of a short-chain fatty alcohol or acid will possess a light feel. Branched esters will feel nongreasy; chemically more "complex" pentaerythrityl esters will have a "cushiony feel" [14]. The structural composition of the ester will also affect its spreading behavior on skin. Branched esters typically have a higher spreading factor. Spreading will begin to decrease as the molecular weight increases. Emollient esters affect the viscosity of the emulsion, either improving texture and formulation aesthetics or detracting from it if incorrectly formulated. When formulating with coated pigments, one must ensure that the selected ester is compatible with the coating. Another consideration is the pH of the finished product. Below a pH of 3.4, esters tend to hydrolyze, resulting in a product that may develop an undesirable odor [16].

TABLE 9.7
Examples of Refractive Indexes (Various Sources)

Material	Refractive Index
Air	1.00
Perspiration	1.33
Polyethylene	1.45
Titanium dioxide	2.51
PMMA	1.49
Silica	1.45
Skin	1.62
Microspheres (general)	1.41–1.53
Propylene glycol dibenzoate (ester)	1.54
Phenyl trimethicone (silicone)	1.46
PPG-3 benzyl ether myristate (ester)	1.465
Dimethicones, cyclomethicones (silicone)	1.375–1.403, 1.394–1.398

Note: PMMA, polymethyl methacrylate; PPG, polypropylene glycol.

Polymers

Polymers are small molecules that are chemically connected in long repeating units. Polymers are ubiquitous in nature. The DNA of all living cells and the protein and starches in our foods as well as the tires of our automobiles are all composed of polymers. The use and function of polymers in cosmetic emulsions are equally diverse. Polymeric emulsifiers, such as those based on silicone or polyacrylic acids, are used as emulsifiers. These polymers have cationic charges that are substantive to skin and impart a smooth, conditioning effect. Other polymers are formulated in emulsions to create the sensation of firming skin, minimize interference pigments and other solid particles rubbing off to clothing, and provide water resistance to sunscreen-containing emulsions. These polymers form a film on the skin's surface (Table 9.8).

FORMULATING EFFECTIVE COSMECEUTICALS FOR AN AGING POPULATION

An aging consumer population seeks products to address fine lines and wrinkles, improve the appearance of an uneven skin tone, smoothen rough-textured skin, and reduce skin dyspigmentation, referred to as "age spots." Advances in molecular biology have enabled research investigators to develop numerous in vitro screening protocols demonstrating the potential of various cosmeceutical ingredients to help improve the appearance of aging skin.

Peptides, Vitamin Derivatives, Botanicals

In vitro data may produce very dramatic results supporting efficacy of cosmeceutical agents. Many of these agents

TABLE 9.8
Examples of Polymers (Various Sources)

Polymer	Type	Potential Application
Acrylates/C10-30 alkyl acrylate cross-polymer	High-molecular-weight polyacrylate	Primary emulsification (o/w)
Carbomer	Acrylic acid	Synthetic thickener
Acrylate/steareth-20 methacrylate copolymers	Acrylic polymer emulsion/anionic	Thickener
PEG-150/decyl alcohol/SMDI copolymer	Hydrophobically modified nonionic polyol	Low-pH formulations, cationic conditioners, o/w sunscreens, cationic silicone emulsions
Caprylic/capric triglyceride sodium acrylate copolymer	Polyacrylic acid	W/o emulsions
PVP/eicosine copolymer	Copolymers of vinylpyrrolidone	Oil soluble, rub resistance in sunscreen
Tricontanyl PVP	Copolymer of vinylpyrrolidone	Oil soluble, rub resistance for pigments and sunscreens

Source: The Personal Care Products Council, in: Gottschalck, T.E., and Bailey, J.E., eds., *International Cosmetic Ingredient Dictionary and Handbook*, 9th ed., The Cosmetic, Toiletry and Fragrance Ass., Inc., Washington, DC, 2002.

Note: PVP, polymer of vinyl pyrrolidine; SMDI, saturated methylene diphenyldisocyanate; w/o, water in oil; o/w, oil in water.

must be properly formulated and should be properly tested in vivo to confirm that they will function as desired to meet consumer expectations. Cosmeceutical agents must be compatible and stable in the vehicle they are formulated in to be effective. For example, peptides are available with variations in the number of amino acids and sequence. The peptide must be designed to have the ability to penetrate skin in order to be effective. One approach is to add a lipophilic chain, such as a palmitate (Table 9.9), to the peptide. A copper peptide is commercially available; to be effective, it must be formulated at significantly higher levels compared with the palmitoyl pentapeptide [17,18].

Published literature supports claims that retinoic acid improves the appearance of wrinkles, promotes collagen formation, and evens skin tone. Retinoic acid has limited stability, and consumers frequently experience dry, irritated skin during product use. To promote stability, formulation exposure to oxygen and light should be minimal. Formulating with an antioxidant and encapsulation of the retinoic acid are other options. The primary package should be designed to be oxygen and light impermeable. Irritation potential may be reduced by formulating with an appropriate retinoic acid derivative. Retinol is better tolerated by skin than *trans*-retinoic acid [19]. Incorporation of anti-inflammatory agents may further mitigate irritation. Sugar amines such as glucosamine and *N*-acetyl-glucosamine can help hydrate skin and reduce fine lines/wrinkles and facial hyperpigmentation. Glucosamine tends to be unstable in products formulated with antioxidants, and at an acidic pH, it may help overcome this problem [20]. Formulations with glycolic acid are associated with an increase in sensitivity to solar exposure and sunburn cell formation in skin. For this reason, products with glycolic acids should also contain sunscreens.

Medicinal and cosmetic use of botanicals has a long history spanning many centuries. Selection of a botanical is influenced by experience passed on from generation to generation. Despite this long history of use, traditional medicine has not been officially recognized by many countries [21]. In recent years, interest has increased regarding use of botanicals in skin care. Data addressing safety, quality, efficacy, and guidelines for formulating with botanicals to achieve optimal benefits are lacking. The formulator of botanical-based products is advised to consult other resources, in addition to information supplied by the manufacturer, such as the World Health Organization [22], *Journal of Nutrition* [23], and other reliable published data.

Two promising groups of botanically derived agents that appear to hold promise as chemotherapeutic treatments for aging skin are polyphenolic antioxidants (catechins and flavonols) and isoflavones. Green tea contains epigallocatein-3-gallate (EGCG), and grape seed contains polyphenolic antioxidants. Silymarin found in milk thistle and genistein found in soybean extract are other examples of useful ingredients for photoaging [24].

Many botanical extracts are available to the formulator. Plant constituents of extracts vary with respect to chemical compounds. Variations in solubility and stability have potential to cause shelf life and stability challenges of the finished product over time. Many extracts have a dark color or an odor that may create aesthetic concerns. Extraction methods intended to lighten color or mitigate odor may remove a compound with the desired activity. To minimize aesthetic and stability concerns, formulators should consult with the extract manufacturer regarding availability of technical information addressing polarity of plant-derived oils and optimal formulation pH range for extracts containing alkaloids. Botanically derived lipids are often not hydrogenated and are subject to oxidation promoting product rancidity [25]. Pharmaceutical-grade extracts are typically five to 10 times stronger than cosmetic-grade extracts. Cosmetic extracts may be aesthetically acceptable in emulsions. They may lack key desirable chemical constituents. Alternatively, pharmaceutical-grade extracts are very resinous, dark in color, and not soluble in many cosmetic formulations [26].

TABLE 9.9
Cosmeceutical Ingredients

Ingredient	Claimed Benefit	Mechanism	Formulation Consideration
Botanicals: soy, green tea, pomegranate, red clover, curcumin, resveratrol (in skin and seeds of grapes)	Soy: skin tone evening, improvement in dyspigmentation	Inhibition of PAR-2 activation by protease inhibitors	Source of soy important
	Green tea: UVB-induced formation of thymine dimers (a marker for DNA damage) inhibited a 5% pretreatment prior to UVB exposure and inhibited keratinocyte damage	Quenching of reactive oxygen species (ROS); also, modulation of NF-κβ pathway, a signal transduction pathway responsive to UV radiation	Green tea extract tested in vivo, applied topically to skin in a solution of ethanol or water. A 5% GTP solution was effective, 10% was optimal (1%–10% solutions demonstrated a dose-dependent response) [22]
Milk thistle (silibinin)	Protect skin from UVR	Antioxidant, free radical scavenger, downregulates chemically induced lipoxygenase, TNFα, and IL-1α in mouse skin	
Hydroxy acids, e.g., alpha, beta, poly, and bionic acids	Photoaging and hyperkeratosis (age spots and hyperkeratotic lesions)	Antioxidant	pH of final product is low, ~4.0; hydrolysis of esters in formulation will occur causing an "off" odor
	Increased dermal thickness	Bionic acid inhibits matrix metalloproteinase enzyme activity responsible for degradation of skin's matrix and structural integrity (wrinkle formation, skin laxity, and telangiectasia)	Sun sensitivity with a and b acids
		Increased production of collagen and fibroblast proliferation	
Peptides, e.g., palmitoyl pentapeptide	Improve appearance of fine lines and wrinkles of the eye area	Stimulation of type I and type III collagens and fibronectin production	Peptide lipidated to penetrate skin
Other peptides, e.g., acetyl hexapeptide-3	Wrinkle reduction (limited data available)	Inhibits calcium-dependent catecholamine release from and assembly of SNARE protein complex	Short amino acid sequence to facilitate cell membrane permeability
Miscellaneous vitamins:			
Vitamin C (ascorbic acid, ascorbyl phosphate)	Wrinkle reduction, improvement in skin tone evening and texture	Improvement in skin collagen, reduced pigment transfer from melanocyte to keratinocyte	Stability, proper formulation pH, penetration into skin
Vitamin B_3 (niacinamide and its esters)	Improved skin tone, reduced dyspigmentation	Antioxidant	Formulate at appropriate pH to avoid hydrolysis
Vitamin E (tocopherol and tocopherol acetate)	Protection against UV-induced effects to skin, reduced inflammation of skin	Antioxidant	Stability against oxidation, oil-soluble forms are less elegant, acetate form is subject to hydrolysis in formulation
Retinoic acid (functional form of vitamin A)	Wrinkle reduction via thickened skin	Increased epidermal thickness and ground substance inhibit collagenase production	Oxygen and light render material unstable. Antioxidants may improve stability
	Reduce appearance of dyspigmentation	Reduces expression of tyrosinase	
Sugar amine, e.g., *N*-acetyl-glucosamine	Moisturization, reduce fine lines/ wrinkles	Precursor of hyaluronic acid, a water-binding component of skin	Tends to be unstable, creating a brown-colored product
	Improve skin tone	Inhibits tyrosinase, thus inhibiting melanin production	

Note: GTP, green tea polyphenol; IL-1α, interleukin 1 alpha; NFκβ, nuclear factor kappa β; PAR-2, proteinase activated receptor-2; SNARE, soluble-*N*-ethylmaleimide activating protein receptor; TNFα, tumor necrosis factor alpha; UVA, ultraviolet A; UVB, ultraviolet B; UVR, ultraviolet radiation.

Notes from an Herbalist: Formulating with Botanical Extracts

A tincture is a solution of soluble plant constituents in a solvent known as the menstrum. Poor filtration, exposure to light, temperature changes from warm to cold, or chemical degradation of extractives can cause precipitation to occur. The precipitate may contain active constituents or inert proteins. Precipitation can be minimized by storage at constant temperature and avoidance of exposure to light. Massive precipitation, development of a marked color change, and "off" odor indicate that the tincture should be discarded. Alkaloids in extracts have diverse medicinal benefits. Acidification of the extraction solvent may increase potency, but efficacy may be neutralized by mixing with tannins. Glycerine is commonly used as an extraction solvent when it is undesirable to use alcohol. This type of extract is referred to as a glycerite. Glycerites tend to be less potent than alcoholic extracts and have a shorter shelf life.

Vegetable oils are good extraction solvents for many plant constituents. Herbalists are concerned that they are also good solvents for pesticides and herbicides. For this reason, it is advisable to formulate with organic certified organic vegetable oils, ideally cold-pressed oils [27].

FUTURE FORMULATION CHALLENGES

Cosmeceutical ingredients have been popular for many years, and new cosmetic active agents are continuously being identified. Many of these active ingredients have excellent in vitro data to support claims but are lacking in vivo data. Further, formulators often formulate the active in an existing prototype rather than employing a strategy of formulation optimization. Consumers have come to expect functional cosmetic products. Products that fail to deliver on consumer expectations are unlikely to succeed long term in the marketplace [28].

Future formulation challenges will be to

- Determine the optimal emulsion system to effectively deliver the desired ingredient to the viable epidermis via the stratum corneum (partition coefficients, penetrant polarity)
- Understand the influence of formulation characteristics on skin delivery (influence of the emulsifier, solubility characteristics of the primary emollient or solvent, and influence of emollients in general)
- Continuously advance regarding knowledge of skin molecular biology, specifically the intended region of product use on the body

Role of Molecular Biology in Selecting Cosmetic Actives for Skin Care

In recent years, techniques developed in the field of molecular biology have been successfully used to screen potential ingredients for skin care application. The fundamental principle of molecular biology is that the body seeks to be at "homeostasis," a general state of well-being. To maintain homeostasis, there is continuous feedback communication within each cell of the body and throughout the body as well. Molecular biology uses various techniques to measure the markers that result from this communication. Typically, these are proteins that can be identified. While numerous protocols exist for all sorts of in vitro testing, there is no specific prescribed battery of molecular testing for a specific application or protein identification. Experts in the field note that some of the reported findings are difficult to reproduce when conducted in different laboratories or, at times, by different individuals in the same laboratory. Minor variations in cell culture media and source of cells used in studies may also lead to variations in laboratory results. For these reasons, test conditions should always be well documented. With these cautions in mind, there are cosmeceutical agents with data obtained from molecular biological studies that are successful in the market place. Examples of these technologies follow.

Vitamins

Various forms of vitamin B_3, specifically niacinamide, are shown to improve skin color, including hyperpigmentation and red blotchiness. Niacinamide inhibits the transfer of pigment-bearing melanosomes from melanocytes to keratinocytes [29]. Certain soy products have been shown to improve the appearance of skin tone by modulating the activity of a protease-activated receptor-2 (PAR-2) in skin. This receptor is involved in cell growth, differentiation, and inflammation [30]. Vitamin C, when properly formulated for stability, has been shown to be effective for skin lightening and collagen stimulation [31].

Peptides

A variety of peptides are available; they serve as signaling molecules, enzyme inhibitors, neurotransmitter inhibitors, or carrier peptides. To be effective, peptides need to be designed to penetrate skin. A comprehensive review of peptides currently available for cosmetic formulations and summary of molecular mechanisms may be found in Ref. 32.

Botanicals

Green tea extracts contain chemical components including flavanols (catechins), flavandiols, flavonoids, and phenolic acids. They have been shown to help protect sun-exposed skin and prevent DNA damage as a result of such exposure. Quercetin, curcumin, and epigallocatechin gallate are varieties of phytochemical polyphenols that are considered bioactive natural products. They act as antioxidants, trace metal complexing compounds, and regulators of cell proliferation. Quercetin is reported protect skin against ultraviolet A (UVA) irradiation. A study published by Sgarbossa et al. [33] indicated the mechanism of antioxidant activity may be similar to the activity of PPG's found in *Echinacea*. Another study by Yao et al. [34] confirmed the conclusion that gene expression and regulation of the antioxidant response mechanism in keratinocytes was confirmed by Yao et al. [34].

More recent studies suggest that they are also modulators of epigenic regulation of gene expression. Epigenics is an emerging area of research that has the potential to help the cosmetic scientist understand how diet and certain cosmetic ingredients can benefit skin on a cellular level. New findings suggest that phytochemical phenolic antioxidants including polyphenols, flavonoids, and nonflavonoid phenols function as potent modulators of epigenome-regulated gene expression [35,36]. A major implication of these findings is that antioxidants provide benefits for skin. It may not be the antioxidant activity that is directly responsible but, perhaps, the ability to modulate gene expression.

Curcumin

Various studies have shown curcumin to have the ability to mitigate oxidative stress and suppress inflammation [35], two factors associated with aging skin. Curcumin used in the referenced study was reported to help protect against singlet oxygen formation associated with UVA exposure, which is thought to product DNA genotoxicity. Numerous studies using molecular biological assays are reported in Ref. 35.

Green Tea

Flavonoids absorb UV light and have direct and indirect cytoprotective activities. There are extensive studies published in the literature about Camellia sinensis, green tea, (-)-epigallocatechin3-gallate that indicate it can help to reduce the risk of carcinogenesis associated with UVB radiation and resulting inflammation to skin [36].

Echinacea and Other Botanicals with Phenylpropanoid Glycosides

Phenylpropanoids have been shown to have antioxidant and radical-scavenging activities. The body has endogenous defense mechanisms to counter excessive levels of reactive oxygen species (ROS). ROS-detoxifying enzymes include superoxide dismutase, catalase, glutathione peroxidase, and heme oxygenase 1 (HMOX1). These defense mechanisms decrease with human aging. Phenylpropanoid glycosides (PPGs) are a class of over 200 plant-derived polyphenols with antioxidant activity by scavenging ROS and reactive nitrogen species. *Echinacea angustifolia* and *Echinacea pallida* are botanicals known to contain PPG. The epidermis is one of the body's primary defense mechanisms against oxidative damage. Keratinocytes in skin express antioxidative, ROS-detoxifying enzymes that help to protect cells against oxidative stress. Molecular assays involving gene expression have shown the biological mechanism of how PPGs signal in skin to activate the antioxidant response.

CONCLUSION

The signaling pathways responsible for the antioxidant response are not completely well-characterized at the molecular level at this time. Several different signaling mechanisms are simultaneously involved. The in vivo relevance of in vitro studies appears to be supported from the existing data.

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10 Confocal Raman Spectroscopy for In Vivo Skin Hydration Measurement

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INTRODUCTION

In 2013, in vivo confocal Raman microspectroscopy of the skin is routinely being applied in human panelist studies. A growing number of research groups have access to the technology and knowledge; the technology is implemented on five continents. Ever since the first Raman spectra of skin was presented, it has been known that these spectra contain unique information on the chemical composition of the skin. The ability to measure the chemical composition of living biological tissues nondestructively is a valuable tool in the skin sciences. Raman spectroscopy has qualities that make it unusually attractive for such measurements. Especially, the ability to measure the chemical composition of tissues noninvasively at defined depths, using confocal optics (optical sectioning), is unique. Because of the complexity of biological tissues, Raman spectroscopy has only in the last decade begun to make significant contributions in skin science. A 2008 review discusses the in vivo applications of Raman spectroscopy in the measurement of the composition of skin, including topically applied compounds and their effects on skin composition, in the context of pharmaceutical applications (such as transdermal drug delivery) [1].

Skin research increasingly depends on more detailed knowledge of the molecular composition of skin and the spatial distribution of skin constituents. On a microscopic scale (the scale of the confocal Raman measurement), the skin is highly heterogeneous. Its molecular composition and structure vary tremendously over different body sites and at different depths below the surface of the skin. In the stratum corneum (SC) especially, concentration gradients (e.g., water gradients, pH gradients, diffusion kinetics) play a role in biochemical or skin physiological processes. The composition of the skin is also affected by skin disorders, environmental factors such as sun exposure, seasonal variation, diets, bathing habits, and cosmetic or medical treatments. Skin treatments may also bring about changes in dimension, such as an increase in SC thickness due to swelling. The spatially complex skin tissue can be excellently studied using Raman spectroscopy with a confocal approach, where spatial resolution can be achieved that is consistent with the size of many features of interest (~5 μm in depth and ~1 μm laterally).

Noninvasive methods are particularly welcome. This is partly because they cause less discomfort for the patient or volunteer subject, but also because noninvasive methods enable investigation of the skin in its natural state without affecting its integrity, morphology, or molecular composition. Noninvasive measurements can be performed repeatedly on the same skin area in vivo and can thus be used to monitor time-dependent changes in the skin brought about by skin treatments.

Caspers et al. [2] presented the first in vivo confocal Raman spectra of human skin and were able to clearly show compositional differences at different depths below the skin surface. For example, large changes in composition were observed near the SC–stratum granulosum interface, from which the SC thickness could be derived in vivo. This was confirmed shortly after by combined confocal microscopy and Raman spectroscopy [3]. (See section "Validation of the Quantitative in vivo Water Concentration Measurement.")

Whereas the aforementioned more general review discusses the measurement of the main chemical composition of the SC [1], this chapter focuses on how measurements of water concentration gradients can be used to study the moisturization process of the skin in its outer layer.

ALTERNATIVE METHODS TO MEASURE SKIN HYDRATION

It has been known for centuries that humans continuously lose water through the skin, for instance, from experiments in which human subjects and their ingested and excreted liquids are carefully weighted. The detailed hydration process of the skin and the dynamic transport of water through the skin have been subject to investigations for decades. Modern methods for studying the moisturization of the skin can be coarsely classified, using the following criteria:

1. The relationship of the measured parameters to the hydration state of the skin (direct or indirect, straightforward or complex)
2. Spatial resolution, parallel and perpendicular to the skin surface
3. The extent to which the measurement influences the skin state (invasiveness and the ability to resample the probed site)
4. The ease of use (both the measurement procedure and the data processing and interpretation)

It is beyond the purpose of this chapter to discuss the features of the various methods in use, and we refer a reader with such an interest to recent review papers by Darlenski et al. [4] and by Byrne [5]. The review by Darlenski et al. focuses on the epidermal barrier function. In the review by Byrne, the emphasis lies more on the details of some common bioengineering methods to evaluate xerotic skin in a broader sense (not limited to hydration only). Yet another review appeared recently, in which Lademann et al. [6] discuss noninvasive in vivo methods for the assessment of dermal penetration of topically applied substances. The paper discusses electron paramagnetic resonance, coherent anti-stokes Raman scattering (CARS) tomography (a multiphoton microscopy technique), and confocal Raman spectroscopy.

The short and simplified classification, using the aforementioned criteria, along with the newest method of confocal Raman spectroscopy, will help in clarifying the quite remarkable position of the latter. The methods included in the comparison are Karl Fischer titration, light microscopy, electron microscopy, electrical methods (capacitance and conductance), transepidermal water loss (TEWL), magnetic resonance imaging (MRI), near-infrared spectroscopy (NIRS), Fourier transform infrared spectroscopy using attenuated total reflection sampling (ATR-FTIR), opto-thermal transient emission radiometry (OTTER), and of course, Raman spectroscopy (Byrne discusses a few more methods [5]). In Table 10.1, these methods are ranked according to their favorability toward the four criteria (from – to ++). The table also contains a short comment. In the right column, some key reference papers are suggested for further reading. The key references are a personal selection by the authors. It will provide the reader with more details, helpful in gaining a better understanding of the different ways in which skin hydration may be measured. The authors by no means claim the selection to be complete or to effectively represent the whole field of science.

Since the hydration of the skin is so closely related to water gradients that reside within the very thin SC, confocal Raman seems unusually suitable, especially when one takes its in vivo applicability into account. Despite its apparent complexity, which we believe may be mainly due to relative unawareness of Raman spectroscopic technologies, the measurement routine is not difficult to master. It is the experience of the authors that an instrument operator (with no prior knowledge of spectroscopy) can be trained in 1 or 2 days, enabling him/her to carry out the in vivo measurements of water depth concentration profiles on human volunteers.

A method that appears to potentially possess comparable features as confocal Raman microspectroscopy is MRI. An impressive spatial resolution of 4 μm (in all directions) has already been demonstrated on very small (single biological

TABLE 10.1
Brief Comparison of Some Features of Current Methods to Assess Skin Hydration

	Water Direct/Indirect	Spatial Resolution	Invasiveness	Ease of Use	Key References
Karl Fisher	++ Direct and absolute	–	– Destructive	– Long preparation	[7]
Light microscopy	+ Swelling can be observed	+ 0.5 μm	– Sections required	– Long preparation	[8,9]
Electron microscopy (SEM and STEM)	+ Indirect	++ <0.01 μm	– Cryosections	– Long preparation, complex instrument	[10–12]
Capacitance and impedance	– Influenced by products	– Probes top 30 μm	+ Contact probe	++ Push button	[13–15]
Conductance	– Influenced by products	– Probes top 1–10 μm	+ Contact probe	++ Push button	[16–18]
TEWL	+ Measures flux	– 0.5 cm	+ Contact probe	++ Push button	[19,20]
MRI	++ Direct	– 70 μm in vivo	++	– Complex instrument	[21]
NIR	+ Difficult to quantify	– Probes top 1–2 mm	++ Noncontact	+ Moderate complex	[22]
ATR-FTIR	+ Difficult to quantify	+/– Probes top 1–2 μm	+ Contact probe	+ Moderate complex	[23]
OTTER	+ Direct, but theoretical modeling required	+ >10 μm	++ Noncontact	+/– Complex instrument	[24]
Confocal Raman	++ Direct, quantitative relative to keratin	+ 1 μm lateral, 5 μm depth	+ Contact probe	+ Moderate complex	This paper

Note: NIR, near-infrared; SEM, scanning electron microscopy; STEM, scanning transmission electron microscopy.

cell) isolated samples [25]. However, the extremely difficult challenges to overcome for large samples, such as human volunteers, will be the size and stability of the magnet and, foremost, the definition and stability of the magnetic field gradient. Whereas the Raman methodology already allows for measurement of the overall chemical composition [1], this is not yet possible for in vivo MRI of large samples. Finally, the cost of ownership of MRI equipment can become very high.

REQUIREMENTS FOR IN VIVO RAMAN METHODOLOGY

Qualitative Description of the Raman Effect

In Raman spectroscopy, a sample of interest (this can be gaseous, liquid, or solid) is illuminated by a laser beam. The light in the laser beam is of single (or very narrow) wavelength nature. The electric component of the electromagnetic light wave, within the laser beam, drives the electron cloud of the molecules present in the sample. The driven (and hence oscillating) electron clouds reemit most of the collided laser light without energy loss, only changing the direction of the ejected photons. This process is referred to as elastic scattering (or Rayleigh scattering). A very small amount of the laser light, however, scatters inelastically; the ejected photons have a different energy than the injected laser photons. The difference in energy is taken up or released by the molecules and is used to promote or demote, respectively, the vibrational energy levels of the molecules. By measurement of the intensity of the reemitted light at different frequencies, a set of Raman signals with distinct shifts in frequency is obtained. In a Raman *spectrum*, the differences in energy between the Raman signals and the energy of the laser photons are plotted versus the intensity of the signals. The differences in energy correspond to transitions in vibrational energy levels (remember that the differences in energy were donated to or taken from the molecules to alter their vibrational energy). In this respect, a Raman spectrum contains the same kind of information as an infrared (IR) spectrum, that is, signals due to molecular vibrations, but the way in which this is obtained (photon scattering) is different from IR spectroscopy (photon absorption). We finally remark that, just as in an IR spectrum, the peaks in a Raman spectrum will correspond very specifically to molecular vibrations of the molecules present. Consequently, a Raman spectrum is highly specific for the molecules present in the volume of sample that is illuminated by the laser light.

In Vivo Raman Methodology

In vivo, Raman spectra are obtained by focusing a laser beam through a microscope and allowing the microscope objective to project the focused laser beam on and below the surface of the skin. Subsequently, the Raman light is measured in the backscattered direction through the same microscope objective. A challenge is to enable Raman measurements on biological tissues with sufficient speed for practical use in in vivo clinical studies. General-purpose Raman instruments, available in most well-equipped analytical laboratories, are not capable of practically useful measurements on skin. Recently, however, Raman instrumentation has been developed, employing advanced technologies, and made commercially available, which is capable of practical use in these demanding applications. Figure 10.1 shows a photo of this first commercially available Raman skin analyzer.

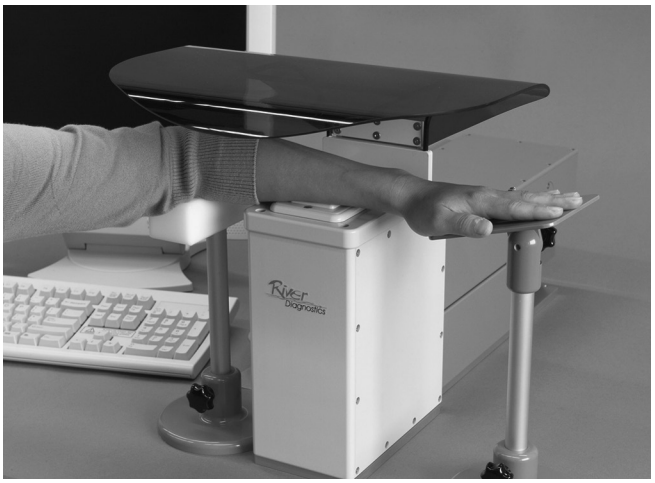

FIGURE 10.1 The RiverD International Model 3510 Skin Composition Analyzer is the first Raman instrument optimized for in vivo analysis of skin.

Most critical factors in an optimized Raman skin analyzer are selection of lasers, choice of optical materials, detector quality, optomechanical stability, and for practical utility, software that is easy to use and can effectively handle the large volumes of data that are generated in in vivo panel studies. Laser safety considerations also create technical requirements that must be met, thereby strongly influencing the overall engineering of a Raman skin analyzer. Indeed, a capable Raman skin analyzer may be thought of as being composed of four components, each of which must meet critical requirements: (1) a laser light source and associated light conditioning optics; (2) a near-infrared (NIR, the optimal wavelength applied in the measurement of skin) optimized microscopic measurement stage; (3) the Raman spectrometer; and (4) specialized operating and data analysis software. Each component, as well as the implications of laser safety, will now be briefly discussed below.

Laser Excitation Source and Optics

First, the laser(s) used must emit light at wavelengths at which no photo(bio)chemical reactions are brought about and at which minimal fluorescence is stimulated in the skin. Fluorescence is a broadband emission signal due to electronic excitations in the molecules. It is not specific to molecular vibrations, and its intrinsic intensity is orders of magnitude higher than that of Raman signals. Therefore, fluorescence signals can obscure Raman signals and should be avoided

where possible. Both requirements place a lower limit on the usable laser wavelength at approximately 660 nm. Second, the Raman-scattered photons must be detected with the highest possible efficiency and the lowest possible noise. State-of-the-art technology for this purpose is a charge-coupled device (CCD) detector for which the detection is limited to wavelengths shorter than about 1100 nm. Detection of a Raman spectrum in the so-called fingerprint spectral region (400–2000 cm^{-1}) therefore sets an upper limit to the laser excitation wavelength of about 900 nm.

Thus, the choice of laser wavelengths is restricted to a "biological and technical window" in the NIR, approximately in the range of 660 to 850 nm. Typically, solid-state diode lasers are applied. For diffraction-limited laser focusing (required for the best spatial resolution), a single-mode laser is required. The laser must be stable in power output and wavelength, and its emission line must be narrow to allow for achievement of high spectral resolution. Unwanted laser diode background radiation or satellite emissions must be removed (optically filtered) before the laser light is injected into the microscope. The laser power out of the measurement device (the microscope) must meet the requirements derived from the laser safety limitations (see below the subsection "Laser Safety Considerations"). Finally, a strict requirement is set for all optical materials in the laser light path; only minimal fluorescence or other background contributions are allowed.

Microscope Measurement Stage

The Raman signal is collected back through the microscope objective, and the microscope must have uncompromised confocal optics. The entire optical train must very efficiently transmit the signal to the spectrometer and, finally, to the detector. The spatial resolution of the microscope must be high compared to the thickness of the SC; otherwise, no information about the distribution of materials (such as water) within this skin layer can be obtained. As objectives designed for microscopy do not meet these requirements, a dedicated objective designed and optimized for Raman spectroscopy using NIR wavelengths (660–950 nm) is required. Also critical, is the absence of any difference in refractive index in the optical path from the objective to the skin. In the skin analyzer (Figure 10.1), this is managed by positioning the microscope objective below a measurement window of identical refractive index to that of the skin and the objective. The space between the objective and the measurement window is filled with a refractive index-matching immersion oil. The sampled skin rests and is locally conveniently fixed on the measurement window. If, on the other hand, a large difference in refractive index is present between the microscope objective and the skin (e.g., by focusing through air), a severe degradation of depth resolution results. The microscope objective must be movable in the axial direction (z axis) under precise control. This allows spectra to be recorded at successive depths in the skin, from which composition depth profiles are obtained. The microscope stage must allow for convenient orientation of human subjects. Usually, an inverted configuration is used. The most common measurement site at present is the volar aspect of the forearm (Figure 10.1).

Raman Spectrometer

A very high laser wavelength rejection and again a high transmission at optical interfaces (low reflection and scattering losses) are required to preserve as many of the information-bearing photons as possible. Of course, the detector must also be of high performance. Any general-purpose Raman spectrometer would benefit from these requirements, but in the measurement of biological samples, the information sought is often in small spectral differences, which require high spectral quality. Furthermore, these high-quality spectra must be routinely obtainable in a time scale compatible with panel studies and the patience of volunteer panelists. Hence, maximizing the signal-to-noise ratio (S/N) by employment of an optimized spectrometer design is of great importance. In a clinical research environment, data recorded today must compare meaningfully to data recorded before or after. Therefore, mechanical and optical stability and measurement repeatability are further important considerations. In clinical environments, where more than one spectrometer is in use, it is further required that results obtained on one skin analyzer will be directly and reliably comparable with results obtained on another skin analyzer. This places very high demands on the accuracy and reproducibility of instrument calibration and correction for instrument response effects.

Software

Data acquisition software for in vivo Raman measurements must have specialized features to handle the often large number of measurements in typical panel studies and to satisfy requirements that are not normally encountered in other types of Raman analysis. For example, the software must enable the operator to quickly select locations of interest on the skin surface. Also, since depth information (usually changes in composition as a function of depth are present) is important, the software must incorporate a reliable and accurate means of locating the skin surface for reference. Further, the software must have minimal data acquisition "dead time" between sequential spectrum acquisitions, to maximize throughput, when thousands of spectra are typically acquired in a day.

Because of the many experimental variables in a typical skin study design, the number of spectra to handle can become very large. Therefore, the data processing software must incorporate special features. In conventional spectroscopic processing software, spectra can be manipulated and analyzed typically one by one or batch by batch. For larger numbers of spectra, typical for in vivo studies, the time, simply to read in each single spectrum and export the result after analysis, can become prohibitive. Even in a batch-processing mode, the time to sort, select, and read in the spectra to

define the batches for analysis can become a bottleneck. Therefore, the software must feature ways to enter the experimental design and use this to select and process the spectra accordingly.

Laser Safety Considerations

The international Laser Safety Standard, IEC 60825-1 (2007), prescribes maximum permissible exposure (MPE) limits for the skin, which are dependent on the wavelength of the laser light and the duration of the exposure. The MPEs for skin are formal limits based on extrapolations of exposure to sunlight and do not represent actual damage thresholds, which may be considerably higher. To provide a "flavor" for MPE magnitude, the configuration of the instrument (Figure 10.1) results in an MPE limit of 30 mW for 785 nm laser excitation and 20 mW for 671 nm excitation. These values are not to be taken as general limits for the wavelengths cited but must be determined for any instrument design intended for in vivo skin analysis.

There must not be any significant risk of eye damage from exposure to the laser beam when working with a Raman instrument for in vivo skin measurements. Practically speaking, laser exposure of the eye is not a difficult risk to manage in a properly designed instrument since the laser beam diverges at a high angle when emerging from the microscope objective, but the risk must nevertheless be properly managed. The instrument (Figure 10.1) operates well within the limits of a class 2M laser device, which means that the instrument is eye-safe. Incidental direct observation of the beam is not an eye hazard, provided that no optical instruments are used to observe the beam. Each instrument is tested for compliance with the class 2M laser device classification.

When these five elements, an appropriate laser light source, microscopic measurement stage, NIR-optimized Raman spectrometer, specialized software, and in vivo laser safety provisions, are combined in a Raman instrument, valuable information hitherto unavailable to researchers becomes accessible.

RAMAN METHODS FOR THE STUDY OF HYDRATION OF THE SKIN

Relationship between the Raman Spectrum of Skin and the Local Water Concentration

In the Raman method for measurement of hydration of the skin, a spectral region, assigned to water present in the skin, is selected. Note that the intensity of all Raman scattered light, hence all Raman signals, depends on the depth at which the light scattering occurs. In other words, Raman signals from greater depths will be weaker. This effect is easily understood; since the skin is not perfectly transparent for the laser and Raman light, it exhibits rather a bit turbid character. Often, in Raman spectroscopy, this effect is compensated for by dividing the measured intensity of the spectral region of interest by the intensity of an intrinsic standard, which may be a carefully selected spectral region from the same Raman spectrum or a generic and abundant constituent of the SC. Since the reference signal is attenuated by exactly the same factor as the analyte signal (e.g., water), the division will cancel out the depth-dependent attenuation. In biological samples, often a signal due to a protein is used. The Mendelsohn group, for example, uses a signal due to phenylalanine to this end [26]. Others use a general signal due to proteins; in the skin, this is mainly keratin, which represents the major dry mass fraction in the SC [27].

In 2000, Caspers et al. [27] published the first in vivo water concentration measurements in skin as a function of depth below the skin surface. In this paper, the method to calculate a water concentration in mass percentages of wet tissue is discussed in detail. It also involves internal normalization of the water signal, in this case by a signal due to keratin. In Figure 10.2, parts of typical Raman spectra, recorded at different depths, of untreated SC skin is shown.

Signals due to protein (keratin) and water are indicated. Furthermore, integration boundaries for the signals due to water and keratin are drawn, after spectral baseline subtraction. From Raman spectral measurement of solutions of protein of known concentration, Casper et al. [27] were able to set up a calibration, equating the ratio of Raman signal intensities due to water (W) and keratin (K) to the mass percentage of water present in the skin (for wet tissue):

$$\text{water(mass\%)} = \frac{W/K}{\left(\frac{W}{K} + R\right)} \times 100\% \qquad (10.1)$$

where R is a calibration constant derived from the measurements of the protein solutions.

Each Raman spectrum thus results in a single local water concentration. Next, the calculated water concentrations are plotted versus depth. Plots of concentrations versus depths are referred to as depth concentration profiles. In Figure 10.3, typical water depth concentration profiles, recorded within a

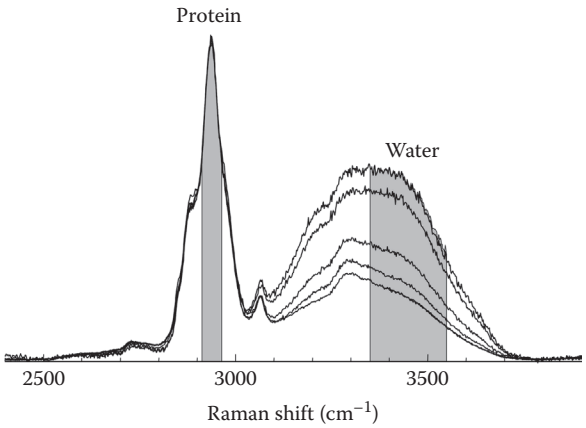

FIGURE 10.2 High-wavenumber part of the spectral baseline-corrected in vivo Raman spectrum of SC on the thenar. Indicated are integration boundaries for signals due to keratin and water.

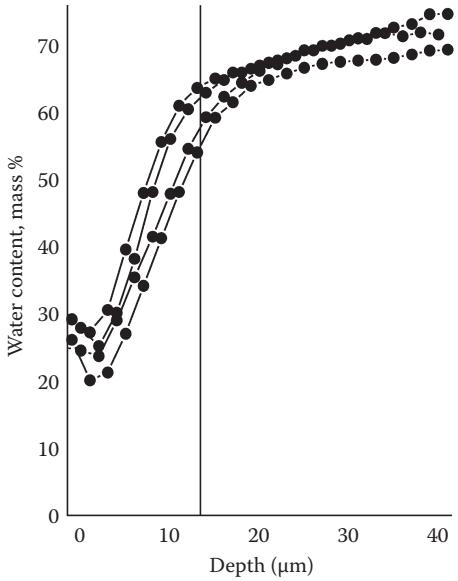

FIGURE 10.3 Measured water concentration versus depth, from confocal Raman measurements, at four locations within a small area (2 × 2 cm^2) on the volar aspect of the forearm. The line indicates the approximate SC–epidermis interface.

2 × 2 cm^2 area on the ventral forearm, are shown. The time required to record a single water depth concentration profile over about 30 μm of skin on the volar forearm (covering the SC and the upper part of the epidermis) can be 15 s or less.

As can be verified from Figure 10.3, the four depth profiles do not coincide. This is caused by the biological heterogeneity of the skin. This implies that for accurate water contents, repeat measurements and averaging must be carried out. In Figure 10.3, the approximate SC–epidermis junction is indicated. At this junction, the water concentration gradient rapidly changes slope. This feature is further discussed below.

In 2005, L'Oréal researchers presented in vivo results on human volar forearm skin, using an in-house-built confocal Raman set-up [28]. The work discusses depth concentration profiles from water, and other components, but for the present purpose, only the water results are highlighted. In their analysis of the Raman spectra, only a ratio between the intensities of signals due to water and a reference signal was used. The ratio chosen was taken from an older paper, on confocal Raman spectroscopy of the cornea [29]. No quantification of the water contents was carried out.

So far, the literature reviewed in this section aims at the measurement, by confocal Raman spectroscopy, of "just" water. However, water present in the skin may affect the tertiary and secondary protein structures through hydrogen bonding to the hydrophilic side chains of the protein. Such water is considered to be different from bulk water ("bound versus unbound water"). Zhang et al. [30] studied extracted pig skin by confocal Raman spectroscopy. The skin was equilibrated in various environments of relative humidities (range, 0%–100%). Among other results, it was found that the measured amounts of absorbed water, by confocal Raman spectroscopy, correlates well with dynamic vapor sorption (DVS) measurements (from the same study). Second, from a detailed analysis of the shape of the Raman spectra, it was concluded that collagen proteins in different relative humidities have similar higher-order structures. But most relevant for this book chapter is the observation of a signal in the Raman spectrum of skin (at 938 cm^{-1}) that appears to offer an experimental handle to the quantification of the amount of bound water.

Validation of the Quantitative *In Vivo* Water Concentration Measurement

To the best of our knowledge, no independent method to quantify in vivo the depth-dependent water concentration in skin exists. Therefore, validation against a "golden standard" is not readily possible. However, there are possibilities for comparison with in vitro methods. If we compare the water depth concentration profiles as proposed by Warner et al. in 1988 [10] to the results (Figure 10.3), the agreement is striking. The two methods are completely independent. Warner et al. obtained their quantitative estimate from an area analysis of scanning transmission electron micrograph (STEM) images of thin cryosections of skin, using the different densities of keratin and ice. The method of Caspers et al. [27], on the other hand, is based on the Raman spectra of prepared solutions of proteins. Both methods result in a concentration in the 20% to 30% range for the outer surface (the upper layer of the SC), increasing to about 70% at the interface with the epidermis (note: the 70% concentration in the method of Warner was an assumption and not a result of his method).

Wu and Polefka have elegantly presented direct validation results for extracted pig skin SC [31]. Samples were equilibrated at different relative humidities and subsequently cut in half. For one half set, the absolute water concentration in the SC was determined with Karl Fischer's titration method. The other half set was analyzed according to the Raman method of Caspers et al. [27]. This approach allowed for a direct correlation of water concentrations from independent analysis methods. The correlation proved remarkably good; an R^2 of 0.989 was found. It was further noted that the precision of the Raman method for water concentrations above 30% was better than for the Karl Fischer method. In their paper, Wu and Polefka also reported correlations of conductance measurements with the Raman measurements. The same paper also covered moisturization efficacy results on pig skin SC (see the "Applications" section).

Boncheva et al. [32] reported a systematic study in which in vivo conductance measurements were correlated to Raman measurements of hydration of the SC. Low- and high-frequency conductance measurements on successively tape-stripped forearm skin were combined with TEWL measurements on the same sites. Assuming that hydration of the SC as measured by confocal Raman spectroscopy provides a "golden standard," it was shown that high-frequency conductance can fairly reliably indicate SC hydration, provided that the barrier function is intact (TEWL < 12 g/m^2h). This holds even for successively tape-stripped skin. Thus, the

combination of tape stripping, TEWL, and high-frequency conductance provides a simple way of estimating hydration at different depths in the top layer of the SC. Note that as an interesting side result, a fair correlation was reported between the amount of protein removed (measured with a spectrophotometric analysis of the protein deposits on the tapes) and the remaining SC thickness (from confocal Raman measurements).

Water Concentration Gradients and Measurement of the SC Thickness

Knowledge of the thickness of the SC is essential in understanding the efficacies of products. Obviously, moisturization of the SC means adding water, and adding water implies adding volume. It is therefore expected that the SC will swell. The changing dimension of the SC under the action of any treatment has consequences for the calculation of efficacies; this applies to not only the degree of moisturization but also, for example, to the content of a constituent of interest before and after application.

Before confocal Raman spectroscopy became available, the shape of the water concentration gradient and its change upon treatment was already known from in vitro experiments or theoretical calculations.

STEM of biopsied and rapidly frozen human epidermis has already been applied for more than 25 years to study the water concentration gradient; see, for example, the work by Warner et al. from 1988 [10]. These results showed that a water concentration gradient must reside in the SC.

In 1984, experimental dynamic water flux measurements of in vitro SC as a function of its water content enabled the calculation of water concentration gradients that must exist in the in vitro SC samples [33]. These calculations were based on Fick's law of diffusion. All profiles were found to be steep and linear in the SC, and the model accurately described the swelling of the SC as a function of the water content and also as a function of the surface water content.

In 1997, Norlén et al. [9] applied light microscopy and confocal laser-scanning microscopy (CLSM) to study the swelling of extracted pieces of human SC. They found that after incubation of dried SC in distilled water for 90 min, the observed swellings were 26.3% ± 16% in the thickness dimension and only 4.1% ± 1.4% in the lateral dimension. Thus, swelling after addition of water to the SC mainly takes place in the thickness direction.

In the original paper by Caspers et al. [27], they pointed out that the shape of the water profiles could be linked to the SC thicknesses. At different body sites, the water concentration profiles changed slopes at different depths. Caspers et al. [34] confirmed the results in 2001 and pointed out that the slope change indicates the SC–epidermis interface. The general appearance of the in vivo water concentration profiles are in agreement with in vitro water concentration profiles as determined by STEM [10]. The SC thicknesses on the thenar and forearm are approximately 110 and 15 μm, respectively. Further and conclusive confirmation of the fact that the steep increase in water concentration occurs at the boundary between SC and living epidermis was presented in 2003 by Caspers et al. [3]. Boundaries, as determined by confocal video microscopy, corresponded precisely to the boundaries as derived from the Raman water concentration profiles.

Recently, a number of groups published estimations of the SC thickness, on the basis of confocal Raman measurements and their corresponding water depth concentration profiles. Sieg et al. [35] studied water depth concentration profiles for forearm skin. They propose to model the profiles with a sigmoid-like function (Weibull function). One of the fit parameters is the location of the steepest gradient; this is indicative of the SC thickness (it is not the thickness itself). Their work is further discussed in the "Applications" section.

In their study of the delivery of retinol to the viable epidermis, by confocal Raman microspectroscopy, Pudney et al. [36] calculated the approximate location of the SC–epidermis boundary from water concentration profiles and from concentration profiles of components of the natural moisturizing factor (NMF) recorded at the same location. By selecting a depth at which 30% of the maximum content of NMF is found and a second depth at which the water concentration is 55% by mass, two closely spaced locations of the interface are obtained. Subsequently, these two estimators are averaged. Although the criteria for depth selection may be arbitrary, their approach allowed for a systematic estimation of the location of the boundary for every individual measurement spot. This information was then used to verify whether the retinol was delivered to the viable cells or not. This latter question is discussed shortly in the section "Hydration of the SC in Drug Delivery Studies."

Egawa et al. [37] propose yet another method to arrive at the location of the SC–epidermis boundary [37]. Their estimation is taken from the depth at which the derivative of the water concentration profile is almost zero, and they coined this as the SC apparent thickness (SCAT; also see the "Application" section).

The criteria discussed above to arrive at the SC thickness are not fully objective and probably do not represent the real thickness. Also, the models lack a physical rationale. Van der Pol et al. [38] first proposed the more objective method for fitting of the water profiles, on the basis of diffusion of water through the SC–epidermis bilayer. The bilayer is thought of as two homogeneous media with two different (but constant) water diffusion coefficients. The water flux is considered constant. This implies that evaporation of water off the surface of the SC is balanced exactly by water transport, by diffusion, from the deeper layers. Under these conditions (Fick's law), the water concentration gradients must be linear in both media, and the experimental water depth concentration profiles can then be modeled simply with two linear functions (one in the SC and one in the epidermis). In other words, the water gradient changes its slope at a depth corresponding exactly to the SC thickness. The model function thus contains four variables; these are the SC thickness, the water concentration at this depth, and the two slopes of the water concentration gradients in both media (SC and epidermis).

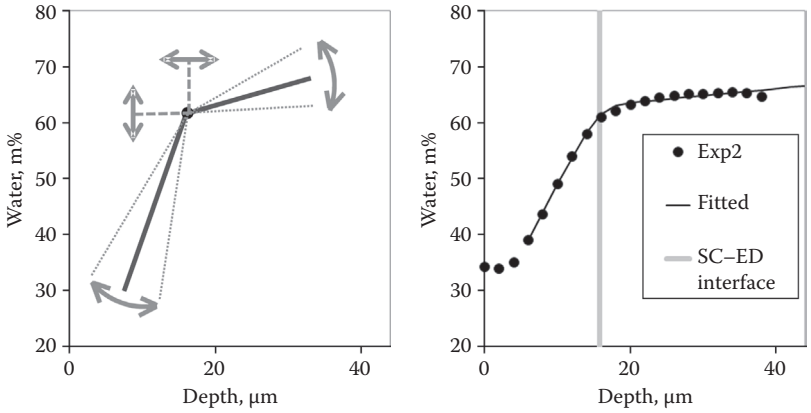

FIGURE 10.4 Model function for water concentration depth profiles. Left: Two linear gradients, connected at the interface, for a certain water concentration (indicated by the dot), of the SC and the epidermis. The double arrows indicate how the model function is varied. After variation, the function is convoluted by the Raman instrument's optical point spread function. Right: Next, the difference between the model function and the experimental profile is calculated. A nonlinear optimization of the four variables results in a satisfactory fit with the experimental profile.

In Figure 10.4, the model function and its variables are explained.

In practice, linear profiles with a sharp discontinuity (Figure 10.4, left image) at the interface cannot be measured. The reason is the limited spatial depth resolution of the Raman microscope of about 5 μm. At each focal depth, some signals from above and below the focal point will contribute to the signal. This "smoothing" effect can be described by a so-called optical point spread function. For the Raman microscope (Figure 10.1), this point spread function can be measured experimentally; it follows quite accurately a Gaussian function with a full width at half maximum of 5 μm or better. In good approximation, it is constant for every measurement situation. Consequently, the model water profile function must be convoluted by the optical point spread function to account for the spatial resolution of the confocal Raman technique. By a nonlinear fitting procedure, the set of four variables that best describes the experimental water profile can be found. An example fit result is also indicated in Figure 10.4 (right image). Hence, each water depth profile results in two water gradients, a water concentration at the SC–epidermis junction and an SC thickness. The described method has been automated and implemented in routine moisturization efficacy studies on human panelists by Bielfeldt et al. [39]. Bielfeldt et al. also take the interpretation of the fitted variables a step further. They propose a method to directly calculate water diffusion constants from combined TEWL measurements and Raman measurements and furthermore present interesting statistical correlations between the results of several techniques. Highly relevant for this review is a presented positive correlation of capacitance readings with the water gradient by confocal Raman spectroscopy, but no correlation of capacitance readings to the total amount of water in the SC, as determined by confocal Raman spectroscopy. Furthermore, a correlation was only found for water content in the lower epidermal layers. These observations were ascribed to a limited penetration depth of the capacitance measurements in addition to a reduction in field density with distance to the measurement probe. Finally, evidence was presented for the critical influence that the environmental humidity may have on skin hydration studies. This latter observation is studied more thoroughly by Zhang et al. [30], albeit for extracted pig and human skin and not in vivo. Their work is discussed in the section "Relationship between the Raman Spectrum of Skin and the Local Water Concentration."

After the first successful validations of SC thickness measurements by confocal Raman spectroscopy against other techniques, skin scientists are now beginning to apply water depth concentration profiles in more advanced studies, for instance, in the field of drug permeation (see section "Hydration of the SC in Drug Delivery Studies"). Förster et al. [40] found good correlations between the amount of SC removed by tape stripping and the remaining SC thickness (from the water depth concentration profiles) for in vitro measurements on extracted pig skin. In other words, a Raman measurement of the SC thickness enables a fair estimation of how much SC has been removed by the tapes. Förster et al. proposed confocal Raman spectroscopy as a tool to verify whether tape stripping was done reliably and actually use this tool in the same paper to assess the quality of SC removal by three methods: tape stripping, trypsinization, and cyanoacrylate stripping. In a second paper, Förster et al. [41] have applied confocal Raman spectroscopy for in vitro permeation studies of drug formulations, using the SC thickness as a control parameter; see also the section "Hydration of the SC in Drug Delivery Studies."

APPLICATIONS

Confocal Raman microspectroscopy is now a tool in the study of epidermal and dermal skin in various skin research groups. For a general review of the role of confocal Raman microspectroscopy in skin science, including the study of

penetration of topically applied materials, see reference [1]. In this section, the published Raman work related to the in vivo study of the moisturization of the skin is highlighted.

Moisturizing the Skin and Efficacy of Skin Moisturizers

A simple way of moisturizing the skin is to wet it with water. In the 2000 paper of Caspers et al. [27], this was demonstrated using a wet towel. The resulting water depth concentration profiles changed dramatically after application of the wet towel. In the SC, the water concentration increased to 50% to 60%, and swelling of the SC was noted.

Chrit et al. [42] studied in vivo the short-term efficacy of a moisturizing cream. Twenty-six volunteers (Caucasian, female, dry skin) received a treatment of the volar forearm site with an emollient without hydrating agent and a treatment with a 3% glycerol-containing cream. A control measurement was included. The normalized water signal was measured at different depths after 1 h of treatment. Signal intensities were then averaged over the depth range 0 to 20 μm. The glycerol-based cream induced a significant increase in average water content as compared with baseline, and at every depth between 0 and 20 μm, the water concentration was higher after the treatment. It is further noted that the shapes of the water depth concentration profiles did not exhibit a clear change of slope at the expected depth of about 15 μm, where the SC–epidermis interface is located. This is most likely caused by low optical resolution of the instrument used. In this study, a so-called dry microscope objective was applied; in other words, there was an air gap in between the objective and the skin. This caused a deterioration of the spatial resolution.

Sieg et al. [35] presented an in vivo 14-volunteer study of forearm skin for a cumulative treatment (3 weeks) with cosmetic moisturizers. The authors calculated the area under the water concentration profiles, for the entire thickness of the SC. During the treatment, the thickness of the SC changed, and this was taken into account. A formulation containing niacinamide was shown to increase the total water content of the SC much more (up to two or three times) than the other tested formulations. The same group produced a paper, by Crowther et al. [43], in which the results were discussed more thoroughly. In addition, a good correlation between SC thicknesses from optical coherence tomography and confocal Raman spectroscopy was reported.

Stamatas et al. [44] presented in vivo confocal Raman spectroscopy data of skin penetration and occlusive potential of two vegetable oils and a paraffin oil. Petrolatum was used as a positive control. The products were applied topically on the forearms of nine volunteers and seven infants, and Raman depth concentration profiles of both the oils and water were acquired before application and at 30 and 90 min after application. It was shown that paraffin and vegetable oils penetrate the top layers of the SC with similar concentration profiles, a result that was confirmed both for adult and infant skin. The three oils tested demonstrated modest SC swelling (10%–20%) compared to moderate swelling (40%–60%) for petrolatum. The swelling was assessed using the method of van der Pol et al. [38]. No statistical difference between the paraffin oil and vegetable oils in terms of skin penetration and skin occlusion was observed.

The already-mentioned work by Wu and Polefka [31] included moisturization experiments using products whose effect was already known. On isolated pig skin, the following products were tested: lotion, commercial soap bar, syndet bar, nonemollient shower gel, and emollient-containing shower gel. As expected, the water content on skin treated with lotion was significantly higher than the nontreated control. Syndet bar-treated skin had significantly higher water content than soap-based bar-treated sites. Sites washed with nonemollient shower gel were more moisturized than soap-based bar-treated samples. Finally, emollient shower gel-treated skin was significantly more hydrated than nonemollient shower gel-washed skin.

The formulations that are studied by confocal Raman spectroscopy for their efficacy in SC and epidermis hydration, in in vivo studies on human volunteers, become more and more complicated. Below, we report on the literature that appeared up to 2012. Note that, not only are the studies rapidly becoming more complicated, but the aims and approaches of the studies are also becoming more diverse.

Manosroi et al. [45] assessed the hydration potential of rice bran extracts entrapped in niosomes in gels or other formulations. Hydration end points derived from corneometry, vapometry, and confocal Raman spectroscopy, on in vivo human skin, all indicated a similar ranking of efficacies (differences to baseline) of the different formulations. However, efficacies by the Raman method were more pronounced and had more contrast between the formulations, lower standard deviations, and a very small difference to baseline for untreated skin.

In a study on 20 healthy volunteers, Mohammed et al. [46] applied niacinamide-containing formulations. They observed larger and more mature corneocytes, decreased inflammatory activity, decreased TEWL, and increased SC thickness (by in vivo Raman) in the treated areas as compared to the control sites.

Tosato et al. [47] applied oil-in-water emulsions on human skin in vivo. The formulations contained plant extracts and antioxidant vitamins, a chemical sunscreen ultraviolet A/B (UVA/UVB) absorber, natural moisturizers from vegetable sources, amino acids, and peptides that act to increase collagen and membrane proteins. After application for up to 60 days, the skin of aged women (aged 60 to 75 years) showed significant changes in lipids, collagen, and water levels. The data from their custom-built confocal Raman setup for in vivo measurements substantiate that the application of cosmetics with active moisturizing and antiaging properties helps to maintain the skin's protective barrier and hydration and slows the intrinsic and extrinsic aging processes.

The reviewed papers on skin hydration, assessed by confocal Raman spectroscopy, so far focused on the magnitude of the hydration. Egawa and Kajikawa [48] also focused on

other aspects of hydration. Using water depth concentration profiles, they showed that it is possible to influence the water distribution and the holding time of water in the SC. Furthermore, they included panelist questionnaires for experienced subjective cutaneous sensations, for different water distributions. The hydration procedure could be adjusted by varying the application time of topically applied water or moisturizing cream and the temperature of the applied water on skin. When only the upper part of the SC was hydrated, volunteers reported "water-rich feelings," whereas when only the middle or lower part of the SC was hydrated, volunteers report "tension." Longer application times were found to result in increased water-penetration depth and amounts. Similar effects were obtained for warmer applied water or warmed skin. In addition, an increased application temperature resulted in a longer holding time of the water in the SC. These results suggest that we can control the depth profile of water content in the SC, with simple adjustments of the moisturization procedure, even in beauty salon practices. In fact, these results are in agreement with results obtained by van der Pol et al. [57] for hot bathing; see section "Hydration Effects in Dysfunctional Skin and Barrier Repair Assessment."

In vivo confocal Raman measurements studying efficacy of much more complicated moisturizing formulations are foreseen in the near future. Although no hydration effects are assessed in their work, Bonnist et al. [49] already use confocal Raman spectroscopy to measure vehicle effects and vehicle partitioning effects in the delivery rates of actives in the SC, for excised pig skin. Whereas the aim was to study skin sensitization (see also the section "Toxicology, Sensitization, and Occupational Hazards") by *trans*-cinnamaldehyde, we believe that similar study designs, including in vivo, will also allow for the quantification of skin hydrating actives.

Water Distribution in the Skin for Different Skin Types

Understanding the hydration processes of the skin requires knowledge of the state of the skin prior to treatment. It is likely that different types of skin will respond differently to equal treatments. Therefore, it is of interest to study differences in water distribution in the skin of human volunteers of different skin types. Such knowledge will no doubt contribute to the development of products targeted to these different skin types.

In 2006, Matsumoto et al. [50] presented the results of a systematic study of the water distribution in the skins of an "old" male Japanese group of volunteers (N = 20, average age 64.0 ± 2.5 years) and a "young" male Japanese group (N = 20, average age 27.8 ± 1.6 years). Water concentration profiles were recorded on untreated areas on the volar aspect of the forearm, down to a depth of 200 μm, which is well in the dermis. Surprisingly, no differences in water concentration profile could be detected in the SC and the epidermis. However, the water content in the upper dermis was found to be significantly lower for the young group. Possibly, the mechanically more worn dermis of the old group contains more damages such as voids. These voids may be filled with water. It was concluded that the water content in the dermis may be a useful parameter for evaluations of aging. The study was later expanded to a total of 2 × 30 volunteers and published as a paper [51].

In the already referenced paper by Egawa et al. [37], the SCATs were measured at different body sites and for different panelist ages (6 male, 9 female). On the forearm, the SCAT tended to be higher for older skin, but at the cheek, no age dependence was found. The volar forearm skin was hydrated with a wet cotton patch, and measurements were done after 15, 50, and 90 min of hydration. A swelling of the SC was observed of 4%, 40%, and 95%, respectively. This finding was in agreement with previously reported swelling of a corneocyte, using cryo-scanning electron microscopy (cryo-SEM) [52]. In a later paper, Egawa and Tagami [53] also addressed the effects of season on the distribution of water in the skin.

Infant skin is a subject in itself. A large panelist study, comparing the barrier function, water-holding, and water-transport properties of a group of infants (N = 124, age 3–12 months) and a group of adults (N = 104, age 14–73 years), was published by Nikolovksi et al. [54]. Capacitance, TEWL, and Raman measurement were employed in this study. The SC was found to be thinner for infants. The capacitance and TEWL values were higher for the infants, and the variations over the infant panelists were larger. Interestingly, as observed in the Raman water depth concentration profile, large differences were also observed in the amount of water that was absorbed after application with a wet-soaked paper towel for only 10 s. Whereas adult skin did not seem to absorb much water, for infant skin, a rapid increase of 5% to 10% by mass of water in the outer 10 μm of the skin was observed. The desorption rate for infants was high initially, followed by a slower rate. Adults only exhibited the slower desorption rate. It was concluded that the way the SC stores and transports water becomes adultlike only after the first year of life.

Fluhr et al. [55] emphasize the role of skin acidification in the development of neonatal skin. They applied confocal Raman spectroscopy for composition analysis (including hydration) and measured TEWL, capacitance, and skin surface pH. Their panelists were grouped according to age, from newborns (days), weeks, months, to years old. In the first 2 weeks after birth, the SC is less hydrated. Evidence was also found for a relation between the formation of an acidic pH and full hydration of the SC, in the first weeks of life. Infants up to 33 months showed a thinner SC (and consequently a steeper water gradient).

In a paper by Chrit et al. [56], an in vitro study on skin models was combined with an in vivo study on human volunteer skin, using the Raman technology. The hydration capacities of 2-methacryloyloxethylphosphorylcholine polymer (pMPC), native or microencapsulated and with or without hyaluronic acid, were investigated. The in vitro experiments on the skin models showed the best hydrating properties for the formulation encapsulated with hyaluronic acid, which also exhibited the longest-lasting efficacy.

In a 26-volunteer in vivo study, using confocal Raman spectroscopy, the formulation encapsulated with hyaluronic acid was tested, and a statistically significant hydration effect was observed.

Hydration Effects in Dysfunctional Skin and Barrier Repair Assessment

The fact that too much exposure of the skin to water may have unbeneficial effects is long known, and it is said to "dry out" the skin. This is already an example of dysfunctional skin. Van der Pol et al. [57] demonstrated in 2005 the effects of hot bathing on the composition and distribution of components (among which is water) in the skin. In this work, an interesting experiment was carried out. Following soaking the forearm in hot water for 30 min, Raman water depth concentration profiles were recorded at the same site (the volunteer did not move his/her arm) every 30 s, after the soaking. First of all, a relatively large swelling of the SC was observed, but more interestingly, within the first 30 min after soaking, the water redistributed over the SC. The water concentration decreased 5% to 10% by mass around a depth of 25 μm and increased a similar amount at a depth of about 10 μm. This phenomenon reflects *dynamically* the reduction of the barrier function (the barrier function is thought to reside at the stratum granulosum) as a result of the intense treatment with hot water.

Another way to arrive at dysfunctional skin is removal of the top part of the SC by sequential tape stripping, thereby disrupting the barrier function. In 2005, Hellemans et al. [58] presented in vivo results on four volunteers using this approach for volar forearm and facial skin. The results illustrated clearly that for untreated skin, facial SC is thinner than SC of the forearm. Moreover, after tape stripping, the remaining thinner SC could be observed easily from the profiles. Remarkably, the remaining thickness of the SC for the face and arm after tape stripping until TEWL = 18 g/m^{2}h is nearly identical. Right after disrupting the barrier, the water concentration over the SC was observed to be higher (as was expected). However, the recovery response after tape stripping between the external water fluxes, determined with TEWL, and the internal water content of the SC differed. A fast initial (4 h) recovery of the TEWL was observed, whereas the internal water content stayed high, even 24 h after stripping. This effect may be explained by the release of the lipid content of the lamellar bodies immediately after barrier disruption. Such a "film of lipids" might keep the internal water content of the SC elevated, which in turn may facilitate the enzymatic processing required for the barrier recovery response.

Cash et al. [59] have investigated the use of confocal Raman measurements to demonstrate efficacy of skin barrier repair using a barrier repair foam product (based on hyaluronic acid and ceramides). Lipid levels and hydration levels improved, as could be concluded from depth concentration profiles.

It is expected that confocal Raman microspectroscopy will find many useful applications in the study of diseased skin and its treatment (e.g., atopic dermatitis [AD] and psoriasis; see also the section "Studies on the Role of Filaggrin Deficiency in AD"). Also, see the review paper by Darlenski et al. [4].

Egawa et al. [60] have investigated SC thickness and hydration in psoriasis using in vivo confocal Raman spectroscopy. They concluded from their measurements that psoriatic skin typically has much thicker SC and a lower water content. From the Raman measurements, they also concluded that lactate, urea, and cholesterol levels were comparable between healthy and psoriatic skin, indicating similar sweat gland activity and decreased NMF and ceramide levels in psoriatic skin.

Studies on the Role of Filaggrin Deficiency in AD

AD is an inflammatory skin disease and one of the most common diseases of childhood. AD is characterized by red, flaky, and severely itching skin lesions, and immunoglobulin E (IgE)-mediated sensitization to food and environmental allergens. The role of filaggrin deficiency in AD is a field of rapidly growing interest. This prompted us to write a paragraph of its own for this topic.

Loss-of-function mutations in the gene encoding for filaggrin have been identified as causative for ichtyosis vulgaris and as the strongest predisposing factor for AD [61]. The role of filaggrin for the barrier function of the skin is closely associated with two important mechanisms of the SC to hold onto water: (1) as a structural protein, filaggrin helps to create the structural integrity of the corneocytes, and (2) filaggrin provides the source for the amino acid fraction of NMF, which is a highly hygroscopic mixture that plays a central role in maintaining hydration of the SC. Filaggrin mutations, found in about 10% of the Caucasian and Asian populations, may therefore have a profound impact on the barrier function of the SC.

Since filaggrin is the source of the amino acid constituents of NMF, filaggrin deficiency as a result of loss-of-function mutations in the FLG gene leads to reduced NMF and thereby compromises the hygroscopic capacity of the SC. It has been known for a long time that lack of SC hydration in itself results in loss of plasticity of the corneocytes and, as a result, loss of integrity of the SC membrane [62].

In the context of AD, the genetic skin barrier impairment is thought to promote the ingress of allergens through the skin, resulting in allergic sensitization as a first step in the development of AD.

The NMF content in normal SC is relatively high and represents 20% or more of the dry weight of the SC. This enables the use of Raman spectroscopy as a noninvasive method to rapidly and accurately measure the NMF content of the SC in vivo.

With the use of in vivo Raman spectroscopy, NMF levels have been shown to correlate very well with FLG-null allele status: loss of function mutations in the filaggrin gene lead to strongly reduced levels of NMF in the SC [62–64]. This effect has also been demonstrated in vitro by high-performance liquid chromatography (HPLC) analysis of pyrrolidone carboxylic acid (PCA) and urocanic acid (UCA) in tape-stripped SC samples [65].

As such, Raman spectroscopy provides a rapid noninvasive method to identify infants at risk for developing AD and people with skin barrier dysfunction as a result of filaggrin mutations. Since it is likely that adults with decreased NMF have modulated SC barrier properties as well, noninvasive assessment of NMF levels may also be useful as a preselection tool for cosmetic panel testing.

Toxicology, Sensitization, and Occupational Hazards

Broding et al. [66] were the first to propose and test in vivo confocal Raman spectroscopy as a risk assessment tool for occupational hazards, sensitization, and toxicology in occupational dermatology. Confocal Raman spectroscopy measurements after application in vivo on human panelists (volar forearm) of 2-butoxyethanol, toluene, and pyrene enabled calculation of mass transports (μg/cm^2) and fluxes (μg/[cm^{2}h]) in and through the SC. Results were in reasonable agreement with in vitro literature data. Relevant for the present review is 2-butoxyethanol, a common solvent component in water-based paints and lubricants. Development of water-based paints is driven for various reasons; one of them is to avoid the use of hazardous and toxic organic solvents. These solvents might be unwantedly inhaled, ingested, or transdermally absorbed (the latter uptake route is usually highly underestimated). However, older in vitro literature data showed that 2-butoxyethanol is delivered in quantities up to 10 times higher when applied to the skin as a solution in water, compared to the pure form. Broding et al. were able to confirm this penetration enhancement of 2-butoxyethanol by water in vivo. To this end, depth concentration profiles of both 2-butoxyethanol and water were measured. Thus, surprisingly, the very solvent (water) that is chosen as a safer alternative to organic solvent enhances the penetration of a minor and equally hazardous component, 2-butoxyethanol, by a tenfold factor.

Bonnist et al. [49] also conclude confocal Raman spectroscopy to have potentials as a risk assessment tool in skin sensitization studies, either in vitro or in vivo. Although no hydration data are presented in their study, the principle of their study would also allow for hydration efficacy measurements as influenced by solvents, vehicle compositions, and even vehicle partitioning effects. Evidence from confocal Raman measurements for vehicle partitioning effects in excised pig skin was presented in this work.

Hydration of the SC in Drug Delivery Studies

Pudney et al. [36] demonstrated that confocal Raman spectroscopy can be used to trace the permeation of actives (in their case, retinol is studied) beyond the SC, into the viable cells. Obviously, this is very relevant for transdermal drug delivery applications. Confocal Raman measurements provide both the depth down to the SC–epidermis interface as well as depth concentration profiles of vehicle and active components. The same group also studied in vivo penetration enhancement effects in delivery vehicles for retinol, using confocal Raman spectroscopy [67].

Förster et al. [41] follow a similar approach as Pudney et al., but their skin samples are extracted from pig skin; hence, the measurements are in vitro. However, they study more vehicle variations and even address lipid organization and fluidization, which is also reflected in the Raman spectra. Relevant to the topic of this review, hydration, is that the depth concentration profile of water again provides the tool to measure the location of the SC–epidermis boundary.

DISCUSSION, CONCLUSION, AND OUTLOOK

In vivo confocal Raman microspectroscopy is a relatively novel method that provides detailed information about the molecular composition of the skin. In this chapter, its application on the study of hydration was reviewed. More and more studies are appearing in which the water contents measured by confocal Raman spectroscopy are compared and validated against the classic methods for hydration quantification. Up to now, the correlation is good. In a number of reviewed papers here, confocal Raman spectroscopy is not only used for measurements of the hydration state of the skin. The other area of application, and this is probably the largest area, lies in the measurement of the chemical composition of skin, including topically applied substances. Together with the fact that more and more research groups are using the technology, this illustrates the growing acceptance of Raman spectroscopy as an extremely useful method in the skin sciences.

Many applications so far have focused on the SC. However, the method is readily capable of measurements to a depth of greater than 150 μm into the skin—well into the dermis.

In the past 15 years, in vivo confocal Raman spectroscopy has made a major leap forward in sensitivity, speed of measurement, and ease of use. Raman technology has now reached a level of refinement where it can be applied in routine clinical studies, and it is. It has become fast enough to perform measurements on numbers of subjects ranging from several up to several dozen per day, depending on the complexity of the study. The user interface has reached a stage of development where routine operation of the equipment by a laboratory technician is practical.

Although the Raman technique has now been shown to be routinely useful in clinical settings, it is, like all measurement techniques, subject to certain limitations. It involves many measurements being made at a single location to generate composition depth profiles, whereas other techniques, such as electrical conductivity, for example, normally take only a single data point at a given location. This means that even with fast instrumentation, Raman measurements may be time consuming compared to other commonly used methods of in vivo skin analysis. That is, however, simply the price paid to obtain much greater information content. A related general issue is that in vivo tissue analysis normally requires considerable replication, by measurement of multiple locations and on multiple subjects, to achieve needed statistical accuracy, given normal biological variability. This is, of course, a characteristic inherent in any human in vivo

measurements and not specific to Raman. Finally, Raman instrumentation for in vivo skin analysis is highly specialized and therefore expensive. However, as in vivo Raman microspectroscopy comes into more general use, the cost of the instruments eventually can be expected to drop as volume efficiencies are realized by manufacturers. These limitations are well compensated by the richness of information achievable and the unique ability to measure the same area of skin repeatedly and with microscopic spatial detail, allowing entirely new kinds of information to be gathered. It can be expected that this detailed and spatially resolved information, and the ability to make these measurements in vivo, will provide insights into the mode of action of skin hydration that have not been previously available.

The conclusion and outlook on the role of in vivo confocal Raman microspectroscopy of skin, in the study of the skin hydration process, is well captured in a citation from the work of Wu and Polefka [31]: "The unique and direct quantitative water content information provided by confocal Raman microspectroscopy offers a whole new perspective for fundamental skin moisturization studies and will play an important role in evaluating moisturizing profiles and the hydration potential of products designed."

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11 Evaluation of the Barrier Function of Skin Using Transepidermal Water Loss (TEWL)
A Critical Overview

Bob Imhof and Gill McFeat

INTRODUCTION

Importance of the Skin Barrier

The epidermal barrier function of the skin resides in the stratum corneum (SC) and is essential to life. It protects us from dehydration, poisoning, and microbial attack. However, the SC is not an impenetrable barrier. Free water from the viable tissues continually diffuses through the SC, evaporating from the SC surface. This is known as transepidermal water loss (TEWL).

TEWL and Skin Barrier Function

The importance of TEWL as a measure of the skin barrier has been recognized for over half a century. TEWL measurement is well established, noninvasive, and widely used to characterize macroscopic changes in skin barrier properties.

It is important to note that TEWL instruments do not measure TEWL directly: TEWL is the diffusion of condensed water through the SC, whereas TEWL instruments measure the water vapor flux in the air immediately above the SC. Of course, TEWL will always be a component of this evaporation flux, but there may be interfering components arising from sweating and/or the evaporation of free surface water (skin surface water loss [SSWL]), neither of which is related to the skin barrier property. At the other end of the scale, the TEWL of a highly compromised skin site may be so high that water accumulates on the skin surface, in which case the measurement will underestimate the true value of TEWL. Often, these interfering components are time dependent, and it is important to ensure that measurements are allowed to settle to steady levels before TEWL is inferred. Care must therefore be taken to control the conditions of measurement to ensure that the measured evaporation flux is TEWL and nothing but TEWL.

This chapter provides a critical overview of TEWL measurement and important factors to consider when using TEWL to evaluate the barrier function of the skin.

MEASUREMENT OF TEWL

The measurement of TEWL has become a standard research method used in dermatology, pharmaceutical, cosmetic, and safety research. This is largely because the measurement procedure itself is straightforward and does not require specialist training yet can provide a rapid and objective assessment of skin barrier function. Consequently, TEWL methods have been used in numerous studies worldwide and have application in formulation, efficacy testing, and claim support for cosmetics and pharmaceuticals [1–3]; safety testing and product research and development (R&D) [4,5]; and clinical diagnosis of disease and response to treatment [6–8]. TEWL has also been used for ex vivo and in vitro testing of materials and products [9,10].

The main commercial instruments that have been developed for the determination of TEWL are listed in Table 11.1. They use three different measurement methods, referred to as open-chamber, unventilated-chamber (closed-chamber), and condenser-chamber (closed-chamber) methods.

Open-Chamber Method

The modern era of TEWL measurement began in the 1970s with the invention of the open-chamber method by Nilsson [11] and its subsequent development into the commercial evaporimeter. Other open-chamber instruments widely used for the measurement of TEWL are listed in Table 11.1.

The measurement chamber comprises a hollow cylinder open at both ends, one end of which is placed into contact with the skin (Figure 11.1). The other end acts as an exhaust allowing the water vapor from the skin to escape into the ambient atmosphere. Under ideal conditions, the air inside the chamber is perfectly still, and water evaporating from the skin surface diffuses through the chamber and into the ambient atmosphere. When water evaporates from the skin surface, the humidity of the air next to the skin increases above ambient humidity, while the humidity at the exhaust opening remains close to that of ambient air. Sensors for relative humidity and temperature are positioned on axis at two locations close to the skin surface, to measure the humidity gradient. According to Nilsson's [11] diffusion gradient measurement principle, this humidity gradient can be used to calculate the flux density (i.e., the amount of water diffusing through the measurement chamber per square meter per hour). An assumption of this method is that the air inside the

TABLE 11.1
Alphabetical List of the Main Commercial TEWL Instruments

Instrument	Method	Manufacturer
AquaFlux	Condenser chamber	Biox Systems Ltd, England
DermaLab	Open chamber	Cortex Technology ApS, Denmark
Evaporimeter[a]	Open chamber	Servo Med AB, Sweden
H4300[a]	Unventilated chamber	Nikkiso-YSI Co Ltd, Japan
RG1	Open chamber	cyberDERM, Inc., USA
Tewameter	Open chamber	Courage & Khazaka GmbH, Germany
VapoMeter	Unventilated chamber	Delfin Technologies Ltd, Finland

[a] No longer manufactured.

measurement chamber is perfectly still so that water vapor transport is by diffusion alone. In practice, these diffusion conditions are easily disturbed by ambient air movements, which cause instrumental readings to fluctuate.

The open-chamber method suffers from two major limitations. First, because the measurement chamber is open, it is vulnerable to disturbance from ambient air movements, which restricts its use to well controlled laboratories, often with shielding boxes. Second, natural convection air movements from skin restrict in vivo measurements to horizontal surfaces [12,13]. However, continuous measurement of evaporation flux is possible, and this is important for ensuring that measurements have settled to steady levels before TEWL is inferred. TEWL measurement times are commonly around 30 s. A postmeasurement instrument recovery period of 2–4 min is recommended in Pinnagoda et al. [12], to allow water vapor in the measurement chamber to evaporate before the next measurement is initiated. This seems excessive, and the more recent advice is to take an equilibrium time into consideration before the next measurement is started [13]. A 1 min postmeasurement delay is typical with a modern Tewameter, for example.

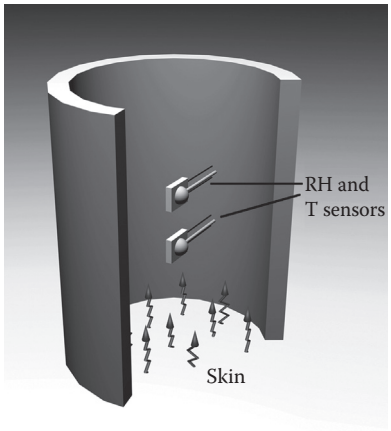

FIGURE 11.1 Schematic illustration of an open-chamber measurement head.

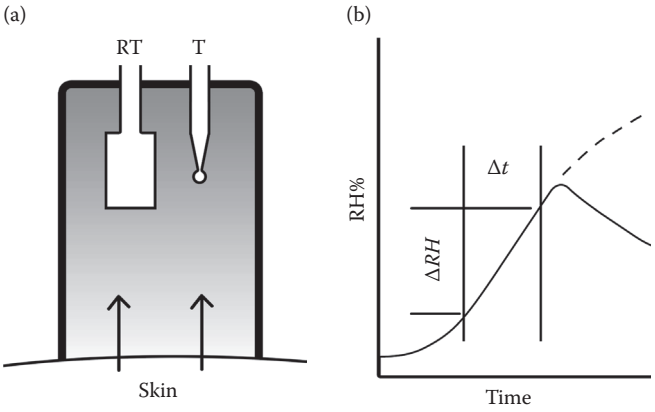

FIGURE 11.2 Schematic illustrations of (a) the measurement chamber and (b) a typical signal of the unventilated-chamber method.

Unventilated-Chamber (Closed-Chamber) Method

The invention of the unventilated closed-chamber method predates that of the open chamber [14]. However, commercial unventilated-chamber instruments for TEWL measurement have only recently been developed, specifically the Nikkiso-YSI Model 4300 [15], which is no longer manufactured, and the portable, battery-powered Delfin VapoMeter [16]. The VapoMeter uses a measurement chamber in the form of a cylinder, equipped with sensors for relative humidity and temperature (Figure 11.2a). The open end of the measurement chamber is placed into contact with the skin. The other end is closed, so that any water vapor emanating from the skin surface is trapped without any means of escape. The mean water vapor flux during the time of contact with the skin is calculated from the rate of increase with time of the humidity and temperature readings [16], and not from a steady-state spatial humidity gradient as in the open-chamber method (Figure 11.2b).

After each measurement is complete, the trapped water vapor in the measurement chamber needs to be allowed to escape; otherwise, the humidity would rise toward saturation level. This need to purge the measurement chamber after every skin contact precludes the use of such instruments for continuous flux measurement, one of the limiting features of unventilated closed-chamber methods. Overall, the time taken to complete a measurement with an unventilated closed-chamber VapoMeter comprises 3 s of premeasurement countdown time followed by 7–16 s of skin contact time followed by 20–90 s of postcontact recovery time.

Condenser-Chamber (Closed-Chamber) Method

The condenser-chamber method, as used in the Biox AquaFlux instrument, also uses a closed chamber to overcome the constraints of the open-chamber method [17]. The measurement chamber, illustrated in Figure 11.3a, is closed at one end by a condenser that is maintained below the freezing point of water by a Peltier cooler. The other end is placed

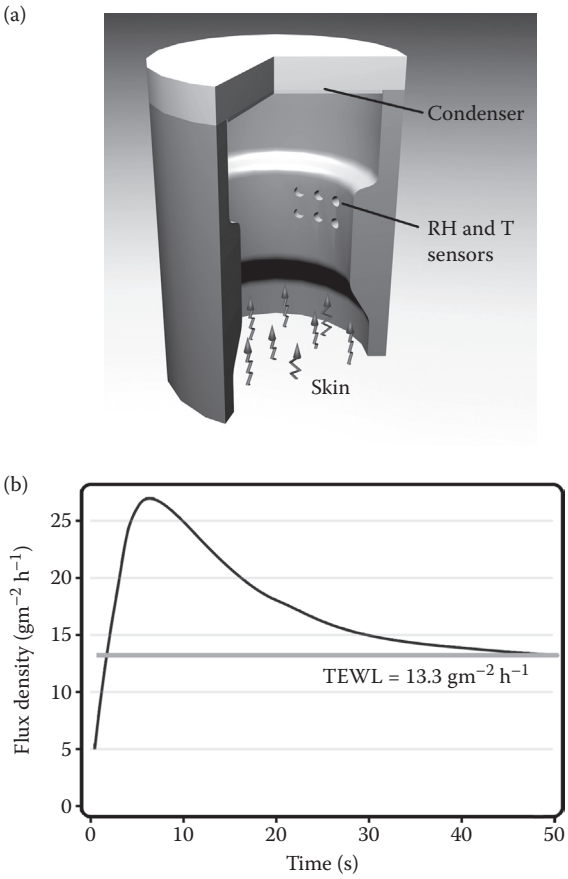

FIGURE 11.3 Schematic illustrations of (a) the condenser-chamber measurement head and (b) flux time-series curve recorded during a typical volar forearm TEWL measurement.

into contact with the skin surface of interest. The condenser continually removes water vapor originating from the skin, storing it as ice. This maintains a low humidity at the condenser, whereas the humidity at the skin surface increases with increasing water evaporation rate. The resulting humidity gradient is calculated from two humidity values measured at two spatially separated points. Relative humidity and temperature sensors mounted in the chamber wall provide one value. A second value comes from the condenser, where the humidity can be calculated from its temperature without the need for a second humidity sensor. This humidity gradient is used to calculate flux density using the same diffusion gradient measurement principle as the open chamber.

The closed-chamber design eliminates disturbance of measurements by external air movements. The removal of water vapor by the condenser enables continuous flux measurements to be made and displayed in real time (Figure 11.3b). This is important for validating measurements by verifying that the evaporation flux has settled to a steady level before TEWL is inferred. For example, sweat gland activity or imperfect sealing of the measurement head against the skin can be identified from anomalies in the displayed flux curves.

The condenser also controls the microclimate humidity within the chamber independently of ambient humidity, thereby removing the need for elaborately controlled environmental conditions. The impact of microclimate on TEWL measurement has been reviewed in detail [18].

METHOD VALIDATION

When the "true" value of the quantity being measured is unknown, the validity of a new measurement method is usually assessed using like-for-like comparisons with an established method. For TEWL measurements, the validity of a new method is typically assessed by comparing equivalent readings with those from an open-chamber instrument. This is because the open-chamber method is seen as a de facto standard, because of both its long history of use and its perceived minimally invasive nature.

Several assessments comparing open-chamber measurements with those from closed-chamber TEWL instruments have recently been published [19–24], with generally good correlation between open-chamber and closed-chamber methods. However, correlation is a poor test of validity because there may be hidden factors that affect either one or both methods being correlated. One such hidden factor is the measurement range of each instrument, that is, the maximum flux density it can measure. For TEWL instruments using the diffusion gradient measurement principle (open chamber and condenser chamber), the maximum flux density is limited by the relative humidity of the air immediately adjacent to the skin surface [18]. A diffusion gradient is established because the skin at one end of such a measurement chamber is a source of water vapor, whereas the atmosphere or the condenser at the opposite end is a sink. A finite water vapor flux causes the humidity immediately adjacent to the skin surface to increase, whereas the humidity at the opposite end is little affected. The measurement range limit is reached as the air immediately adjacent to the skin surface approaches saturation at 100% relative humidity. For unventilated-chamber instruments, the nonsteady-state nature of this method makes it difficult to predict its measurement range. However, it is generally acknowledged that such instruments respond to higher flux densities than diffusion gradient instruments. To add to the complexity, irrespective of measurement method, measurement range is not constant but, rather, depends on temperature (relative humidity decreases as temperature increases) and the diameter of the orifice in contact with the skin (smaller orifice diameters can be used to increase measurement range by attenuating the flux entering the measurement chamber).

The comparisons presented in Figure 11.4, use the nonparametric Bland–Altman mean difference method [25], where the means of equivalent pairs of readings with the two instruments are plotted on the *x*-axis and the differences between them on the *y*-axis. This is a more sensitive method of comparison than the standard correlation plot because it shows deviations between equivalent readings (the differences) against the assumed best estimate of the "true value" of the quantity being measured (the means).

The mean flux density shown in Figure 11.4 is restricted to the range of 0–80 gm^{-2} h^{-1} to remain well within the

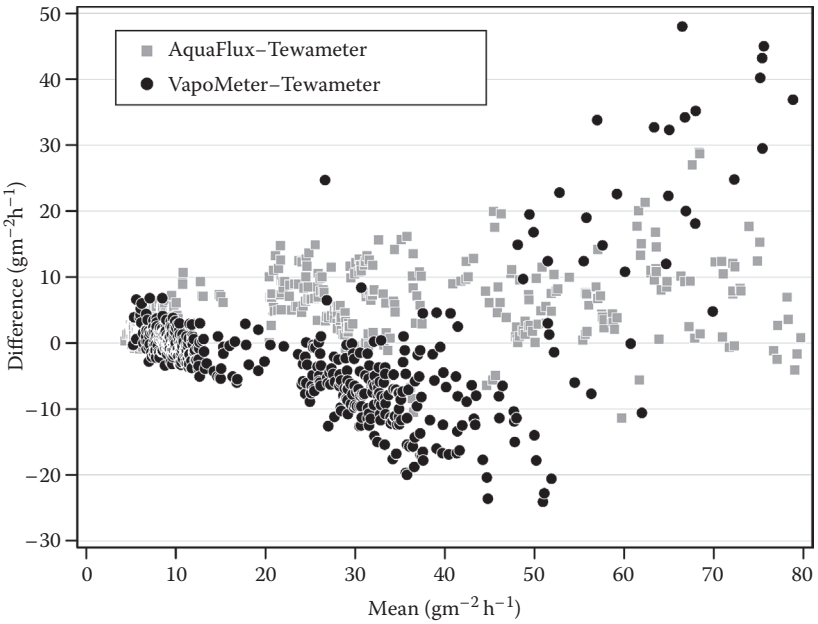

FIGURE 11.4 Bland–Altman mean difference comparisons of in vivo AquaFlux and VapoMeter TEWL measurements. (From Angelova-Fischer, I. et al., *Dermatologie in Beruf und Umwelt*, 57, 2009; and Steiner, M. et al., *Skin Res. Technol*., 17, 2011.)

measurement range of all three instruments used in the tests. Systematic bias is evident between the open-chamber Tewameter TM300 readings and those of the two closed-chamber instruments. For the condenser-chamber AquaFlux, there is a flux-independent offset of ~4.0 $gm^{-2}h^{-1}$, which indicates a calibration difference. For the unventilated-chamber VapoMeter, there are flux-dependent trends, with increasingly negative differences in the range of 0–40 $gm^{-2}h^{-1}$, followed by a reversed trend above ~50 $gm^{-2}h^{-1}$.

Significant differences in the repeatability of in vivo TEWL measurements are also evident in the data presented in Figure 11.4. Of course, the scatter in these readings cannot be attributed solely to the instruments under test since they include (1) the scatter in the reference Tewameter readings and (2) skin variability, where probe repositioning errors and the different diameters of skin included in the measurement (standard measurement orifice diameters are 10 mm for the Tewameter, 7 mm for the AquaFlux, and 11 mm for the VapoMeter) contribute to the scatter. Nevertheless, the scatter of AquaFlux–Tewameter differences (standard deviation, ~4.6 $gm^{-2}h^{-1}$) is approximately half that of the equivalent VapoMeter-Tewameter differences (standard deviation, ~9.2 $gm^{-2}h^{-1}$).

IN VIVO TEWL MEASUREMENTS FOR THE EVALUATION OF SKIN BARRIER FUNCTION

The development of closed-chamber instruments such as the AquaFlux and the VapoMeter are transforming skin barrier measurement. First, they overcome the main limitation of open-chamber systems, namely, their vulnerability to disturbance from external air movements. This makes it possible to perform measurements away from the well-controlled laboratory environment, in the workplace or clinic. Second, they are more versatile because they can readily be adapted to different measurement tasks by fitting purpose-designed measurement caps and adaptors. For example, both the AquaFlux and the VapoMeter have O-ring caps that allow them to measure water loss from curved hard surfaces, such as fingernails. Other designs are available for scalp measurement along a hair parting without shaving, for areas with high TEWL such as the axilla or lips and for otherwise difficult-to-reach sites such as the nasolabial fold and so forth. There is also a large orifice cap that can be used for sampling over a greater skin area, which is useful for studying heterogeneous skin such as the male face. Furthermore, quick-fit measurement caps that can be easily removed and cleaned are important in studies where infection or cross-contamination can occur.

IN VITRO TEWL MEASUREMENTS FOR THE EVALUATION OF SKIN BARRIER FUNCTION

Membrane Integrity Testing

Membrane permeability is a critical parameter in in vitro percutaneous absorption studies. Indeed, OECD Guidelines stipulate that barrier integrity should be tested before permeation experiments are carried out [26,27]. TEWL measurements are a recognized alternative approach to tritiated water flux for such measurements. TEWL methods have the unique advantage of testing membranes under conditions that resemble normal in vivo skin, where the donor side is dry and the acceptor side is wet. Recent studies have shown that TEWL measurements are a good alternative to tritiated water flux measurements for assessing full-thickness skin barrier

function and the AquaFlux was found to be particularly sensitive to changes in barrier function [23,28].

Quick-fit measurement adaptors, which are a feature of closed-chamber technology, can also improve the reliability of membrane integrity tests with purpose-designed adaptors [29] replacing improvised couplings commonly used with open-chamber instruments [30,31]. An example of such a coupling is shown in Figure 11.5.

Such couplings have the advantages of (1) performing the test in situ, in the same apparatus as will subsequently be used for the permeation experiment, (2) exposing the whole area of the membrane to the test, (3) sealing reliably to the Franz cell donor chamber, and (4) not requiring contact with the membrane itself. The consistency of this measurement geometry makes it possible to calibrate the system of probe, coupling, and donor cell, so that in vitro Franz cell TEWL readings can be interpreted on the same scale as in vivo skin TEWL readings [32].

There is a need, prior to membrane integrity testing by the TEWL method, to acclimatize the membranes to ambient conditions of temperature and humidity. Of course, there can be no sweat gland activity in vitro, but membranes are often stored in conditions that are far removed from those that would mimic normal (dry on top, wet underneath, steady-state TEWL) in vivo skin conditions. Furthermore, even miniscule quantities of moisture anywhere within the donor chamber will produce a substantial and sustained evaporation flux that is unrelated to membrane barrier function. With a condenser-chamber instrument, donor-side moisture and poor membrane acclimatization will produce a large initial flux density (TEWL + SSWL) that decays with time as donor-side moisture evaporates and steady-state transmembrane diffusion conditions are established. A more efficient procedure is to acclimatize before measurement, but this can take a long time if the air in the donor chambers is stagnant. It is important, therefore, to produce adequate air movement in the vicinity of the donor chamber openings to ensure rapid drying by turbulent mixing of enclosed air with the moving ambient air.

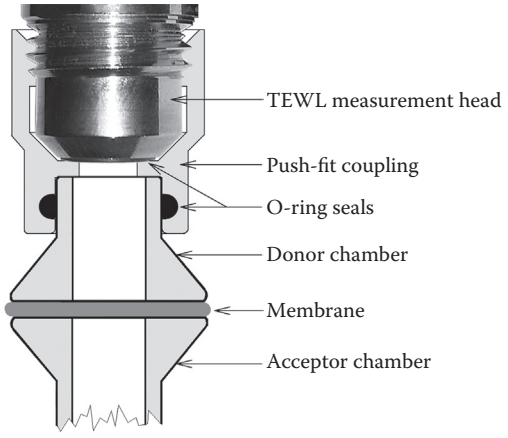

FIGURE 11.5 Coupling between a condenser-chamber instrument and the donor chamber of a Franz cell.

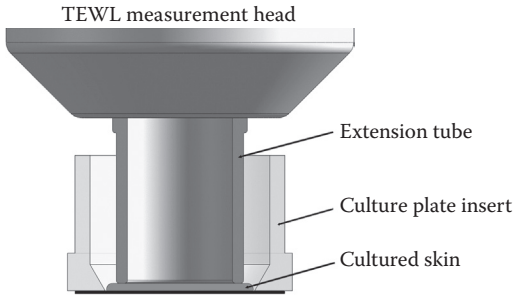

FIGURE 11.6 Coupling between a condenser-chamber instrument and a cultured skin sample maintained in a tissue culture plate insert.

Cultured Skin Barrier Function Measurement

The AquaFlux can also be adapted for TEWL measurements on cultured skin samples maintained in tissue culture inserts. In this case, contact with the cultured skin sample cannot be avoided, because the culture medium is exposed around the periphery of the sample, and this is a source of water vapor flux that is unrelated to barrier function. The approach adopted is to make contact with the sample surface in situ using a sterilizable stainless steel extension tube clamped to an AquaFlux measurement head to reach down into the insert, as illustrated in Figure 11.6. It is important to control the contact pressure onto the sample surface to minimize disturbance while still ensuring a leak-free seal. This is achieved by using (1) a purpose-designed TEWL probe holder with precise rack and pinion control of the vertical position of the probe and (2) a foam rubber cushion below the culture plate to soften the contact and accommodate small angular misalignments.

Skin culture barrier function measurement by the TEWL method has the same need for prior acclimatization of the samples to ambient conditions of temperature and humidity as membranes used in Franz cells. The same criteria apply: dry on top, wet underneath, steady-state TEWL. Evaluating the barrier property of cultured skin has the potential to complement current in vitro methods used in toxicity testing of raw materials and for screening of new actives, thereby reducing or replacing in vivo testing.

FACTORS AND VARIABLES THAT CAN INFLUENCE IN VIVO TEWL MEASUREMENTS

A number of factors can influence TEWL measurements and need to be carefully considered and controlled, where possible. These have been comprehensively reviewed and may be related to individuals, environment, instrument, or procedural variables [33]. Many of the instrument-related and procedural factors can be controlled or minimized by using well-developed measurement protocols, using best practices described in more detail in the following section. Person-related factors are more difficult to control. Table 11.2 lists the main person-related factors and whether they have been observed to influence TEWL measurements.

TABLE 11.2
Influence of Person-Related Factors on In Vivo TEWL Measurement

Factor	Impact on TEWL [References]
Biological	
Thermal and emotional sweating	Yes [39–41]
Age, sex, ethnicity	Conflicting evidence [13,35,41–45]
Skin physiology (thickness, hydration, appendages, constituents)	Yes [7]
Skin type according to Fitzpatrick scale	Yes [42]
Anatomical location of sample site	Yes [12,13,15,35,41,46–48]
Skin temperature	Yes [13,40,41]
Skin health	Yes [15,49]
Circadian rhythm	Yes [41,50]
Hormonal influences	Yes [51]
External Factors	
Lifestyle factors (e.g., smoking, alcohol consumption, caffeine, diet, occupation)	Yes [52–54]
Skin cleansing	Yes [55,56]
Chemical exposure	Yes [57,58]
Mechanical skin damage	Yes [59,60]
Occlusion	Yes [46,61–63]

Biological heterogeneity and Instrument Scatter

The inherent biological variation that is observed in any in vivo study can mean that large sample sizes are required for studies and that results can be difficult to interpret. Instrument scatter is another potential source of variability. In order to maximize the signal-to-noise ratio of any experiment, it is important that instrumental scatter is as low as possible, especially when measuring potentially small but clinically meaningful differences in TEWL.

Instrumental repeatability can be measured in isolation by using a steady in vitro flux source in place of in vivo skin. The repeatability of AquaFlux and VapoMeter instruments was measured this way using a novel inverted wet-cup flux source [18]. The main findings, from 200 repeat measurements for each instrument, is that the scatter of AquaFlux readings (coefficient of variation, CV = 0.93%) is approximately 10 times smaller than that of the VapoMeter (CV = 10.3%). These values are compatible with the manufacturers' published specifications. Identifying the main source of variability in this way can be invaluable when interpreting skin measurements.

The presence of significant anatomical variation in TEWL has been well documented [12,34,35] and may be due to the degree of vasculature in the underlying tissue; musculature in the limb; skin tonicity; and/or variations in the size, structure, and arrangement of the constituent corneocytes [36]. The anatomical variation in TEWL needs to be considered in the design and interpretation of any comparative study involving different skin sites.

In addition, when designing study protocols, post-treatment delay time is an important factor to consider. Aqueous formulations applied to the skin result in an immediate increase in evaporation flux (SSWL, not TEWL). In contrast, application of water-free substances such as petrolatum will reduce TEWL values due to occlusion. Care also needs to be exercised to ensure that topical residues do not enter the measurement chamber or cross-contaminate skin sites.

GUIDANCE FOR THE MEASUREMENT OF TEWL WHEN EVALUATING BARRIER FUNCTION

Guidelines have been published for the measurement of TEWL in highly controlled settings [12,13]; these were developed before the introduction of the new generation of closed-chamber instruments. More recently, international guidelines on in vivo TEWL measurement in nonclinical settings (e.g., workplace) have been published [33]. These contain information to help reduce measurement errors and achieve consistency among users and, importantly, include best practices for measuring TEWL using closed-chamber systems. The main recommendations are detailed below, and a summary can be found in Table 11.3.

Instrument Type

For TEWL measurements, it is recommended that a closed-chamber type instrument be used in the workplace because this design is not influenced by ambient air movement.

Ambient Conditions

According to the guidelines, a study participant should be acclimatized to the measurement environment for at least 15–30 min prior to TEWL measurement. Ambient room

TABLE 11.3
Main Instrumental Factors that can Influence In Vivo TEWL Measurement According to the TEWL Guidelines and Their Relevance to the Different Types of TEWL Instrument

		Closed Chamber	
Guideline Criteria	**Open Chamber**	**Unventilated**	**Condenser**
Skin acclimatization	Essential	Essential	Essential
Prior-to-use instrument stabilization	Yes	Yes	Yes
Recommended for use in nonclinical settings	No	Yes	Yes
Immune to ambient air movements	No	Yes	Yes
Immune to probe heating	No	No	Yes
Immune to contact pressure	No	Yes	Yes
All surface orientations	No	Yes	Yes
Postmeasurement recovery delay	Yes	Yes	No

Sources: Pinnagoda, J. et al., *Contact Dermat.*, 22, 1990; Rogiers, V., *Skin Pharmacol. Appl. Skin Physiol.*, 14, 2001; Du Plessis, J. et al., *Skin Res. Technol.*, 19, 2013; Serup, J., *Acta Derm. Venereol. Suppl.* (Stockh.), 185, 1994.

temperature should be 20°C–22°C, and relative humidity, 40%–60%.

Calibration and Quality Control

Only calibrated TEWL instruments should be used for measurements. With most instruments, this can only be performed by the manufacturer who recommends regular calibration, usually once a year. Only the condenser-chamber AquaFlux can be calibrated by users with the same independently verified droplet method as used by the manufacturer [18].

Probe Heating

Some instruments have a temperature-dependent response, and earlier guidelines recommend use of an insulating glove to avoid handling the probe. For the closed-chamber systems, the AquaFlux probe shows no significant effect upon heating, unlike the VapoMeter. According to De Paepe et al. [19], holding the VapoMeter between both hands, which caused its temperature to increase by ~6ºC, resulted in an increase of volar forearm TEWL readings from a baseline of 7 ± 2 gm^{-2} h^{-1} to 15 ± 6 g^{-2} h^{-1} [19]. The latest recommendation is that the VapoMeter probe always be handled with an insulating glove or other indirect means [33].

Contact Pressure

With open-chamber instruments, contact pressure has been found to influence measurements [11,37], which makes it important to ensure that the contact pressure is light and consistent from measurement to measurement. In contrast, closed-chamber instruments have been shown to have minimal sensitivity to contact pressure [18,19], which obviates the need for controlling this variable beyond ensuring a leak-free seal between the measurement chamber and the skin.

Surface Orientation

Open-chamber measurements need to be restricted to horizontal surfaces because of interference from natural convection air movements. For closed-chamber instruments, external air movements do not interfere with measurements. However, for the VapoMeter, Cohen et al. [38] found that measurements vary with orientation, although unpublished data from the manufacturer (private communication) contradict this. For the AquaFlux, the probe can be used with all surface orientations with little effect on sensitivity, providing the probe is held correctly, with the sensors remaining above the chamber axis [18].

SUMMARY

The importance of TEWL as a measure of the skin barrier is well recognized, and instruments are now available for the rapid and reliable evaluation of skin barrier function. The open-chamber method is well established and has been used extensively in recent years despite its vulnerability to external air movements. The new generation of closed-chamber instruments extend application away from highly controlled laboratory environments, with portability a key feature of the VapoMeter and accuracy a major strength of the AquaFlux. However, results observed with any instrument are only meaningful if potentially confounding variables are recognized, controlled, or otherwise allowed for. Under these conditions, TEWL measurement is undoubtedly an excellent method for the evaluation of skin barrier function.

ACKNOWLEDGMENTS

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12 Percutaneous Penetration Enhancers
An Overview

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INTRODUCTION

Skin is an optimal interface for systemic drug administration. Transdermal drug delivery (TDD) is the controlled release of drugs through intact and/or altered skin to obtain therapeutic levels systematically and to affect specified targets for the purpose of, for example, blood pressure control, pain management, and others. Dermal drug delivery (DDD) is similar to TDD except that the specified target is the skin itself [1]. TDD has the advantages of bypassing gastrointestinal incompatibility and hepatic "first pass" effect; reduction of side effects due to the optimization of the blood concentration time profile; predictable and extended duration of activity; patient-activated/patient-modulated delivery; elimination of multiple dosing schedules, thus enhancing patient compliance; minimization of interpatient and intrapatient variability; reversibility of drug delivery allowing the removal of drug source; and relatively large area of application compared with the mucosal surfaces [1]. After nearly four decades of extensive study, the success of this technology remains limited, with many problems waiting to be solved, one of which is the challenge of low skin permeability hindering the development of TDD for macromolecules. To overcome the skin barrier safely and reversibly while enabling the penetration of macromolecules is a fundamental problem in the field of TDD and DDD.

Several technological advances have been made in the recent decades to overcome skin barrier properties [2]. Examples include physical means such as iontophoresis, sonophoresis, and microneedles; chemical means such as penetration enhancers (PEs); and biochemical means such as liposomal vesicles and enzyme inhibition. We overview physical and biochemical means of penetration enhancement and focus on the common chemical PEs (CPEs). We discuss the classification and mechanisms of CPEs, their applications in TDD, and trends and development in penetration enhancement.

PHYSICAL PENETRATION ENHANCEMENT

Physical means of penetration enhancement mainly incorporate mechanisms to transiently circumvent the normal barrier function of stratum corneum (SC) and to allow the passage of macromolecules. Although the mechanisms are different, these methods share the common goal to disrupt SC structure to create "holes" big enough for molecules to permeate. Table 12.1 summarizes the commonly investigated technologies of physical penetration enhancement. Two of the better-known technologies are iontophoresis and sonophoresis, and the holes created by these methods are generally believed to be of nanometer dimensions, permissive of transport of small drugs [3]. A new and exciting technology for macromolecule delivery is microneedle-enhanced delivery. These systems uses an array of tiny needlelike structures to create transport pathways of microns' dimensions and should be able to permit transport of macromolecules, possibly supramolecular complexes and microparticles. These systems have greatly enhanced (up to 100,000-fold) the penetration of macromolecules through skin [4], while also offering painless drug delivery [5,6]. Microneedles directly intervene with the skin but do not penetrate deep enough to touch nerve endings below the epidermis, increasing permeation and offering a painless alternative for application [7]. Using thermal energy as a source to create microchannels within the SC, thermal ablation has become a focal part of permeation enhancement. A direct relationship between temperature and permeation exists using thermal ablation, with an increase of thermal temperature between 100°C and 300°C permeation gradually increasing tenfold, up to 300°C [8]. This can attributed to the lipid–protein layer disruption within the SC. Similar to iontophoresis, electroporation is a method that uses short bursts of high-voltage electrical pulses to disturb the SC, awakening the skin's channels to allow permeation [7]. Other methods like the use of jet injections, delivering particles across the skin using high pressure, and laser usage have also been developed but remain in development due to high cost and intricate technology [7]. Baris et al. have found a synergistic relationship between low-frequency sonophoresis and CPEs. They investigated the mechanism of action between these two forms of enhancers and write that low frequency sonophoresis (LFS) increases the penetration and dispersion of the CPE to the localized transport region by collapsing the cavitation microjets. Furthermore, amphiphilic CPEs are greater than nonamphiphilics due to the absorption of the amphiphilic monomers or CPEs to the surface of the cavitation bubbles directly depositing themselves into the skin [9]. The amphiphilic CPEs offer higher upside given the dual

TABLE 12.1
Physical Methods of Penetration Enhancement

Method	Definition	Mechanism(s)	Examples of Drugs	Reference
Ionotophoresis	The electrical driving of charged molecules into tissue by passing a small direct current through a drug-containing electrode in contact with skin	1. Electrical repulsion from the driving electrode drives charged molecules. 2. The flow of electric current enhances skin permeability. 3. Electroosmosis affects uncharged and large polar molecules.	Calcitonin, transnail delivery of salicylic acid; transdermal delivery of peptides, proteins, and oligonucleotides	[4,10–13]
Electroporation	A method of reversibly permeabilizing lipid bilayers by the application of an electric pulse	Application of short (microsecond to millisecond) electrical pulses of 100–1000 V/cm creates transient aqueous pores in the lipid bilayers.	Methotrexate, timolol, fentanyl, tetracaine, nalbuphine, cyclosporin A	[14–20]
Sonoporation	Ultrasound-mediated delivery of therapeutic agents into biological cells	1. (Low-energy frequency): disturbs the lipid packing in SC by cavitation. 2. (Shock waves): increase free volume space in bimolecular leaflets thus enhancing permeation	Insulin, cutaneous vaccination, transdermal heparin delivery, transdermal glucose monitoring, delivery of acetyl cholinesterase inhibitors for the treatment of Alzheimer's disease, treatment of bone diseases and Peyronie's disease, dermal exposure assessment	[4,21,22]
Microneedle-enhanced delivery systems	A method using arrays of microscopic needles to open pores in SC, thus facilitating drug permeation	Bypasses the SC and delivers drugs directly to the skin capillaries. Also has the advantage of being too short to stimulate the pain fibers.	Oligonucleotide, insulin, protein vaccine, DNA vaccine, methyl nicotinate	[3,6]

Note: SC, stratum corneum.

mechanisms that are possible. The physical methods offer alternatives to oral and injection drug delivery with high upside, and potential growth in the field remains.

BIOCHEMICAL PENETRATION ENHANCEMENT

Biochemical means of penetration enhancement include using prodrug molecules [20], chemical modification [21], enzyme inhibition [22], and the usage of vesicular systems or colloidal particles [23]. Among these strategies, special formulation approaches, based mainly on the usage of colloidal carriers, are most promising. Liposomes (phospholipid-based artificial vesicles) and niosomes (nonionic surfactant vesicles) are widely used to enhance drug delivery across the skin. In addition, proliposomes and proniosomes, which are converted to liposomes and niosomes upon simple hydration, are also used in TDD [24]. Generally, these colloidal carriers are not expected to penetrate into viable skin. Most reports cite a localizing effect whereby the carriers accumulate in SC or other upper skin layers [4]. A new type of liposome called a transferosome has been introduced [25,26]. Transferosomes consist of phospholipids, cholesterol, and additional "edge activators"—surfactant molecules such as sodium cholate. The inventors claim that 200 to 300 nm transferosomes are ultradeformable and squeeze through pores less than one-tenth of their diameter and are thus able to penetrate intact skin. Penetration of these colloidal particles works best under in vivo conditions and requires a hydration gradient from the skin surface toward the viable tissues to encourage skin penetration under nonoccluded conditions.

In addition, ethosomes, which are liposomes high in ethanol content (up to 45%), penetrate skin and enhance compound delivery to deep skin strata or systematically. The mechanism suggested is that ethanol fluidizes both ethosomal lipids and lipid bilayers in the SC, allowing the soft, malleable vesicles to penetrate through the disorganized lipid bilayers [27]. In recent years, new information on the interactions between surfactants and its contact with the skin has been reported. Once the surfactant makes contact with the skin, it binds to the skin's deeper proteins by denaturing the surface proteins, causing the SC to swell. It thereby disorganizes the intercellular lipids of the skin, that is, the fluid lipids, and causes the removal of the calcium ions or surrounding ions, resulting in a reduction of corneocyte adhesion, leading to accessibility of deeper proteins [28].

In general, six potential mechanisms of action of these colloidal carriers were proposed [4]:

1. Penetration of SC by a free drug process—drug releases from vesicle and then penetrates skin independently
2. Penetration of SC by intact liposomes

3. Enhancement due to release of lipids from carriers and interaction with SC lipids
4. Improved drug uptake by skin
5. Different enhancement efficiencies control drug input
6. The role of protein requires elaboration

CPEs

Substances that help promote drug diffusion through the SC and epidermis are referred to as PEs, accelerants, adjuvants, or sorption promoters [29]. PEs have been extensively studied given their advantages such as design flexibility with formulation chemistry and patch application over large areas. PEs improve drug transport by reducing the resistance of SC to drug permeation. To date, none of the existing CPEs have proven to be ideal. In particular, the efficacy of PEs toward the delivery of high-molecular-weight drugs remains limited. Attempts to improve enhancement by increasing the potency of enhancers inevitably lead to a compromise on safety issues. Achieving sufficient potency without irritancy has proved challenging. Nanostructured lipid carriers, nanoemulsions, and oil solutions "have received increasing attention during the past few years, because of having several advantages such as ease of manufacturing, thermodynamic stability, enhanced drug solubilation and increased drug permeation rate" [30]. Tsai and his colleagues created a hesperetin-carrying microemulsion that showed improve permeation in comparison to nonmicroemulsion aqueous solutions [33]. A similar oil-based nanocarrier system of ropinirole, used to treat Parkinson's, was found to "have good pharmacokinetic features which can replace oral dosage form for the same and a sufficient manipulation of stratum corneum barrier was found" [31]. The solubility involving mircoemulsion/nanoemulsion is the reason for its increased permeation effects with delivering drugs. The effect is from the "partitioning of the drug between the internal oil phase and the external aqueous phase" [32]. An increase in drug solubility within the external phase will progress the partitioning effect from the internal to the external phase; the drug will be able to diffuse more easily to be discharged [32]. Emulsifiers can explain the increase in permeation due to the "enhanced partitioning of the actives into the skin" [33]. The actives mentioned, including the "micelles and liquid crystalline phases," affect the solubility properties of the active ingredient, changing the thermodynamic movement [33]. Otto concluded that "emulsifiers arranged in liquid crystalline structures in the water phase enhanced the skin penetration of the active ingredients" [33]. Specifically, a recent study by Degim found multiwalled and double-walled carbon nanotubules to have permeation effects as drug carriers [34]. While their penetration enhancement is through "absorption and subsequent desorption (depot effect)," the carbon nanotubules do not penetrate the skin. However, Degim did use the carbon nanotubules to "provide a high loading and enhanced transdermal penetration for especially hydrophobic drugs" [34]. Additional information has been published on the advantages of nanostructured lipid carriers. Using idbenone (IDB), Li using a comparative study between nano-structured lipid carriers (NLCs), nanoemulsions, and oil solutions to determine which improved chemical stability and enhanced skin delivery. He found NLCs to "achieve a significant improvement with respect to chemical stability of IDB, skin permeation, and formulation stability compared to NE and oil solution" [35]. NLCs will continue to progress in the transdermal field and can be effectively used as a carrier for topical drugs. A chemically complex mixture of arginine and chitosan was formed to see what value it may have as an enhancer in the presence of the adefovir, an "acylic nucleoside phosphonate used as a broad-spectrum antiviral that is highly effective against herpes-, retro-, and hepadviruses" [36]. In the enhancement study conducted by Lv using *N*-arginine chitosan, a peptide, they found that the "*N*-Arg-CS simulated arginine-rich cell penetration peptides have potential as a novel transdermal enhancer" [36]. The arginine-rich cell peptides offer an alternative method to disrupt the molecular protein side chains within the SC, creating specific methods for enhancement.

CLASSIFICATION OF CPEs

The diverse physicochemical properties and variation in mechanisms of action of compounds investigated for their penetration enhancement effects made a simple classification scheme for PEs difficult to set up. Hori et al. [37] proposed a conceptual diagrammatic approach based on Fujita's data [38] for the classification of PEs. In this approach, they determined organic and inorganic values for PEs, and the resultant plot of organic versus inorganic characteristics grouped PEs into distinct areas on the diagram—area I encloses enhancers, which are solvents; area II designates PEs for hydrophilic drugs; and area III contains PEs for lipophilic compounds. On the other hand, Lambert et al. [39] grouped most PEs into three classes—solvents and hydrogen bond acceptors (e.g., dimethylsulfoxide, dimethylacetamide, and dimethylformamide), simple fatty acids and alcohols, and weak surfactants containing a moderately sized polar group (e.g., Azone, 1-dodecylazacycloheptan-2-one)—whereas Pfister et al. [29] classified PEs as either polar or nonpolar. To date, there is no consensus as to which classification to adopt. Table 12.2 classifies commonly investigated PEs based on the chemical classes to which the compounds belong [44]. Only representative compounds are listed to avoid an exhaustive list. Note that a perfect classification is yet to be developed, and the key lies in a comprehensive understanding of the mechanisms and the physicochemical parameters of CPEs.

Whether natural or synthetic, considerable effort has been put into finding CPEs with low toxicity and irritancy. For example, terpenes, a naturally made enhancer derived from plant oils, have the beneficial properties of being a low-irritancy substance while significantly increasing permeation [45]. In a study, conducted by Chantasart, using oxygen-rich terpenes including menthol, methone, thymol, cineole, and carbacrol, oxygen-containing terpenes did increase permeation of lipophilic compounds [45]. The findings indicated the mechanisms of enhancement for terpenes and alcohols could be similar if not the same. Additionally, a review from Sapra found the benefits of terpenes having "high percutaneous

TABLE 12.2
Chemical Penetration Enhancers

Category and Examples	Cosolvent/Vehicle	Mechanism	Examples of Drugs (33)	Comment	Reference
		Sulfoxides			
DMSO		1. Increase lipid fluidity 2. Promote drug partitioning	DMSO: theophylline, salicylic acid, hydrocortisone, testosterone, scopolamine, antimycotics, fluocinolone acetonide, flufenamic acid		[45,46]
DCMS		Protein–DCMS interactions, resulting in a change in protein conformation, creating aqueous channels	DCMS: methotrexate, naloxone, pyridostigmine bromide, hydrocortisone, progesterone	DCMS enhances polar drugs more effectively	
		Alkanones			
N-heptane, *n*-octane, *n*-nonane, *n*-decane, *n*-undecane, *n*-dodecane, *n*-tridecane, *n*-tetradecane, *n*-hexadecane		Extensive barrier alteration of SC	Propanolol, diazepam		[47]
		Alcohols			
Alkanol: E, propanol, butanol, 2-butanol, pentanol, 2-pentanol, hexanol, octanol, nonanol, decanol, BA		1. Low-molecular-weight alkanols (C_3 6) may act as solubilizing agents 2. More hydrophobic alkanols may extract lipids from SC, leading to increased diffusion	E: tacrine, metrifonate, dichlorvos, ketolorac, nitroglycerin, tazifylline, betahistine, cyclosporin A		[65–67]
Fatty alcohol: Caprylic, decyl, LA, 2-lauryl, myristyl, cetyl, stearyl, oleyl, linoleyl, linoleyl alcohol			LA: buprenorphine		[69]
		Polyols			
PG, PEG, ethylene glycol, diethylene glycol, triethylene glycol, dipropylene glycol, G, propanediol, butanediol, pentanediol, hexanetriol	43_3 enhancement of diazepam and 86_3 enhancement of midazolam maleate seen in PG and 5% Azone in a PG:ethanol:water (2:2:1) vehicle	PG may solvate a-keratin and occupy hydrogen bonding sites, reducing drug-tissue binding	PG: 5-fluorouracil, tacrine, ketorolac, isosorbide dinitrate, clonazapem, albuterol, verapamil, betahistine, estradiol, dihydroergotamine, methotrexate, steroids, midazolam maleate, diazepam PEG: terbutaline G: diazepam, terbutaline, 5-fluorouracil	Inclusion of 2% Azone or 5% oleic acid to PG produced a more bioactive formulation	[70,71]
		Amides			
Urea, DMA, diethyltoluamide, DMF, dimethyloctamide, dimethyldecamide		Urea: hydration of SC, keratolytic, creating hydrophilic diffusion channels DMA/DMF: Low conc.: partition to keratin, High conc.: increase lipid fluidity, disrupt lipid packaging	Urea: ketoprofen, 5-fluorouracil DMA/DMF: griseofluvin, beta-methasone 17-benzoate, caffeine	Urea analogues in PG enhanced permeability of 5-fluorouracil 6_3	[69,72]
Biodegradable cyclic urea: 1-alkyl-4-imidazolin-2-one			Indomethacin	Comparable to or better than Azone	[73]

Pyrrolidone derivatives: 1M2P, 2-pyrrolidone, 1-lauryl-2-pyrrolidone, 1-methyl-4-carboxy-2-pyrrolidone, 1-hexyl-4-carboxy-2-pyrrolidone, 1-lauryl-4-carboxy-2-pyrrolidone, 1-methyl-4-methoxycarbonyl-2-pyrrolidone, 1-hexyl-4-methoxycarbonyl-2-pyrrolidone, 1-lauryl-4-methoxycarbonyl-2-pyrrolidone, NMP, *N*-cyclohexylpyrrolidone, *N*-dimethylaminopropylpyrrolidone, *N*-cocoalkypyrrolidone, *N*-tallowalkylpyrrolidone		Interact with both keratin in the SC and lipids in the skin structure	1M2P: griseofulvin, theophylline, tetracycline, ibuprofen, betamethasone 17-benzoate NMP: prazosin		[69,74]
Biodegradable pyrrolidone derivatives: fatty acid esters of *N*-(2-hydroxyethyl)-2-pyrrolidone					[42]
Cyclic amides: 1-dodecylazacycloheptane-2-one (Azone), 1-geranylazacycloheptan-2-one, 1-farnesylazacycloheptan-2-one, 1-geranylgeranylazacyclo-heptan-2-one, 1-(3,7-dimethyloctyl) azacycloheptan-2-one, 1-(3,7,11-trimethyldodecyl) azacycloheptan-2-one, 1-geranylazacyclohexane-2-one, 1-geranylazacyclopentan-2,5-dione, 1-farnesylazacyclopentan-2-one	Azone: enhancer effect can be increased by use of a cosolvent such as PG	Azone: 1. Affects lipid structure of SC 2. Increases partitioning 3. Increases membrane fluidity	Azone: 5-fluorouracil, antibiotics, glucocorticoids, peptites, clonazepam, albuterol, estradiol, levonorgestrel, HIV protease inhibitor (LB-71148), betahistine, dihydroergotamine	Azone: significant accelerant effects at low conc. (1%–5%), can be applied undiluted to skin without significant discomfort, effective for both hydrophilic and hydrophobic drugs	[75–77]
Hexamethylenelauramide and its derivatives					[78]
Diethanolamine, triethanolamine Fatty acids					[70]
Linear: LIA, valeric, heptanoic, pelagonic, caproic, CA, LAA, myristic, stearic, OA, caprylic		Selective perturbation of the intercellular lipid bilayers OA: decreases the phase transition temperatures of the lipid, increasing motional freedom or fluidity of lipids	Naloxone, mannitol, betamethasone 17-benzoate, hydrocortisone, acyclovir, nitroglycerin OA: galanthamine, estradiol, levonorgestrel CA: buprenorphine, albiterol LAA: buprenorphine, betahistine	Among stearic, oleic, and linoleic acids, maximum enhancement was observed with linoleic acid	[69,79,80]
Branched: isovaleric, neopentanoic, neoheptanoic, neononanoic, trimethyl hexanoic, neodecanoic, isostearic					

(*continued*)

TABLE 12.2 (Continued)
Chemical Penetration Enhancers

Category and Examples	Cosolvent/Vehicle	Mechanism	Examples of Drugs (33)	Comment	Reference
		Fatty Acid Esters			
Aliphatic: Isopropyl *n*-butyrate, isopropyl *n*-hexanoate, isopropyl *n*-decanoate, IPM, isopropyl palmitate, octyldodecyl myristate		IPM: direct action on SC, permeating into liposome bilayers, increasing fluidity Aliphatic: increase diffusivity in the SC and/or the partition coefficient Alkyl: increase lipid fluidity (similar to DMSO)	IPM: galanthamine, ketorolac, chlorpheniramine, dexbrompheniramine, diphenhydramine, theophylline, pilocarpine, verapamil EA: levonorgestrel, 17b-estradiol, hydrocortisone, 5-fluorouracil, nefedipine		[81,82]
Alkyl: EA, butyl acetate, methyl acetate, methylvalerate, methylpropionate, diethyl sebacate, ethyl oleate					
		Surfactants			
Anionic: Sodium laurate, sodium lauryl sulfate, sodium octyl sulfate		Alter the barrier function of SC, allowing removal of water-soluble agents that normally act as plasticizers		Greater damage and permeation enhancement with anionic surfactants than with nonionic surfactants	[83,84]
Cationic: Cetyltrimethylammonium bromide, tetradecyltrimethylammonium bromide, octyltrimethylammonium bromide, benzalkonium chloride, octadecyltrimethylammonium chloride, cetylpyridinium chloride, dodecyltrimethylammonium chloride, chexadecyltrimethylammonium chloride, zwitterionic surfactants, hexadecyl trimethyl ammoniopropane sulfonate, oleyl betaine, cocamidopropyl hydroxysultaine, cocamidopropyl betaine	Significant increases in the flux of lidocaine from saturated systems in PG–water mixtures	Adsorb at interfaces and interact with biological membranes, causing damage to skin		Cationic surfactants are more destructive to skin than anionic surfactants.	[84–86]
Nonionics: Polyxamer (231, 182, 184), polysorbate (20, 60), Brij (30, 93, 96, 99), Span (20, 40, 60, 80, 85), Tween (20, 40, 60, 80), Myrj (45, 51, 52), Miglyol 840	Polysorbate 20 and 60 increased lidocaine flux in the presence of PG	Emulsify sebum, enhancing the thermodynamic activity of coefficients of drugs	Tween 80: ketoprofen Polysorbate 20, 60: lidocaine		[69,88,89]

Bile salts: Sodium cholate, sodium salts of TC, glycolic and desoxycholic acids			TC: elcatonin and vit. D_3, estradiol and vit. D_3,		[90]
Lecithin					[91]
Terpenes					
Hydrocarbons: D-limonene, a-pinene, b-carene	1. Increases diffusivity of drugs within SC due to disruption of intercellular lipid barrier 2. Opens new polar pathways within and across the SC		5-Fluorouracil, aspirin, haloperidol	Hydrocarbon terpenoids were least effective; oxides moderately effective; and the alcohols, ketones, and cyclic ethers most effective accelerants of 5-fluorouracil permeation	[40,92,93]
Alcohols: a-terpineol, terpinen-4-ol, carvol					
Ketones: Carvone, pulegone, piperitone, menthone					
Oxides: Cyclohexene oxide, limonene oxide, a-pinene oxide, cyclopentene oxide, 1,8-cineole					
Oils: Ylang ylang, anise, chenopodium, eucalyptus					
Organic Acids					
Salicylic acid and salicylates (including their methyl, ethyl, and propyl glycol derivatives), citric and succinic acid					[94]
Cyclodextrins					
HPbCD DIMEB	Higher penetration of liarzole in DIMEB with PG/oleic acid compared with HPbCD	Form inclusion complexes with lipophilic drugs and increase their solubility in aqueous solutions	Liarzole		[95,96]
Proprietary chemical enhancers					[97]
Alkyl-2-(*N*,*N*-disubstituted amino)-alkanoate ester (NexAct)					
2-(*n*-nonyl)-1,3-dioxolane (SEPA)			Ibuprofen, ketoprofen, alprostadil, testoterone		

Source: Polat et al., *J Pharm Sci* 2011, 100(2): 512–529.

Note: BA, benzyl alcohol; CA, capric acid; conc., concentration; DCMS, decylmethylsulfoxide; DIMEB, 2,6-dimethyl-b-cyclodextrin; DMA, dimethylacetamide; DMF, dimethyformamide; DMSO, dimethylsulfoxide; E, ethanol; EA, ethyl acetate; G, glycerol; HPbCD, 2-hydroxypropyl-b-cyclodextrin; IPM, isopropyl myristate; LA, lauryl; LAA, lauric acid; LIA, linoleic acid; NMP, *N*-methyl-pyrrolidone; OA, oleic acid; PEG, polyethylene glycol; PG, propylene glycol; SC, stratum corneum; TC, taurocholic; vit., vitamin; 1M2P, 1-methyl-2-pyrrolidone.

ability, reversible effect on the lipids of the SC, minimal percutaneous irritancy at low concentration and good evidence of freedom from toxicity" [46]. These findings were in agreement with an elegant study conducted by Sarah Ibrahim, who, along with colleagues, found that "the ability to elucidate a structure relationship of chemical permeation enhancers would facilitate the selection of enhancers" [47]. Her study concluded, "A general correlation was found between EMax and permeation enhancement induced by fatty acid deposition from a volatile solvent" [47]. The study concluded with the usage of solid fatty acids as PEs. However, the fatty acids may become dissolute, raising concern as a limiting factor for future research [47]. More literature has been made available on the permeation effects of fatty acids and their conjugates. Known for having positive permeation effects but negative if not detrimental effects on the skin itself, that is, lipid extraction in the SC and damage to epidermal cells, these compounds were modified through esterification to reduce irritancy [48]. Using the conjugates of unsaturated fatty acids, oleic acid, linoleic acid, and α-linoleic acid in the monoester or diester form, Ben-Shabat found increased permeation in the delivery of lidocaine. Lidocaine is a common anesthetic and antiarrhythmic drug. The conjugate monoester and diester of oleic and linoleic acids had the same enhancement effects as their father fatty acids with reduced skin irritation over a 7-day period [48]. Due to their high level of irritancy, fatty acid conjugates do offer an interesting alternative but require further insight. Kandimalla et al. observed how saturated and unsaturated fatty alcohols (SFALs and USFALs) exhibit biphasic flux behavior in the presence of melatonin, a chemical involved with sleep rhythms [49]. The rationale behind using a patch is to ensure that if the drug is administered, the patient will have a constant delivery of melatonin to prevent any disruption in sleeping patterns. A study by Kanimalla et al. found a correlation between first-phase melatonin delivery and enhancer length, but the same could not be said for the second phase, where enhancer length had an inverse relationship with melatonin flux. The discussion concluded that the reasons for this effect might be due to the fact that an increase in polarity of the skin barrier decreases the skin partitioning efficient for melatonin [49]. The researchers suggest using short-chain SFALs (nonanol or decanol) or USFALs with a low degree of unsaturation to achieve prolonged drug penetration [49]. Building on the theme of naturally occurring substances, cholic acid, an important human bile, along with 14 other derivatives of the 5B-cholic acid, have been found to have properties consistent with penetration modifiers [50]. The study conducted found beneficial properties from cholic acid and its derivatives; two compounds were found to have antiproliferative effect on cancer cells without affecting normal cells, indicating minimal cytotoxic side effects. Of the 14 other chemical derivatives studied, 10 compounds demonstrated minimal cytotoxicity, leaving open the possibility of using them as enhancers. Despite only having minimal to marginal penetration enhancement, a direct relationship can be taken away from this study between the solubility/lipophilicity and enhancement effects [50].

Size continues to be a factor in developing reliable PEs that can effectively permeate through the SC. The most "potent chemical enhancers … penetrate to a significant extent due to their small molecular weight causing skin irritation" [51]. Dendrimers have been found to have enhancement properties and are rather large polymers with "core-shell architecture having a large number of surface functional groups" [51]. Venugati studied the "structure–activity relationship of [poly(amidoamine)] dendrimers in the skin as a function of their surface charge, generation, and concentration" [51]. The usage of polyamidoamine (PAMAN) dendrimers with hydroxyl and carboxyl functional groups is to "enhance the permeation of hydrophilic molecules in a charge, size, and concentration dependent manner" [51]. Despite having conclusive findings for dendrimers to increase permeation, the compounds are charge, size and concentration dependent, requiring extensive research for a specific molecule. A separate study was conducted by Borowska et al. to find if G3 and G4 polyamidoamine dendrimers can help the transdermal delivery of methoxypsoralene in vivo. Their study found the specific G3 and G4 dendrimers to be "effective enhancers of transdermal delivery of 8-MOP …. resulting in higher concentration within the epidermis and dermis" [52]. As we come to understand the importance of size, charge, and concentration, many drugs in the future will have specific modes of action. The mechanisms of CPEs to permeate the epidermis and dermis will be helpful to understand how effective drugs can be developed to create the largest desired effect.

MECHANISM OF CPEs

The mechanisms of action proposed for commonly seen CPEs are listed in Table 12.2. Basically, transdermal penetration of most drugs is a passive diffusion process [70]. There are three major potential routes for penetration—appendageal (through sweat ducts and/or hair follicles with associated sebaceous glands), transcellular permeation through the SC, or intercellular permeation through the SC [4]. The appendageal route usually contributes negligibly to steady-state drug flux given its small available fractional area of 0.1%. This route may be important for short diffusional times and for ions and large polar molecules, which have low penetration across SC. The intact SC thus comprises the predominant route through which most molecules penetrate. Kanikkannan et al. [71] suggested three pathways for drug penetration through the skin: polar, nonpolar, and both. The mechanism of penetration through the polar pathway is to cause protein conformational change or solvent swelling, whereas the key to penetrate via the nonpolar pathway is to alter the rigidity of the lipid structure and fluidize the crystalline pathway. Some enhancers may act on both polar and nonpolar pathways by dissolving the skin lipids or denaturing skin proteins. On the other hand, Ogiso and Tanino [72] proposed the following mechanisms for the enhancement effect: (1) an increase in the fluidity of the SC lipids and reduction in the diffusional resistance to permeants; (2) the removal of intercellular lipids and dilation between adherent cornified cells; (3) an increase in the thermodynamic activity of drugs in vehicles; and (4) the

exfoliation of SC cell membranes, the dissociation of adherent cornified cells, and elimination of the barrier function. Ogiso et al. [73] also proposed examples of PEs with different relative enhancement capabilities due to differences in the chemical structure and other parameters. In their study, the relative ability to enhance transdermal penetration of indomethacin into hairless rat skin was studied. The results are summarized in Table 12.3 [69]. Furthermore, Kanikkannan et al. [71] proposed that on the basis of the chemical structure of PEs (such as chain length, polarity, level of unsaturation, and presence of specific chemical groups such as ketones), the interaction between the SC and PEs may vary, contributing to the different mechanisms in penetration enhancement. Schroter et al. [53] have studied the lipid structure of the SC using Fourier synthesis, to develop a reasonable idea of organization. The study focused on three main lipids and produced an SC lipid model of CER[EOS(]/CER[AP(]/CHOL[BA(behemic acid)], with a ratio of 23:10:33:33% w/w]. This ratio of the SC should be further studied to develop drugs specifically engineered to permeate through the specific lipids. They found the lipid CER[EOS] to exist in short-periodicity phase due to the ceramide[AP] arrangement and to extend into the next two bilayers [53]. This extension will be helpful to deliver drugs, but knowing the complete structure of the lipids bilayers will aid the development of delivering drugs directly to the blood stream. As Venuganti et al. [52] have studied dendrimers, they have found that the mode of action for these compounds is different depending on the charge. A cationic dendrimer will react with the hydrophilic heads of ceramides and fatty acids to change the lipid bilayer. However, dendrimers have also been found to react with the hydrophilic heads of phospholipids and change the flux of the lipid membranes [52]. The mechanisms altering the hydrophilic heads of dendrimers and phospholipids cause channels to be opened. These channels, once opened, allow the permeation of drugs. Knowing how these drugs affect the SC is most important in developing greater enhancers. A separate study by Kear et al. [54] found dendrimers, specifically hydroxypropyl-β-cyclodextrin, to not extract lipids in the SC. Instead they believe that the dendrimer increased the solubility of the drug, therefore increasing the thermodynamic driving force for permeation [54]. This thermodynamic force greatly affects the lipid structure, allowing the permeation. Despite these findings, debate remains on how dendrimer mechanisms function due to differences in molecular weight, concentration, and sensitivity. Always relying on the differences in charges, molecular weight, concentration, and sensitivity, the development of each drug will always be different, but the mechanisms of action may be similar once studied. Ammonium carbamate, a relatively new chemical enhancer, has been found to have low toxicity and low dermal irritation properties, making it ideal for usage. Michael Novotny et al. [55] found two mechanisms of action for ammonium carbamate, which start with the breakdown of the carbamate polar head within the SC lipids [55]. Novotny et al. found that with a neutral or alkaline pH on the skin, the compound ammonium carbamate will only diffuse through the intact SC via a lateral diffusion route [55]. The lateral diffusion route is one of the quickest-acting modes of action within the lipid bilayers. Following this route of action within the lipid structure, carbon dioxide is formed and then released through the SC, causing a disturbance within the lipid structure, thus opening drug diffusion pathways [55]. From this action, a separate chemical is formed, Dodecyl-6-aminohexanoate (DDEAC), which helps aid the permeation of the drug, becoming an active enhancer in the process. As was hypothesized before this study, permeation through the lipids proves to be affected by the pH levels. The T12 mode of action is ideal to occur with a pH of ~5 or lower; the hypothesis was found to be correct when T12 activity decreased with an increase of pH to ~9 [55]. Gillet et al. [56] developed a study to see if altering the surface charges of the delivery vehicle will affect permeability within the dermis and epidermis. Using betamethasone and betamethasone dipropionate as the model drugs inside the liposomes, they found that using negatively charged liposomes did increase permeation within the epidermis compared to positively and neutral liposomes [56]. Another study by Ibrahim et al. [57] tried to uncover the mechanisms of commonly used PEs to determine which would be best for usage. They first determined that if an enhancer uses the lipoidal route across the SC, enhancement is related to enhancer solubility within the lipids. A direct relationship between lipid fluidization and amount of enhancer within the SC lipids exists [57]. Additionally, enhancement is independent of the

TABLE 12.3
Examples of Penetration Enhancers with Different Relative Enhancement Capabilities Due to Differences in the Chemical Structure and Other Parameters

Mechanisms	Comparison
Extraction of intercellular lipids and dilations between cornified cells, permitting percutaneous passage of polar substances	1-dodecylazacycloheptane-2-one (Azone) > *n*-octanol > D-limonen > oleic acid > cineol
Increase in partitioning into skin	1-dodecylazacycloheptane-2-one > *n*-octanol > cineol > D-limonen > oleic acid > isopropyl myristate > monooleate
Increase in the fluidity of SC lipids and reduction in diffusional resistance	1-dodecylazacycloheptane-2-one > isopropyl myristate > monoolein > oleic acid > cineol, sodium oleate
Increase in thermodynamic activity in vehicles	*n*-octanol > sodium oleate > D-limonen > monoolein > cineol > oleyl oleate > isopropyl myristate

enhancer configuration, that is, alkyl chain length and polar head group. Ibrahim tested this further through Differential scanning calorimetry (DSC) and Attenuated Total Reflectance Fourier Transform Infrared spectroscopy (ATR-FTIR) analyses, concluding that the major mechanism of enhancement is through the fluidization of the lipids in the SC, while lipid extraction is not a major mechanism [57]. He et al. [58] have examined the mechanisms of chitosans and their derivatives including *N*-trimethyl chitosan (TMC) and mono-*N*-carboxylmethyl chitosan (MCC). Through the usage of ATR-FTIR, these results indicated that chitosan and its derivatives, TMC and MCC, could in fact increase permeation in a variety of ways. The chitosan's mechanisms were described in four different ways: changing the secondary structure of the keratin in the SC, increasing saturation or water content in the SC, decreasing the HaCaT cell membrane potential, and enhancing the cell membrane fluidity [61]. This study may be the first to examine the methods of mechanisms involving chitosans, leaving room for more literature and research. Otto et al. [59] have hypothesized that if the solubility of the active ingredient is greater than the solubility in the SC, penetration will decrease. Moreover, if the solubility of the penetrants in the SC is greater than the formulation, then penetration will increase [59]. The study by Watkinson et al. [60] found this hypothesis to hold some truth for a formulation involving ethanol in delivering ibuprofen. They found that flux increased with an increase in ethanol but pure ethanol had no effect on penetration of ibuprofen [60]. The solubility effect ethanol has on ibuprofen is the main reason for enhancement and permeation. Increasing the solubility of ibuprofen may be the mechanism of enhancement for ethanol. While this chapter has discussed the role SC lipids have for skin barrier function, recent literature has found that the proteins existing in the epidermis may play an important role in the skin barrier function as well [61]. The keratinocytes in the epidermis tested by Wato et al. included the intercellular proteins involucrin, keratin 10, desmoglein-1, claudin-1, and E-cadherin. Using immunohistochemical studies, Wato et al. [61] determined two separate methods of mechanisms involving the proteins depending on what type of enhancer was used and their site of action. Citing the usage of fatty esters, alcohols, and surfactants in the study, mechanisms were proposed for fatty esters and alcohol. Fatty esters appear to change the upper epidermal layer proteins of keratin 10 and involucrin; alcohols changed the lower epidermal layers proteins of demoglein-1, claudin-1, and E-cadherin [61]. Using this information and insight into the effect that these enhancers have on different proteins should lead to their usage together as possibly having synergistic properties. A comprehensive understanding of the mechanisms of action and a judicious selection of CPE would be helpful in the successful development of TDD and DDD products.

Synergistic Combinations

Synergistic compounds have become a new form of TDD that could become the best form of delivery in comparison to using single chemical enhancers alone. Research by Krande et al. [109] has examined the possible routes and modes of enhancement through combining chemicals to create a synergistic relationship. The relationship of synergy follows one that indicates the "extent of interaction" between two PEs, that is, the ratio of penetration enhancement obtained by the mixture to the weighted sum average of the penetration enhancement taken from the individual components of the mixture [109]. This relationship can be explained in mathematical terms with the equation provided. The systems explained by Krande include solvent mixtures, microemulsions, eutectic mixtures, intricate vesicles, and inclusion complexes, all used to perform together, in harmony, to create a more powerful form of enhancement. These chemicals in combination have a synergistic relationship together, one that cannot be described as A plus B equals C but instead A plus B equals C^2. Thus, this method of enhancement delivers the drug to the skin in a very positive and powerful way.

Krande has developed a special equation involving the formulation of two permeation enhancers at total concentration with the weight fraction, adding to the equation the enhancement ratios of pure components of A and B (the two permeation enhancers) at the same total concentration of Y.

$$\text{The equation of } S \text{ (synergy)} = \frac{E_{\mathrm{A+B}}^{\mathrm{X,Y}}}{\left\{X\left[E_{\mathrm{A}}^{\mathrm{Y}} + (1-X)E_{\mathrm{B}}^{\mathrm{Y}}\right]\right\}}$$

This equation stands to assume that any value obtained for synergy, *S*, greater than 1 will have a superior skin permeabilization; any value less than or equal to 1 will have no change in permeabilization but instead will have a decreased interaction with the skin. After developing this equation, Krande dedicated his review to all permeation enhancers whose *S* had a value greater than 1 for transdermal drug usage. The chemicals in combination help to overcome individual problems that made be associated with one enhancer and offer greater enhancement possibilities. The positives associated can be explained through the differences in enhancement some of the synergistic combinations can have on the skin layers. For example, with a combination of enhancers, a single enhancer may affect the lipids within the SC, while the other enhancer can disrupt the corneocytes within the same layer, opening up many routes of enhancement for interhydrophobic and intrahydrophilic pathways for drug permeation. An alternative combination may disrupt the lipid bilayers/corneocytes, while the other enhancer can alter the partitioning of the drug in the SC [109]. Additionally, not only can they affect different layers of the skin, but also, another synergistic combination may stabilize the drug and prevent it from metabolizing in the skin, while the other enhancer disrupts and creates diffusion pathways for the drug. The possibilities for different types of formulations and combinations are endless, infinite with the information we now posses about CPEs. The sophistication of the combinations will allow for high-potency enhancers to disrupt the SC, and with the aide of a low-disruption enhancer, the epidermis can be left unscathed. Using

a form of yin and yang in developing these synergistic typical drugs could be very effective. Klang et al. [110] have studied sucrose stearate-based nanoemulsions with γ-cyclodetrin-based systems and found very positive effects. The aim of Klang's study was to find a characterization of blank and drug-loaded nanoemulsions in terms of physiochemical formulation parameters, microscopic appearance, and long-term stability [110]. The aide of γ-cyclodetrin to the lecithin system allowed for the structure, which has a high potential for self-aggregation, to have an accelerated release of the drug. The effect of the γ-cyclodetrin on different emulsions was found to increase permeation; the increase was greater for lecithin-based emulsions then sucrose stearate-based emulsions. With information already known about individual enhancers, the possibilities for creating synergistic chemicals for transdermal delivery remain the greatest asset to improve permeation.

TDD APPROVED BY THE FOOD AND DRUG ADMINISTRATION

There has been an increased focus on the potential of TDD, as evident from the increase in the number of patents as well as scientific publications on TDD systems. Many drugs have been evaluated for TDD in prototype patches, in in vitro permeation studies using mouse, rat, or human skin, or have reached varying stages of clinical testing. Examples are listed in Table 12.2. Despite a wide array of TDD systems undergoing research and development, only a small percentage of the drugs reach the market successfully because of three limitations: difficulty of penetration through human skin, skin irritation and allergenicity, and clinical need. In addition, it is generally accepted that the best drug candidates for passive adhesive transdermal patches must be nonionic; must have low molecular weight (<500 Da), adequate solubility in oil and water (log P in the range 1–3), and a low melting point (<200°C); and must be potent (dose <50 mg/day and ideally <10 mg/day) [74–76]. Given these operating parameters, the number of drug candidates that fit the criteria may seem low. Nevertheless, with the development of novel technologies, such a constraint may be overcome.

Since the introduction of a TDD for scopolamine in 1981, several new products have been introduced. The US TDD market approached $1.2 billion in 2001 and was based on 11 drug molecules: fentanyl, lidocaine, prilocaine, nitroglycerin, estradiol, ethinyl estradiol, norethindrone acetate, testosterone, clonidine, nicotine, and scopolamine [77]. Barry [4] reported that 40% of drug delivery candidate products that were under clinical evaluation and 30% of those in preclinical development in the United States were TDD or DDD systems.

Examples of Food and Drug Administration (FDA)-approved transdermal patches and their applications are given in Table 12.4. Despite a plethora of candidate CPEs to choose from, all currently available TDD products adopt

TABLE 12.4
Examples of FDA-Approved Transdermal Patches, Their Applications, and the Mechanisms/Compounds Used for Penetration Enhancement

Drug	Application(s)	Example of Commercially Available Product(s)	Penetration Enhancement Effect and PEs
Scopolamine	Motion sickness	Transderm Scop	Occlusive effect
Fentanyl	Moderate-to-severe chronic pain	Duragesic	Occlusive effect
Lidocaine	Anesthesia	Lidoderm	Occlusive effect, urea, propylene glycol
Prilocaine	Anesthesia	EMLA anesthetic disc	Occlusive effect, polyoxyethylene fatty acid esters
Testosterone	Hormone replacement therapy	Androderm	Occlusive effect, glycerol monooleate
Estradiol/norethindrone acetate	Hormone replacement therapy	Combipatch	Occlusive effect, silicone, oleic acid, dipropylene glycol
Estradiol	Symptomatic relief of postmenopausal symptoms and prevention of osteoporosis	Alora, Climera, Esclim, Vivelle, Vivelle-Dot	Occlusive effect; Climera: fatty acid esters; Vivelle: 1,3-butylene glycerol, oleic acid, lecithin, propylene glycol, dipropylene glycol; Vivelle-Dot: oleyl alcohol, dipropylene glycol
Norelgestromin/ethinyl estradiol	Contraception	Ortho Evra	Occlusive effect, lauryl lactate
Nitroglycerin	Angina pectoris	Nitro-Dur, Nitrodisc, Transderm-Nitro	Occlusive effect, fatty acid esters
Clonidine	Hypertension	Catapres-TTS	Occlusive effect
Nicotine	Smoking cessation	Nicoderm CQ	Occlusive effect
Methyphenidate	Attention deficit hyperactive disorder	Daytrana	Occlusive effect
Selegiline	Depression	Emsam	Occlusive effect
Oxybutynin	Urge/urinary incontinence	Oxytrol	Occlusive effect

Note: EMLA, eutectic mixture of local anesthetic; PEs, penetration enhancers.

skin occlusion as the primary mechanism for penetration enhancement, perhaps due to its simplicity and convenience and the following effects on SC [78,79]: an increase in SC hydration and a reservoir effect in penetration rates of the drug due to hydration, an increase in skin temperature from 32°C to 37°C, and the prevention of accidental wiping or evaporation (volatile compound) of the applied compound.

FUTURE TRENDS

The protective function of human SC imposes physicochemical limitations to the type of molecules that can traverse the barrier. As a result, commercially available products based on TDD or DDD have been limited. Various strategies have emerged over the last decade to optimize delivery. Approaches such as the optimization of formulation or of drug-carrying vehicle to increase skin permeability do not greatly improve the permeation of macromolecules.

On the contrary, physical or mechanical methods of enhancing delivery have been more promising. Improved delivery has been shown for drugs of differing lipophilicity and molecular weight, including proteins, peptides, and oligonucleotides, using electrical (iontophoresis and electroporation); mechanical (abrasion, ablation, and perforation); and other energy-related techniques such as ultrasound and needleless injection [80].

Another strategy for penetration enhancement is to exploit the synergistic effects offered by combined techniques. Karande et al. [81] reported the discovery of synergistic combinations of PEs (SCOPE), which allow permeation of 10 kDa macromolecules with minimal skin irritation using a high-throughput screening method. Kogan and Garti [51] also showed that the combination of several enhancement techniques led to synergetic drug penetration and decrease in skin toxicity. In essence, the possibilities seem endless in the field of TDD and DDD.

CONCLUSION

TDD would prevent problems associated with the oral route as well as the inconvenience and pain associated with needle delivery and has thus competed with oral and injection therapy for the accolade of the innovative research area for drug delivery. Yet there remains a paucity of candidates for TDD or DDD to be marketed. The reasons are twofold: (1) most candidate drug molecules have permeation rates through the skin too low to ever reach a clinically satisfactory plasma level; (2) risk of skin irritation and allergic contact dermatitis may be increased by skin occlusion [79,82] and/or the application of potent PEs [81]. The ideal characteristics of PEs include the following [28]:

- Be both pharmacologically and chemically inert
- Be chemically stable
- Have a high degree of potency with specific activity, rapid onset, predictable duration of activity, and reversible effects on skin properties
- Show chemical and physical compatibility with formulation and system components
- Be nonirritant, nonallergenic, nonphototoxic, and noncomedogenic
- Be odorless, tasteless, colorless, cosmetically acceptable, and inexpensive
- Be readily formulated into dermatological preparations, transdermal patches, and skin adhesives
- Have a solubility parameter approximating that of skin [83]

Future studies on the mechanisms of penetration enhancement, the metabolic processes of chemicals within the skin, skin toxicity, as well as the development of novel technologies will improve our knowledge on penetration enhancement. While the current TDD and DDD technologies still offer significant potential for growth, next-generation technologies will enable a much broader application of TDD to the biopharmaceutical industry.

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13 Human Skin Buffering Capacity
An Updated Overview

Jacquelyn Levin and Howard I. Maibach

INTRODUCTION

The acidic character of skin was first mentioned by Heuss [1] and later by Schade and Marchionini [2], who introduced the term "acid mantle" for skin's acidic outer surface pH. The importance of skin's acidic character has been recognized as playing a crucial role in permeability barrier homeostasis, skin integrity/cohesion, and immune function [3–5]. Given the importance of the acidic skin pH for normal function and defenses, it is important for skin to be able to resist acidic/alkaline aggression to some extent (i.e., have buffering capacity) [6]. As we age and in skin disease, the baseline skin pH can increase, and our ability to buffer the change in skin pH can decrease [7]. This increase in pH and impaired skin buffering capacity can lead to dysfunctional barrier homeostasis and skin integrity/cohesion, increased likelihood for skin infection, and increased sensitivity/irritation to topically applied products [8].

This chapter briefly reviews the basic science of pH and buffering capacity and the deleterious effects of increased pH in the skin. In more detail, we discern which components of the stratum corneum (SC) are most likely responsible for skin buffering capacity and present the results of recent experimentation in this arena.

HOW DO WE DEFINE THE pH AND BUFFERING CAPACITY OF SKIN? HOW DO WE MEASURE THE BUFFERING CAPACITY OF SKIN?

Skin pH is defined and measured by the concentration of hydronium ions. A buffer is a chemical system that can limit changes in pH when an acid or a base is added. Buffer solutions consist of a weak acid and its conjugate base. A buffer has its optimum capacity when about 50% of the buffer is dissociated, or in other words, the pH of the environment is equal to its pKa of the buffering system [6,9]. The pKa is defined as the negative of the common logarithm of the acid dissociation constant (Ka) and is a measure of the strength of the acid.

When dilute aqueous acid or alkaline solutions come into contact with healthy skin, the change in pH is generally temporary, and the original skin pH is rapidly restored, indicating that skin has significant buffering capacity. Therefore, the buffering capacity of skin can be assessed by exposure of the skin to acids and alkali substances and monitoring the temporal changes in skin pH. This type of assessment of skin buffering capacity is termed an acid/alkali aggression (or resistance) test. Alkali/acidic resistance tests were commonly used in the 1960s to detect workers who were likely to develop occupational diseases in certain chemical work environments [6]. A mild variation of the alkali/acidic resistance tests, also called acid/alkali neutralization test, assesses how quickly the skin is able to buffer applied acids/bases without the occurrence of skin corrosion [10]. Experiments involving the repetitive applications of acid or base to skin demonstrate that the skin's buffering capacity is limited and may be overcome, as illustrated by the long time required for skin pH to return to baseline [10].

HOW DOES THE INCREASE IN SKIN pH AFFECT SKIN FUNCTION?

In a multicenter study concerning the measurement of natural skin pH, the average skin surface pH was 4.9, with a 95% confidence interval between 4.1 and 5.8 [9]. The ideal acidity for the SC is a pH of approximately 5.4 [11]. It is well known that an increased skin pH is detected in elderly skin starting anywhere from age 50 to 80 years [11], and in patients who develop skin disease such as atopic dermatitis [12]. Most likely, this decreased acidity in elderly skin is due to less efficient mechanisms for skin acidification and, more specifically, decreased NA+/H+ antiporter (NHE1) expression. The NHE1 is one of three of highly studied mechanisms for maintaining skin acidity and is assumed to be the predominant mechanism for maintaining skin acidity [13], while in atopic dermatitis, skin pH is thought to be altered due to multiple mechanisms such as decreased filaggrin and its breakdown [12].

Elevation of the skin pH alters multiple functions. Those discussed here include impairment of permeability barrier homeostasis, decreased skin integrity/cohesion, and increased susceptibility for microbial infection.

Impaired Permeability Barrier Homeostasis

An acidic pH is critical for permeability barrier homeostasis, in part because of two key lipid-processing enzymes,

B-glucocerebrosidase and acid sphingomyelinase, which generate a family of ceramides from glucosylceramide and sphingomyelin precursors and exhibit low pH optima [5]. An increased skin pH results in defective lipid processing and delayed maturation of lamellar membranes [13]. These lipids form multilamellar sheets amidst the intracellular spaces of the SC critical to the SC's mechanical and cohesive properties, enabling it to function as an effective water barrier [13].

This delayed barrier function allows easier penetration of topically applied products and delays barrier recovery after injury or insult to the skin [13,14].

Decreased Skin Integrity and Cohesion

An acidic pH also clearly promotes SC integrity and cohesion. In a neutral pH environment, there is an enhanced tendency for the SC to be removed by tape stripping (integrity) as well as an increased amount of protein removed per stripping (cohesion) [13]. The impaired SC integrity/cohesion is due to pH-dependent activation of serine proteases, which exhibit neutral pH optima [14]. Serine proteases become activated in the increased pH of skin and lead to the premature degradation of corneodesmosomes and, hence, increased desquamation [13,14].

Increased Susceptibility for Skin Infections

The acidic pH of the SC restricts colonization by pathogenic flora and encourages persistence of normal microbial flora. Pertinently elderly skin, intertriginous areas, and chronically inflamed skin display an increased skin pH [2] and, hence, reduce resistance to pathogens [11].

In summary, skin can have abnormalities in SC integrity/cohesion, permeability barrier homeostasis, and immune function due to increased skin pH. These abnormalities are attributable to the pH-mediated increase in serine protease-mediated degradation of corneodesmosomes, defect in lipid processing, and decrease in antibacterial activity, respectively.

WHAT COMPONENTS OF THE SC CONTRIBUTE TO THE BUFFERING CAPACITY OF SKIN?

Lipid Content/Sebum Production

Early experimentation hypothesized that the sebum contributes to the buffering capacity of skin in two ways: First, it protects the epidermis against the influence of alkali by slowing down the exposure and penetration of acids or alkalis applied to the skin [15,16], and second, the fatty acids in sebum may act as buffer system [17]. Lincke et al. [18] refuted this second hypothesis by demonstrating that the sebum had no relevant acid and a negligible alkali buffering capacity of around pH 9. Further challenging the hypothesis, a quicker neutralization was observed on delipidized skin than untreated skin [15,17]. However, the increase in buffer capacity after lipid removal was temporary and limited to the first few minutes.

Due to the negligible buffering capacity of sebum and to standardize experimentation (and limit interindividual and intraindividual variability), today, most neutralization experiments are performed after cleansing the skin with solvents, which remove most of the sebum, including fatty acids. However, assuming that the presence of sebum does slow down the penetration of topical insults, skin with a decreased amount of sebum will have a buffering system that is more easily overwhelmed with exposure to acids or alkalis.

Water

Vermeer et al. [19] first demonstrated the importance of water-soluble constituents to the skin's buffering ability. Vermeer et al. extracted the water-soluble constituents of the SC before experimentation by soaking the skin in water. He then demonstrated a significantly reduced neutralization capacity, indicating that water-soluble substance constituent(s) of the skin are major contributors to the buffering capacity [19].

The significance of water-soluble constituents of the epidermis to the buffering capacity of skin further supports the theory of minimal contribution from the sebum of skin due to its lipid-soluble nature [15].

Proteins

We have learned from experimentation that free amino acids (AAs) in the water-soluble portion of the epidermis play a significant role in the neutralization of alkalis within the first 5 min of alkali exposure [18]. In addition, Piper [20] found a good buffering capacity of skin at a pH between 4 and 8, with an optimum at 6.5, corresponding well to the pKa of AA in the skin.

Despite the general agreement about the role of AAs in the neutralization of alkalis, the AAs, which are the key buffering agents, remain an open question. There is, however, information on the AA composition of the upper SC as reported by Spier and Pascher [21]. Spier and Pascher reported that the free AAs of the SC accounts for 40% of the water-soluble substances extracted from the SC removed by tape stripping [18,21,22]. From the AAs present, 20%–32% was serine, and 9%–16% was citrulline. Aspartic acid, glycine, threonine, and alanine were 6%–10%. The smallest concentration of AAs accounted for glutamic acid at 0.5%–2%.

It is our hypothesis that the water-soluble, free AAs on the skin surface may originate from five possible sources.

Eccrine Sweat

Sweat contains 0.05% AAs, which remain on the surface of the skin after evaporation. The specific AAs found in sweat were not investigated.

Degradation of Skin Proteins

Degradation of skin proteins, including proteins constituting the desmosomes, may be a source for AAs such as serine, glycine, and alanine.

Hair Follicles

Citrulline is recognized as a constituent of protein synthesized in the inner root sheath and medulla cells of the hair follicle. Specific proteases release citrulline. Citrulline is also found in the membrane of the corneocytes [23].

Keratin Hydrolysates

The contribution of keratin to the buffering capacity of skin remains questionable. Keratin is an amphoteric protein with the ability to neutralize acids and alkalis in vitro [8–11,16,23,24] and hence may participate in skin's buffering capacity. Scales scraped from normal skin have been shown to bind small amounts of alkali in vitro [21,25]; however, Vermeer and coworkers showed that water-soluble constituents of the epidermis participate more in skin's buffering capacity than the insoluble constituents of the skin, such as keratin.

While insoluble keratin filaments on the skin may have limited buffering capacity [15,22], keratin hydrolysates and free AAs might contribute to the water-soluble portion of the epidermis. Yet, the AA composition of keratin [26,27] does not correspond with AA composition found in the water-soluble portion of the SC [18], which implies that keratin is not a major contributor to the pool of free AAs.

Despite little evidence of keratin's role in the buffering capacity, a modifying action of keratin is assumed [16]. Without an intact keratin layer, neither a physiological surface pH nor normal neutralization capacity can be maintained [28]. Further research remains to be conducted to determine keratin's role in the buffering capacity of the epidermis.

Keratohyalin Granule Histidine-Rich Protein

The pool of free AAs, urocanic acid, and pyrrolidone carboxylic acid in mammalian SC has been shown to be derived principally or totally from the histidine-rich protein of the keratohyalin granules. The time course of appearance of free AAs and breakdown of the histidine-rich protein are similar, as are the analyses of the free AAs and the histidine-rich protein. Quantitative studies show that between 70% and 100% of the total SC-free AAs are derived from the histidine-rich protein [25].

Eccrine Sweat Glands

Eccrine sweat initially accelerates the neutralization of alkalis [10,19]. Spier and Pascher [21] suggest that the main buffering agents of sweat are lactic acid and AAs (as discussed above). The lactic acid–lactate system in sweat has a highly efficient buffering capacity at a pH between 4 and 5 [16]; however, lactic acid's role in the buffering capacity of skin has not been established through experimentation [19].

SC Thickness

Differences in thickness of the SC may explain the interindividual differences in buffering capacity [29]. It has been demonstrated that the thicker the SC, the better the buffering [10,19]. This increase in buffering capacity in thick skin is likely secondary to thick SC providing a better barrier for acids/bases [29]. Current technology allowing more accurate SC measurement may help in clarifying this point [30].

CO_2

Little is known about the role of CO_2/HCO_3^- in skin's buffering capacity. Burckhardt's [10] studies were the first to suggest that the CO_2 diffusing from the epidermal layer may be responsible for neutralizing alkali in contact with skin. He demonstrated [10] that when a 5 min alkali neutralization experiment is repeated subsequently several times on the same skin area, the neutralization times become longer and eventually reaches a constant value. He suggested that the shorter neutralization times at the beginning were due to acids present on the skin surface rapidly neutralizing the alkali and that after successive alkali exposure, the endogenous acids on the skin surface are consumed and diffusing carbon dioxide takes over the role of neutralizing the alkali.

Burckhardt's hypothesis made over fifty years ago concerning the role of carbon dioxide as a buffering agent in skin was accepted by others despite the rather weak experimental evidence presented in the original paper [18,20,31,32]. In the original publication, the skin pH neutralization time after lipid removal (with soaps or neutral detergents) was postulated to be the consequence of a greater diffusion of CO_2 but never proven or quantified.

Knowing that several authors considered CO_2 a relevant contributor in alkali neutralization without having quantitative data to sustain their hypothesis, Vermeer et al. [19] demonstrated that CO_2 is unlikely of great importance for alkali neutralization on skin during the first few minutes of the neutralization process. However, Piper [20] analyzed the neutralization process for up to 1 h and concluded that, for the first half hour, alkalis are neutralized on the skin by the skin's own amphoteric substances (such as AAs) but that in the second half hour, diffusing carbon dioxide takes over. According to Piper, "the longer the contact between skin and alkali, the greater the importance of CO_2." Supporting these discoveries are the relatively limited activity of the Krebs cycle and the low level of carbon dioxide detected in the epidermis, suggesting that a minimal amount of CO_2 would be available for neutralization [20]. Further studies are needed to help to clarify the relevance of CO_2 in skin's buffering capacity.

Clearly, the above studies fail to provide quantitative support for their conclusions concerning CO_2 as a relevant buffering agent. More likely, the constant neutralization time after successive alkali exposure may be related to the destruction of the skin barrier [19]. Whether the destruction of the skin barrier and the subsequently increased diffusion

of CO_2 may contribute to the neutralization of alkali is to yet be determined. However, recent experimentation discussed elsewhere in detail has shown that the dermis has considerable buffering capacity [33].

WHAT RECENT EXPERIMENTATION HAS BEEN PUBLISHED REGARDING HUMAN SKIN BUFFERING CAPACITY?

A majority of the research presented prior to this section is half a century old. In this section, we present more recent investigations in regard to human skin buffering capacity.

In 2008, Ayer and Maibach [34] assessed the possibility of using an in vitro model for evaluating skin buffering capacity. In this study, a model base, NaOH, is topically applied to cadaver skin in three different concentrations (0.1, 0.05, and 0.025 N). pH readings were taken at baseline; immediately after base application; and then at 5, 10, 15, 20, and 25 min after application. After the last reading, the solution was removed, and pH readings were taken immediately and after 5, 10, 15, 20, and 25 min. This procedure was repeated three times using the same skin samples. Unexposed cadaver skin and cadaver skin with deionized water were used as a control.

Ayer and Maibach found a significant difference in the buffering capacity of the cadaver skin with successive applications ($P < .0001$). The different NaOH solutions all demonstrated buffer capacity for the two initial successive applications and then showed a significantly diminished buffering ability with the third remaining subsequent application of base. The controls showed no significant variation in pH throughout the experimentation. These results imply a decrease of skin buffering capacity with successive applications of NaOH. Interestingly, the decrease in buffering capacity after successive applications did not differ between the concentrations of base solution used [34].

After the removal of the alkali, the cadaver skin slowly returned towards baseline skin pH. The ability to restore pH in this experiment did not decrease with successive applications or differ between the three NaOH strengths. Therefore, it is a possibility that the buffering capacity of the skin was overwhelmed in the first portion of the experiment when successive applications of base were applied, and only when the base was removed for a significant period of time were the mechanisms to restore skin pH able to take effect [34].

Ayer and Maibach [34] offer a good introductory in vitro model adequate for future experimentation and investigation into skin buffering capacity. In 2009, Zhai et al. [35] used the same in vitro model as introduced by Ayer and Maibach [34], to measure the skin buffering capacity against hydrochloric acid (HCl, a model acid) and sodium hydroxide (NaOH, a model base) at concentrations of 0.025, 0.05, and 0.1 N. This experimental design not only serves to verify the reproducibility of the in vitro model proposed by Ayer and Maibach in 2008 [34] but also provides a comparison of human skin buffering capacity upon exposure to acids and bases. pH values of all solutions used were also reported in this experimentation. This is helpful for future experimentation where buffering capacity of chemically different acids and bases can be compared and may discern if the chemical composition of the acid or base in addition to the pH of the solution affects skin buffering capacity.

The results of this experiment showed changes in pH after applications of base or acid that correlated with the increasing concentration of acid or base in the applied solution (i.e., the highest concentration of acid or base caused the largest change in pH). This is not in accord with the previously mentioned study by Ayer and Maibach [34], which observed no evidence of a difference between the three strengths of NaOH and suggested that lower concentrations should be examined. Also, in contrast to the experiment by Ayer and Maibach, both controls, a phosphate buffer solution (pH = 7.46) and water (pH = 7.41), significantly elevated skin pH ($P < .05$) following the washing procedure. This increase in pH may be explained by their alkaline characteristic when compared with untreated acidic skin. Other studies have also shown that the use of plain tap water increases skin pH up to 6 h after application before returning to its "natural" value [35,36]. The reason for the difference between the two experiments remains unclear.

When comparing pH patterns of acids and bases after solution removal, Zhai et al. [35] reported a significant difference. The authors found that the skin pH normalized relatively faster with acid application when compared with base application, and for all cadaver skin exposed to a base, the change in the pH values was significantly greater ($P < .05$) at all time points postwashing compared to the acid-exposed skin. This may imply different inherent buffering mechanisms [35] and perhaps different skin tolerability between bases and acids.

In 2012, Zheng et al. [33] investigated the buffering capacity in three skin layers: the SC, viable epidermis, and dermis. Zheng et al. used the same in vitro technique on cadaver skin as used in the experiment by Zhai et al. [35] and Ayer and Maibach [34] to evaluate the buffering capacity of different skin layers. Viable epidermis was exposed by removing the SC using 40 continuous tape strippings. The dermis was exposed by heat separating the epidermis in the water bath for 30 s at 60ºC [37].

NaOH and HCl solutions at 0.025, 0.05, and 0.1 N were applied to the skin layers (3.18 mL/cm^2). After 30 min, the skin was washed with 1 mL deionized water. Transepidermal water loss (TEWL) and pH measurements were conducted at baseline (before contact with acid or base) and at 0, 10, and 30 min postexposure, and they were continued at 0, 10, and 30 min postwashing [33].

The dermis demonstrated lower pH values at 0 and 10 min post-NaOH exposures in comparison with the intact SC ($P < .001$ at each concentration); different NaOH concentrations presented a similar trend. This observation indicates that within a short time span post-base exposure, the dermis demonstrates the strongest buffering capacity among all three layers [33]. At 30 min post-base dosing, intact skin demonstrated the strongest buffering capacity, while the

dermis was ranked the weakest ($P < .01$ compared with the other two layers). At 30 min postwashing, the intact skin's pH values declined faster than the other layers ($P < .01$ at 0.1 N). However, at that time, the layers did not present a significant difference in the pH values compared with the blank control's pH reading ($P > .05$) [33].

The ability of HCl to modify skin pH was comparable with that of NaOH. For HCl, similar to NaOH, the dermis demonstrated the strongest buffering capacity, while at 30 min, intact skin presented the greatest buffering ability [33].

These results from Zheng et al. [33] revealed that skin buffering capacities of different skin layers differ substantially from each other. Future experimentation may help expose specific reasoning and/or mechanisms involved as well as help establish clinical relevance.

Taking a look at these three experiments discussed above, using similar in vitro models to investigate skin buffering capacity, the results demonstrate that cadaver skin retains its buffering capacity and mechanisms involved in restoring skin pH. Cadaver skin before experimentation is stored frozen at −25°C. Freezing the skin at such a temperature could compromise the buffering capacity when compared to in vivo living skin [35]. This might be the limitation of cadaver skin (especially the frozen skin), but it needs to be further investigated to discern how great the difference could be.

The authors feel that the reproducibility of the model also needs to be investigated further as each of the three experiments had slightly conflicting results. The first experiment by Ayer and Maibach [34] found no significant difference in pH values between the three concentrations of base, while Zhai et al. [35] found significant differences. Also, while Zhai et al. [35] found a significant difference in the amount of pH change after exposure to acid versus base, Zheng et al. [33] found similar modifications in the skin pH. In addition, there were also differences among the controls of each experiment. Whether these differences are from a lack of reproducibility or due to the slight differences in the methodology or statistical analysis remains unclear.

DISCUSSION AND CONCLUSION

The pH of skin is a physiochemical property that influences the health of skin in terms of skin barrier homeostasis, skin integrity/cohesion, and susceptibility to infection, to name a few. Because of the importance of skin pH in skin health, the skin must have mechanisms that participate in buffering capacity to help decrease alterations in skin pH.

Skin's exquisite buffering capacity has been widely studied in vitro and in vivo, yet further research needs to be completed to better understand the exact mechanisms that are responsible for the buffering capacity of skin.

The experimentation reviewed here suggests that AAs are primarily responsible for the neutralization capacity of skin. The exact sources of the AAs as well as the types of AAs that are primarily responsible for the neutralization capacity still remain rather speculative. Additional components of the epidermis such as sebum and CO_2 seem not to significantly participate as buffering agents of the epidermis, yet they still may play a role in the protection of skin from the harm of acids and bases.

In the previous edition of this book, we presented the idea that the mechanisms responsible for the buffering capacity of skin and the endogenous mechanisms for restoring and maintaining skin pH may be related. This theory is supported by the discovery that 70%–100% of AAs of the SC are derived from the degradation of histidine-rich protein in keratohyalin granules, which is also one of the essential pathways involved in maintaining skin pH [3]. This theory is further supported by the increased pH and decreased buffering capacity that is seen in certain skin disease [26] and in elderly skin [7].

From more recent experimentation, we have learned that skin pH and its buffering capacity can be easily measured utilizing an in vitro model, and this model may partially replicate the response of in vivo skin buffering capacity. While this model has limitations and cannot completely replace in vivo studies, the in vitro model may be beneficial for pharmacologic and toxicologic studies, as well as for defining mechanisms.

Taken together, we interpret this rich experimental literature as leading the way to utilization of contemporary methods to further refine our insight into skin's buffering capacity. This capacity, when fully understood, may lead to the potential not only for decreasing the threat of exogenous acids and bases to aged skin but also for establishing experimental bases for optimal pH in many cosmetic, pharmacologic, metabolic, and toxicologic situations.

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14 Skin pH and Skin Flora

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INTRODUCTION

The skin, being the largest organ, covers the entire exterior of the body and thus forms a protective barrier between the human body and its environment. It has a complex and dynamic microbial ecosystem; some areas are moist like a rainforest, and others are dry like a desert. The tough and dry exterior signifies the physical character of the skin covering most parts of the body. The uppermost layer of the skin is a multilayered structure called the stratum corneum (SC). The top three to five layers of SC undergo progressive desquamation. The morphology and thickness of SC is different at various body sites [1–3]. The skin maintains characteristic physicochemical features such as structure, hydration, temperature, pH, oxygen, carbon dioxide gradients, and so forth. Changes in any of these features impact the overall physiology of the skin. The acidic nature of the skin was discovered in 1892 by Heuss [4] and was later validated in 1928 by Schade and Marchionini [5], who underlined acidity as its protective feature and called it the "acid mantle." Current literature indicates that the skin surface pH ranges from 4.0 to 7.0, but it is mostly acidic, between 5.4 and 5.9 [6].

The skin surface pH plays an important role in skin physiology and directly or indirectly influences various other factors such as composition of SC lipids, SC hydration, barrier function of the skin, and the skin's microbiota [7–15]. The acidic pH of skin provides optimal pH for enzymes, for example, glucocerebrosidase [16] and phospholipases [17], to work on extracellular lipids and on a vitamin A–esterifying enzyme [18]. Conversely, the acidic pH of the skin has also been shown to accelerate the repair process of barrier function when damaged with acetone or extensive tape stripping [19]. Also, the acidic skin pH has been shown to correlate with enhanced resistance against sodium lauryl sulfate (SLS)–induced irritant dermatitis [15,20].

An intraday variation (circadian rhythm) of skin pH was reported at some body sites, for example, shin, forearms, and axilla [21,23]. The skin pH was higher (pH 5.3) in the afternoon and lower (pH 4.9) at night [21–23]. Investigations on seasonal differences in skin surface pH are limited [24]. During summer, the pH of the skin surface is usually 0.5 units below the pH values during the rest of the year [25].

Acidic pH of the skin is the result of the physiology of the human body, which, in turn, regulates endogenous skin flora [15,26,27]. The skin further provides a habitat for resident microbiota, which, under normal conditions, protects skin from pathogenic organisms. Soon after birth, bacteria start to colonize the skin and other body sites. Despite wide variations in environmental conditions, the skin is capable of maintaining a stable microbial ecosystem [28]. Skin tends to be cooler than normal body temperature, slightly acidic, and mostly dry, whereas most bacteria prefer neutral pH, temperature of 37°C, and moisture for optimal growth. Therefore, skin's microenvironment greatly dictates the microbial spectrum and population density. Some of the resident bacteria play an active role in maintaining the acidic pH of the skin and preventing colonization by pathogenic bacteria.

ORIGIN OF THE SKIN pH

It is now well accepted that the "acid mantle" of the SC is very important for normal skin physiology and its bacterial flora. What makes the skin surface "acidic" is still not fully understood [14]. Many endogenous and exogenous factors have been proposed to influence the skin pH. For example, eccrine and sebaceous secretions, anatomic sites, moisture, proton pumps, genetic predispositions, and age [10,11,14]. Active and passive energy bioenergetic processes have also been suggested as sources for the acidic pH of the skin [11,29,30]. For example, lactic acid, produced by a passive metabolic process, acidifies the superficial layers of skin [31]. Other important components of passive metabolic processes include free fatty acids, cholesterol sulfate, urocanic acid, and pyrrolidone carboxylic acid, which also contribute to the skin's acidic pH [32]. Active proton pumps (e.g., the sodium/hydrogen anion exchanger proteins or NHE1) present in the membranes of the lamellar bodies are responsible for acidification of the intracellular space in the lower layers of SC [33]. Free fatty acids generated by lipases of bacterial and/or pilosebaceous origin are partly implicated in the genesis of the acid mantle [34].

The pores of the skin are made up of a combination of sebaceous and sudoriferous glands. When in balance, the combined excretion of oil and sweat from these pores has a pH of about 5.5. However, occlusive dressing has been shown to significantly increase the skin surface pH, moisture content, and bacterial density [35], indicating the role of endogenous factors in these changes. Exogenous factors such as skin cleansers, cosmetics, occlusive treatments, and topical antibiotics/antiseptics have been shown to alter the skin surface pH [36–39]. Altered skin pH has been associated with dermatological conditions such as irritant contact dermatitis, atopic dermatitis, ichthyosis, acne vulgaris, and *Candida albicans* infections [31,40–42].

TABLE 14.1
Gender Differences in Skin pH

	pH		
Anatomical Sites	**Female**	**Male**	**Reference**
Forehead	5.4–5.8	5.1–5.5	62
Forehead	5.73	5.51	65
Axilla	5.80–5.94	6.58–6.67	70
Volar forearm	4.8–5.8	4.3–4.7	54
Volar forearm	5.60–5.88	4.76–4.93	67
Volar forearm	4.97–6.09	5.44–6.16	63
Back of wrist	5.84	5.56	65
Back skin surface	5.43–5.73	4.96–5.12	22

There could be many factors affecting the overall pH of the skin depending on the subject, body sites, and other biochemical factors. The skin of newborns and small infants differs from that of adults with regard to some characteristics [43,44]. The pH of newborn skin is higher (e.g., 6.6 ± 0.25) than that of adults [45–48]. The pH of the skin differs at various anatomical sites (Table 14.1); for example, the superficial pH on the nose was the lowest among the regions tested [49]. Regions with higher *Staphylococcus epidermidis* concentrations are slightly more acidic; also slightly higher pH was reported in areas with higher moisture, such as intertriginous areas (axillae, inguinal and submammary folds, and finger webs) [6,14,50].

A slight person–to–person variation in skin pH occurs because not everyone's skin is exposed to the same conditions, such as weather and harsh detergents. In a large multicenter study, the skin surface pH of the volar forearm was assessed before and after refraining from showering and cosmetic product application for 24 h [15]. The average pH dropped from 5.12 ± 0.56 to 4.93 ± 0.45. The authors concluded that the "natural" skin pH is, on average, 4.7, which is below the generally reported pH range between 5.4 and 5.9. Interestingly, the study also suggested that showering with plain tap water in Europe, which has a pH of around 8.0, could increase the skin pH for >4 h [15]. The skin surface pH varied at different locations (Table 14.2), but also, the lipid composition in the SC differs as a function of skin region and could influence the pH profile across the SC [51,52]. Other reports [29,31,53,54] suggest that the pH of the skin follows a sharp gradient across the SC, which is possibly involved in controlling enzymatic actives and skin renewal [55].

AGE, RACE, AND GENDER DIFFERENCES

Reports on the differences and/or similarities in the skin surface pH among various ages, races, and genders are scarce. The newborn baby's skin pH, recorded to be neutral, soon becomes acidic within a month [56]. The higher skin pH in infants is well documented [45–48] and may be associated with the different chemical composition of the skin lipids [44]; however, within a month, the baby's skin attains an acidic pH similar to adult skin. The available literature on skin surface pH indicates that the pH remains constant between 18 and 60 years of age [21,57,58]. Men and women over the age of 80 years showed increased pH values [57,59]. In the older age group over 70 years of age, the mean pH value of the forehead was measured to be 5.6 as opposed to a pH value of 5.3 in the younger age group [59]. Anatomical differences in pH have also been reported (Table 14.2), which also influence the microbial composition and density as stated below and summarized in Table 14.3. In one of the studies [57], among 89% of the subjects, the skin surface pH on the cheek was higher than that on the forehead. In subjects younger than 80

TABLE 14.2
pH Values on Human Skin at Various Locations as Reported in Selected Literature

Skin Surface pH	**Location**	**Reference**
4.0–5.5	Forehead	50,61
4.0–5.5	Forehead and cheek	59
4.1–4.2	Forearm	72
4.4	Volar forearm	63
4.4–5.1	Volar forearm	66
4.5–5.6	Forehead	142
4.2–4.5	Forearm	142
5.5–5.8	Forehead	65
5.56–5.96	Back of wrist	65
4.8–5.0	Volar forearm	64
4.93–5.12	Volar forearm	15
5.0–5.4	Volar forearm	67
5.0–5.5	Ventral forearm	30
5.4–5.9	Lower arm	6
5.5–5.8	Forearm	63

TABLE 14.3
Normal Skin Microflora in Area With High Density

Bacteria	**Area**
Staphylococcus epidermidis	Upper trunk
Staphylococcus hominis	Glaborous skin
Staphylococcus capitis	Head
Staphylococcus saccharoliticus	Forehead/antecubital
Staphylococcus saprophyticus	Perineum
Micrococcus luteus	Forearm
Corynebacterium xerosis	Axilla, conjuctiva
Corynebacterium minutissimum	Intertriginous (e.g., axilla)
Corynebacterium jeikeium	Intertriginous (e.g., axilla)
Propionibacterium acnes	Sebaceous gland, forehead
Propionibacterium granulosum	Sebaceous gland, forehead, axilla
Propionibacterium avidum	Axilla
Brevibacterium spp.	Axilla, toe webs
Dermabacter spp.	Forearm
Acinetobacter spp.	Dry area
Pityrosporum spp.	Uppermost part of sebaceous gland follicle

years, the average pH ranged from 4.0 to 5.5 on the forehead and from 4.2 to 5.9 on the cheek [57]. In another study, facial pH at different sites did not differ significantly between subjects with and without acne [60]. Unlike in women, in men, the area close to the wrist had significantly lower pH values compared with the proximal sites [61].

Skin pH has been reported to vary with race, gender, and genetic background. Black people have a lower skin surface pH compared to Caucasians [58,68], which has been attributed to pigmentation effects [29]. Gupta et al. [65] measured the skin surface pH of 55 brown–skinned Indians comprising 30 males and 25 females in the age range of 12–58 years. Indian skin was slightly more alkaline, though the data are not definitive because the groups tested were small [65]. The average pH values on the forehead of males and females were 5.51 and 5.73, respectively.

The differences between male and female skin surface pH have not yet been fully established. Published studies show contradictory results (Table 14.1). In most studies, skin pH was significantly more acidic in men when compared to women [60,64,68–71], while other studies [63] showed the reverse situation, that is, more acidic pH for women rather than men, and others showed no gender differences [21,57,58,61,72]. A study conducted in India found that male skin was slightly but significantly more acidic than female skin. The same study [65] reported that the pH values at the axilla, umbilicus, palm, foot, sole, and cheek were consistently higher than those at the scalp, forehead, retroauricular and popliteal fossae, anterior arm, anterior forearm, posterior neck, back, dorsum of the hand, anterior leg, and anterior thigh. The highest pH was recorded in the axilla (5.98 for male and 6.00 for female). The study notes that the high density of both sweat glands and bacterial flora leads to a high skin pH, whereas lower pH was observed in areas with a high concentration of sebaceous glands and bacterial flora.

In the underarm region, the skin surface pH is significantly different between men and women; more acidic pH values were found in women than men [71]. The baseline pH value before washing was 6.58 ± 0.63 (right armpit) and 6.67 ± 0.65 (left armpit) in men versus 5.80 ± 0.53 (right armpit) and 5.94 ± 0.62 (left armpit) in women. Interestingly, washing of armpits with pure tap water further increased the difference between male and female pH values [71], the reasons for which remained unclear and warrant a validation study. The pH difference between right and left armpit was not statistically significant [71] or similar to some earlier reports of no difference in skin pH between the dominant and nondominant forearms or hands [61,62].

One of the prevalent hypotheses about the role of skin pH is its putative importance in antimicrobial defense [63,73]. Possible explanations include the following: (1) The top layer of the skin is very dry and densely packed, which makes this first line of defense inhospitable to many bacteria. (2) Salty secretions from sweat glands create an environment that is hyperosmotic and thus unfavorable for bacteria. (3) Normal flora grow best at a more acidic pH, whereas pathogenic bacteria, such as *Staphylococcus aureus*, grow best at neutral pH [74]. A more acidic pH helps to protect skin against colonization by nonresident and pathogenic bacteria because many of them survive well in a narrow pH range near neutral.

The acidic condition of the skin is caused by secretions from sweat glands, skin oil, and the breakdown of fatty acids by *S. epidermidis*. Thus, resident microflora are partly responsible for the acidic pH of skin. A multicenter study also found that the acidic pH of the skin surface (4.0–4.5) keeps the resident bacteria attached to the skin, whereas an alkaline pH [8–9] increased the dispersal of bacteria from the skin [11,15,27]. The importance of pH for antimicrobial function is further supported by neonatal eczematous and atopic skin, which displays a neutral pH [41,75,76].

SKIN FLORA

The skin makes up the largest organ (about 2 m^2 skin surface in an average human adult) and provides an intricate habitat for a complex microbial ecosystem comprising resident and transient microflora: mainly bacteria [77,78] and, to a lesser extent, fungal and possibly viral agents. The bacteria–skin relationship can be commensal, symbiotic, or parasitic relative to the host's overall physical and immune status. Persistent colonization is the result of alterations in the host's immune status, leading to a significant impact on the balance of the bacteria–skin relationship.

The acid mantle, levels of mineral and moisture, and use of skin cleansers and cosmetics influence the growth and maintenance of resident flora. In turn, the state of resident flora influences the acquisition of transient bacteria [77]. This acid mantle, a fine film with a slightly acidic pH on the surface of the skin, provides a protective barrier to the skin. The microbial population dynamics on various parts of the skin are determined by the anatomical location, the amount of sebum and sweat production, local pH, humidity, temperature, light exposure, and so forth [71,79]. Host factors such as age, immune status, hormonal status, and other habits also influence the composition and density of the skin flora [80,81]. The development of bacterial flora on skin from birth to adulthood has not been systematically studied. Recently, it has been reported that the microbial colonization begins immediately after birth [82] but is not fully established within a few weeks or months and evolves over the first year and beyond [83]. During the prenatal stage, the skin remains sterile but soon becomes colonized by bacteria after birth. Not all bacteria are welcome onto the skin. Skin allows the colonization and growth of those bacteria that protect the host from pathogenic bacteria both directly and indirectly. These bacteria can act by producing antibiotics (e.g., bacteriocin) or toxic metabolites, inducing a low reduction oxidation potential, depleting essential nutrients, preventing attachment of competing bacteria, inhibiting translocation, and degrading toxins [81,82].

Microbial status on skin can be temporary or transient, short–term resident and long–term resident biota. Establishment of a resident status depends on the ability of the bacteria to adhere to the skin epithelium, grow in a relatively

dry and acidic environment, and establish a relationship that is more mutualistic than commensalistic [11,15,84]. Bacterial adhesion or detachment from the skin could be mediated by (1) specific interactions via lectin or sugar binding, (2) hydrophobic interactions and (3) electrostatic interactions [85,86]. Hand washing with a skin cleanser containing microbial antiattachment ingredients has also been shown to prevent bacterial adherence to skin, which may work via electrostatic interaction [87]. A recent study [15] where washing of the volar forearm with 1% lactic acid (pH 3.0) was compared with washing with 1% sodium carbonate (pH 11.0) solution. The study revealed that under acidic conditions, the dispersal rate of the resident bacteria from the volar forearm was much lower than under alkaline conditions, suggesting the role of electrostatic interaction between bacteria and positive charges of the skin under acidic pH. The difference in dispersal rate under acidic and alkaline pH has not been fully understood. Various explanations [15] are put forward for high dispersal rate under alkaline conditions: (1) under alkaline conditions, both keratins and the bacterial surfaces are negatively charged, resulting in repulsion; (2) the net negative charge of the keratins created by alkaline treatment would lead to the swelling of the skin, which may open up the sponge–like corneocytes, allowing the bacteria to diffuse to the surface. A laboratory–based study has shown that washing hands with plain soap spreads bacteria on the entire hands. It has also been reported that repeated washing did not diminish the number of bacteria [88]; therefore, the practice of rigorous preoperative washing of the hands in hospitals has been questioned [89,90]. Because of the inefficacy of washing regimens, especially in health care settings, selection of an effective skin cleanser for routine hand hygiene is very important [90,91].

Bacterial species commonly isolated from normal skin includes *Staphylococcus, Micrococcus*, *Corynebacterium*, *Brevibacterium*, *Propionibacterium*, and *Acinetobacter* [79,81,92,93]. *S. aureus*, *Streptococcus pyogenes*, *Escherichia coli*, and *Pseudomonas aeruginosa* are transient colonizers [93,94]. The Gram–negative bacteria are the minor constituents of the normal skin flora, and *Acinetobacter* is one of the few Gram–negative bacteria commonly found on skin. The presence of *E. coli* on the skin surface is indicative of fecal contamination. Yeasts are uncommon on the skin surface, but the lipophilic yeast *Pityrosporum ovale* is occasionally found on the scalp. Racial and gender differences are not fully examined [95].

MOLECULAR ANALYSIS OF SKIN MICROFLORA

Recent advances in genomic approaches have led to the identification of a much greater diversity of organisms than one could find using the conventional culture–based methods [96–98,100–102]. Gao et al. [96], using molecular techniques, have identified 182 species of bacteria on human forearm skin, of which 8% were unknown species that had never been described before. This study also shed some light on the gender differences in skin microbiota, the microbial mix, and the possible role of pH [61]. Roughly half of the bacteria identified in the samples represented the genera *Propionibacterium*, *Corynebacterium*, *Staphylococcus*, and *Streptococcus*, which are generally considered as the resident flora of human skin. Among the six individuals sampled, only four species of bacteria were present in all: *Propionibacterium acnes*, *Corynebacterium tuberculostearicum*, *Streptococcus mitis*, and *Finegoldia* AB109769. Interestingly, three bacterial species were found only in the male subjects: *Propionibacterium granulosum*, *Corynebacterium singulare*, and *Corynebacterium appendixes* [96]. A recent study on skin microbiota also suggested "*Staphylococcus*, *Corynebacterium, Propionibacterium, Micrococcus, Streptococcus, Brevibacterium, Acinetobacterium*, and *Pseudomonas*" as the resident human skin bacteria [102]. Overall, the skin microflora are diverse in nature. One survey using 16S ribosomal RNA (rRNA) gene phylotyping, identified 19 phyla and 205 genera from 20 distinct skin sites of 10 healthy volunteers [101]; using broad–range 16S rRNA genes, PCR–based sequencing of randomly selected clones identified eight phyla and 91 genera from the superficial volar forearms of six healthy subjects. In this study, *Actinobacterium*, *Firmicutes*, and *Proteobacterium* accounted for 94.6% of the clones. Using a pyrosequencing–based method, palmar surfaces of the hands of 51 healthy young adult volunteers were surveyed and shown to harbor more than 25 phyla [103]. This study observed a pronounced intrapersonal and interpersonal variation in bacterial composition; women had significantly higher diversity than men, and microbial composition was significantly affected by handedness [104].

Using a multiplexed bar–coded pyrosequencing approach, Fierer et al. [99] investigated skin flora of 18 different skin sites among healthy adults and identified the same three phyla (*Actinobacteria*, *Firmicutes*, and *Proteobacteria*) accounting for over 82% of the sequences. It is interesting to note that many skin bacteria are emerging as multidrug–resistant pathogens, and genetic profiling of the antibiotic–resistant reservoir would have greater epidemiologic and clinical implications [105,106].

There are certain limitations in utilizing the genomic technique in current form; for example, it does not discriminate between 16S rRNA derived from living versus dead organisms. Similarly, it does not differentiate between the transient and resident members of the skin microbiota. Another area that needs more in–depth research is the status of cutaneous microbial communities in health and disease conditions. In the past, dermatological studies using culture–based techniques were able to find associations between certain skin ailments and microbes [107,108]. Only recently, has research work in this area gained momentum. For example, characterization of skin's microbial communities in healthy individuals and in patients with psoriasis [109], atopic dermatitis [110], and acne [111] has been reported. Dekio et al. [110] investigated the skin microbiota in 13 patients with atopic dermatitis and 10 healthy controls, using terminal restriction fragment length polymorphism (RFLP) analysis of bacterial 16S rRNA genes.

The skin surface pH influences various factors for the growth of resident and pathological microorganisms

[7,11,71,112]. The acidic pH of the skin is regarded as one of the major factors in making the skin a less favorable habitat for bacteria [113]. A high density of bacteria was found in skin areas with less acidic pH, such as the genitocrural area, anal regions, toe webs, submammary fold, and axillae [55,71]. Those areas of the skin that are relatively dry and exposed have lower pH and lower microbial population density as well. For example, volar forearm skin has a bacterial population about 10^2–10^3 cfu/cm^2 (colony forming unit/cm^2) [63], compared to 10^5 cfu/cm^2 in the relatively moist underarm area [78]. Artificial occlusion of the forearm skin leads to significant changes in skin pH and in the composition and density of bacterial species [35,63]. For example, before occlusion, the skin pH value was 4.38, and after 5 days of occlusion, the pH increased to 7.05 [63]. Similarly, the average bacterial count before occlusion was 1.8×10^2 cfu/cm^2, which increased to 4.5×10^6 cfu/cm^2 on the fifth day [63]. It is evident that a moist skin environment promotes bacterial growth and colonization. The distribution and composition of bacterial species on the skin varies at different body sites (Table 14.3). In intratrigenous areas, the skin surface pH is somewhat higher, which, in turn, favors higher bacterial density [92,93].

The normal flora also act as a barrier to prevent invasion and growth of pathogenic bacteria [34,114]. The relevance of normal skin flora as a defensive barrier can be articulated with the finding that intensive use of antimicrobial skin cleansers could lead to an increased susceptibility to skin infections by Gram–negative bacteria [115–117]. A healthy growth and maintenance of the resident bacteria effectively prevent the colonization by transient bacteria (e.g., *E. coli*, *Pseudomonas*, coagulase–positive *S. aureus*, *C. albicans*). The skin's antimicrobial defenses include the mechanical rigidity of the SC, its low moisture content, SC lipids, lysozyme, acidity (pH 5), and defensins [29,118–120]. Studies suggest that increased enzyme activity of phospholipase A2 is related to the formation of the acid mantle in the SC [29,31].

PROTECTIVE ROLE OF ACIDIC pH OF SKIN

Besides other physicochemical roles of the skin pH, it is now generally accepted that the normal skin surface pH has a beneficial role in relation to skin microflora (Table 14.4). Acidic pH of the skin (pH 4.0–4.5) helps the resident bacterial flora remain attached to the skin and prevents cutaneous invasion by pathogenic microorganisms [7,8,15], whereas alkaline pH (8.0–9.0) is reported to promote dispersal of the bacteria [15]. Acids produced by bacteria also contribute to the local protective mechanisms. For example, *S. epidermidis*, *P. acnes*, *P. ovale*, and *Corynebacterium* produce lipases and esterases that break triglycerides to free fatty acids, leading to a lower skin surface pH and thereby creating an unfavorable environment for skin pathogens. Acidic pH of the skin also facilitates production of natural antimicrobial peptides, attributed to wound healing, and regulates the keratinization and desquamation processes [9,54,121–125]. The skin flora also produce proteinaceous or lipidic antibacterial compounds termed "bacteriocins." These bacteriocins are involved in controlling/regulating bacterial competition for survival in this microenvironment. For example, a bacteriocin—Pep 5—produced by *S. epidermidis* is particularly active against other staphylococci, specifically *S. aureus* [126]. Interestingly, the acidic pH of the skin boosts the activity of these antibacterial lipids and peptides, possibly by enhancing the interaction with the bacterial membrane [26,122,125,126].

TABLE 14.4
Effects of Skin pH on Skin Microflora

Effects	Reference
Acidic pH (4–4.5) keeps the resident flora attached to the skin.	15
Alkaline pH (8–9) promotes dispersal of bacteria from the skin.	
Less acidic pH promotes bacterial growth, especially Gram-negative bacteria and propionibacteria.	73,75
Skin *Candida* infection is more inflammatory when the SC was buffered to pH 6.0 versus 4.5, indicating that pH may mediate immune reaction to infections.	130
High pH in the axilla promotes high bacterial growth and malodor.	136
Acidic pH boosts the activity of antibacterial lipids and peptides.	26,121,124,125
Acidic pH facilitates production of natural antimicrobial peptides, wound healing, and regulating keratinization and desquamation processes.	9,54,122,123,124

EFFECTS OF THE SKIN pH ON SKIN FLORA AND PATHOLOGY

Cutaneous pH plays an important role in maintaining the normal bacterial flora of the skin and in preventing cutaneous invasion by pathogens [26,127]. The acidic pH of the skin surface has long been regarded as the result of exocrine secretions of the skin glands, which, in turn, are involved in regulating the skin flora. Furthermore, the pH gradients in deeper layers of skin indicate a close relationship among the barrier function, a normal maturation of SC, and desquamation [14,19,30]. Initially, work done in test tubes clearly demonstrated the effect of pH on bacterial growth [128,129]. The study found that *S. aureus* grew equally well at a pH of 5, 6, and 7; normal micrococci showed somewhat, but not significantly, better growth at a pH of 6 and 7 than at a pH of 5. On the other hand, aerobic diphtheroids grew significantly better at a pH of 7 than at lower pH levels [130]. The acidic pH of skin provides a balanced environment for the resident bacteria. Changes in the skin pH and other organic factors play a role in certain skin pathogenesis and in their prevention and treatment (Table 14.4).

P. acnes is a classical example of how a slight increase in the skin pH can facilitate the resident bacteria's becoming pathogenic. Under a pH of 5.5, growth of *P. acnes* is at its minimum; however, a slight shift toward alkaline pH would make it a more favorable environment, resulting in increased growth of this organism [129,131]. As mentioned earlier, prolonged occlusion of skin significantly affects the growth of the normal skin flora, skin pH, and the rate of transepidermal water loss (TEWL) and carbon dioxide emission [35,63].

A study has shown the relationship between a change in skin pH and its consequences in atopic dermatitis, particularly disturbances in skin barrier function and increased colonization with *S. aureus* [132]. However, other studies [122,133] have suggested that in atopic dermatitis, increased colonization by *S. aureus* and other bacteria could be associated with a decrease in sphingosine and ceramide production. In atopic eczema, not only was the skin surface pH significantly higher than in normal healthy skin [41,134], but also, the growth of *S. aureus* and exotoxin production were increased, which have been shown to induce eczema on intact skin [135].

Changes in the skin pH from acidic to alkaline could also be a risk factor for the development of candidal infections [130]. A laboratory–based study, where the right and left forearms were, respectively, buffered at a pH of 6.0 and 4.5, inoculated with a suspension of *C. albicans* and occluded for 24 h, showed more pronounced skin lesions with the higher pH, suggesting that the higher pH may increase yeast virulence and/or modulate the host's defense capability [66]. Yosipovitch et al. [67] found that the pH values in the intertrigenous skin among 50 non-insulin–dependent diabetic patients were significantly higher than in normal healthy volunteers and attributed the higher pH as a risk factor for candidal infection [66]. Patients on dialysis also showed significant increase in their skin surface pH.

In moist intertrigenous area such as the axilla, the pH is physiologically higher than other skin regions [78,92,121], which promotes the growth of local flora. It has been established that underarm odor is created by the action of indigenous bacteria on axillary apocrine gland secretions [78]. Application of a deodorant product showed significant reduction in axillary pH, which, in turn, inhibited the growth of underarm bacteria [136].

EFFECTS OF SKIN CLEANSERS AND COSMETICS ON SKIN pH AND FLORA

As mentioned earlier, there are many external factors that influence the skin surface pH. Some of the external factors include the use of soap, detergents, and cosmetic products. Long–term usage of these agents has been shown to alter the skin surface pH and, to some extent, affects the skin microflora at least for a short duration [37,136,137]. Alterations in skin pH could cause irritation or interfere with the keratinization process as well [11,138].

Frequent hand washing with soap may damage the skin and facilitate more bacterial colonization. In fact, water–and–soap washing of damaged skin was not effective in reducing bacterial contamination [139]. Use of an alkaline soap with a pH of 10.5–11.0 resulted in higher skin surface pH and marked increase in the number of *Propionibacterium*, but the counts of coagulase–negative *Staphylococci* were not changed much [48,113]. In acne–prone young adults, washing of facial skin with an alkaline cleansing agent was reported to cause more inflammatory reaction than the acidic syndet bar [42]. On the other hand, washing with an acidic skin cleanser (pH of 5.5) similar to the normal skin pH in adults increased the skin surface pH but significantly less than the alkaline soap [48,74,115,140]. At the forehead, there was a clear correlation between bacterial counts and the skin pH, both with *Propionibacterium* and *Staphylococci*, but on the forearm only, *Propionibacterium* count was higher with higher pH. The skin surface pH was significantly higher when neutral preparations were used. The number of propionibacteria was significantly linked to the skin pH [74]. The use of synthetic detergents with pH similar to the skin surface pH led to a rise in the skin surface pH for a shorter duration [36,42], and such temporary changes in skin pH were limited to the top layers of the SC [55].

Korting et al. [113] were among the first to examine the effect of different skin cleansing treatments on the bacterial flora and the skin surface pH in healthy volunteers [37,113] using a crossover clinical design. Volunteers in one group washed their foreheads and forearms with an alkaline soap twice daily for 1 min, and the other group used an acidic soap (synthetic detergent–based bar [syndet]). After 4 weeks, both groups switch their soaps, respectively, in a crossover fashion. The skin pH and bacterial density were determined at the beginning of the study and at the end of every week [113]. The pH was increased when alkaline soap was used first, and the pH dropped with the changeover to syndet. When syndet was used first, the pH dropped slightly and then increased when alkaline soap was used. Long–term use of syndet lowered the skin pH by about 0.3 units. In general, washing with alkaline soap resulted in an increase in skin pH and a marked increase in propionibacteria without any significant change in counts of coagulase–negative staphylococci.

A study by Lambers et al. [15] found the natural skin surface pH to be below 5, which is at the lower end of many studies reported to date in the literature. They assessed the pH on the volar forearm before and after refraining from showering and using any cosmetic products on the skin for 24 h. The baseline pH before taking a shower was 5.12 ± 0.56. After 24 h without any product application or contact with water, the pH value dropped to 4.93 ± 0.45. On average, the authors estimated the natural pH value of the volar forearm skin to be 4.7 [15], which is in contrast to general assumption that the average skin pH ranges between 5.0 and 6.0. Interestingly, the study also found that plain tap water with a pH of around 8.0 as generally found in Europe could increase the skin pH up to 6 h after application. It is important to note that with the advancement in skin research, new

ingredients are being introduced in skin cleansers to protect skin's natural physiology.

CONCLUSION

Since the first report in 1892 by Heuss [4] on the acidic nature of skin, significant progress has been made in the field of skin biochemistry/microbiology, yet a number of areas still remain to be fully explored. The exact origin of the skin's acidity is still being investigated, but published studies [10,11,32,33] appear to indicate that several endogenous factors, including the presence of lactic acid, free fatty acids, urocanic acid, and pyrrolidone carboxylic acid in sweat and sebaceous secretions are involved. The skin is the primary organ protecting the human body from external physical and chemical assaults. Overall, the skin surface is acidic, with subtle differences between races and genders. It is not yet clear whether the skin has an inherently acidic pH that provides a hospitable environment for certain organisms or whether the organisms are attracted by other factors to colonize the skin. In recent years, tremendous progress has been made in investigating the human microbiome using high–throughput pyrosequencing and other genomic techniques. Human skin microbiome studies have revealed high diversity in skin–associated bacterial communities on the human hand surface. Interestingly, women's hands showed significantly higher diversity than men. The microbial community's composition is also affected by handedness, gender, and time since last hand washing. These variations created a challenge in defining what constitutes a "healthy" bacterial community, which warrants further studies, including host and site specificities of the bacterial species on skin and their role (if any) in the pathogenesis of skin diseases. These new insights and understanding of the skin flora will advance our knowledge about microbe–skin interplay in the genesis of skin diseases, which could eventually lead to novel therapeutic approaches.

The acidic pH of the skin provides an optimal environment for resident bacteria and their enzymatic activities. Together, the acidic pH of the skin and the resident flora of the skin play an important role in maintaining skin health. The acidic pH of skin is a key factor in barrier function [14,19] and plays a key role in the mutualistic relationship with resident microflora [80,141–143]. It is well recognized that an increased skin surface pH may be associated with the pathogenesis or the severity of many skin disorders, including acute eczema, irritant contact dermatitis, atopic dermatitis, ichthyosis, acne vulgaris, and *C. albicans* infections [11,20,26]. It is becoming more evident that the repeated use of alkaline skin cleansing products, detergents, and even hard water (pH 8.0) can adversely affect the natural skin pH and disturb the normal flora. To maintain the normal physiology and microflora of the skin, use of cosmetics and skin cleansing products that do not alter the skin pH or adversely affect the skin flora should be considered. Skin cleansing formulations with known functional actives and ingredients would help to deliver skin health benefits. Additionally, more research on potential role of prebiotics and probiotics in regulating healthy skin flora and in maintaining optimal skin biochemistry is needed.

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15 The "Magic" Effects of Dermatologic and Cosmetic Vehicles

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INTRODUCTION

Topical treatment of the skin is as old as the evolution of man. Instinctively, we try to treat a skin injury or irritation with cooling or soothing substances. Even animals lick their wounds, trusting instinctively in the healing power of saliva. When did this archaic pattern of treatment take the gigantic leap from folk medicine to modern treatment and care of the skin?

Patients and consumers apply a wide spectrum of preparations to their diseased or healthy skin that range in their physicochemical nature from powders through semisolids to liquids and even patches. Over the centuries, a myriad of concoctions have appeared and evolved as science has refined and new materials useful in product formulation have been discovered. Despite this rational progress, formulators, marketers, clinicians, and consumers attach extraordinary importance and, not seldom, *magic* effects to both the vehicle (type of formulation, e.g., cream, liposome, ointment, solution) and the individual ingredients of the topical preparations. The recent hype around the blemish balm (BB) creams is a good example for how many tasks are incorporated into a product and how high the expectations of consumers may become. BB creams are promoted as *magic* multitaskers or all-in-one cosmetic products to replace serum, moisturizer, primer, foundation, and sunscreen and contain further cosmetic actives such as hyaluronic acids or vitamin C [1].

The following text examines some aspects of the evolution of topical pharmaceutical and cosmetic vehicles, their application, and future use in dermatology and cosmetics.

TOPICAL TREATMENT—A BRIEF RETROSPECTIVE

In the classical European tradition of medicine (stretching, roughly speaking, from Ancient Greece to the early Renaissance), local treatment of the skin with medicines made no sense under the prevailing theory of humoral pathology. All skin diseases were believed to be secondary symptoms of circumstantial relevance, at most, to the diagnosis of internal disorders. Physicians left the treatment of diseased or damaged skin to the barber–surgeons (internal medicine was the domain of the true physician or *medicus*, while surgery was left to the barber–surgeon or *chirurgus* who, because he was not actually a doctor, was inferior in rank).

The transition from classical to modern, scientific medicine probably began during the Renaissance (15–16th centuries), but it took centuries for effective topical treatments to become widely accepted. The treatment of syphilis with mercury ointments illustrates this point. This treatment was probably common enough in the fifteenth century (it had been taken over from Arabic medicine), but the rationale behind it was still derived from humoral theories of pathology. In medicine's subsequent turbulent development of the nineteenth century, almost every conceivable substance was used as a dermatological agent—for example, crushed scorpions, human excrement, and pulverized Egyptian mummies, to name only the most extreme. Presumably, some success was achieved from these efforts.

It was only in the nineteenth century that a smooth transition from such empirical treatment to a more cognitive approach to topical therapy was achieved. Antiparasitic, disinfectant, and antifungal agents were the first milestones in this medical evolution, even though these topical agents often exacted a heavy toll in the form of adverse effects [2]. With publication of the *Histopathologie der Haut* (*Histopathology of the Skin*), the Hamburg dermatologist Paul Gerson Unna (1850–1929) radically altered the entire approach to dermatology by placing the focus on the skin as an organ [reviews: Refs. 3,4,5]. He also defined the requirements for acceptance of any effective topical agent—for example, it should not have painful adverse effects. This book finally dispelled any remnants of humoral thinking and thus founded a modern approach for the treatment of the skin and its diseases.

In the shadow of these cardinal changes in dermatology, drug uptake through the skin was another issue of heated debate. In the last century, some scientists declared the skin as totally impermeable, a shield against the external world [7]. However, this extreme view did not persist as Bourget [8], and others, published data as early as 1893 showing that acute rheumatoid arthritis could be treated with topical salicylic acid. He also stated that the amount of salicylic acid absorbed was vehicle dependent. In 1904, Schwenkenbecher [9] reported in a review article with more than 100 references from the penultimate century that the skin allowed some substances to permeate much better than others. Simple

experiments using clear end points such as death of the animal led to useful generalizations. It was shown that lipid-soluble agents were more likely to permeate the skin than water-soluble ones, a rule that still holds today. Moreover, while topical application with the base form of alkaloids could result in death of the animal, the salts were shown to be innocuous; for example, strychnine was lethal, but strychnine chloride was not. A useful generalization that can even be applied today is that polar electrolytes and ionized salts permeate the skin poorly. Other disputed topics at that time (which are still under investigation today) were drug uptake through appendages and the use of an electrical potential difference to force charged chemicals through the skin [10–14].

In the past, physicians often prescribed topical products for treatment of skin diseases. However, it was not until the mid-1940s and early 1950s that a topically administered drug was made commercially available to treat a systemic disease [15,16]. The forerunner of the current transdermal products was the nitroglycerin ointment. Thirty years later, in the early 1980s, a more sophisticated product appeared on the market in the form of a transdermal therapeutic delivery system [17–20]. The "artfully created" therapies and dosage forms of the past have fallen out of use and have been replaced by experimentally tested and proven therapeutics housed in more elegant delivery systems (vehicles). Nevertheless, strong threads of the ancient art remain in today's dermatological treatment. Intuition as well as trial and error often surrogate for science in topical product development and in dermatological practice. At this point, we still do not have a complete understanding of the function of topical products. In fact, we are only starting to understand the topical availability of the drugs in these systems and the bioequivalence of similar dosage forms of the same drug.

The word *cosmetics* derives from Greek, meaning "technique of dress and ornament," "skilled in ordering or arranging." Archaeological evidence of cosmetics dates at least from ancient Egypt and Greece. A separation of medicine and cosmetics was first mentioned by Henri de Mondeville at the beginning of the fourteenth century. In his textbook on surgery, he makes a distinction between pathological changes of the skin that need medical therapy and beautifying treatments (*Cyrurgia* [1312]). A spectacular peak of using cosmetic preparations was reached during regency of the "Sun King," Louis XIV of France, when powdering and the use of fragrance was "chic" or, rather, a necessity to cover bad smells and dirt. The next major peak was reached with the industrialization and the increased understanding of chemical synthesis. From there, new ingredients with innovative properties were synthesized, and today, the cosmetic formulator has an infinite array of possibilities for new creations delivering extraordinary and magic effects.

DIFFERENCE BETWEEN A DERMATOLOGIC AND A COSMETIC PRODUCT

The ultimate question is how do dermatologic products differ from cosmetic products. The difference is defined by the *intended use of the product* housing the actives. Most regulatory bodies define *drugs* as "products intended for use in the diagnosis, cure, mitigation, treatment, or prevention of disease" and "products intended to affect the structure or any function of the body of man." The latter statement is somewhat challenging since even water could alter the structure and function of skin (moisturizer). *Cosmetics* are defined as "products intended to be rubbed, poured, sprinkled, or sprayed on, introduced into, or otherwise applied to the human body … for cleansing, beautifying, promoting attractiveness, or altering the appearance." Among the products included in this definition are skin moisturizers, perfumes, lipsticks, fingernail polishes, eye and facial makeup preparations, cleansing shampoos, permanent waves, hair colors, and deodorants, as well as any substance intended for use as a component of a cosmetic product.

This seems straightforward; however, some products meet the definitions of both drugs and cosmetics. This may happen when a product has two intended uses. For example, a shampoo is a cosmetic because its intended use is to cleanse the hair. A shampoo for antidandruff treatment is a drug because its intended use is to treat dandruff and all respective regulatory requirements must be fulfilled to acquire this status. This principle also holds true, for example, for "essential oils." For example, a fragrance marketed for promoting attractiveness is a cosmetic. But a fragrance marketed with certain "aromatherapy" claims, such as assertions that the scent will help the consumer sleep or quit smoking, meets the definition of a drug because of its intended use. Similarly, massage oils that are simply intended to lubricate the skin and impart fragrance are cosmetics, but if the product is intended for a therapeutic use, such as relieving muscle pain, it is a drug. The intended use becomes visible by the claims stated on the product labeling, in advertising, or in promotional materials. Furthermore, different approval, manufacturing, and labeling requirements apply [21].

Cosmeceuticals

The term *cosmeceuticals* was coined in 1961 by Reymond Reed and popularized by Albert Kligman in the late 1970s [22]. Cosmeceuticals are considered to be hybrids between cosmetics and pharmaceuticals that are then intended to enhance the health and beauty of skin. The term has initiated a heated debate about the meaning and function of a cosmeceuticals ever since. Cosmeceuticals are often close to pharmaceuticals regarding well-documented and apparent effect on the skin [23], but their regulatory environment is totally different. Currently, regulators worldwide do not recognize any such category. A product can be a drug, a cosmetic, or a combination of both, but the term *cosmeceutical* has no meaning under the law. The belief that the safety and efficacy is the same compared to pharmaceuticals is therefore wrong and leads to many misconceptions. As a result, the confusion among dermatologists, pharmacists, marketing specialists, and consumers is still ubiquitous.

Over the past few years, a number of big industry players have been pulled over by advertising watchdogs over claims made in adverts, many being cited for misleading claims. Regulatory bodies have warned cosmetic manufacturers, saying that they should stop promoting unsubstantiated claims. The call for additional regulation is not far to protect the public from the wide range of potentially misleading claims and terms used by the cosmetic industry on their product labels.

TOPICAL DELIVERY OF ACTIVES

The visibility of a skin injury or a dermatosis led to the reasonable assumption that the direct (*topical*) application of a therapeutic active (drug) to the target tissue holds many advantages. Treatment of inner organs or the skin with a *systemically* applied drug leads to drug distribution throughout the entire organism, and only a small fraction of the totally delivered drug will reach the target site.

Treatment by topical (local) application means that there is intimate contact between the therapeutic active and the target tissue, and the risks of developing systemic adverse effects are minimized.

Today, a considerable endeavor is being undertaken in topical dosage form design to improve therapy by

- Replacing oral treatment of, for example, psoriasis with methotrexate [24], acitretin [25], and cyclosporine [26], or to replace oral treatment of infective conditions with acyclovir [27,28], to reduce possible systemic adverse effects of the compounds and to improve local availability
- Enhancing drug delivery [29]
- Targeting drug delivery to the skin [30]
- Enhancing drug retention [31]
- Hindering delivery of toxic chemicals to the skin [32]

Drug delivery to (penetration) or through (permeation) the skin is aimed at targeting the drug to three anatomical locations, namely,

- The skin itself (topical delivery, local delivery, dermal delivery)
- The deeper tissues, such as joints or musculature (*regional delivery*)
- The systemic circulation (*transdermal delivery*)

The same laws of nature and similar principles apply to formulate vehicles for delivering cosmetic actives. The target site of cosmetic actives is preferably located in the uppermost layers of the skin, and pharmacological and immunological effects must be excluded.

THE VEHICLE

Patients and consumers apply a wide spectrum of preparations to their diseased or healthy skin that range in their physicochemical nature from powders to semisolids to liquids and even patches. Over the centuries, a myriad of concoctions have appeared and evolved as medical science has refined and new materials useful in product formulation have been discovered.

In dermatology, the drug is rarely applied to the skin in the form of a pure chemical but, instead, is normally incorporated into a carrier system—the vehicle. The term *vehicle* in this context is relatively young and was developed only when it became possible to assign a specific (therapeutic) effect to a chemical substance. At this point, it became common to distinguish between "active" and "inactive" ingredients. Until the beginning of the last century, no clear difference was made between an "active" agent and the "inactive" carrier system components (Table 15.1).

Many dermatological topical therapies have been developed empirically by clinicians using their favorite mixtures and remedies, the so-called magistral formulations. Although many formulations listed in the older official monographs have been tried and tested over many years, properly controlled reevaluations of these products have led to major deletions in more recent editions. Stricter controls over the quality of ingredients, active agents, and the conditions of manufacture (and economical factors) have contributed to the significant reduction in the number of these formulations. Modern vehicles are frequently tailor-made and chosen as carefully as the therapeutic or cosmetic actives for which they are intended. Formulators develop

TABLE 15.1
Selected Ingredients from Classic and Modern Medicine

Medicine	Solid	Semisolid	Liquid
Classic	Sulfur	Human fat	Water
	Powdered bone	Animal fat	Wine (distillation was unknown until 1359)
	Various woods	Honey	Olive oil, aromatic oils
Modern	Talcum	Yellow wax (cera flava)	Water
	Titan oxide	Petrolatum	Ethanol
	Bentonite	Lanolin	Isopropyl myristate
			Propylene glycol

Note: Due to the rationale of classic medicine, no distinction was made between an "active" agent and the "inactive" ingredients.

these preparations in terms of stability, compatibility, and patient or consumer acceptability of the vehicle.*

The observation that the type of vehicle and the excipients can affect the percutaneous absorption of an active added another dimension to the formulation development process. Studies on the percutaneous absorption of therapeutic actives such as corticosteroids have yielded much information on vehicle effects, and the vasoconstrictor test became a frequently used tool for determining how the vehicle affects the topical availability of the steroid [33–36]. The same fundamental laws apply to the delivery of cosmetic actives. However, formulatory development steps must be taken to prevent excessive absorption of the cosmetic actives (e.g., sunscreens). In general, for the delivery of cosmetic actives, similar delivery systems (e.g., liposomes, nanoemulsion) are being used, and their names are often fancifully incorporated into product and brand marketing activities.

Modern formulation development is based upon the stability and compatibility of ingredients and active agents (Table 15.2), cosmetic acceptability, usage criteria of the vehicle (Table 15.3), and bioavailability of the actives at the target site (Table 15.4). Depending on the pharmaceutical or cosmetic product profile, the character of the requirements may vary significantly—for example, microbiological product stability and ingredient characterization.

Thus, the "ideal" vehicle for both pharmaceutical and cosmetic application should fulfill many different criteria; it should be easy to apply and remove, nontoxic, nonirritant, nonallergenic, chemically stable, homogenous, bacteriostatic, cosmetically acceptable, and pharmacologically inert, and should readily release the active agents for enhanced, controlled, or targeted delivery.

Classification of Dermal Formulations

Many attempts have been made to produce a comprehensive classification of dermatologic formulations [40–43]. A formulation may be classified by its pharmaceutical nomenclature used in pharmacopoeias (e.g., cream, ointment, gel, paste); by the principle of the structural matrix (e.g., emulsion, liposome, gel, suspension, transdermal patch); or by associated appearance (e.g., paint, milk, foam, shake). However, no uniform and comprehensive classification is currently available. Due to nonuniform definitions and the interchangeable nomenclature, it seems unlikely that a general classification will emerge in the near future. In a clinical setting, a further approach is used in which an expected "in-use" effect (Figure 15.5) such as hydrating, lubricating, protecting, or drying is defined. The attributes *drying* and *protecting* are often defined for pastes, and their clinically distinctive features are the quality to (1) absorb exudates because of their powder and other absorptive component content (hydrophilic cream pastes) or to (2) protect the skin against physical effects from fluids or light (Pasta zinci, British Pharmacopea 90) [44].

The simplest classification consists of an initial division of the topical preparation into liquid, semisolid, or solid (powder) systems that may be defined as monophasic, diphasic, or tri(multi)phasic matrices (Table 15.5). Katz et al. [43] proposed a more complex classification that is based on the association of aqueous, oil, powder, and emulsifier materials that form a variety of topical products (Figure 15.1). Comprehensive classifications have been proposed (e.g., semisolid pastes) for some specific dermatological formulations, which attempt to describe in more detail the complexities of the matrix comprising the dosage form (Figure 15.2). A very practical classification is based

TABLE 15.2
Some Pharmaceutical–Technological Criteria for Pharmaceutical Semisolids

Stability of active drugs and ingredients
Rheological properties—consistency, extrudability
Loss of water and other volatile components
Phase changes—homogeneity/phase separation, "bleeding"
Particle size and particle size distribution of dispersed phase
Apparent pH
Microbial contamination/sterility (in unopened containers and under conditions of use)
Enhanced or controlled drug release from the vehicle

Source: Flynn, G.L., Topical Drug Absorption and Topical Pharmaceutical Systems, in: Banker, G.S., and Rhodes, C.T., editors, *Modern Pharmaceutics*, Marcel Dekker, Inc., New York, Basel, 1990.

TABLE 15.3
Cosmetic and Usage Criteria for Topical Vehicles

Visual appearance of product
Odor, color
Sampling and dispensing characteristics: ease of removal from container
Application properties, texture (stiffness, grittiness, greasiness, adhesiveness)
Residual impression after application, permanency on the skin

Sources: Clark, R., Cosmetic Creams and Lotions, in: Hibbott, H.W., editor, *Handbook of Cosmetic Science. An Introduction to Principles and Applications*, Pergamon, Oxford, 1963. Sherman, P., *Rheol Acta*, 10, 1971.

TABLE 15.4
Biopharmaceutical Criteria for Topical Vehicles

Enhanced drug delivery and drug retention in the skin
Controlled drug delivery and drug retention in the skin
Targeted drug delivery and drug retention in the skin

* In some parts of Europe, the term galenic is used to paraphrase the field of research and development of pharmaceutical carrier systems, including topical dermatologic formulations. According to The Oxford English Dictionary (second edition, 1992), the term galenic pertains to vegetable preparations, as distinguished from chemical remedies. In Anglo-Saxon countries, the term is not used in this context.

TABLE 15.5
Simple Classification System for Topical Dermatologic Vehicles

System	Monophasic	Diphasic	Tri(multi)phasic
Liquid	Nonpolar solution	Emulsion (o/w, w/o)	Emulsion (o/w/o, o/w/o)
	Often designated as oil	Often designated as milk, lotion, shake, etc.	Often designated as milk, lotion, shake, etc.
	Polar solution	Suspension	Suspension
	Often designated as paint, lotion, etc.	Often designated as paint, shake, etc.	Often designated as paint, shake, etc.
Semisolid	Water-free	Emulsion (o/w, w/o)	Emulsion with powder (o/w, w/o)
	Polar or nonpolar ointment	Often designated as washable (o/w), nonwashable	Often designated as cream pastes
	Water-containing polar or nonpolar gel	(w/o), or ambiphilic (o/w, w/o) cream	
		Suspension	
		Often designated as paste	
Solid	Powder	Transdermal patch	Transdermal patch

Note: It is obvious that this classification system is a raw simplification of the diversity of external formulations. It does not account for many of the newer external formulations (e.g., liposomes, microcapsules, etc.).

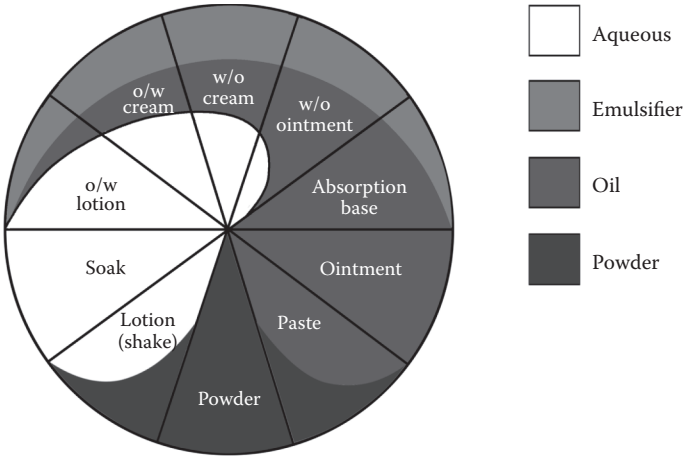

FIGURE 15.1 Diagrammatic representation of how aqueous, oil, powder, and emulsifier materials combine to form a variety of topical products. Note that the term *w/o ointment* is misleading since ointments are defined as monophasic systems, whereas creams (emulsions) are defined as diphasic or multiphasic systems (see Table 15.5). (From Katz, M., Design of Topical Drug Products: Pharmaceutics, in: Ariëns, E.J., editor, *Drug Design*, Academic Press, New York, 1973.)

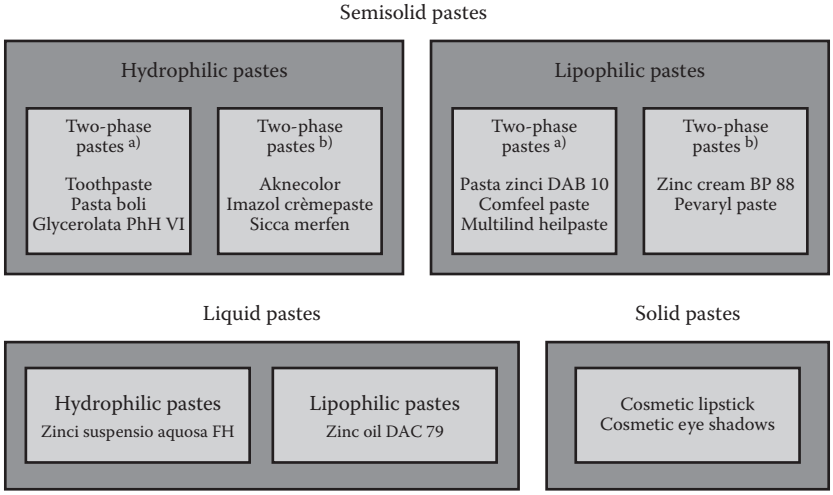

FIGURE 15.2 Classification of pastes: (a) the two-phase pastes consist of two immiscible components, one (the dispersed or inner phase, powder) being suspended in the other (the continuous or outer phase); (b) the three-phase pastes consist of a two-phase emulsion (oil in water or water in oil) with high concentrations of incorporated powder (cream pastes). (From Juch, R.D. et al., *Dermatology*, 189, 1994.)

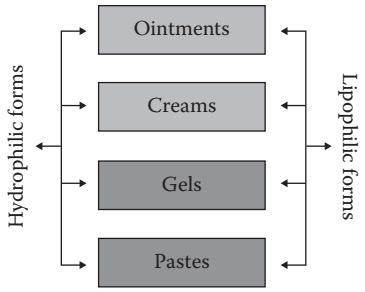

FIGURE 15.3 The pharmacopoeial nomenclature used to classify topical formulations.

on the pharmaceutical nomenclature method used in pharmacopoeias (Figure 15.3).

A recently proposed, simple description of the vehicle distinguishes between the sum of the ingredients in which the pharmaceutical or cosmetic active is presented to the skin and the principle of the structural matrix of the vehicle [45] (Figure 15.4). The structural matrix can be considered as monophasic (e.g., solution) or multiphasic systems (e.g., cream, cream paste). This simplification is helpful when considering vehicle effects, discussed later. Clinical textbooks tend to combine the vehicle nomenclature, the principle of the structural matrix, and the expected performance (Figure 15.5), leading to further confusion. In addition, many imaginative, surprising, or even unexpected word creations by marketers of the cosmetic industry make a comprehensive classification of vehicles most likely unapproachable.

Vehicle Effect

Despite the fact that the "ideal" vehicle should be "inert," both clinicians and consumers expect that application of a topical drug product should provide several effects in addition to the requirement that the vehicle should readily release the active moiety for the therapeutic or cosmetic effect. These additional effects, some of which emerged from traditional dermatological treatments, rely both on the physical and on the chemical (pharmacological) properties of the ingredients (Table 15.6).

In topical dermatological treatment and care, formulators, producers, legislators, clinicians, and consumers attach extraordinary importance to both the principle of the structural matrix (the type of formulation, e.g., cream, liposome, ointment, solution) and the individual ingredients of the topical preparations (Figure 15.4). This importance, although admittedly correct, not only applies to topical preparations but also holds true for most pharmaceutical preparations. The visibility of the application site (target organ); the tactile sensations when the topical preparation is applied to the skin; and the appearance, ease of use, and consistency of preparations probably led to the distinctive importance of the formulation type and the ingredients of the vehicle. These circumstances have led to extensive discussions on the "vehicle effect" where the various interest groups have different conceptions and different expectations of the formulation. The issue concerning whether the principle of the structural matrix or the ingredients of the preparations are responsible for a particular effect (e.g., enhanced, targeted, delayed, or prevented) is currently under debate [30,54–57]. In clinical practice, the question is often asked whether creams, gels, ointments, or liposomes are better in delivering a drug to the skin in terms of promoting the drug absorption and therapeutic effect. Certainly, cosmetic aspects of the delivery system, a result of the structural matrix, may have an influence on compliance and are therefore of clinical relevance. However, drug delivery to the skin is controlled by the vehicle excipients as these affect partitioning into and diffusion through the stratum corneum [45]. It remains speculative whether one can assign a specific effect to a particular structural feature of a vehicle, keeping in mind that the structural matrix changes once it is

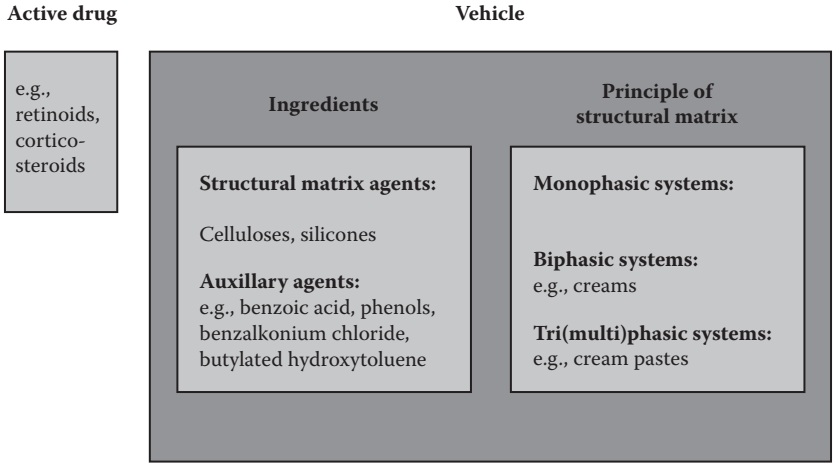

FIGURE 15.4 Principle of topical preparations: example of a topical preparation comprised of individual ingredients that form a structural matrix. (From Smith, E. et al., Topical Dermatological Vehicles: An Holistic Approach, in: Bronaugh, R.L., and Maibach, H.I., editors, *Topical Absorption of Dermatological Products*, Marcel Dekker, New York, Basel, 2002.)

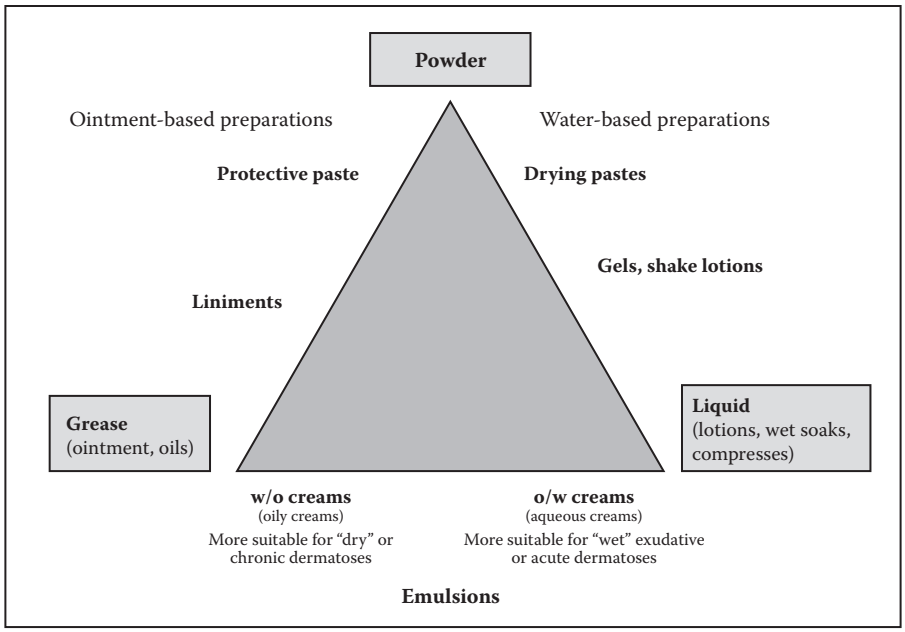

FIGURE 15.5 Clinical textbooks tend to combine the pharmaceutical nomenclature, the principle of the structural matrix, and the expected performance. (From Griffiths, W.A. et al., Topical Therapy, in: Rook, A. et al., editors, *Textbook of Dermatology*, Blackwell Scientific Publications, London, 1992, and Polano M.K., *Topical Skin Therapeutics*, Churchill Livingstone, Edinburgh, London, 1984.)

applied to the skin (see section titled "Metamorphosis of the Vehicle").

It is often stated that effects of formulation or vehicle on the rate and extent of drug absorption are much greater with topical drug delivery than with any other route of drug administration. For example, comparing alternative topical formulations of the same drug, differences in the extent of absorption through the skin between 10- and 50-fold (and greater) have been reported [58–61]. To put this into perspective, 50%–100% (up to onefold) differences in the extent of drug absorption via the oral route are rare [62–64]. The data presented in Table 15.7 show a broad potency range (I–V) of various marketed 0.5% betamethasone dipropionate products. One would expect the same drug in the same concentration formulated into different vehicles to have approximately the same potency. It remains uncertain to what extent the matrix or the excipients are responsible for the potency differences between these formulations of the same active entity, although excipient effects will probably predominate (Figure 15.4).

In the cosmetic world, additional challenges regarding vehicle properties need to be considered. The BB cream is a good example of how many tasks can be incorporated into a product and how high the expectations of a consumer may become. *BB cream* stands for *blemish balm*, or *blemish base*, and, in Western markets, *beauty balm*. It is a cosmetic item sold mainly in East and Southeast Asia, although larger beauty brands are increasingly introducing BB creams to Western luxury markets and mass-market retailers. BB creams are promoted as *magic* multitaskers or all-in-one facial cosmetic products to replace serum, moisturizer, primer, foundation, and sunscreen and contain further cosmetic actives such as hyaluronic acids or vitamin C. It is said to be able to moisturize, protect against the sun, soothe skin, prime skin, and give full coverage, depending on the brand. An even newer trend has appeared at the horizon—the CC cream. *CC* stands for "color correcting." This hype clearly illustrates how expectations are created and the meaning and function of the vehicle and the cosmetic actives fuse (Figure 15.6).

TABLE 15.6
Vehicle Effects in Addition to the Drug Effect and to the Requirement that the Vehicle Should Readily Release the Drug for Optimal Absorption

Chemical (Pharmacological) Effect	Physical Effect
Analgesic	Cleansing
Astringent	Protecting
Bacteriostatic	Lubricant
Antipruritic	Hydrating/drying

Sources: Juch, R.D. et al., *Dermatology*, 189, 1994. Lorincz, A.L., The Pharmacology of Topically Applied Medications, in: Sternberg, T.H., and Newcomer, V.D., editors, *The Evaluation of Therapeutic Agents and Cosmetics*, McGraw-Hill, New York, 1964. Heisel, E.W., Principles of Local Dermatologic Therapy, in: Criep, L.H., editor, *Dermatologic Allergy: Immunology, Diagnosis and Management*, Saunders, Philadelphia, London, 1967. Obata, M., and Tagami, H.A., *J. Soc. Cosmet. Chem*., 41, 1990. Fowler, J.F., *Am. J. Contact Dermatitis*, 3, 1992. Wolf, R., *Dermatology*, 189, 1994. Hills, R.J. et al., *Br. J. Dermatol.,* 130, 1994. Gabard, B. Testing the Efficacy of Moisturizers, in: Elsner, P. et al., *Bioengineering of the Skin: Water and Stratum Corneum*, CRC Press, Boca Raton, 1994.

TABLE 15.7
Comparative Potency of Various 0.5% Betamethasone Dipropionate Products

Drug Product	Vehicle Type	Potency Group (US)
Diprolene	Ointment	I
Diprolene AF	Optimized cream	I
Diprosone	Ointment	II
Diprosone	Cream	III
Diprosone	Lotion	V

Source: Cornell, R.C., and Stoughton, R.B., *Arch. Dermatol.*, 121, 1985.

Note: It remains uncertain to what extent the matrix or the excipients are responsible for the differences between formulations, although excipient effects will probably predominate in defining (1) the chances of stratum corneum permeability and (2) the different drug delivery rates from the vehicle.

FIGURE 15.6 BB creams are good examples of how many tasks may be incorporated into a product. *BB cream* stands for *blemish balm*, or *blemish base*, and, in Western markets, *beauty balm*. BB creams are promoted as *magic* multitaskers or all-in-one facial cosmetic products to replace serum, moisturizer, primer, foundation, and sunscreen and contain further cosmetic actives such as hyaluronic acids or vitamin C. They are said to be able to moisturize, protect against the sun, soothe skin, prime skin, and give full coverage, depending on the brand. This hype clearly illustrates how expectations may be created and the meaning and function of the vehicle and the cosmetic actives fuse.

Vehicle Interactions

The potential for large differences in the extent of drug absorption between topical dermatologic formulations, as is evident from the data in Table 15.7, is due to the complex interactions between the drug, the vehicle, and the skin that control partitioning into and diffusion through the skin barrier. Three types of interactions have been described in this regard [41].

- *Vehicle–drug interactions* include the thermodynamic activity of the drug in the vehicle that is related to solute drug concentration in the vehicle (Cv) and the thermodynamic activity of the drug.
- *Vehicle–skin interactions* include the wide area of permeation/penetration enhancers. Basically, these are vehicle components that interact with the stratum corneum to bring about enhancements in drug solubility or drug diffusion or both.
- *Drug–skin interactions* include skin metabolism and binding of the drug by the skin.

Formulations in which significant *vehicle–skin or vehicle–drug interactions* occur are probably the most common.

Many pharmaceutical and cosmetic ingredients are known to have pronounced effects on reducing the skin barrier function (vehicle–skin interaction) as well as influencing the partitioning of both the pharmaceutical and cosmetic actives from the vehicle into the stratum corneum (vehicle–drug interaction).

Vehicle Ingredients

The use of ingredients in therapeutic and cosmetic formulations is strongly regulated to ensure safety of the final product. Nevertheless, in recent years—in particular, in cosmetic products for the mass market—certain ingredients have drawn criticism from consumer organizations as being dangerous and should therefore be avoided. Propylene glycol is depreciated as an ingredient used in deicing solutions for airplanes, petrolatum is qualified as a carcinogenic gasoline distillate, and so forth. The list of these criticisms can be lengthened indefinitely, and sound scientific argumentation and explanation most often fade away. To remain competitive in the marketplace, a reformulation is often unavoidable. The reformulated products—with ingredients usually less well-known—are then promoted as innovative and new creations with claims such as "without propylene glycol" and so forth, forcing other market players to follow.

After product application (see paragraph Metamorphosis of the Vehicle) many vehicles—depending on the ingredients they are made of—pass through a process of considerable change (metamorphosis). Hence, the sum of the remaining ingredients after application on the skin—and *not* the initial vehicle composition or principle of the structural matrix in its initial form—determines the fate of therapeutic and cosmetic actives and, hence, their final effect.

CHOICE OF VEHICLE

From the forgoing, it is obvious that an enormous empirical theorem on the choice and use of dermatologic and cosmetic vehicles is available. Nevertheless, many dermatologic textbooks, pharmacopoeias, and formularies do not elucidate the rationale behind the choice and use of different dermatologic vehicles. The choice of a vehicle in a particular disease or in a particular patient often follows recommendations that either are based on a classification of external preparations [40,41,43,46,66] or follow a few simple factors compiled by Schaefer et al. [67]. The recommendation by Schaefer—as outlined below—is practical for both the prescriber and the formulator since it avoids the use of the unsatisfactory and often confusing classifications of topical preparations. In the cosmetic world, the choice of vehicle is most likely driven by the current marketing stories and the sensory qualities of the products.

Acuity and Type of Disease

It is a basic dermatological precept that the more acute the dermatosis, the blander the treatment. The application of cooling vehicles and the use of frequent wet compresses, with or without drugs, remain an indispensable part of the management of acute or exudative dermatoses. The principle of "wet-on-wet" and the use of occlusive ointments for dry or chronic dermatoses have become axiomatic. As the condition improves, a "wet" dermatosis may subsequently be treated with either a drying paste or an oil/water (o/w) cream, and a "dry" dermatosis may be treated with a hydrous ointment, water/oil (w/o) cream, or even an occlusive ointment [40,68,69].

Skin Type, Skin Status

Depending on environmental conditions, ethnic origin [70,71] gender, age, localization, and state of disease, different skin conditions are treated with respect to oiliness or humidity. Formulators and clinicians try to select an appropriate vehicle that is adjusted to the skin type or skin status of the patient or the consumer. The capability of vehicles to alter the physical and chemical state of the skin can be attributed to their influence on the lipid and water content, or the lipid composition of the skin [53,72,73]. Vehicles with more hydrophilic properties are suitable for oily and normal skin conditions, whereas vehicles with more lipophilic properties are suitable for dry skin conditions.

Localization of the Disease

Chemicals applied to different anatomical regions of the body permeate to varying extents [74,75]. The quantity of a topical preparation that may be applied to the different body regions is a highly variable parameter [76,77]. Most importantly, the clinical reactivity of the same dermatosis at different locations may vary markedly [78,79]. Furthermore, it can be noted that national or ethnographic preferences may exist, as this has been demonstrated for other pharmaceutical carrier systems. Table 15.8, based on empiricism rather than scientific investigation, may give an overview of current dispensing practice. However, for the prescriber and the consumer, it is generally only important to distinguish between vehicles with hydrophilic and lipophilic features and to avoid vehicles with high alcohol concentrations in "wet" and acute dermatosis.

Environmental Factors

Topical preparations are tested in the pharmaceutical and cosmetic industry with respect to their stability under various, carefully defined, climatic conditions [80]. Practical tests to study the stability problems of vehicles or vehicle constituents that are encountered during use are rarely available. Preparations formulated for certain temperature climates can become useless in more extreme climatic conditions due to decomposition of vehicle constituents or due to changes of the structural matrix (breaking of the emulsion, viscosity changes). Protective topical vehicles (e.g., sunscreens used in tropical temperatures) should be designed and selected appropriately. The conditions of usage of the topical preparation should therefore be kept in mind.

TABLE 15.8
Localization, Skin Status, and Vehicle Used in Different Locations

Localization	Status	Forms
Hairy skin	Dry	Solutions, w/o cream
	Oily	Solution, gel
Face	Dry	w/o cream
	Oily	Solution, o/w cream
Ear	Oily	o/w cream
Body/extremities	Dry	Ointment, w/o cream
	Oily	o/w cream
Intertriginous area	Humid	Drying pastes, o/w cream
Hands/feet	Dry	During the day: o/w or w/o cream
	Oily	During the night: ointment, w/o cream
Nail	Dry	Solution, lacquer

Cosmetic Consideration

Cosmetic or aesthetic criteria such as visual appearance, odor, application properties, residual impression after application or permanency (substantivity) of the vehicle, and actives on the skin are important factors that influence consumer acceptance and patient compliance. These are important criteria to avoid the transfer of consumer allegiance to an alternative, competitor's product, yet the power of appealing promises of the cosmetic industry may move mountains.

The characterization of the topical preparation (ointment, cream, lotion, liposome, etc.) is often associated with the expected effectiveness, with the severity of a disease, or with the appearance of the formulation. In everyday life, ointments are often associated with adhesiveness/stickiness and are used for more severe diseases, whereas creams, lotions, and similar products represent ease of use and are used in less severe diseases. However, in a more recent investigation, higher efficacy rates for the spray and foam formulations of clobetasol propionate for the treatment of psoriasis in comparison to traditional vehicles was reported. The clinical use treatment outcome was associated with the patient adherence to treatment [81].

METAMORPHOSIS OF THE VEHICLE

In experimental, clinical, and everyday life situations, most vehicles (structural matrix and ingredients) undergo considerable changes after they are removed from the primary container and are applied to the skin (Figure 15.7). Subsequently, the initial structural matrix and the quantitative composition of the vehicle will most likely change during and after the mechanical agitation associated with application of the product (e.g., rubbing) and/or evaporation of ingredients (phase inversion). As a consequence of these processes, the thermodynamic activity of an active within the formulation will also change. An important influence on the rate of active transfer from the vehicle into the skin is the thermodynamic activity of the active within the formulation. The maximum active transfer into the skin takes place when it is saturated at the vehicle–skin interface (thermodynamic activity = 1). Otherwise, the rate of active transfer across the interface is proportional to its degree of saturation (concentration/solubility). Rapid evaporation of the volatile components of some vehicles results in an appreciable increase in solute active concentration in the vehicle. Evaporative concentration first leads to saturation and then to supersaturation (thermodynamic activity > 1) that, although generally a transient condition, results in delivery exceeding that achievable with a saturated solution [82,83]. This evaporative concentration effect may even force the active out of the solution and superimpose a dissolution dependency in the delivery rate. We are only beginning to research the complicating effects of vehicle metamorphosis on the entire clinical picture. Dynamic in situ eutectic formation for topical drug delivery [84] or a moisture-activated bioadhesive drug delivery system for skin and mucous membranes [85] illustrate the potential of the phenomenon of the metamorphosis of the vehicle.

FUTURE OF TOPICAL VEHICLES

During the last two decades, increased attention has focused on novel drug delivery systems for extending the control over the release and penetration/permeation of drugs into the skin. Phospholipid vesicular carriers have gained recognition as potential colloidal drug delivery systems for topical drugs. Liposomes not only may act as "drug transporters" but also may show localizing effects whereby vesicles can accumulate

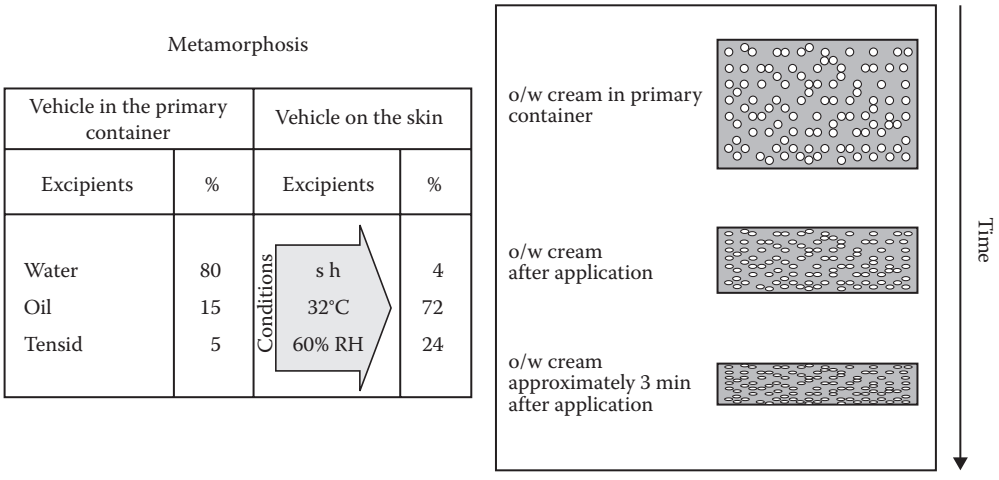

FIGURE 15.7 In clinical situations, most dermatologic vehicles (structural matrix and ingredients) undergo considerable changes after they are removed from the primary container and are applied to the skin. This process is termed metamorphosis of the vehicle. The initial structural matrix of the vehicle will most likely change during and after application of the product and/or evaporation of ingredients. Evaporative concentration first leads to saturation and then to supersaturation that, although generally a transient condition, results in delivery exceeding that achievable with a saturated solution.

drugs in the stratum corneum and thereby reduce systemic effects [86]. Transfersomes are said to be ultradeformable by the incorporation of "edge activators" such as sodium cholate and are thus reportedly able to squeeze through "pores" in the stratum corneum. This is a potentially promising field for systemic drug therapy through the intact skin. Ethosomes contain relatively high concentrations of ethanol, when compared with conventional liposomes, which facilitates significantly enhanced drug delivery both to deep skin strata and systemic circulation. Formulations based on microemulsions show very interesting characteristics for topical administration. These "critical solutions" combine the properties of emulsions and solutions [87], having an ultralow interfacial tension and a small droplet diameter of 10–140 nm. It is reported that microemulsions could increase both dermal and transdermal drug delivery due to different mechanisms [86]. Microsponge delivery systems are usually blended into formulated products such as gels or creams and are primarily used as reservoirs for releasing active ingredients over a prolonged period of time or as receptacles for absorbing undesirable substances, such as excess skin oil. Solid lipid nanoparticles (SLNs) show great promise of success as carrier systems for topical and cosmetic applications, having evolved from the liposome generation. These extremely small particles composed of biocompatible lipids may form an occlusive film on the skin that reduces transepidermal water loss and enhance the penetration of drugs through the stratum corneum by increased hydration. More distinctive advantages of SLNs are due to the solid matrix structures of particles, which make modulation of drug release possible and reduce chemical transformation of unstable drugs. SLNs also have apparent physical sunscreen potential by scattering light efficiently [89]. Recently, an advanced type of lipid nanoparticle with a solid matrix, called a nanostructured lipid carrier (NLC), has been investigated. Some of the potential problems of SLNs, like low drug loading capacity and severe drug expulsion during storage, could effectively be avoided in NLCs by combination of very different lipid molecule types [90].

Unfortunately, the authors of many publications documenting vehicle effects claim a direct association between the structural matrix in its initial form (e.g., liposome, emulsion, etc.) and the therapeutic or cosmetic effect, obscuring the fact that the vehicle may undergo a dramatic metamorphosis after application. It is most doubtful that the structural elements in its initial form (e.g., liposome) remain intact and "active" after evaporation of volatile ingredients.

CONCLUSIONS

The topical treatment of diseased and healthy skin has a long and controversial history. After dropping the theory of humoral pathology and new scientific insights, it became possible to assign a specific (therapeutic) effect to a chemical substance. It became common to distinguish between "active" and "inactive" ingredients—the latter forming the *vehicle*.

Due to the multifaceted complexity of topical vehicles, no uniform and comprehensive recommendations or guidelines are currently available for the development and the use of topical dermatologic and cosmetic formulations. As a rule of thumb, one may only recommend that more hydrophilc formulations are suitable for mainly hydrophilic sites of the skin (or wet [acute] dermatosis), whereas more lipophilc formulations are more suitable for mainly dry skin sites (dry [chronic] dermatosis).

More recently, the term "cosmeceutical" opened up a heated debate on the delineation of dermatologic and cosmetic products. Cosmeceuticals are considered to be hybrids between dermatologics and cosmetics that are then intended to enhance the health and beauty of the skin. Even though the delineation is clearly and logically based on the product's intended use (with [dermatologics] and without [cosmetics] healing promise), it is misunderstood and misused. To protect the patient and the consumer, regulators worldwide quite rightly do not recognize any such category.

In topical dermatological treatment and care, formulators, producers, regulators, clinicians, and consumers attach extraordinary importance and, not seldom, *magic* effects to both the principle of the structural matrix (type of formulation) and the individual ingredients of the topical preparations. The recent hype in the world of cosmetics around the BB creams is a good example of how many tasks may be incorporated into a product and how high the expectations of consumers may become.

An obvious phenomenon and the concomitant consequences when applying dermatologic and cosmetic vehicles are still often overlooked. In experimental, clinical, and everyday life situations, most vehicles (structural matrix and ingredients) undergo considerable changes after they are removed from the primary container and are applied to the skin (metamorphosis of the vehicle). Subsequently, the initial structural matrix and the quantitative composition of the vehicle ingredients will most likely change during and after the mechanical agitation associated with application of the product (e.g., rubbing) and/or evaporation of ingredients. Research documenting vehicle effects often claim a direct association between the structural matrix in its initial form (e.g., liposome, emulsion, etc.) and the therapeutic or cosmetic effect, obscuring the fact that the vehicle may undergo a dramatic metamorphosis after application. It is most doubtful that the structural elements in its initial form (e.g., liposome) remain intact and "active" after evaporation of volatile ingredients.

More diverse and molecularly complex classes of dermatologic and cosmetic vehicles are continuously being researched and refined. The scientific progress has been remarkable when one considers the simple emulsion mixtures that were commonplace in dermatological therapy 20 years ago and still persist to this day in some commercial products. It is hoped that the results of current and future research endeavors will foster the emergence of more innovative topical formulations, applying engineered bioavailability control systems, with broader applications in topical therapeutic and cosmetic vehicles.

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16 Dissolution of Materials in Contact with Skin Film Liquids

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INTRODUCTION

The human skin serves many purposes, one of which is to provide a barrier between the external environment and our internal tissues and organs. Throughout the course of our everyday lives, the skin is exposed to exogenous materials, some of which may be beneficial and others harmful. Examples of exposure sources include consumer articles such as cosmetics [1], textiles [2,3] and carpeting [4], or prophylaxes [5,6]; medical devices for topical therapeutic drug delivery [7] or noninvasive diagnostics [8,9]; and occupational agents such as latex gloves [10,11] and chemicals [12]. These exposures may be intentional and involve direct application onto the skin (e.g., use of cosmetics, topical drugs, textiles, jewelry) or be the result of unintentional contact (e.g., the settling of chemicals from air onto skin or splashing of liquids onto skin in occupational environments).

Exogenous materials first come into contact with the outer superficial layer of the skin called the stratum corneum (SC). The SC is approximately 8 to 20 μm thick except on the palms of the hands and soles of the feet, where it is on the order of 80 to 200 μm thick. The SC is comprised of dead (keratinized) corneocyte cells, which are tightly joined together by an intercellular lipid glue composed mainly of ceramides, free fatty acids (FFAs), and cholesterol [13]. Directly beneath the SC is the viable epidermis, which is composed of live keratinocytes. The viable epidermis serves many roles in skin physiology, including the regeneration of the SC and homing and maturation of skin immune cells [14]. The dermis, a thick layer of connective tissue that contains vasculature to provide nutrition to the overlying epidermis, contributes to the immunological activity of the skin and gives structural support to hair follicles and sweat glands [15].

The extent to which an exogenous material will interact with the skin is dependent, in part, on the composition and properties of the skin surface film liquids (SSFL). The SC is coated with a cosolvent of mainly aqueous sweat and oily sebum. Sweat is secreted by sweat glands, which are tubular structures that consist of a coiled portion and a duct portion. The coiled portion (located in the dermis) functions to secrete a precursor sweat solution. The duct portion (located in the epidermis) functions to modify the precursor sweat solution by reabsorbing ionic constituents (e.g., sodium) prior to the solution reaching the skin surface [16–18]. Once on the skin surface, the glandular secreted sweat mixes with by-products from skin surface maturation and desquamation processes and metabolites from skin bacteria. The second main component of SSFL is the oily sebum, which is formed by pilosebaceous units that consist of a sebaceous gland physically connected via a sebaceous duct to a hair follicle [19]. The sebaceous gland consists of undifferentiated, differentiated, and mature sebocyte cells. Undifferentiated sebocyte cells are pushed through the maturation zone in the gland, where they are filled with freshly synthesized lipids to develop into differentiated sebocyte cells. These differentiated cells pass through the zone of necrosis in the gland, where they swell in volume by accumulating more lipids to form mature sebocyte cells. This increase in mature sebocyte cell volume causes the cell to disintegrate, and it releases the sebum into the sebaceous duct. The glandular sebum is secreted via hair follicles onto the skin surface, where it mixes with epidermally derived lipids [19,20].

The interaction of exogenous materials that come into contact with human skin is often investigated using artificial SSFL and in vitro test methods as a surrogate for in vivo measurements. The term *artificial SSFL* is used in this chapter as a synonym for terms such as *artificial sweat*, *artificial perspiration*, *synthetic perspiration or sweat*, *sweat simulant*, *simulated sweat*, *artificial sebum*, *soil*, and so forth. Table 16.1 provides a synopsis of in vitro studies that utilized artificial SSFLs and were published from 1935 to 2013. While extensive, this list may not be totally comprehensive since it is limited to publications in English or for which abstracts were available in the English language. From Table 16.1, it is clear that use of artificial SSFLs has broad application to elastomers, metals, textiles, organic chemicals, currency, and numerous other materials encountered daily.

Specific materials studied in the last 75 years include consumer products (cosmetics, fabrics, carpeting, jewelry, etc.), medical devices, workplace or environmental contaminants, coins, and so forth. Often, these in vitro tests are used to understand the therapeutic value of medical devices (e.g., transdermal drug delivery); for product stewardship/consumer safety (e.g., prevention of skin reactions); for occupational toxicology (e.g., identification of skin hazards); and/or for compliance with regulations such as the European Union Nickel Directive (188]. Of the studies listed in Table 16.1, 69% (124 of 179) were published after the year 2000. Much of this recent growth has been focused on evaluation of functional coatings for textiles and metal coatings (thin

TABLE 16.1
In Vitro Studies of the Interaction of Exogenous Materials with Artificial Skin Surface Film Liquids (1935 to 2013)

Category	Exogenous Material	Reference	Synopsis
Elastomers	Silicone, polyurethane	(21)	Exposure to sebum affected tear load and extension of both elastomers
	Silicone	(22)	Accelerated aging in sweat and sebum yielded minimal changes in elastomer
	Chlorinated polyethylene	(23)	Elastomer hardened in sweat and lost weight and changed color in sebum
	Bromobutyl rubber	(24)	Sweat did not cause significant changes in swelling, tensile strength, or cracking
	Silicone	(25)	Elastomer hardened in sweat and lost weight and changed color in sebum
	Silicone	(26)	Significant degradation of mechanical properties in sweat and sebum
Humans	Skin	(27)	Sebum reabsorbed into the stratum corneum in low-sebum-production regions
	Human sweat	(28)	Developed analytical method for specific cations and amino acids in human sweat
	Surfactant proteins	(29)	Addition of skin-derived surfactant proteins lowered surface tension of sebum
Illicit drugs	Hair	(30)	Sweat facilitated penetration of cocaine into hair
	THC	(31)	Developed sensitive method for detection of THC in sweat patches
Leather	Shoes	(32)	Human foot perspiration degraded chrome-tanned leather
	Shoes	(33)	Loss of sulfates and chromic oxide increased as sweat temperature increased
	Shoes	(34)	Decomposition of leather influenced by lactic acid component of sweat
	Shoes	(35)	Loss of sulfates and chromic oxide attributed to sweat model in buffers
	Shoes	(36)	Developed a simplified sweat to enable rapid testing of articles
	Shoes	(37)	Sodium lactate largely influenced migration of chrome from leather
	Gloves	(38)	Chrome- and vegetable-tanned leathers released chromium(VI) into sweat
	Clothing	(39)	Decreased shrinkage temperature and increased shrinkage ratio from sweat
	Clothing	(40)	Chrome content of leathers declined rapidly over 4 days then reached equilibrium
	Clothing	(41)	Low levels of Cr, Fe, and Cu leached from metal dyes
Metals	Steel	(42)	Sweats used to evaluate efficacy of cleaning solutions
	Mild steel	(43)	NaCl in sweat mainly responsible for corrosion of mild steels
	Telephone switches	(44)	Pyruvic acid corroded unpassivated Zn whereas lactic acid corroded passivated Zn
	Cutlery, steels, coins	(45)	Ni released from consumer products, stainless steel, and US coins in sweat
	Danish coins, buttons	(46)	Ni released from Danish coins and buttons from jeans in sweat
	Gold foil	(47)	Dissolution increased in presence of amino acid thiol ligands at higher pH values
	Coins, paperclips, thread	(48)	Cu dissolution increased with higher Na concentration in sweat
	Stainless steels	(49)	Studs, clasps, and earrings released nickel in sweat
	Ni wire	(50)	Ni release increased as sweat pH decreased and temperature and oxygen increased
	Ni alloys	(51)	Measureable Ni release from 9 of 11 alloys immersed in sweat
	Stainless steels	(52)	Classified stainless steels according to their pitting potential in sweat
	Ni disks	(53)	Ni corroded in oxygenated sweat
	Jewelry	(54)	Dissolution increased as sweat pH decreased
	Ni alloys	(55)	Ni corroded in oxygenated sweat
	Steel discs, screws, etc.	(56)	Chromium(VI) leached from yellow chromated articles in sweat
	Steels, Ni-plated pieces	(57)	More Ni released from plated pieces and AISI 303 SS than 304, 316, and 430 SSs
	Ni alloys	(58)	Ni release increased with the ratio of sweat volume to alloy surface area
	Steels, Ni-plated pieces	(59)	More Ni released from plated pieces and AISI 303 SS than 304, 316, and 430 SSs
	Jewelry, clothes rivets	(60)	Ni release varied among test articles and did not correlate with DMG test
	Lead powder	(61)	More Pb dissolved in sweat than in NaCl or lactic acid at same concentration
	Cr-contaminated soil	(62)	Sweat pH had varying effect on dissolution of Cr and was sample dependent
	Steels	(63)	Dissolution of Ni, Cr, and Fe increased with time in sweat
	Steels	(64)	Mid-sulfur content AISI 304L, 304L+Ca, 304L+Cu SSs released low levels of Ni
	Cu–Ni alloys	(65)	Corrosion layer of insoluble Cu species formed on the surface of alloys in sweat
	Cr alloys, steels	(66)	Chromium(III) but not(VI) leached from Ni–Cr alloy, SS, Cr–Ni steel in sweat
	Tools	(67)	Two-thirds of tools tested released Ni above safe threshold
	Jewelry	(68)	Cu and Zn, but not gold, were released from gold-containing materials in sweat
	Cu, Ni, and Zn alloys	(69)	Thickness of corrosion layer formed on alloys increased with increased Cu content
	Cr salts	(70)	Sweat used as patch test vehicle and reactions elicited to both chromium(III) and chromium(VI)
	Steel alloys	(71)	Low levels of Ni released from AISI 305, 321, 316L SS alloys into sweat
	ZrN_xO_y thin films	(72)	ZrO_2 films had higher corrosion resistance than ZrN_xO_y films in sweat
	Nb thin films	(73)	Nb thin films on Al, Ti alloy, and SSs imparted corrosion resistance in sweat

(*continued*)

TABLE 16.1 (Continued)
In Vitro Studies of the Interaction of Exogenous Materials with Artificial Skin Surface Film Liquids (1935 to 2013)

Category	Exogenous Material	Reference	Synopsis
Metals	Ti thin films	(74)	Ti thin films on SSs imparted corrosion resistance independent of film thickness
	Co powder	(75)	Co powder oxidized in sweat to produce ions that permeated through skin
	Cr salts	(76)	Methionine reduced chromium(VI) to chromium(III), latter complexed with lactic acid
	Stainless steel	(77)	Developed method to deposit sweat and sebum onto steel for microbial growth
	ZrN_xO_y thin films	(78)	Presence of ZrN_xO_y thin film on steel inhibited corrosion
	Medical devices	(79)	Bi film microelectrode was sensitive for measuring Co and Ni ions in sweat
	Stainless steels	(80)	Ni-free steels for biomedical applications released only traces of Ni ions
	Cr salts	(81)	Chromium(VI) but not chromium(III) in sweat permeated through human and porcine skin
	Jewelry	(82)	Metals such as Pb, Zn, Ni, Cu, and Fe released from jewelry into sweat
	Co, Ni, Cr powders	(83)	Co and Ni (but not Cr) particles oxidized in sweat and ions permeated human skin
	Ni particles	(84)	Ni oxidization in sweat influenced by particle physicochemical properties
	Cu–Ni–Zn alloy	(85)	More Ni than Cu or Zn dissolved from alloy in sweat
	Cu–Ni–Zn alloy, Cu alloy	(86)	Surface layer formed in sweat and was influenced by metal/chloride ion complexes
	Medical device	(87)	AgCl coating on sensor degraded by hydrogen ions in acidic sweat
	Mg alloys	(88)	NaCl and lactic acid in sweat corroded NiP coatings, but urea inhibited corrosion
	Steel, Co–Cr–Mo alloy	(89)	SS released Ni and Cr (but not Co), whereas alloy released Co and Cr (but not Ni)
	Cr powder	(90)	Use of cleanser stopped permeation of Cr ions but resulted in buildup in the skin
	TiC_xO_y thin films	(91)	Thin films exhibited high corrosion resistance
	Gold–indium alloy	(92)	Blue–gold alloy formed an indium oxide surface layer that changed color in sweat
	TiN_x thin films	(93)	TiN_x films rich in N suitable for use as an electrode for electroencephalography
	Hard metal alloys	(94)	All disks released Co, Cr, and Ni into sweat; Co levels high enough to elicit ACD
	Co, Ni, Cr powders	(95)	Co and Ni ion permeation higher for damaged human skin compared to intact skin
	Guitar strings	(96)	Fe, Sn, Mn, and low levels of Ni released from guitar strings in sweat
	Croatian coins	(97)	Amounts of Ni released from coins exceeded EU safe level
	Medical devices	(98)	Passive film on an Ni electrode weakened in the presence of Cl^-
	Fe and Cr alloys	(99)	Low levels of Fe, Cr, and Ni leached from alloy particles into artificial sweat
	TiC_xO_y thin films	(100)	Immersion in sweat caused changes in film surface chemistry
	Pb salts	(101)	Accumulation of Pb in skin lower when sweat was vehicle compared to water
	Bottom ash	(102)	Low levels of Al, As, and Ba leached from ash into sweat
	Silver alloys	(103)	Tarnish of alloys in sweat induced by Cl^- and OH^- ions
	Guitar strings	(104)	Low levels of Fe, Sn, Mn, Si, and Ni leached from strings into sweat
	Metal buttons	(105)	Varying amounts of metals released from buttons; Ni exceeded EU safe level
	Guitar strings	(106)	Varying amounts of metals released from guitar strings
	Hard metal powders	(107)	Co and W powders generated varying levels of ·OH radicals in sweat
	Hard metal powders	(108)	Vitamin E in sebum inhibited dissolution of Co and W from sintered particles
	Gold alloys	(109)	Varying corrosion resistance in sweat that depended on alloy composition
	Gold alloys	(110)	Corrosion behavior of alloys in sweat varied with alloy composition
	Be powders, alloys, tools	(111)	Dissolution of Be from all articles was greater in sweat with pH 5.3 than at pH 6.5
	Mg alloys	(112)	Strongly corroded by lactic acid and NaCl (pitting); urea inhibited corrosion
	Mg alloys	(113)	Alloys corroded by sweat
	Medical devices	(8)	Ni dissolution from an electrode varied with pH
	Medical devices	(9)	Passive film on 304L SS broken down by Cl^-
	Bronze alloy	(114)	Sweat degraded passive indium tin oxide layer on white bronze alloys
	Cosmetics	(1)	Chromium(VI) released from commercially available cosmetic powders
	Be ore powders	(115)	Dissolution of Be from bertrandite ores faster in sweat at pH 5.3 than pH 6.5
	Guitar strings	(116)	Release of Ni from guitar strings influenced by composition of sweat
	Metallic glass	(117)	Mg, Zn, and Ca release in sweat higher for crystalline alloy than amorphous alloy
	Stainless steel	(118)	0.15% Ce in SS improved corrosion resistance and antibacterial performance
	Stainless steel	(119)	0.15% La in SS improved corrosion resistance and antibacterial performance
	Ni powders	(120)	More Ni released from metal powder than oxide powders
	Co powders	(121)	Co permeation higher for damaged human skin compared to intact skin
Minerals	Cataplasms	(7)	Ionic composition of sweat influenced cataplasm swelling
	Glazes	(122)	Wollastonite crystals on surfaces corroded, which led to sebum build up
	Peloids	(123)	Several metals shown to leach from peloids into sweat

(*continued*)

TABLE 16.1 (Continued)
In Vitro Studies of the Interaction of Exogenous Materials with Artificial Skin Surface Film Liquids (1935 to 2013)

Category	Exogenous Material	Reference	Synopsis
Money	Swedish coins	(124)	Ni released from Swedish coins in sweat
	European coins	(125)	Varying amounts of Ni released from coins; some amounts above EU safe level
	Euro coins	(126)	High Ni release observed for Euro coins
	Coins	(127)	PMTA provided better antitarnish performance than BTA for brass in sweat
	Swedish and Euro coins	(128)	Ni released from Euro coins in sweat
	Polish and Euro coins	(129)	Temperature-dependent release of Ni from coins in sweat exceeded EU safe level
Organics	Cosmetics	(130)	Skin softener acted on skin by imparting structural effects on skin lipids
	Rubber (natural) gloves	(131)	Household and surgical gloves released thiurams and carbonates in sweat
	Rubber (natural) products	(132)	Shoes, scuba mask, and gloves released EBT, MBT, and MBTS in sweat
	Rubber (natural) products	(133)	Sweat a more sensitive patch test vehicle for thiurams than petrolatum
	PAHs	(134)	Sweat a more realistic vehicle for PAH permeation through skin than an oil
	Model development	(135)	All sebum models yielded mixtures of solid and liquid lipids at skin temperature
	Therapeutic drugs	(136)	Vehicle miscibility with sebum important for follicular drug delivery
	MPA	(137)	Release of MPA into sweat depended upon how it was incorporated into petrolatum
	Triethanolamine	(138)	Controlled penetration of aqueous triethanolamine into artificial sebum
	Soaps	(139)	Carbopols penetrated into sebum and could influence skin cleansing
	Rubber (natural) gloves	(140)	Amount of sweat-extracted ZDEC linearly correlated with observed cytotoxicity
	Rubber (natural) gloves	(141)	Amount of sweat-extracted ZDEC varied with changes in sweat pH
	Rubber prophylaxis	(5)	Variable amounts of nitrosamines released from condoms into sweat
	IPA, MEK	(142)	IPA and MEK partitioning increased by NaCl and sodium lactate but not urea or NH_3
	Skin	(143)	Unsaturated FAs alter Ca dynamics and induce abnormal follicular keratinization
	Rubber (natural) products	(144)	Amount of sweat-extracted ZDEC higher than other accelerators
	Soaps	(145)	Microemulsions of triglycerides, co-oils, and salts important for cleanser delivery
	Cosmetics	(146)	Varying penetration of alcoholamines in sebum in artificial pilosebaceous unit
	Rubber (natural)	(147)	Developed precise and accurate method for detection of ZDEC in sweat
	Rubber (natural) gloves	(148)	Double layer of gloves prevented migration of mitomycin C into sweat
	Transdermal drugs	(149)	Compound-dependent flux through sebum observed for model drug compounds
	VOCs	(150)	Salts in sweat increased partitioning of hydrophillic and hydrophobic VOCs
	Munitions contaminants	(151)	Hydration of skin with sweat increased absorption of munitions relative to dry skin
	Therapeutic drugs	(152)	Partitioning of model drugs in sebum an important parameter for drug delivery
	Latex gloves	(10)	NDMA, NDEA, and NDBA released from gloves into artificial sweat
	Mechanical skin model	(153)	Developed model for research on friction, adhesion, etc., of liquids and emulsions
	Rubber	(154)	Low levels of PAHs released from rubbers with varying amounts of carbon black
	Therapeutic drugs	(155)	Sebum composition influenced partitioning of model drug compounds
	Model development	(156)	Developed a chemically stable lipid formulation to mimic human sebum
	BTEXs	(12)	Salts in sweat increased partitioning coefficient for BTEXs
	Rubber prophylaxis	(6)	NDMA, NDEA, and NDBA released from condoms into artificial sweat
	Model development	(157)	Developed a sweat model that included amino acids and vitamins
	Model development	(158)	Developed a chemically stable lipid formulation to mimic human sebum
	Latex products	(11)	Developed method to detect nitrosamines released from latex products into sweat
	Toys	(159)	Release of phthalates from toys influenced by lactic acid and urea in sweat
	Artificial sebum	(160)	Developed a microdispensing system for development of artificial fingerprints
Textiles	Cotton	(161)	Identified simplified sweat to test dye fastness on cotton textiles
	Cotton	(162)	At high humidity, unsaturated lipids caused more yellowing than saturated lipids
	Terry cloth	(163)	Developed test to measure foam generated by a laundry detergent formulation
	Percale weave fabric	(164)	Developed test to measure brightener buildup and detergency
	PET, TFEP	(165)	Sebum removal from polymers depended on wash water temperature and detergent
	Cotton, nylon, Dacron	(166)	$^{14}C/^{3}H$-labeled sebum used to measure detergency of soiled textiles
	Cotton, cotton/polyester	(167)	^{14}C-labeled sebum used for studying soiled textiles
	Cotton, nylon, Dacron	(168)	Developed test to measure the yellowing of textiles from sebum residue
	Cotton	(169)	Warm water more effective than cold water in removal of sebum from cotton
	Cotton, cotton/polyester	(170)	Tristearin bound more strongly to textiles than other sebum lipids
	Cotton	(171)	Transfer of aldicarb (a pesticide) increased for sebum soiled textile over time
	Carpeting, polyester	(172)	Extraction of PFOA into sweat influenced by physical differences in fibers

(*continued*)

TABLE 16.1 (Continued)
In Vitro Studies of the Interaction of Exogenous Materials with Artificial Skin Surface Film Liquids (1935 to 2013)

Category	Exogenous Material	Reference	Synopsis
Textiles	Cellulose	(173)	More hydrolysable formaldehyde extracted in sweat than free formaldehyde
	Carpeting	(4)	Sweat facilitated transfer of chlorpyrifos from carpet to skin and into epidermis
	Cotton	(174)	Wash temperature and cycle influenced disinfection of textiles with sweat
	Cotton	(175)	ZnO-finished fabrics leached Zn in acid sweat but were durable in alkaline sweat
	Cotton	(176)	Fixed model dye to cotton and demonstrated its controlled release in sweat
	Flax, linen	(177)	Antibacterial growth on linens evaluated after soaking in artificial sweat
	Cotton	(178)	Cu and Zn leached into sweat with less Ag and almost no Cr released
	Polyester/cotton	(179)	Extraction of dye from textiles higher in sweat at pH 8.0 than at pH 5.5 or 6.8
	Cotton	(180)	More silver dissolved in sweat with pH 6.5 or 8.0 than in sweats with acidic pHs
	Textiles	(181)	Amounts of metals extracted in sweat lower than Oko Tex Standard 100
	Cotton, bamboo, silk	(182)	Release of resveratrol (antibacterial) highest for cotton in alkaline sweat
	Cotton	(183)	Trace levels of Fe detected on textiles saturated with sweat
	Cotton	(184)	Amounts of Cd that leached into sweat below Oko Tex Standard 100
	DBE 209 flame retardant	(185)	DBE migration into sweat increased for UV-degraded and thermally degraded textiles
	Cotton	(2)	More silver dissolved in artificial sweat at pH 8.0 than pH 5.5
	Nylon	(3)	Varying amounts of Cr leached from nylon with black textile dyes
	Cellulose	(186)	Copper from unfixed and fixed dyes released into sweat
	Polyester	(187)	Ag particles and ions released into acid sweat but mostly ions in alkaline sweat

Note: ACD = allergic contact dermatitis; Ag = silver; AgCl = silver chloride; AISI = American Iron and Steel Institute; Al = aluminum; As = arsenic; Ba = barium; Be = beryllium; Bi = bismuth; BTA = benzotriazole; BTEX = benzene, toluene, ethylbenzene, o-xylene, m-xylene, and p-xylene; C = carbon; Ca = calcium; Cd = cadmium; Ce = cerium; Cl– = chloride ion; Co = cobalt; Cr = chromium; Cr^{3+} = chromium(III) ion; Cr^{6+} = chromium(VI) ion; Cu = copper; DBE 209 = decabromodiphenyl ether; DMG = dimethylgloxime; EBT = ethylbutyl thiourea; EU = European Union; FAs = fatty acids; Fe = iron; IPA = isopropyl alcohol; La = lanthanum; MBT = 2-mercaptobenzothiazole; MBTS = 2,2-dithio-bis-benzothiazole; MEK = methylethyl ketone; Mg = magnesium; Mn = manganese; Mo = molybdenum; MPA = maleopimaric acid; N = nitrogen; Na = sodium; NaCl = sodium chloride; Nb = niobium; NDBA = N-nitrosodibutylamine; NDEA = N-nitrosodiethylamine; NDMA = N-nitrosodimethylamine; NH_3 = ammonia; Ni = nickel; OH = hydroxyl; P = phosphorus; PAHs = polycyclic aromatic hydrocarbons; Pb = lead; PET = polyethylene terephthalate; PFOA = perfluorooctanoic acid; PMTA = 1-phenyl-5-mercaptotetrazole; Si = silica; Sn = tin; SS = stainless steel; THC = tetrahydrocannabinol; TFEP = tetrafluoroethylene and hexafluoropolypropylene copolymer; Ti = titanium; TiC_xO_y = titanium oxycarbide; TiN_x = titanium nitride; UV = ultraviolet light; VOCs = volatile organic compounds; W = tungsten; ZDEC = zinc diethyldithiocarbamate; Zn = zinc; ZnO = zinc oxide; ZrN_xO_y = zirconium oxynitride; ZrO_2 = zirconium oxide.

films) to enhance product performance and aesthetics while minimizing potential for adverse skin reactions.

Previously, Stefaniak and Harvey [189] critically reviewed the composition of several artificial SSFL models and compared their composition to human SSFL properties. From that review, it was concluded that the concentrations of individual constituents and pH of artificial sweat and amounts of artificial sebum lipids were not always within ranges reported for human SSFL. Additionally, nearly all artificial SSFL formulations lacked many of the constituents in human SSFL, especially amino acids and vitamins. Given the recent proliferation in reports on the behavior of exogenous materials in contact with artificial SSFLs, it was felt that an updated critical review was needed. Specifically, in this chapter, the composition of human SSFL and artificial SSFL models will be summarized and compared; the importance of test material characterization will be discussed; and available dissolution literature will be summarized and reviewed. Finally, advantages and limitations of in vitro testing approaches are described.

PROPERTIES OF HUMAN SSFL

In this chapter, the properties of human SSFL described are limited to nonexercise-induced eccrine sweat secreted by glands in most skin surfaces. Apocrine sweat secreted by glands in certain body regions (e.g., axillae, pubis) is beyond the scope of this review. The sebum composition of SSFL is summarized for various body regions. On the skin surface, sweat and sebum exist as a cosolvent; sebum lipids are present in both the solid and liquid phase [19,135,190].

The weighted average skin temperature calculated from over 1500 published measurements of various anatomical sites is 33.0°C, with a range of 27.6°C to 45.0°C [191–197]. The temperature of the skin surface varies with anatomical region, level of activity, climate, and many other factors [194–196]. For example, the skin temperature of appendages (fingers, etc.) tends to be cooler than the body torso. Hence, the choice of temperature for in vitro studies should be selected to accurately reflect the temperature of the anatomical region that is anticipated to contact the exogenous material of interest.

Human Sweat

The composition of human sweat is highly variable, which makes it difficult to define "normal" concentrations. Some factors that influence the composition of sweat include body region, age, gender, ethnicity, diet, activity level, and acclimatization [198–201]. Additionally, the technique used to collect sweat and analyze constituents contributes to variability in reported constituent concentrations [202,203].

Sweat pH varies with anatomical region, which is an important consideration when designing in vitro dissolution studies. The pH of the liquid coating on the outer surface of the SC is the product of both endogenous (i.e., phospholipid–FFA pathway, sodium–proton transporters, and possibly the histidine–urocanic acid pathway) and exogenous (i.e., sweat and sebum secretions and their degradation products) factors [204]. Endogenous factors that may influence skin surface pH include anatomical position (e.g., wrist or face); skin health (disease, etc.); age; and chronological rhythms. Exogenous factors that may influence skin surface pH include hygiene practices; use of topical products (i.e., lotions, barrier creams, and cosmetics); and seasonality (winter vs. summer) [205]. Using data from several studies, a weighted average sweat pH is 5.9, with range of 2 to 8 [43,64,141,206,207].

Tables 16.2 through 16.12 summarizes the concentrations of adult human sweat constituents from the published literature reviewed for this chapter. Wherever possible, a weighted average concentration was calculated from study data. If sufficient data were available, a range is also reported to capture variability. In most cases, these range values represent an individual measurement, though in a few instances, a value may be a subject average. If sufficient data were not available to calculate a weighted average, only a range is provided. Note that the composition of human sweat in the first two columns of Table 16.2 is repeated on each page of the table to facilitate the comparison with artificial sweats (described below).

Human sweat contains over 60 different constituents that, for the purposes of this chapter, are categorized as electrolytes, "other" ionic constituents, organic acids, amino acids, nitrogenous substances, and vitamins (and miscellaneous substances). Sweat electrolytes include the cations calcium (Ca^{2+}), potassium (K^+), magnesium (Mg^{2+}), and sodium (Na^+) and the anions chloride (Cl^-), phosphate (PO_4^{2-}), and bicarbonate/carbonate (HCO_3^-/CO_3^{2-}) [28,191,206,210,224–246]. The average concentration of sweat Na^+ is slightly higher than that of Cl^-, though both constituents are an order of magnitude greater than the other sweat electrolytes. The ratio of sweat Na^+ to Cl^- ranges from 0.97 to 1.43, with a median of 1.11 [227]. The ratio of sweat Na^+ to K^+ may be as high as 15 in unacclimatized men but decreases to 5 with adaptation [227]. One review reported that the ratio of sweat Cl^- to K^+ is about 9 [190]. Measured concentrations of sweat electrolytes are highly variable, with concentrations that span two to six orders of magnitude.

Additional cations and anions in human sweat that were categorized as ionic constituents include bromide (Br^-), copper (Cu^{2+}), fluoride (F^-), iron (Fe^{2+}), iodide (I^-), manganese (Mn^{2+}), phosphorous (P^{3+}), sulfur (S^{2-}), sulfate (SO_4^{2-}), and zinc (Zn^{2+}) [7,227,228,231,233,234,237–239,241,243,246–251]. Note that the concentrations of these ionic constituents listed in Table 16.2 have units of micromolar (μM), which is a factor of 1000 lower than the primary electrolytes. Among these ionic constituents, concentrations were greatest for P^{3+}, S^{2-}, and SO_4^{2-}. The Fe^{2+} content of sweat is attributed to epidermal desquamation, not gland-derived sweat [190]. Stauber and Florence [241] reported that persons who were occupationally exposed to cadmium, nickel, and lead secreted trace levels of these metals in sweat; average measured concentrations were 0.02, 1.6, and 0.06 μM, respectively. Sears et al. [252] reviewed available literature and reported that individuals with higher exposure or body burden excreted more lead, arsenic, cadmium, and/or mercury via sweat than urine.

Organic acids in human sweat include acetic, butyric, hexanoic, isobutyric, isovaleric, lactic, propionic, and pyruvic acids. Additionally, sweat contains glucose [18,224,228,230,236,239,242,246,253–257]. Among organic acids in sweat, lactic acid is present at the highest concentration, followed by pyruvic acid [234,258]. The high concentration of these two acids is thought to contribute to the acidity of the skin film [18].

Quantitative data for 18 different amino acids have been reported in the literature: alanine (Ala), arginine (Arg), aspartic acid (Asp), citrulline (Cit), glutamic acid (Glu), glycine (Gly), histidine (His), isoleucine (Ile), leucine (Leu), lysine (Lys), methionine (Met), ornithine (Orn), phenylalanine (Phe), serine (Ser), threonine (Thr), tryptophan (Trp), tyrosine (Tyr), and valine (Val) [28,225,256,258–260]. Itoh and Nakayama [261] reported that sweat also contains cystine (oxidized form of cysteine) and proline, though quantitative data on their concentrations are lacking. Among these amino acids, Met and cystine contain a thiol (−SH) functional group, which has high binding affinity for metal ions such as silver [262,263] and may promote dissolution of gold [47].

Urea and ammonia (NH_3) are the predominant nitrogenous substances in sweat, with traces of creatine, creatinine, and uric acid also present [28,206,224,230,234,239,242,248,253,254,256,258,259,264]. Both sweat urea and NH_3 concentrations are at least a factor of 2 higher than blood plasma and can vary among different regions of the body [227].

Vitamins and miscellaneous compounds in human sweat include ascorbic acid, choline, dehydroascorbic acid (DHA), folic acid (folate), inositol, nicotinic acid (niacin), *p*-aminobenzoic acid (PABA), pantothenic acid, pyridoxine, riboflavin, and thiamine [190,224,234,258,265–269]. Generally, these constituents are present at very low concentrations in sweat.

Human Sebum

Human sebum, the oily phase of SSFL, is a mixture of nonpolar lipids that can be grouped as cholesterol esters (CEs), free cholesterol (CH), FFA, squalene (SQ), triglycerides

TABLE 16.2
Comparison of Human and Artificial Sweats (Part I)[a]

Constituent	Human[b]	(34)	(161)	(161)	(161)	(161)	(208)	(209)
Ca^{2+}	2.8 (0.07–12)							
Cl^-	33.3 (0.02–282)	42.8	59.9	74.8	855.6	855.6	171.1	154.0
K^+	8.4 (1.0–40)							
Mg^{2+}	0.5 ($1.6E^{-5}$–8)							
Na^+	40.7 (4.3–1305)	42.8	62.7	62.7	855.6	855.6	185.2	615.4
PO_4^{2-}	(0.1–1.1)		1.4	1.4			7.0	0.5
HCO_3^-/CO_3^{2-}	(0.3–20.0)						41.6	
Br^{-c}	(2.3–6.3)							
Cu^{2+c}	2.6 (0.7–1180)							
F^{-c}	(3.7–95)							
Fe^{2+c}	4.3 (3.9–190)							
I^{-c}	(0.04–0.1)							
Mn^{2+c}	(0.4–1437)							
P^{3+c}	83.8 (1.0–1550)							
S^{2-c}	(44–2298)							
SO_4^{2-c}	(72.9–1978)							
Zn^{2+c}	16.5 (0.4–104)							
Acetic acid[c]	128 (59–415)		3500	8700		2.6×10^3		
Butyric acid[c]	2.4 (0.5–6.0)	5400						
Glucose[c]	272.2 (11.1–1421)		800	800				
Hexanoic acid[c]	0.9 (0.2–3.5)							
Isobutyric acid[c]	0.8 (?–2.8)							
Isovaleric acid[c]	1.1 (0.2–4.5)							
Lactic acid	19.8 (3.7–57.8)	13.9						465.7
Propionic acid[c]	3.5 (1.2–7.4)							
Pyruvic acid	1.5 (0.06–1.6)							
Alanine	8.6							
Arginine	1.1 (0.3–1.2)							
Aspartic acid	10.6							
Citrulline[d]	0.4							
Glutamic acid	3.1							
Glycine	27.1							
Histidine	3.0							
Isoleucine	1.2							
Leucine	1.4							
Lysine	0.6							
Methionine	(1.0–2.0)							
Ornithine	2.6							
Phenylalanine	0.9							
Serine	35.8							
Threonine	2.9							
Tryptophan[c]	65.4 (19.6–90.6)							
Tyrosine	1.0							
Valine	1.3							
NH_3	4.2 (0.5–8.0)	1.5		26.4	8.6		83.3	
Creatine	(0.2–1.6)							
Creatinine[c]	80.4 (0.9–115)							
Urea	9.8 (2.0–38.8)		8.3	8.3				27.8
Uric acid[c]	37.5 (4.3–148.7)							
Ascorbic acid[c]	1.8 (1.4–62.5)							
Choline[c]	(0.03–0.2)							
DHA[c]	1.8 (?–11.5)							

(*continued*)

TABLE 16.2 (Continued)
Comparison of Human and Artificial Sweats (Part I)[a]

Constituent	Human[b]	(34)	(161)	(161)	(161)	(161)	(208)	(209)
Folic acid[c]	(0.01–0.02)							
Inositol[c]	(0.8–2.0)							
Nicotinic acid[c]	4.1 (0.1–8.1)							
PABA[c]	(0.01–0.1)							
Pantothenic acid[c]	0.2 (0.1–0.4)							
Pyridoxine[c]	(0.002–0.01)							
Riboflavin[c]	0.1							
Thiamine[c]	2.1							

Note: Ca^{2+} = calcium; Cl^- = chloride; K^+ = potassium; Mg^{2+} = magnesium; Na^+ = sodium; PO_4^{2-} = phosphate; HCO_3^-/CO_3^{2-} = bicarbonate/carbonate; Br^- = bromide; Cu^{2+} = copper; F^- = fluoride; Fe^{2+} = iron; I^- = iodide; Mn^{2+} = manganese; P^{3+} = phosphorus; S^{2-} = sulfur; SO_4^{2-} = sulfate; Zn^{2+} = zinc; DHA = dehydroascorbic acid; PABA = p-aminobenzoic acid.

[a] All values in mM unless specified otherwise.

[b] Human sweat: average is a weighted average; (range) is the minimum and maximum values reported in the literature reviewed for this chapter.

[c] Values for constituents in μM.

[d] Not a weighted average (only one study).

TABLE 16.3
Comparison of Human and Artificial Sweats (Part II)[a]

Constituent	Human[b]	(42)	(42)	(42)	(42)	(42)	(210)	(43)
Ca^{2+}	2.8 (0.07–12)							
Cl^-	33.3 (0.02–282)	136.9	171.1	51.3	119.8	89.8	669.4	85.6
K^+	8.4 (1.0–40)							
Mg^{2+}	0.5 (1.6E^{-5}–8)							
Na^+	40.7 (4.3–1305)	136.9	179.5	52.7	119.8	89.8	342.2	85.6
PO_4^{2-}	(0.1–1.1)		8.3					
HCO_3^-/CO_3^{2-}	(0.3–20.0)							
Br^{-c}	(2.3–6.3)							
Cu^{2+c}	2.6 (0.7–1180)							
F^{-c}	(3.7–95)							
Fe^{2+c}	4.3 (3.9–190)							
I^{-c}	(0.04–0.1)							
Mn^{2+c}	(0.4–1437)							
P^{3+c}	83.8 (1.0–1550)							
S^{2-c}	(44–2298)							
SO_4^{2-c}	(72.9–1978)			700				
Zn^{2+c}	16.5 (0.4–104)							
Acetic acid[c]	128 (59–415)	1.7×10^3					4.3×10^3	
Butyric acid[c]	2.4 (0.5–6.0)	2.7×10^3					5.4×10^3	
Glucose[c]	272.2 (11.1–1421)							
Hexanoic acid[c]	0.9 (0.2–3.5)							
Isobutyric acid[c]	0.8 (?–2.8)							
Isovaleric acid[c]	1.1 (0.2–4.5)							
Lactic acid	19.8 (3.7–57.8)		11.1	22.2	44.4	33.3	179.8	10.0
Propionic acid[c]	3.5 (1.2–7.4)						3.5×10^3	
Pyruvic acid	1.5 (0.06–1.6)							
Alanine	8.6							
Arginine	1.1 (0.3–1.2)							
Aspartic acid	10.6							
Citrulline[d]	0.4							

(*continued*)

TABLE 16.3 (Continued)
Comparison of Human and Artificial Sweats (Part II)[a]

Constituent	Human[b]	(42)	(42)	(42)	(42)	(42)	(210)	(43)
Glutamic acid	3.1							
Glycine	27.1							
Histidine	3.0							
Isoleucine	1.2							
Leucine	1.4							
Lysine	0.6							
Methionine	(1.0–2.0)							
Ornithine	2.6							
Phenylalanine	0.9							
Serine	35.8							
Threonine	2.9							
Tryptophan[c]	65.4 (19.6–90.6)							
Tyrosine	1.0							
Valine	1.3							
NH_3	4.2 (0.5–8.0)						327.2	
Creatine	(0.2–1.6)							
Creatinine[c]	80.4 (0.9–115)							
Urea	9.8 (2.0–38.8)			33.3	16.7	12.5	83.3	16.7
Uric acid[c]	37.5 (4.3–148.7)							
Ascorbic acid[c]	1.8 (1.4–62.5)							
Choline[c]	(0.03–0.2)							
DHA[c]	1.8 (?–11.5)							
Folic acid[c]	(0.01–0.02)							
Inositol[c]	(0.8–2.0)							
Nicotinic acid[c]	4.1 (0.1–8.1)							
PABA[c]	(0.01–0.1)							
Pantothenic acid[c]	0.2 (0.1–0.4)							
Pyridoxine[c]	(0.002–0.01)							
Riboflavin[c]	0.1							
Thiamine[c]	2.1							

Note: Ca^{2+} = calcium; Cl^- = chloride; K^+ = potassium; Mg^{2+} = magnesium; Na^+ = sodium; PO_4^{2-} = phosphate; HCO_3^-/CO_3^{2-} = bicarbonate/carbonate; Br^- = bromide; Cu^{2+} = copper; F^- = fluoride; Fe^{2+} = iron; I^- = iodide; Mn^{2+} = manganese; P^{3+} = phosphorus; S^{2-} = sulfur; SO_4^{2-} = sulfate; Zn^{2+} = zinc; DHA = dehydroascorbic acid; PABA = p-aminobenzoic acid.

[a] All values in mM unless specified otherwise.

[b] Human sweat: average is a weighted average; (range) is the minimum and maximum values reported in the literature reviewed for this chapter.

[c] Values for constituents in μM.

[d] Not a weighted average (only one study).

TABLE 16.4
Comparison of Human and Artificial Sweats (Part III)[a]

Constituent	Human[b]	(44)	(44)	(211)	(212)	(124)	(213)	(214)
Ca^{2+}	2.8 (0.07–12)							
Cl^-	33.3 (0.02–282)	171.1	171.1	51.3	172.5	85.6	172.5	1711
K^+	8.4 (1.0–40)							
Mg^{2+}	0.5 ($1.6E^{-5}$–8)							
Na^+	40.7 (4.3–1305)	171.1	171.1	65.4	185.2	85.6	185.2	1711

(*continued*)

TABLE 16.4 (Continued)
Comparison of Human and Artificial Sweats (Part III)[a]

Constituent	Human[b]	(44)	(44)	(211)	(212)	(124)	(213)	(214)
PO_4^{2-}	(0.1–1.1)				7.0		7.0	
HCO_3^-/CO_3^{2-}	(0.3–20.0)				41.6			
Br^{-b}	(2.3–6.3)							
Cu^{2+b}	2.6 (0.7–1180)							
F^{-c}	(3.7–95)							
Fe^{2+c}	4.3 (3.9–190)							
I^{-c}	(0.04–0.1)							
Mn^{2+c}	(0.4–1437)							
P^{3+c}	83.8 (1.0–1550)							
S^{2-c}	(44–2298)							
SO_4^{2-c}	(72.9–1978)			7000				
Zn^{2+c}	16.5 (0.4–104)							
Acetic acid[c]	128 (59–415)							
Butyric acid[c]	2.4 (0.5–6.0)							
Glucose[c]	272.2 (11.1–1421)							
Hexanoic acid[c]	0.9 (0.2–3.5)							
Isobutyric acid[c]	0.8 (?–2.8)							
Isovaleric acid[c]	1.1 (0.2–4.5)							
Lactic acid	19.8 (3.7–57.8)	5551		26.6		11.1	9.4	
Propionic acid[c]	3.5 (1.2–7.4)							
Pyruvic acid	1.5 (0.06–1.6)		5678					
Alanine	8.6							
Arginine	1.1 (0.3–1.2)							
Aspartic acid	10.6							
Citrulline[d]	0.4							
Glutamic acid	3.1							
Glycine	27.1							
Histidine	3.0				1.2		1.2	
Isoleucine	1.2							
Leucine	1.4							
Lysine	0.6							
Methionine	(1.0–2.0)							
Ornithine	2.6							
Phenylalanine	0.9							
Serine	35.8							
Threonine	2.9							
Tryptophan[c]	65.4 (19.6–90.6)							
Tyrosine	1.0							
Valine	1.3							
NH_3	4.2 (0.5–8.0)				83.3			
Creatine	(0.2–1.6)							
Creatinine[c]	80.4 (0.9–115)							
Urea	9.8 (2.0–38.8)			33.3		16.7		
Uric acid[c]	37.5 (4.3–148.7)							
Ascorbic acid[c]	1.8 (1.4–62.5)							
Choline[c]	(0.03–0.2)							
DHA[c]	1.8 (?–11.5)							
Folic acid[c]	(0.01–0.02)							
Inositol[c]	(0.8–2.0)							
Nicotinic acid[c]	4.1 (0.1–8.1)							
PABA[c]	(0.01–0.1)							
Pantothenic acid[c]	0.2 (0.1–0.4)							
Pyridoxine[c]	(0.002–0.01)							

(*continued*)

TABLE 16.4 (Continued)
Comparison of Human and Artificial Sweats (Part III)[a]

Constituent	Human[b]	(44)	(44)	(211)	(212)	(124)	(213)	(214)
Riboflavin[b]	0.1							
Thiamine[b]	2.1							

Note: Ca^{2+} = calcium; Cl^- = chloride; K^+ = potassium; Mg^{2+} = magnesium; Na^+ = sodium; PO_4^{2-} = phosphate; HCO_3^-/CO_3^{2-} = bicarbonate/carbonate; Br^- = bromide; Cu^{2+} = copper; F^- = fluoride; Fe^{2+} = iron; I^- = iodide; Mn^{2+} = manganese; P^{3+} = phosphorus; S^{2-} = sulfur; SO_4^{2-} = sulfate; Zn^{2+} = zinc; DHA = dehydroascorbic acid; PABA = p-aminobenzoic acid.

[a] All values in mM unless noted otherwise.

[b] Human sweat: average is a weighted average; (range) is the minimum and maximum values reported in the literature reviewed for this chapter.

[c] Values for constituents in μM.

[d] Not a weighted average (only one study).

TABLE 16.5
Comparison of Human and Artificial Sweats (Part IV)[a]

Constituent	Human[b]	(214)	(214)	(215)	(47)	(47)	(48)	(48)
Ca^{2+}	2.8 (0.07–12)						0.7	0.7
Cl^-	33.3 (0.02–282)			669.4			17.1	13.7
K^+	8.4 (1.0–40)						2.8	2.8
Mg^{2+}	0.5 ($1.6E^{-5}$–8)							
Na^+	40.7 (4.3–1305)			342.2			19.4	15.5
PO_4^{2-}	(0.1–1.1)						1.4	1.4
HCO_3^-/CO_3^{2-}	(0.3–20.0)							
Br^{-c}	(2.3–6.3)							
Cu^{2+c}	2.6 (0.7–1180)							
F^{-c}	(3.7–95)							
Fe^{2+c}	4.3 (3.9–190)							
I^{-c}	(0.04–0.1)							
Mn^{2+c}	(0.4–1437)							
P^{3+c}	83.8 (1.0–1550)							
S^{2-c}	(44–2298)							
SO_4^{2-c}	(72.9–1978)						400	400
Zn^{2+c}	16.5 (0.4–104)							
Acetic acid[c]	128 (59–415)			4.3×10^3				
Butyric acid[c]	2.4 (0.5–6.0)							
Glucose[c]	272.2 (11.1–1421)						100	100
Hexanoic acid[c]	0.9 (0.2–3.5)							
Isobutyric acid[c]	0.8 (?–2.8)							
Isovaleric acid[c]	1.1 (0.2–4.5)							
Lactic acid	19.8 (3.7–57.8)	5551		179.8			24.9	24.9
Propionic acid[c]	3.5 (1.2–7.4)							
Pyruvic acid	1.5 (0.06–1.6)		5678					
Alanine	8.6				100			
Arginine	1.1 (0.3–1.2)						0.8	0.8
Aspartic acid	10.6							
Citrulline[d]	0.4							
Glutamic acid	3.1							
Glycine	27.1							
Histidine	3.0					100	0.6	0.6
Isoleucine	1.2							
Leucine	1.4						0.4	0.4
Lysine	0.6							

(*continued*)

TABLE 16.5 (Continued)
Comparison of Human and Artificial Sweats (Part IV)[a]

Constituent	Human[b]	(214)	(214)	(215)	(47)	(47)	(48)	(48)
Methionine	(1.0–2.0)							
Ornithine	2.6							
Phenylalanine	0.9							
Serine	35.8							
Threonine	2.9						0.7	0.7
Tryptophan[c]	65.4 (19.6–90.6)							
Tyrosine	1.0							
Valine	1.3						0.5	0.5
NH_3	4.2 (0.5–8.0)			327.2			4.1	4.1
Creatine	(0.2–1.6)							
Creatinine[c]	80.4 (0.9–115)							
Urea	9.8 (2.0–38.8)			83.3			16.1	16.1
Uric acid[c]	37.5 (4.3–148.7)							
Ascorbic acid[c]	1.8 (1.4–62.5)							
Choline[c]	(0.03–0.2)							
DHA[c]	1.8 (?–11.5)							
Folic acid[c]	(0.01–0.02)							
Inositol[c]	(0.8–2.0)							
Nicotinic acid[c]	4.1 (0.1–8.1)							
PABA[c]	(0.01–0.1)							
Pantothenic acid[c]	0.2 (0.1–0.4)							
Pyridoxine[c]	(0.002–0.01)							
Riboflavin[c]	0.1							
Thiamine[c]	2.1							

Note: Ca^{2+} = calcium; Cl^- = chloride; K^+ = potassium; Mg^{2+} = magnesium; Na^+ = sodium; PO_4^{2-} = phosphate; HCO_3^-/CO_3^{2-} = bicarbonate/carbonate; Br^- = bromide; Cu^{2+} = copper; F^- = fluoride; Fe^{2+} = iron; I^- = iodide; Mn^{2+} = manganese; P^{3+} = phosphorus; S^{2-} = sulfur; SO_4^{2-} = sulfate; Zn^{2+} = zinc; DHA = dehydroascorbic acid; PABA = p-aminobenzoic acid.

[a] All values in mM unless noted otherwise.

[b] Human sweat: average is a weighted average; (range) is the minimum and maximum values reported in the literature reviewed for this chapter.

[c] Values for constituents in μM.

[d] Not a weighted average (only one study).

TABLE 16.6
Comparison of Human and Artificial Sweats (Part V)[a]

Constituent	Human[b]	(48)	(50)	(216)	(57)	(217)	(218)	(219)
Ca^{2+}	2.8 (0.07–12)	0.7						
Cl^-	33.3 (0.02–282)	20.6	51.3	85.6	51.3	154	133.9	87.9
K^+	8.4 (1.0–40)	2.8						
Mg^{2+}	0.5 ($1.6E^{-5}$–8)							
Na^+	40.7 (4.3–1305)	22.6	65.4	85.6	65.4	154	68.4	99.7
PO_4^{2-}	(0.1–1.1)	1.4						14.1
HCO_3^-/CO_3^{2-}	(0.3–20.0)							
Br^{-c}	(2.3–6.3)							
Cu^{2+c}	2.6 (0.7–1180)							
F^{-c}	(3.7–95)							
Fe^{2+c}	4.3 (3.9–190)							
I^{-c}	(0.04–0.1)							
Mn^{2+c}	(0.4–1437)							
P^{3+c}	83.8 (1.0–1550)							

(*continued*)

TABLE 16.6 (Continued)
Comparison of Human and Artificial Sweats (Part V)[a]

Constituent	Human[b]	(48)	(50)	(216)	(57)	(217)	(218)	(219)
S^{2-}[c]	(44–2298)							
SO_4^{2-}[c]	(72.9–1978)	400	7000		7000			
Zn^{2+}[c]	16.5 (0.4–104)							
Acetic acid[c]	128 (59–415)			7800			8700	
Butyric acid[c]	2.4 (0.5–6.0)						1.8×10^3	
Glucose[c]	272.2 (11.1–1421)	100						
Hexanoic acid[c]	0.9 (0.2–3.5)							
Isobutyric acid[c]	0.8 (?–2.8)							
Isovaleric acid[c]	1.1 (0.2–4.5)							
Lactic acid	19.8 (3.7–57.8)	24.9	22.2		22.2		40.0	
Propionic acid[c]	3.5 (1.2–7.4)							
Pyruvic acid	1.5 (0.06–1.6)						7.1	
Alanine	8.6							
Arginine	1.1 (0.3–1.2)	0.8						
Aspartic acid	10.6							
Citrulline[d]	0.4							
Glutamic acid	3.1							
Glycine	27.1							
Histidine	3.0	0.6						2.4
Isoleucine	1.2							
Leucine	1.4	0.4						
Lysine	0.6							
Methionine	(1.0–2.0)							
Ornithine	2.6							
Phenylalanine	0.9							
Serine	35.8							
Threonine	2.9	0.7						
Tryptophan[c]	65.4 (19.6–90.6)							
Tyrosine	1.0							
Valine	1.3	0.5						
NH_3	4.2 (0.5–8.0)	4.1					65.4	
Creatine	(0.2–1.6)							
Creatinine[c]	80.4 (0.9–115)							
Urea	9.8 (2.0–38.8)	16.1	33.3	8.3	33.3		16.7	
Uric acid[c]	37.5 (4.3–148.7)							
Ascorbic acid[c]	1.8 (1.4–62.5)							
Choline[c]	(0.03–0.2)							
DHA[c]	1.8 (?–11.5)							
Folic acid[c]	(0.01–0.02)							
Inositol[c]	(0.8–2.0)							
Nicotinic acid[c]	4.1 (0.1–8.1)							
PABA[c]	(0.01–0.1)							
Pantothenic acid[c]	0.2 (0.1–0.4)							
Pyridoxine[c]	(0.002–0.01)							
Riboflavin[c]	0.1							
Thiamine[c]	2.1							

Note: Ca^{2+} = calcium; Cl^- = chloride; K^+ = potassium; Mg^{2+} = magnesium; Na^+ = sodium; PO_4^{2-} = phosphate; HCO_3^-/CO_3^{2-} = bicarbonate/carbonate; Br^- = bromide; Cu^{2+} = copper; F^- = fluoride; Fe^{2+} = iron; I^- = iodide; Mn^{2+} = manganese; P^{3+} = phosphorus; S^{2-} = sulfur; SO_4^{2-} = sulfate; Zn^{2+} = zinc; DHA = dehydroascorbic acid; PABA = p-aminobenzoic acid.

[a] All values in mM unless noted otherwise.

[b] Human sweat: average is a weighted average; (range) is the minimum and maximum values reported in the literature reviewed for this chapter.

[c] Values for constituents in μM.

[d] Not a weighted average (only one study).

TABLE 16.7
Comparison of Human and Artificial Sweats (Part VI)[a]

Constituent	Human[b]	(219)	(63)	(63)	(63)	(63)	(134)	(220)
Ca^{2+}	2.8 (0.07–12)							
Cl^-	33.3 (0.02–282)	87.9	171.1	51.3	85.6	1711		109.4
K^+	8.4 (1.0–40)							
Mg^{2+}	0.5 ($1.6E^{-5}$–8)							
Na^+	40.7 (4.3–1305)	117.6	171.1	51.3	85.6	1711	35.2	101.6
PO_4^{2-}	(0.1–1.1)	32.0					17.6	16.0
HCO_3^-/CO_3^{2-}	(0.3–20.0)							
Br^{-c}	(2.3–6.3)							
Cu^{2+c}	2.6 (0.7–1180)							
F^{-c}	(3.7–95)							
Fe^{2+c}	4.3 (3.9–190)							
I^{-c}	(0.04–0.1)							
Mn^{2+c}	(0.4–1437)							
P^{3+c}	83.8 (1.0–1550)							
S^{2-c}	(44–2298)							
SO_4^{2-c}	(72.9–1978)							
Zn^{2+c}	16.5 (0.4–104)							
Acetic acid[c]	128 (59–415)							
Butyric acid[c]	2.4 (0.5–6.0)							
Glucose[c]	272.2 (11.1–1421)							
Hexanoic acid[c]	0.9 (0.2–3.5)							
Isobutyric acid[c]	0.8 (?–2.8)							
Isovaleric acid[c]	1.1 (0.2–4.5)							
Lactic acid	19.8 (3.7–57.8)							
Propionic acid[c]	3.5 (1.2–7.4)							
Pyruvic acid	1.5 (0.06–1.6)							
Alanine	8.6							
Arginine	1.1 (0.3–1.2)							
Aspartic acid	10.6							
Citrulline[d]	0.4							
Glutamic acid	3.1							
Glycine	27.1							
Histidine	3.0	2.4						23.9
Isoleucine	1.2							
Leucine	1.4							
Lysine	0.6							
Methionine	(1.0–2.0)							
Ornithine	2.6							
Phenylalanine	0.9							
Serine	35.8							
Threonine	2.9							
Tryptophan[c]	65.4 (19.6–90.6)							
Tyrosine	1.0							
Valine	1.3							
NH_3	4.2 (0.5–8.0)							
Creatine	(0.2–1.6)							
Creatinine[c]	80.4 (0.9–115)							
Urea	9.8 (2.0–38.8)							
Uric acid[c]	37.5 (4.3–148.7)							
Ascorbic acid[c]	1.8 (1.4–62.5)							
Choline[c]	(0.03–0.2)							
DHA[c]	1.8 (?–11.5)							
Folic acid[c]	(0.01–0.02)							

(*continued*)

TABLE 16.7 (Continued)
Comparison of Human and Artificial Sweats (Part VI)[a]

Constituent	Human[b]	(219)	(63)	(63)	(63)	(63)	(134)	(220)
Inositol[c]	(0.8–2.0)							
Nicotinic acid[c]	4.1 (0.1–8.1)							
PABA[c]	(0.01–0.1)							
Pantothenic acid[c]	0.2 (0.1–0.4)							
Pyridoxine[c]	(0.002–0.01)							
Riboflavin[c]	0.1							
Thiamine[c]	2.1							

Note: Ca^{2+} = calcium; Cl^- = chloride; K^+ = potassium; Mg^{2+} = magnesium; Na^+ = sodium; PO_4^{2-} = phosphate; HCO_3^-/CO_3^{2-} = bicarbonate/carbonate; Br^- = bromide; Cu^{2+} = copper; F^- = fluoride; Fe^{2+} = iron; I^- = iodide; Mn^{2+} = manganese; P^{3+} = phosphorus; S^{2-} = sulfur; SO_4^{2-} = sulfate; Zn^{2+} = zinc; DHA = dehydroascorbic acid; PABA = p-aminobenzoic acid.

[a] All values in mM unless noted otherwise.

[b] Human sweat: average is a weighted average; (range) is the minimum and maximum values reported in the literature reviewed for this chapter.

[c] Values for constituents in μM.

[d] Not a weighted average (only one study).

TABLE 16.8
Comparison of Human and Artificial Sweats (Part VII)[a]

Constituent	Human[b]	(70)	(7)	(7)	(7)	(7)	(30)	(72)
Ca^{2+}	2.8 (0.07–12)		1.5	29.9	59.8	119.7		
Cl^-	33.3 (0.02–282)	85.6	53.0	153.8	213.6	333.3	74.0	144.4
K^+	8.4 (1.0–40)		7.5	10.0	10.0	10.0	5.0	16.1
Mg^{2+}	0.5 ($1.6E^{-5}$–8)		1.0	2.0	2.0	2.0		
Na^+	40.7 (4.3–1305)	94.5	50.0	93.9	93.9	93.9	74.0	128.3
PO_4^{2-}	(0.1–1.1)		7.4	10.0	10.0	10.0		
HCO_3^-/CO_3^{2-}	(0.3–20.0)							
Br^{-c}	(2.3–6.3)							
Cu^{2+c}	2.6 (0.7–1180)							
F^{-c}	(3.7–95)							
Fe^{2+c}	4.3 (3.9–190)							
I^{-c}	(0.04–0.1)							
Mn^{2+c}	(0.4–1437)							
P^{3+c}	83.8 (1.0–1550)							
S^{2-c}	(44–2298)							
SO_4^{2-c}	(72.9–1978)		1000	2000	2000	2000		
Zn^{2+c}	16.5 (0.4–104)							
Acetic acid[c]	128 (59–415)							
Butyric acid[c]	2.4 (0.5–6.0)							
Glucose[c]	272.2 (11.1–1421)							
Hexanoic acid[c]	0.9 (0.2–3.5)							
Isobutyric acid[c]	0.8 (?–2.8)							
Isovaleric acid[c]	1.1 (0.2–4.5)							
Lactic acid	19.8 (3.7–57.8)	9.0					9.1	11.1
Propionic acid[c]	3.5 (1.2–7.4)							
Pyruvic acid	1.5 (0.06–1.6)							
Alanine	8.6	11.2						

(*continued*)

TABLE 16.8 (Continued)
Comparison of Human and Artificial Sweats (Part VII)[a]

Constituent	Human[b]	(70)	(7)	(7)	(7)	(7)	(30)	(72)
Arginine	1.1 (0.3–1.2)							
Aspartic acid	10.6							
Citrulline[d]	0.4							
Glutamic acid	3.1							
Glycine	27.1	13.3						
Histidine	3.0							
Isoleucine	1.2							
Leucine	1.4							
Lysine	0.6							
Methionine	(1.0–2.0)	6.7						
Ornithine	2.6							
Phenylalanine	0.9							
Serine	35.8	9.5						
Threonine	2.9	8.4						
Tryptophan[c]	65.4 (19.6–90.6)							
Tyrosine	1.0							
Valine	1.3							
NH_3	4.2 (0.5–8.0)							
Creatine	(0.2–1.6)							
Creatinine[c]	80.4 (0.9–115)							
Urea	9.8 (2.0–38.8)	16.7					22.0	16.7
Uric acid[c]	37.5 (4.3–148.7)							
Ascorbic acid[c]	1.8 (1.4–62.5)							
Choline[c]	(0.03–0.2)							
DHA[c]	1.8 (?–11.5)							
Folic acid[c]	(0.01–0.02)							
Inositol[c]	(0.8–2.0)							
Nicotinic acid[c]	4.1 (0.1–8.1)							
PABA[c]	(0.01–0.1)							
Pantothenic acid[c]	0.2 (0.1–0.4)							
Pyridoxine[c]	(0.002–0.01)							
Riboflavin[c]	0.1							
Thiamine[c]	2.1							

Note: Ca^{2+} = calcium; Cl^- = chloride; K^+ = potassium; Mg^{2+} = magnesium; Na^+ = sodium; PO_4^{2-} = phosphate; HCO_3^-/CO_3^{2-} = bicarbonate/carbonate; Br^- = bromide; Cu^{2+} = copper; F^- = fluoride; Fe^{2+} = iron; I^- = iodide; Mn^{2+} = manganese; P^{3+} = phosphorus; S^{2-} = sulfur; SO_4^{2-} = sulfate; Zn^{2+} = zinc; DHA = dehydroascorbic acid; PABA = p-aminobenzoic acid.

[a] All values in mM unless noted otherwise.

[b] Human sweat: average is a weighted average; (range) is the minimum and maximum values reported in the literature reviewed for this chapter.

[c] Values for constituents in μM.

[d] Not a weighted average (only one study).

TABLE 16.9
Comparison of Human and Artificial Sweats (Part VIII)[a]

Constituent	Human[b]	(221)	(222)	(5)	(142)	(77)	(28)	(40)
Ca^{2+}	2.8 (0.07–12)						0.04	
Cl^-	33.3 (0.02–282)	85.6	1711	88.5	80.1	481.7	2.0	85.0
K^+	8.4 (1.0–40)			4.0		15.0	0.7	5.0
Mg^{2+}	0.5 ($1.6E^{-5}$–8)						0.01	
Na^+	40.7 (4.3–1305)	85.6	1711	81.2	133.6	443.1	1.0	80.1
PO_4^{2-}	(0.1–1.1)							
HCO_3^-/CO_3^{2-}	(0.3–20.0)					7.5		
Br^{-c}	(2.3–6.3)							
Cu^{2+c}	2.6 (0.7–1180)							
F^{-c}	(3.7–95)							
Fe^{2+c}	4.3 (3.9–190)							
I^{-c}	(0.04–0.1)							
Mn^{2+c}	(0.4–1437)							
P^{3+c}	83.8 (1.0–1550)							
S^{2-c}	(44–2298)							
SO_4^{2-c}	(72.9–1978)			2100		19.3		
Zn^{2+c}	16.5 (0.4–104)							
Acetic acid[c]	128 (59–415)					8.6		
Butyric acid[c]	2.4 (0.5–6.0)							
Glucose[c]	272.2 (11.1–1421)							2000
Hexanoic acid[c]	0.9 (0.2–3.5)							
Isobutyric acid[c]	0.8 (?–2.8)							
Isovaleric acid[c]	1.1 (0.2–4.5)							
Lactic acid	19.8 (3.7–57.8)		666	33.3	54.1			27.8
Propionic acid[c]	3.5 (1.2–7.4)							
Pyruvic acid	1.5 (0.06–1.6)							
Alanine	8.6							
Arginine	1.1 (0.3–1.2)						0.002	
Aspartic acid	10.6							
Citrulline[d]	0.4							
Glutamic acid	3.1							
Glycine	27.1							
Histidine	3.0						0.02	
Isoleucine	1.2							
Leucine	1.4							
Lysine	0.6						0.20	
Methionine	(1.0–2.0)							
Ornithine	2.6						0.06	
Phenylalanine	0.9							
Serine	35.8							
Threonine	2.9							
Tryptophan[c]	65.4 (19.6–90.6)							
Tyrosine	1.0							
Valine	1.3							
NH_3	4.2 (0.5–8.0)	150.7		7.5	1.0		0.2	
Creatine	(0.2–1.6)							
Creatinine[c]	80.4 (0.9–115)							
Urea	9.8 (2.0–38.8)			3.3	8.6	207.0		7.5
Uric acid[c]	37.5 (4.3–148.7)							
Ascorbic acid[c]	1.8 (1.4–62.5)							
Choline[c]	(0.03–0.2)							
DHA[c]	1.8 (?–11.5)							
Folic acid[c]	(0.01–0.02)							

(*continued*)

TABLE 16.9 (Continued)
Comparison of Human and Artificial Sweats (Part VIII)[a]

Constituent	Human[b]	(221)	(222)	(5)	(142)	(77)	(28)	(40)
Inositol[c]	(0.8–2.0)							
Nicotinic acid[c]	4.1 (0.1–8.1)							
PABA[c]	(0.01–0.1)							
Pantothenic acid[c]	0.2 (0.1–0.4)							
Pyridoxine[c]	(0.002–0.01)							
Riboflavin[c]	0.1							
Thiamine[c]	2.1							

Note: Ca^{2+} = calcium; Cl^- = chloride; K^+ = potassium; Mg^{2+} = magnesium; Na^+ = sodium; PO_4^{2-} = phosphate; HCO_3^-/CO_3^{2-} = bicarbonate/carbonate; Br^- = bromide; Cu^{2+} = copper; F^- = fluoride; Fe^{2+} = iron; I^- = iodide; Mn^{2+} = manganese; P^{3+} = phosphorus; S^{2-} = sulfur; SO_4^{2-} = sulfate; Zn^{2+} = zinc; DHA = dehydroascorbic acid; PABA = p-aminobenzoic acid.

[a] All values in mM unless noted otherwise.

[b] Human sweat: average is a weighted average; (range) is the minimum and maximum values reported in the literature reviewed for this chapter.

[c] Values for constituents in μM.

[d] Not a weighted average (only one study).

TABLE 16.10
Comparison of Human and Artificial Sweats (Part IX)[a]

Constituent	Human[b]	(148)	(175)	(175)	(175)	(88)	(88)	(88)
Ca^{2+}	2.8 (0.07–12)							
Cl^-	33.3 (0.02–282)	14.9	136.9	85.6	8.6	85.6	85.6	
K^+	8.4 (1.0–40)	3.0			1.4			
Mg^{2+}	0.5 (1.6E^{-5}–8)							
Na^+	40.7 (4.3–1305)	13.0	136.9	120.8	58.5	85.6	85.6	
PO_4^{2-}	(0.1–1.1)			17.6				
HCO_3^-/CO_3^{2-}	(0.3–20.0)	1.1			51.4			
Br^{-c}	(2.3–6.3)							
Cu^{2+c}	2.6 (0.7–1180)							
F^{-c}	(3.7–95)							
Fe^{2+c}	4.3 (3.9–190)							
I^{-c}	(0.04–0.1)							
Mn^{2+c}	(0.4–1437)							
P^{3+c}	83.8 (1.0–1550)							
S^{2-c}	(44–2298)							
SO_4^{2-c}	(72.9–1978)							
Zn^{2+c}	16.5 (0.4–104)							
Acetic acid[c]	128 (59–415)							
Butyric acid[c]	2.4 (0.5–6.0)							
Glucose[c]	272.2 (11.1–1421)							
Hexanoic acid[c]	0.9 (0.2–3.5)							
Isobutyric acid[c]	0.8 (?–2.8)							
Isovaleric acid[c]	1.1 (0.2–4.5)							
Lactic acid	19.8 (3.7–57.8)	11.0	22.2			55.5		55.5

(*continued*)

TABLE 16.10 (Continued)
Comparison of Human and Artificial Sweats (Part IX)[a]

Constituent	Human[b]	(148)	(175)	(175)	(175)	(88)	(88)	(88)
Propionic acid[c]	3.5 (1.2–7.4)							
Pyruvic acid	1.5 (0.06–1.6)							
Alanine	8.6							
Arginine	1.1 (0.3–1.2)							
Aspartic acid	10.6							
Citrulline[d]	0.4							
Glutamic acid	3.1							
Glycine	27.1							
Histidine	3.0							
Isoleucine	1.2							
Leucine	1.4							
Lysine	0.6			3.4				
Methionine	(1.0–2.0)							
Ornithine	2.6							
Phenylalanine	0.9							
Serine	35.8							
Threonine	2.9							
Tryptophan[c]	65.4 (19.6–90.6)							
Tyrosine	1.0							
Valine	1.3							
NH_3	4.2 (0.5–8.0)							
Creatine	(0.2–1.6)							
Creatinine[c]	80.4 (0.9–115)							
Urea	9.8 (2.0–38.8)		16.7			16.7		
Uric acid[c]	37.5 (4.3–148.7)							
Ascorbic acid[c]	1.8 (1.4–62.5)							
Choline[c]	(0.03–0.2)							
DHA[c]	1.8 (?–11.5)							
Folic acid[c]	(0.01–0.02)							
Inositol[c]	(0.8–2.0)							
Nicotinic acid[c]	4.1 (0.1–8.1)							
PABA[c]	(0.01–0.1)							
Pantothenic acid[c]	0.2 (0.1–0.4)							
Pyridoxine[c]	(0.002–0.01)							
Riboflavin[c]	0.1							
Thiamine[c]	2.1							

Note: Ca^{2+} = calcium; Cl^- = chloride; K^+ = potassium; Mg^{2+} = magnesium; Na^+ = sodium; PO_4^{2-} = phosphate; HCO_3^-/CO_3^{2-} = bicarbonate/carbonate; Br^- = bromide; Cu^{2+} = copper; F^- = fluoride; Fe^{2+} = iron; I^- = iodide; Mn^{2+} = manganese; P^{3+} = phosphorus; S^{2-} = sulfur; SO_4^{2-} = sulfate; Zn^{2+} = zinc; DHA = dehydroascorbic acid; PABA = p-aminobenzoic acid.

[a] All values in mM unless noted otherwise.

[b] Human sweat: average is a weighted average; (range) is the minimum and maximum values reported in the literature reviewed for this chapter.

[c] Values for constituents in μM.

[d] Not a weighted average (only one study).

TABLE 16.11
Comparison of Human and Artificial Sweats (Part X)[a]

Constituent	Human[b]	(88)	(88)	(183)	(103)	(157)	(111)	(12)
Ca^{2+}	2.8 (0.07–12)					11.3	2.3E-4	
Cl^-	33.3 (0.02–282)	85.6		100.0	85.6	33.7	33.2	80.1
K^+	8.4 (1.0–40)					8.5	6.1	
Mg^{2+}	0.5 (1.6E^{-5}–8)					0.082	0.082	
Na^+	40.7 (4.3–1305)	85.6		100.0	85.6	27.7	30.7	133.6
PO_4^{2-}	(0.1–1.1)					0.31	0.31	
HCO_3^-/CO_3^{2-}	(0.3–20.0)					2.5	3.0	
Br^{-c}	(2.3–6.3)					2.3	2.3	
Cu^{2+c}	2.6 (0.7–1180)					0.94	0.94	
F^{-c}	(3.7–95)					11	11	
Fe^{2+c}	4.3 (3.9–190)					9.8	9.8	
I^{-c}	(0.04–0.1)					0.07	0.07	
Mn^{2+c}	(0.4–1437)					11	11	
P^{3+c}	83.8 (1.0–1550)					13	13	
S^{2-c}	(44–2298)					2300	2300	
SO_4^{2-c}	(72.9–1978)				1000	420	420	
Zn^{2+c}	16.5 (0.4–104)					13	13	
Acetic acid[c]	128 (59–415)					130	130	
Butyric acid[c]	2.4 (0.5–6.0)					2.4	2.4	
Glucose[c]	272.2 (11.1–1421)					170	170	
Hexanoic acid[c]	0.9 (0.2–3.5)					0.9	0.9	
Isobutyric acid[c]	0.8 (?–2.8)					0.8	0.8	
Isovaleric acid[c]	1.1 (0.2–4.5)					1.1	1.1	
Lactic acid	19.8 (3.7–57.8)		55.5		12.5	14.0	14.0	54.1
Propionic acid[c]	3.5 (1.2–7.4)					3.5	3.5	
Pyruvic acid	1.5 (0.06–1.6)					0.18	0.18	
Alanine	8.6					0.36	0.36	
Arginine	1.1 (0.3–1.2)					0.78	0.78	
Aspartic acid	10.6					0.34	0.34	
Citrulline[d]	0.4					0.40	0.40	
Glutamic acid	3.1					0.37	0.37	
Glycine	27.1					0.39	0.39	
Histidine	3.0					0.52	0.52	
Isoleucine	1.2					0.17	0.17	
Leucine	1.4					0.21	0.21	
Lysine	0.6					0.15	0.15	
Methionine	(1.0–2.0)						1.5	
Ornithine	2.6					0.15	0.15	
Phenylalanine	0.9					0.13	0.13	
Serine	35.8							
Threonine	2.9					0.45	0.45	
Tryptophan[c]	65.4 (19.6–90.6)					60	60	
Tyrosine	1.0					0.17	0.17	
Valine	1.3					0.25	0.25	
NH_3	4.2 (0.5–8.0)					5.3	5.2	1.0
Creatine	(0.2–1.6)					0.015	0.015	
Creatinine[c]	80.4 (0.9–115)					84	84	
Urea	9.8 (2.0–38.8)	16.7	16.7		16.7	10.0	10.0	8.6
Uric acid[c]	37.5 (4.3–148.7)					59	59	
Ascorbic acid[c]	1.8 (1.4–62.5)					10	10	
Choline[c]	(0.03–0.2)					26	26	
DHA[c]	1.8 (?–11.5)					11	11	
Folic acid[c]	(0.01–0.02)					0.016	0.016	

(*continued*)

TABLE 16.11 (Continued)
Comparison of Human and Artificial Sweats (Part X)[a]

Constituent	Human[b]	(88)	(88)	(183)	(103)	(157)	(111)	(12)
Inositol[c]	(0.8–2.0)					1.6	1.6	
Nicotinic acid[c]	4.1 (0.1–8.1)					4.1×10^3	2.9	
PABA[c]	(0.01–0.1)					0.071	0.071	
Pantothenic acid[c]	0.2 (0.1–0.4)					11300	0.23	
Pyridoxine[c]	(0.002–0.01)					0.01	0.006	
Riboflavin[c]	0.1					2000	0.013	
Thiamine[c]	2.1					500	0.020	

Note: Ca^{2+} = calcium; Cl^- = chloride; K^+ = potassium; Mg^{2+} = magnesium; Na^+ = sodium; PO_4^{2-} = phosphate; HCO_3^-/CO_3^{2-} = bicarbonate/carbonate; Br^- = bromide; Cu^{2+} = copper; F^- = fluoride; Fe^{2+} = iron; I^- = iodide; Mn^{2+} = manganese; P^{3+} = phosphorus; S^{2-} = sulfur; SO_4^{2-} = sulfate; Zn^{2+} = zinc; DHA = dehydroascorbic acid; PABA = p-aminobenzoic acid.

[a] All values in mM unless noted otherwise.

[b] Human sweat: average is a weighted average; (range) is the minimum and maximum values reported in the literature reviewed for this chapter.

[c] Values for constituents in μM.

[d] Not a weighted average (only one study).

TABLE 16.12
Comparison of Human and Artificial Sweats (Part XI)[a]

Constituent	Human[b]	(98)	(98)	(98)	(98)	(223)	(116)
Ca^{2+}	2.8 (0.07–12)						
Cl^-	33.3 (0.02–282)	30.0	60.0	90.0	120.0	514.4	154.0
K^+	8.4 (1.0–40)					17.7	
Mg^{2+}	0.5 ($1.6E^{-5}$–8)						
Na^+	40.7 (4.3–1305)	30.0	60.0	90.0	120.0	559.1	154.0
PO_4^{2-}	(0.1–1.1)						
HCO_3^-/CO_3^{2-}	(0.3–20.0)					8.8	
Br^{-c}	(2.3–6.3)						
Cu^{2+c}	2.6 (0.7–1180)						
F^{-c}	(3.7–95)						
Fe^{2+c}	4.3 (3.9–190)						
I^{-c}	(0.04–0.1)						
Mn^{2+c}	(0.4–1437)						
P^{3+c}	83.8 (1.0–1550)						
S^{2-c}	(44–2298)						
SO_4^{2-c}	(72.9–1978)					22.4	
Zn^{2+c}	16.5 (0.4–104)						
Acetic acid[c]	128 (59–415)					10	
Butyric acid[c]	2.4 (0.5–6.0)						
Glucose[c]	272.2 (11.1–1421)						
Hexanoic acid[c]	0.9 (0.2–3.5)						
Isobutyric acid[c]	0.8 (?–2.8)						
Isovaleric acid[c]	1.1 (0.2–4.5)						
Lactic acid	19.8 (3.7–57.8)						11.1
Propionic acid[c]	3.5 (1.2–7.4)						

(*continued*)

TABLE 16.12 (Continued)
Comparison of Human and Artificial Sweats (Part XI)[a]

Constituent	Human[b]	(98)	(98)	(98)	(98)	(223)	(116)
Pyruvic acid	1.5 (0.06–1.6)						
Alanine	8.6						
Arginine	1.1 (0.3–1.2)						
Aspartic acid	10.6						
Citrulline[d]	0.4						
Glutamic acid	3.1						
Glycine	27.1						
Histidine	3.0						
Isoleucine	1.2						
Leucine	1.4						
Lysine	0.6						
Methionine	(1.0–2.0)						
Ornithine	2.6						
Phenylalanine	0.9						
Serine	35.8						
Threonine	2.9						
Tryptophan[c]	65.4 (19.6–90.6)						
Tyrosine	1.0						
Valine	1.3						
NH_3	4.2 (0.5–8.0)						
Creatine	(0.2–1.6)						
Creatinine[c]	80.4 (0.9–115)						
Urea	9.8 (2.0–38.8)					240.1	16.7
Uric acid[c]	37.5 (4.3–148.7)						
Ascorbic acid[c]	1.8 (1.4–62.5)						
Choline[c]	(0.03–0.2)						
DHA[c]	1.8 (?–11.5)						
Folic acid[c]	(0.01–0.02)						
Inositol[c]	(0.8–2.0)						
Nicotinic acid[c]	4.1 (0.1–8.1)						
PABA[c]	(0.01–0.1)						
Pantothenic acid[c]	0.2 (0.1–0.4)						
Pyridoxine[c]	(0.002–0.01)						
Riboflavin[c]	0.1						
Thiamine[c]	2.1						

Note: Ca^{2+} = calcium; Cl^- = chloride; K^+ = potassium; Mg^{2+} = magnesium; Na^+ = sodium; PO_4^{2-} = phosphate; HCO_3^-/CO_3^{2-} = bicarbonate/carbonate; Br^- = bromide; Cu^{2+} = copper; F^- = fluoride; Fe^{2+} = iron; I^- = iodide; Mn^{2+} = manganese; P^{3+} = phosphorus; S^{2-} = sulfur; SO_4^{2-} = sulfate; Zn^{2+} = zinc; DHA = dehydroascorbic acid; PABA = p-aminobenzoic acid.

[a] All values in mM unless noted otherwise.

[b] Human sweat: average is a weighted average; (range) is the minimum and maximum values reported in the literature reviewed for this chapter.

[c] Values for constituents in μM.

[d] Not a weighted average (only one study).

(TGs), and wax esters (WEs) [190,270–274]. Among these lipid classes, FFA, WE, TG, and SQ represent approximately 95% of sebum lipids. At human skin surface temperature, sebum has a specific gravity of 0.91 to 0.93 [19,135,190]. Sebum spreads itself on the skin surface as a relatively thin, unevenly distributed sheet that ranges in thickness from <0.05 μm in sebum-poor areas to >4 μm in sebum-rich areas such as the face [275]. Sebum secretion rates vary among facial and anatomical regions [195,276], with age [277], and with climatic season [278]. The composition of sebum is relatively constant within a person but varies among persons [190,279–281].

As summarized in Table 16.13, CE and CH are present in relatively lower amounts than other sebum lipids, but concentrations are variable. The mean CE concentration is 2.4% [19,149,155,283], and that of CH is 2.2% [19,149,155,

TABLE 16.13
Comparison of Human and Artificial Sebums

Artificial	CE	CH	FFA	SQ	TG	WE
(42)[a]					0.4	
(163)		5.0	30.0	5.0	35.0	25.0
(164)[b]		5.0	30.0	5.0	25.0	25.0
(165)[c]			30.0		30.0	
(168)[b]		5.0	30.0	5.0	35.0	25.0
(167)[c]		8.0	30.0	8.0	46.0	
(166)[c]		7.0	50.0		35.0	
(169)[c]		5.0	30.0	8.0	50.0	
(170)[c]		8.0	30.0	8.0	46.0	
(211)[a]					0.4	
(130)[c]	3.0		19.9	12.2	41.8	20.3
(50)[a]			0.001		0.04	
(27)	6.0		37.0	15.0	34.0	8.0
(21)			98.0		2.0	
(134)			0.01		0.02	
(135)[d]			17.0	13.0	43.0	27.0
(136)			50.0	13.0	10.0	27.0
(282)	3.0	1.0	5.0	12.0	53.0	26.0
(139)		4.0	24.0	12.0	33.0	22.0
(143)			40.0		10.0	
(77)[c]		4.2	31.9	4.2	29.4	8.4
(145)	2.3	1.5	29.3	12.2	23.5	31.2
(29)	2.0	1.0	35.0	15.0	42.0	
(152)	2.4	1.2	11.4	15.0	45.0	25.0
(156)			17.0	12.4	44.7	25.0
(155)	2.0	3.0	14.0	15.0	46.0	20.0
(158)	1.9	3.0	27.6	10.3	32.1	24.3
Human[e]	2.4	2.2	24.4	12.6	40.1	25.3
	(1.5–3.5)	(0.6–9.5)	(2.3–39.0)	(3.3–19.9)	(14.8–63.6)	(19.0–31.7)

Note: CE = cholesterol esters; CH = free cholesterol; FFA = free fatty acids; SQ = squalene; TG = triglycerides; WE = wax esters; Vit E = vitamin E.
All values in wt.%.

[a] Remainder of formulation is artificial sweat.

[b] Compositions of Spangler and Cross (164) and Spangler et al. (168) are the same; however, ratios of saturated to unsaturated triglycerides, fatty acids, and wax esters differ.

[c] Remainder of formulation is alcohols or constituents not present in human sebum.

[d] Seven formulations each having different carbon chain length constituents and ratios of saturated to unsaturated triglycerides, fatty acids, and wax esters.

[e] Values are average (range).

248,258,283,284]. The theoretical CE content of sebum is 3.0%, and that of CH is 1.5% [285]. In normal human sebum, both CE and CH are derived from degeneration of cells on the skin surface [286], sebaceous gland production, and esterification of cholesterol to CEs by skin bacteria [287]. Using gas chromatography analysis, Haahti et al. [288] determined that CH is a chief component of unsaponifiable materials in sebum.

Bacterial enzymatic hydrolysis of TGs on the skin surface yields FFAs, which are thought to contribute to the acidic pH of the skin film liquids [190,289]. The mean FFA content of sebum on the skin surface is 24%, with range a range of 2% to 39% [19,149,248,258,290]. Some skin surface-derived FFAs have a 16-carbon chain length for both saturated and unsaturated fatty acids rather than an 18-carbon chain length, which differentiates them from the fatty acids synthesized in other human tissues or obtained from diet [291]. The major FFAs in sebum have 14-, 16-, or 18-carbon-length chains [135,288,292]. Approximately 21 different lipids constitute 88% of FFAs on skin, with the balance composed of over 200 different fatty acids. Among FFAs on skin, 37% are considered "biologically valuable": palmitic (25.3%), myristic (6.9%), stearic (2.9%), oleic (1.9%), and linoleic (0.5%) [293].

SQ is a 30-carbon hexaene lipid constituent of human sebum at an average concentration of 12.6% [19,149,155,

248,258,283,284,290]. Clinical measurements of sebum SQ content are in good agreement with the theoretical predicted amount of 12.0% calculated by Strauss et al. [285].

TGs (neutral fats) are the primary constituents of sebum secreted via hair follicles onto the skin surface. Once on the skin surface, bacterial lipases hydrolyze certain TGs to small amounts of monoglycerides and diglycerides and FFAs [19,135,277,291,294]. Pure human sebum TGs have unsaturated and saturated components [135] mixed at a ratio of 1:2 [295]. The average TG concentration on the skin surface is approximately 40% but may be as high as 64% of sebum lipids [19,149,155,248,283,290,294].

The long-chain fatty alcohols of sebaceous WEs have similar unsaturated structure (i.e., types of carbon chain branching and double bond location at an even number of carbons from the carboxyl group) to those of the fatty acids. WE carbon chains that are saturated (reduced to alcohols) are subjected to extensive elongation, with the end result being a predominant chain length of 20 carbons [291]. The average WE content in human sebum was 25%, with a range of 19% to 32% [19,149,155,248,283,284,290]. Strauss et al. [285] estimated that the theoretical concentration of unsaturated and saturated WEs in human sebum is 26.0%.

In addition to the major lipid categories summarized above, sebaceous glands secrete vitamin E (α-tocopherol) onto the skin surface [296,297]. Though vitamin E is only present in trace amounts, it is a potent antioxidant that protects the skin from wrinkling, erythema, and other effects of environmental oxidants such as ultraviolet light, air pollution, and chemicals. For this reason, vitamin E is often added to cosmetics and personal care products such as lotions and emollients intended for direct application to skin.

PROPERTIES OF ARTIFICIAL SKIN FILM LIQUIDS

Sweat and sebum exist as a cosolvent on the skin surface; however, investigators have been unable to reproduce this in vivo property of SSFL with in vitro models because of immiscibility of model constituents. Often, one SSFL component or the other is used for in vitro studies, though some investigators have attempted to add trace amounts of sebum to sweat models [42,50,134,211]. For this reason, compositions of artificial sweats and sebums are critically reviewed separately in this section.

Among the in vitro studies listed in Table 16.1 that reported the temperature of the artificial sweat or sebum, the average value was 35.0°C, which is in good agreement with the average whole-body human skin surface temperature (33.0°C). In some studies, extreme temperature values (range, 4°C to 100°C) were used to investigate effects of temperature on dissolution or expedite reaction kinetics. For example, Hemingway and Molokhia [50] reported that the rate of nickel dissolution from wire increased by a factor of 2.5 for every 10°C rise in temperature over the range of 10°C to 40°C. Smolik et al. [129] also observed temperature-dependent dissolution of nickel from coins. Colin-Russ [33,35] and Roddy and Lollar [37] observed that the decomposition of leather (loss of sulfates and chromic oxide) increased as artificial sweat temperature increased.

Artificial Sweats

Table 16.2 summarizes the compositions of 76 artificial sweat formulations used in dissolution studies published from the 1940s to 2013 (listed in chronological order). Note that not all studies that were listed in Table 16.1 were included in this summary table. Some of the historical studies listed in Table 16.1 did not report the exact composition of artificial sweat or did not include quantitative units, which precluded calculation of model composition. Additionally, each unique formulation of artificial sweat is listed only once in Table 16.2 and is attributed to the earliest published article to use that formulation; for brevity, all subsequent studies that used a given formulation were not listed. For example, the formulation first published by Pedersen et al. [124] is listed only once, though it was subsequently specified for use in European Standard 1811: "Reference test method for release of nickel from products intended to come into direct and prolonged contact with the skin" [220], ISO 24348: "Ophthalmic optics—Spectacle frames—Method for the simulation of wear and detectable nickel release from metal and combination spectacle frames" [298], and the German standard DIN 53160-2010—"Determination of the colourfastness of exogenous materials for common use—Part 2: Test with artificial sweat" [299] and has been used in more than 55 experimental studies.

Table 16.2 illustrates that most artificial sweat formulations to date have omitted several of the ionic constituents, organic acids, amino acids, and vitamins present in human sweat. Generally, artificial sweat formulations have been simplified solutions of a few select electrolytes (Na, Cl), a representative organic acid (lactic acid), and nitrogenous substances (NH_3, urea) in varying concentrations. The average pH of artificial sweats used in these in vitro studies was 5.9, with a range of 1.6 to 9.5, which is in good agreement with human sweat pH data. The pH of artificial sweat can be an important experimental variable. For example, the dissolution of sulfate from leather [35], chromium(VI) in some soils [62], gold under certain solvent conditions [47], silver from nanoparticle-finished textiles [2,180,187], resveratrol (antibacterial compound) from textiles [182], and dye from cotton and polyester textiles [179] increases as pH increases. In contrast, dissolution of nickel from certain nickel-containing stainless steels, wires, and earrings [50,54,57,59,64]; beryllium ores and metal, oxide, and copper alloys [111,115]; and zinc from ZnO-finished fabrics [175] increases as pH of the solvent decreases. For substances such as 2-mercaptobenzothiazole, dissolution from rubber materials may increase or decrease with pH depending on the test material matrix [132]. In some cases, dissolution of zinc diethyldithiocarbamate from rubber gloves [141], nickel from plated stainless steels [57,59], and cobalt and tungsten from fine powders [108] is independent of artificial sweat pH.

Sodium and Cl^- were the most frequently used electrolytes in artificial sweat formulations, followed by PO_4^{2-} and K^+. The dissolution of copper decreases with increasing Na^+ and Cl^- concentration in artificial sweat [48], whereas dissolution of chromium(III) increases with increasing concentration of these electrolytes [66]. The presence of Cl^- is a main driver of the dissolution of steels [8,43,300] and is believed to cause tarnish on silver alloys [103]. Stauber et al. [61] reported that more lead dissolved from powders in an artificial sweat than in a solution of Na^+ or Cl^- alone at the same concentration. The partitioning coefficient of isopropyl alcohol and methyl ethyl ketone is increased in the presence of Na^+ and Cl^- [142]. Song et al. [88] reported that Na^+ and Cl^- corroded nickel phosphate coatings on an alloy. Most studies omitted Ca^{2+}, Mg^{2+}, and HCO_3^-/CO_3^{2-} from their sweat formulations. The concentrations of Na^+, Cl^-, K^+, and Mg^{2+} used in artificial sweats were generally within the range of values reported for human sweat, whereas concentrations of Ca^{2+}, PO_4^{2-}, and HCO_3^-/CO_3^{2-} were generally outside the range of human sweat.

With the exception of SO_4^{2-}, all other cations and anions categorized as ionic constituents have been omitted from historical artificial sweat formulations. Based on human data reported by Stefaniak and Harvey [189], we successfully formulated artificial sweats to better mimic human sweat and demonstrated their chemical stability over time. These formulations contained all 10 ionic constituents in human sweat [111,157]; concentrations of these constituents were selected to be within the ranges for humans. The influence of specific ionic constituents on dissolution has not been investigated to date.

Early formulations of artificial sweat included acetic, butyric, and lactic acids as representative organic acids. Due to its malodorous property, butyric acid was subsequently dropped from most artificial sweat formulations, and lactic acid has been used as a representative organic acid constituent. Concentrations of acetic, butyric, and pyruvic acids and glucose in most artificial sweats greatly exceeded human sweat values. The concentrations of lactic acid used in artificial sweat formulations were generally within the range for human sweat. Among organic acids, butyric and pyruvic acids have negligible effect on the dissolution of stainless steels and nickel [52,53] or on the release of chrome from chrome-tanned leather [33,34,36,76]. Lactic acid is known to increase the deterioration and/or discoloration of chrome from chrome-tanned leather [33,34,36] and dissolution of mild steel [43] but not dissolution of nickel wire [50]. Lactic acid strongly corroded magnesium alloys [112] and nickel phosphate coatings on an alloy [88], whereas urea acted as a corrosion inhibitor. Release of phthalates from toys is influenced by the presence of lactic acid in artificial sweat [159]. Underwood et al. [44] reported that pyruvic acid corroded unpassivated zinc telephone parts, whereas lactic acid corroded passivated parts.

Amino acids have often been omitted from artificial sweat formulations. When included in formulations, the selection of amino acids and their biologically relevant concentrations has been a subject of discussion among researchers. Lidén et al. [68] reported that gold did not dissolve in a simple artificial sweat consisting of Na^+, Cl^-, urea, and lactic acid. However, Möller et al. [301] questioned these results because the artificial sweat formulation used in that study lacked amino acids, specifically, sulfur-containing amino acids. Previously, Brown et al. [47] reported dissolution of gold in oxygenated solutions containing 100 mM of the sulfur-containing amino acids cysteine and glutathione. As noted above, of these amino acids, cysteine is the only one that has been identified in human sweat, though quantitative data on its concentration are lacking. Lidén and Nordenadler [302] and Flint [303] noted that the choice of amino acids and the high concentrations used by Brown et al. [47] may not be biologically relevant for the skin surface. Other researchers have noted that amino acids may adsorb onto metal surfaces and act as corrosion inhibitors or complex with metal ions and thereby act as corrosion promoters [66]. Van Lierde et al. [76] reported that Met was important in the reduction of chromium(VI) leached from leather to chromium(III) in artificial sweat. Among the 18 amino acids listed in Table 16.2, 7 (Asp, Cit, Glu, Ile, Phe, Trp, Tyr) have been omitted from historical artificial sweat formulations. When included, concentrations of the other 11 amino acids have generally been similar to measured values for human sweat, with the exception of the formulations used by Brown et al. [47] and Hirokawa et al. [28]. The original formulations created by Harvey et al. [157] and Stefaniak et al. [111] matched human sweat amino acid concentrations based on data available at the time [189]; however, these values should be adjusted to match concentrations reported in more recent publications (Table 16.2).

Artificial sweat formulations have generally only included NH_3 and urea as representative nitrogenous substances. In these formulations, NH_3 concentrations often greatly exceeded levels in human sweat, whereas urea concentrations were similar to human sweat. The influence of nitrogenous substances on dissolution has not been well studied, though in one report [43], urea had a negligible effect on the dissolution of mild steel. Cheng et al. [142] reported that the partitioning coefficient of isopropyl alcohol and methyl ethyl ketone was not influenced by artificial sweat urea and NH_3. Release of phthalates from toys was influenced by the presence of urea in artificial sweat [159]. In an attempt to better mimic the composition of human sweat, Harvey et al. [157] included creatine, creatinine, and uric acid in their artificial sweat formulation.

None of the artificial sweat formulations listed in Table 16.2, except for those developed by Harvey et al. [157] and modified by Stefaniak et al. [111], contained any vitamins. Initially, they formulated an artificial sweat that included vitamins at levels reported for human sweat [189]. However, these vitamin concentrations included historical data from the Kuno [228] monograph on human perspiration. As noted by Kuno [228], data reported in that monograph for niacin, pantothenic acid, riboflavin, and thiamine in human sweat were biased because of flawed sweat collection and analysis

techniques. Subsequently, the data for these vitamins were omitted, vitamin concentrations in human sweat were recalculated (Table 16.2), and Stefaniak et al. [111] revised the artificial sweat formulation to better mimic the actual human sweat concentrations of these four vitamins.

Artificial Sebums

CEs are the lipid class most commonly omitted from artificial sebums. When included, CE concentrations were generally representative of human sebum (Table 16.13). Free CH has been included in about half of the published artificial sebums; levels used in these formulations were consistent with human sebum. The frequent omission of CE and CH from artificial sebum may reflect their minor contribution to total skin film lipids, and as such, most formulations are composed of FFAs, SQ, TGs, and WEs (Table 16.13). The FFA content of most artificial sebums is within ranges for human sebum (2% to 39%), but some formulations contained as little as 0.001% or as much as 98% FFA. As noted above, FFAs on the skin surface are a mixture of saturated and unsaturated compounds. To mimic the biologically valuable unsaturated FFAs (oleic acid, linoleic acid) [293], researchers have used myristoleic acid (14-carbon chain length), palmitoleic acid (16-carbon chain length), and oleic acid or sodium oleate (18-carbon chain length) [27,50,135,136,143,171]. According to Collins [43], on an equal mass concentration basis, oleic acid caused just 5% of the corrosion of steel observed in a solution of sodium chloride. McLendon and Richardson [162] observed that unsaturated FFAs and TGs caused more yellowing of cotton textiles than saturated FFAs and TGs. Katsuta et al. [143] reported that unsaturated FFAs in a model sebum altered the calcium dynamics in epidermal keratinocytes and induced abnormal follicular keratinization. To mimic the biologically valuable saturated FFAs (palmitic, myristic, and stearic acids), researchers used lauric acid (12-carbon chain length), myristic acid (14-carbon chain length), palmitic acid (16-carbon chain length), and stearic acid or sodium stearate (18-carbon chain length) [27,50,135,136,139,143,171]. Among the artificial sebum formulations listed in Table 16.13, most included SQ; levels used were consistent with human sebum. Of the six major lipid classes that compose human sebum, only TGs were included in all artificial sebum formulations. Among the formulations listed in Table 16.13, all but five contained TGs at levels consistent with human sebum. These exceptions were studies in which the investigators attempted to disperse small amounts of TGs in aqueous sweat to better mimic SSFL. To mimic unsaturated TGs, researchers often used triolein, trimyristolein, or tripalmitolein, and to mimic saturated TGs, they often used trimyristin (14-carbon chain length), tripalmitin (16-carbon chain length), or tristearin (18-carbon chain length). In one study, the tristearin component of an artificial sebum was observed to have higher binding affinity for cotton and polyester textiles relative to other lipids [170]. Nearly all artificial sebum formulations listed in Table 16.13 contained WE levels consistent with human sebum. In the cosmetics industry, oleyl oleate is often used to mimic unsaturated WEs, whereas myristyl myristate, palmityl palmitate, palmitic acid myristyl ester, and stearyl stearate are used to mimic 14- to 18-carbon-chain-length saturated WEs [135,136]. Other investigators have used lanolin (sheep sebum with paraffin wax and water added) to mimic both unsaturated and saturated WEs in artificial human sebum [27,139]. Human sebum is composed of wax monoesters, which are only a minor component of lanolin. Only two artificial sebum formulations have included vitamin E [156,158], an important antioxidant present at low levels on the skin surface. Both investigators reported better long-term chemical stability of model sebum formulations when 0.1% to 1% by weight vitamin E was included in the formulation. In a study of cemented tungsten carbide powders, the presence of vitamin E in sebum suppressed the dissolution of cobalt and tungsten carbide from sintered (chemically bonded) particles, a process that involves production of hydroxyl free radicals [107,108].

TEST MATERIAL CHARACTERIZATION

In addition to the composition of SSFL, the properties of the exogenous material itself will have an influence on the extent of interaction with the skin. For example, Wang et al. [117] reported that the release of Mg^{2+}, Zn^{2+}, and Ca^{2+} was higher for a crystalline alloy relative to an amorphous alloy in artificial sweat. Hence, characterization of the physical, chemical, and/or mechanical properties (e.g., tear load, tensile strength) of a test material is critical for understanding the underlying mechanisms of interaction (e.g., dissolution or partitioning) and potential mechanisms of therapeutic or toxic action of its bioaccessible constituents. Unfortunately, characterization of test materials has often been overlooked in historical dissolution studies. More recent studies of thin films, functional textiles, and particles have placed more emphasis on characterization. One good example of a comprehensive characterization strategy is given by Mazinanian et al. [120]. In this study, multiple confirmatory and complementary techniques were used to assess the physicochemical properties of the test materials. A list of characterization methods used in previous studies is given in Table 16.14; however, a description of the underlying principles of these methods is beyond the scope of this chapter. Physical characterization methods include variations of microscopy (electron, atomic force, fluorescence, and light) to image surface and cross-sectional morphology of test materials (textiles, metal films, and powders); ellipsometry to measure film thickness; gas adsorption to measure surface area; and pycnometry to measure powder density. Chemical characterization methods can be used to identify and/or quantify masses of specific analytes and compounds in a wide range of materials. Atomic spectroscopy (atomic absorption, atomic emission, mass spectroscopy, etc.) can provide quantitative information on the bulk elemental metal composition of test materials provided that a suitable sample preparation technique is available. High-performance liquid chromatography or liquid chromatography coupled with mass spectroscopy is used to quantify

TABLE 16.14
Instruments and Methods for Characterization of Physicochemical and Mechanical Properties of Materials

Instrument/Method	Property	Applications
Atomic spectroscopy	Elemental composition	Leathers, metals, money, textiles
AFM	Surface and cross-sectional morphology	Metals
Auger spectroscopy	Surface chemistry	Metals
Color difference meter	Color, reflectance	Textiles
DSC	Phase transition temperatures	Elastomers, glass-like metals, organic chemicals
Durometer	Hardness	Elastomers
EDX (with electron microscopy)	Elemental composition	Metals, minerals
Ellipsometry	Film thickness	Metals
EPMA (with electron microscopy)	Elemental composition	Metals
Extensometer	Tensile strength	Elastomers
Fluorescence microscopy	Morphology	Textiles
FTIR	Compound identification	Textiles
Gas adsorption	Surface area	Metals (powders)
HPLC	Compound mass	Organic chemicals, textiles
Langmuir–Adams trough	Surface tension	Human surfactant
Light microscopy	Surface morphology	Metals, minerals
LC-MS	Compound mass	Illicit drugs
NMR	Compound mass	Organic chemicals
PIXE (with electron microscopy)	Elemental composition	Metals
Pycnometry	Density	Metals (powders)
Raman spectroscopy	Surface composition	Metals
RBS	Elemental composition	Metals
SEM	Surface morphology and microstructure	Sebum, metals, money, textiles
Spectrophotometry	Color changes	Elastomers
TLC	Compound mass	Organic chemicals
Universal testing apparatus	Tear load and strength	Elastomers
UV-vis spectroscopy	Analyte mass	Metals, organic chemicals
Viscometer	Viscosity	Organic chemicals
XPS	Surface chemistry	Metals
XRD	Crystalline phases	Metals, minerals, organic chemicals, textiles

Note: AFM = atomic force microscopy; DSC = differential scanning calorimetry; EDX = energy-dispersive x-ray analysis; EPMA = electron probe microanalysis; FTIR = Fourier Transform infrared ATR spectroscopy; HPLC = high-performance liquid chromatography; LC-MS = liquid chromatography-mass spectroscopy; NMR = nuclear magnetic resonance spectroscopy; PIXE = particle-induced x-ray emission spectroscopy; RBS = Rutherford backscattering spectrometry; SEM = scanning electron microscopy; TLC = thin layer chromatography; UV-vis spectroscopy = ultraviolet-visible spectroscopy; XPS = x-ray photoelectron spectroscopy; XRD = x-ray diffraction.

masses of organic compounds and is often employed in research on pharmaceuticals and consumer products (cosmetics, lotions, personal care products). X-ray diffraction is a useful technique for identification and quantification of the crystallinity of constituents in test materials such as minerals, metals, and textiles (identification of phases, calculation of crystallite size). To evaluate the bulk chemistry of specific regions or aspects of a test material, techniques such as electron microscopy analysis can be augmented with energy-dispersive x-ray analysis, electron probe microanalysis, or particle-induced x-ray emission spectroscopy. The preceding chemical characterization methods are limited to bulk chemistry of a test material. In many cases, the interaction of a test material surface with SSFL is of interest. Auger spectroscopy and x-ray photoelectron spectroscopy are two techniques that permit identification and quantification of elements on surfaces; information on binding energies can be used to identify chemical compounds. Characterization of mechanical properties is of particular interest for elastomers, textiles, and metals. Available instruments include color difference meters to assess changes in textiles following immersion in artificial sweat or sebum, a durometer to determine hardness, and an extensometer to evaluate tensile strength.

SUMMARY

The extent to which an exogenous material will interact with the skin is dependent on the composition of the SSFLs and

the properties of the exogenous material itself. Artificial SSFLs are widely utilized for in vitro investigation of the potential behavior of materials that come into contact with human skin (Table 16.1). While in vitro testing with artificial SSFLs can provide valuable information for development of effective therapeutics, consumer safety, and so forth, there are limitations of these approaches of which researchers should be cognizant:

- Artificial sweat (Table 16.2) and sebum (Table 16.13) formulations are intended to mimic in vivo conditions, but they often lack many of the constituents present in human sweat and sebum or use concentrations that are not within ranges for human sweat. Numerous studies reviewed in this chapter indicate that specific constituents (e.g., Cl^-, lactic acid) influence the dissolution of certain materials. Hence, researchers should carefully consider the composition of their artificial SSFL formulation to ensure that it is appropriate for the intended purpose and relevant to human skin conditions for the anatomical region that will contact the study material.
- All in vitro tests are static and hence do not mimic the dynamic nature of the skin surface. The temperature of the skin, pH of sweat, and concentration of sweat constituents varies with activity level (and other factors). These transient fluctuations are not accounted for in current test systems but may be important for dissolution and partitioning of materials.
- A suitable model of the cosolvent behavior of sweat and sebum on the skin surface is lacking. As noted above, some investigators have attempted to disperse small amounts of lipids in artificial sweat, but the lipids proved immiscible [50]. An alternative approach is to dissolve the artificial sebum lipids in an organic solvent, deposit onto a container or material surface, allow the organic solvent to evaporate, and immerse in artificial sweat [111]. Recent developments with spray technologies may permit more precise coating of test materials with sebum lipids [77,160]. Komesvarakul et al. [145] have reported preparation of microemulsions of TGs in aqueous solutions; however, a method to reproduce the in vivo cosolvent sweat-and-sebum system is still needed to understand dissolution and partitioning of compounds released from materials that come into contact with the skin surface.
- All artificial SSFL models lack enzymes and bacteria, which are important for maintenance of skin microflora and hydrolysis of sebum lipids and help to regulate skin surface pH. This shortcoming precludes the accurate representation of organic acid and lipid chemistry dynamics on the skin surface.
- Detailed characterization of study material properties (physical, chemical, mechanical, etc.) is needed to understand mechanisms of interaction with skin surface cells.
- Accounting for just the properties of SSFL and the properties of a material that may contact the skin is only part of the picture. The status of the skin barrier itself (e.g., hydration status, integrity) may be altered in diseased skin, dry skin, solvent-exposed skin, and so forth, and barrier integrity is an important factor in permeation across the SC [95,121].

For more than 75 years, researchers have relied on in vitro testing with artificial SSFLs to understand the interaction of a wide variety of materials that may come into contact with the skin. While these tests can provide valuable insights with regard to the therapeutic value of medical devices and for product stewardship/consumer safety, occupational toxicology, compliance with regulations (e.g., European Union Nickel Directive), and other purposes, the composition and concentration of SSFLs used by investigators has not always been representative of human skin conditions. Data on human SSFL composition and concentration ranges reported in this chapter can aid in the design of meaningful artificial SSFLs for future studies.

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17 Skin Care Occlusive Ingredients *Predicting Occlusion's Effects Using Partition Coefficients*

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INTRODUCTION

Occlusion refers to the impervious-to-water covering of the skin directly or indirectly by various means, including tape, gloves, impermeable dressings, or even transdermal devices [1]. Certain topical vehicles, for example, petrolatum or paraffin, contain fats and/or polymer oils that may generate occlusive effects by reducing water loss [2]. The epidermis of healthy skin provides an efficient barrier against the infiltration of exogenous and potentially harmful substances, and the stratum corneum typically has a water content of 10%–20% by weight. Skin occlusion increases the water content of the stratum corneum up to 50%, and even a short-time occlusion (30 min) results in significantly increased hydration [3–5]. By increasing stratum corneum hydration, occlusion influences percutaneous absorption by altering the partitioning between the chemical penetrant and the skin, swelling corneocytes and possibly altering the intercellular lipid phase organization, increasing skin surface temperature, and increasing blood flow [4–6].

In general, occlusion is widely utilized to enhance the penetration of applied drugs in clinical practice; however, occlusion does not increase the percutaneous absorption of all chemicals [3–4]. In fact, evidence suggests that skin occlusion is more complex than previously thought as it can induce changes in epidermal lipid content, DNA synthesis, epidermal turnover, skin pH, epidermal morphology, sweat glands, and Langerhans cell stresses [7–21]. This overview focuses on what effect occlusion has on the in vitro and in vivo percutaneous absorption of compounds of varying lipophilicities/hydrophilicities. As few cosmetic ingredients have been studied, other chemical classes noted here may provide guidance for the formulator.

METHODS

Studies and prior reviews of the effects of occlusion on the in vitro and in vivo percutaneous penetration of penetrants of varying liphophilicities/hydrophilicities were identified in the MEDLINE, PubMed, Embase, and Science Citation Index databases using the terms *occlusive, occluded, occlusion,* in vitro*, skin,* and *percutaneous absorption/penetration* to generate as broad of a search as possible. The search occurred between July 23, 2012, and August 10, 2012. From the results generated, abstracts were subsequently scrutinized to identify articles dealing primarily with in vitro models of the skin involving occlusion. Moreover, after the identification of relevant articles, their references were examined to find additional sources of information.

RESULTS OF IN VITRO EXPERIMENTS

After examining the research articles generated by the search results, five original research articles were obtained that used in vitro occlusion models and provided insight regarding the role of partition coefficients in predicting occlusion's effects on percutaneous penetration; articles that dealt with occlusion and percutaneous penetration but did not shed light on how the lipophilicity/hydrophilicity of a compound could affect occlusion efficacy were excluded. The log of the octanol–water partition coefficients (log Kow) reported here were taken from the publications cited, or they were taken from values obtained from The PubChem Project (http://pubchem.ncbi.nlm.nih.gov/) and LOGKOW, a data bank of evaluated octanol–water partition coefficients (http://logkow.cisti.nrc.ca/logkow/).

Gummer and Maibach [22] investigated the in vitro percutaneous penetration of methanol and ethanol through full-thickness, excised guinea pig skin at varying volumes and under various occlusive conditions. Though neither compound demonstrated an increase in penetration with increasing dose volume, they determined that occlusion significantly enhanced penetration ($P < .01$) when compared to nonocclusion (Table 17.1). Also, the nature of the occlusive material greatly affected both the penetrated amounts of the compounds as well as the profiles of the amount penetrating per hour. Methanol showed both a greater penetration rate and a greater total penetration than ethanol even though both alcohols have similar octanol–water coefficients. The larger molecular size of ethanol may explain why it penetrated excised guinea pig skin more slowly.

Treffel et al. [23] compared the in vitro permeation profiles of two compounds with different physiochemical properties, citropten (lipophilic) and caffeine (amphiphilic), under

TABLE 17.1
In Vitro Penetration of 14C-Labeled Methanol and Ethanol through Guinea Pig Skin

		Penetration: % of Applied Dose ± SD	
Volume of Alcohol (μL)	**Occlusive Device**	**Methanol**	**Ethanol**
50	None	0.48 ± 0.09	0.94 ± 0.14
100	None	1.33 ± 0.30	0.38 ± 0.04
200	None	1.40 ± 0.07	0.29 ± 0.01
100	Parafilm	13.2 ± 2.7 (ER = 9.9 ± 3.0)	8.10 ± 0.43 (ER = 21 ± 2.5)
100	Gel Bond	34.8 ± 1.8 (ER = 26 ± 6.1)	23.5 ± 1.6 (ER = 62 ± 7.8)
100	Hill Top Chamber	44.2 ± 3.0 (ER = 33 ± 7.8)	27.10 ± 2.54 (ER = 71 ± 10)

Source: Gummer, C.L., and Maibach, H.I., *Food Chem. Toxicol.*, 24, 1986.

Note: Occlusion enhanced penetration of both chemicals compared to nonocclusion. ER, enhancement ratio (penetration of occluded chemical divided by penetration of nonoccluded chemical under otherwise identical conditions including dosing); SD, standard deviation.

occluded versus nonoccluded conditions over a 24 h period using an in vitro model involving human abdominal skin [23]. The data demonstrated that occlusion increased the permeation of citropten (partition coefficient = 2.17) 1.6 times over nonoccluded conditions ($P < .05$), but occlusion did not enhance the permeation of caffeine (partition coefficient = 0.02). Their results bolster the idea that occlusion does not enhance the percutaneous penetration of all compounds, especially hydrophilic compounds [3].

Roper et al. [24] determined the percutaneous absorption of 2-phenoxyethanol (lipophilic compound, log Kow = 1.16) applied in methanol through nonoccluded rat and human skin in vitro in two diffusion cell systems over 24 h [25]. Under nonoccluded conditions, 2-phenoxyethanol was greatly lost by evaporation, but once occlusion was applied, evaporation was decreased, and total absorption increased.

Taylor et al. [26] studied the effect occlusion exerts upon the in vitro percutaneous penetration of the model penetrant linoleic acid (log Kow = 7.05) dissolved in solvents of two different volatilities, ethanol and cyclomethicone [27]. Using porcine skin, nonocclusion resulted in a greater skin concentration of linoleic acid dissolved in an ethanolic vehicle when compared to occlusion ($P < .05$); similar statistically significant trends were observed when linoleic acid was dissolved in the less volatile organic solvent cyclomethicone, that is, nonocclusion resulted in greater percutaneous absorption of linoleic acid in cyclomethicone than occlusion. Then the authors compared these studies with the percutaneous penetration of glycerol, a hydrophilic molecule, dissolved in an aqueous solution. They did not find statistically significant differences in the concentrations of glycerol in the skin and the receptor cell when comparing the occluded and nonoccluded conditions. The authors attributed the results of this study, which revealed that occlusion did not enhance the percutaneous penetration of the lipophilic compound linoleic acid, to the increase in the concentration gradient of linoleic acid enabled by nonocclusion due to the unimpeded evaporation of the volatile solvents, which provided a greater driving force for percutaneous absorption than occlusion, which, in turn, prevented evaporation. They found these findings to be consistent with experiments conducted by Stinchomb et al. [28], which revealed that by increasing the volatility of solvents, one can increase the concentration of the penetrant in the donor phase and enhance the deposition and delivery of the penetrant into the skin.

Brooks and Riviere [29] used isolated perfused porcine skin flap (IPPSF) topical experiments to study the percutaneous absorption of 14C-labeled phenol (log Kow = 1.50) versus para-nitrophenol (PNP, log Kow = 1.91) at two concentrations (4 vs. 40 μg/cm^2) in two vehicles (acetone versus ethanol) under occluded versus nonoccluded conditions for 8 h in order to determine if dose, vehicle, or occlusion had significant effects on percutaneous penetration (Tables 17.2 and 17.3) [30,31]. For phenol, occlusion enhanced the absorption, penetration into issues, and total recoveries when compared to nonoccluded conditions. Phenol's absorption and penetration into tissues was greater when dissolved in an ethanol vehicle compared to the acetone vehicle under nonocclusive conditions, but phenol's absorption and penetration under occlusive conditions was greater with acetone than with ethanol. Phenol in acetone had a greater percentage of applied dose penetration into tissues at a low dose than a high dose, which could suggest a fixed absorption rate for the penetrant. (This was also seen for PNP but only under occlusion.) With regard to PNP, none among its dose, vehicle, or occlusion had a significant effect on total recovery of labeled PNP. These findings led the authors to conclude that absorption of phenol and PNP are dependent upon the vehicle, occlusion, and penetrant.

RESULTS OF IN VIVO EXPERIMENTS

Feldmann and Maibach [32] were the first to correlate the increased pharmacologic effect of hydrocortisone under occlusive conditions with the pharmacokinetics of the penetration of 14C hydrocortisone through normal skin.

TABLE 17.2
Effects of Dose, Vehicle, and Occlusion on Percutaneous Penetration of Phenol

	Dose (μg/cm²)	Penetration (% Dose)
	Nonoccluded phenol in acetone, 40 μg/cm²	
Mean ± SD	40.0 ± 0.00	2.60 ± 0.03
	Nonoccluded phenol in EtOH, 40 μg/cm²	
Mean ± SD	40.0 ± 0.00	8.49 ± 3.80
	Occluded phenol in acetone, 40 μg/cm²	
Mean ± SD	39.50 ± 0.35	12.21 ± 2.06 (ER = 4.70 ± 0.794)
	Occluded phenol in EtOH, 40 μg/cm²	
Mean ± SD	0.20 ± 0.35	8.42 ± 3.23 (ER = 0.99 ± 0.584)
	Nonoccluded phenol in acetone, 4.0 μg/cm²	
Mean ± SD	4.0 ± 0.00	3.88 ± 1.25
	Nonoccluded phenol in EtOH, 4.0 μg/cm²	
Mean ± SD	4.0 ± 0.00	6.24 ± 1.42
	Occluded phenol in acetone, 4.0 μg/cm²	
Mean ± SD	5.17 ± 0.53	17.06 ± 2.04 (ER = 4.40 ± 1.51)
	Occluded phenol in EtOH, 4.0 μg/cm²	
Mean ± SD	5.01 ± 0.62	10.09 ± 1.91 (ER = 1.62 ± 0.479)

Source: Brooks, J.D., and Riviere, J.E., *Fundam. Appl. Toxicol.*, 32, 1996.
Note: ER, enhancement ratio (penetration of occluded chemical divided by penetration of nonoccluded chemical under otherwise identical conditions including dosing); SD, standard deviation.

Following the topical application of 14C hydrocortisone to the ventral forearm of human volunteers, the rate and extent of 14C-labeled excretion was measured. The application site was either nonoccluded or occluded with plastic wrap. For the nonoccluded condition, the application site was washed 24 h postapplication, while for the occluded skin condition, the plastic wrap was left in place for 96 h postapplication before the site was washed. The urine for both conditions was collected for 10 days. The percentage of the applied dose excreted into the urine after 10 days was 0.46 ± 0.2 (mean ±

TABLE 17.3
Effects of Dose, Vehicle, and Occlusion on Percutaneous Penetration of PNP

	Dose (μg/cm²)	Penetration (% Dose)
	Nonoccluded PNP in acetone, 40 μg/cm²	
Mean ± SD	43.43 ± 1.25	33.41 ± 3.82
	Nonoccluded PNP in EtOH, 40 μg/cm²	
Mean ± SD	40.93 ± 1.65	31.67 ± 4.19
	Occluded PNP in acetone, 40 μg/cm²	
Mean ± SD	45.13 ± 3.53	24.47 ± 5.08 (ER = 0.732 ± 0.174)
	Occluded PNP in EtOH, 40 μg/cm²	
Mean ± SD	43.28 ± 1.94	7.20 ± 1.58 (ER = 0.23 ± 0.058)
	Nonoccluded PNP in acetone, 4.0 μg/cm²	
Mean ± SD	3.28 ± 0.11	14.19 ± 0.94
	Nonoccluded PNP in EtOH, 4.0 μg/cm²	
Mean ± SD	4.38 ± 0.21	13.32 ± 3.10
	Occluded PNP in acetone, 4.0 μg/cm²	
Mean ± SD	4.433 ± 0.232	28.845 ± 5.171 (ER = 2.033 ± 0.388)
	Occluded PNP in EtOH, 4.0 μg/cm²	
Mean ± SD	3.95 ± 0.05	9.04 ± 2.59 (ER = 0.68 ± 0.25)

Source: Brooks, J.D., and Riviere, J.E., *Fundam. Appl. Toxicol.*, 32, 1996.
Note: ER, enhancement ratio (penetration of occluded chemical divided by penetration of nonoccluded chemical under otherwise identical conditions including dosing); SD, standard deviation.

TABLE 17.4
Total Excretion Data Summary for Topical Application of 14C Hydrocortisone to the Ventral Forearm of Human Volunteers

Method of Topical Administration	Total Excretion (% of Dose)	Ratio to Unmodified Skin
Unmodified	0.46%	1×
Occluded	4.48%	10×
Stripped	0.91%	2×
Stripped and occluded	14.91%	32×

Source: Feldmann, R.J., and Maibach, H.I., *Arch. Dermatol.*, 91, 1965.
Note: The total excretion is the total amount of 14C hydrocortisone excreted in urine after 10 days reported as the percentage of the dose applied.

SD) for the nonoccluded condition and 4.48 ± 2.7 for the occluded condition (Table 17.4). The occlusive condition significantly increased (tenfold) the cumulative absorption of hydrocortisone compared to the nonoccluded condition ($P = .01$). The authors noted that the difference of application duration (24 h exposure for the nonoccluded site versus 96 h exposure for the occluded site) could affect absorption as measured by the cumulative amount of drug excreted into urine, but the significant difference observed in the percentage of dose absorbed at 12 and 24 h between occluded and nonoccluded conditions could not be explained by differences in application duration.

Maibach and Feldmann [33] then later studied the effect of occlusion on the percutaneous penetration of pesticides. They applied 14C-radiolabeled pesticides to the forearm of volunteers, and the rate and extent of 14C-labeled urinary excretion was determined using sensitive methods that allowed the doses to be in micrograms, far below the toxic range of any pesticide. From their experiments, it is evident occlusion has a variable effect on penetration; at a minimum, occlusion increased the penetration of azodrin approximately threefold, while at the other extreme, it increased the penetration of malathion almost tenfold (Table 17.5 and Figure 17.1). In general, as the octanol–water partition coefficients increased, occlusion had a greater effect on enhancing penetration, though enhancement by occlusion peaked for malathion and then decreased as the octanol–water partition coefficients further increased. In order to understand how occlusion duration affects penetration, the authors of the study then documented the effects of occluding malathion under variable amounts of time (Table 17.6). As the occlusion duration increased, the penetration of malathion increased as well; by 2 h of occlusion, the penetration had almost doubled, and by 8 h, penetration increased almost fourfold. There have been few, if any, experiments besides this one that have documented the effect of occlusion duration on percutaneous penetration.

Guy et al. [34] studied how occlusion impacts the percutaneous absorption of a variety of steroids (progesterone, testosterone, estradiol, and hydrocortisone) in vivo. In the control studies, they applied the 14C-radiolabeled steroids dissolved in acetone to the ventral forearm of volunteers and

TABLE 17.5
Effect of Occlusion on the Penetration of Pesticides

Compound	Log Kow	Control (Nonocclusion)	Occlusion (24 h)	ER
Diquat	−3.05	0.4%	1.4%	3.5
Baygon	0.14	19.6%	68.8%	3.5
Azodrin	1.03	14.7%	33.6%	2.3
Guthion	2.75	15.9%	56.1%	3.5
Malathion	2.89	6.8%	62.8%	9.2
Lindane	3.55	9.3%	82.1%	8.8
Parathion	3.9	8.6%	54.8%	6.4
Dieldrin	5.4	7.7%	65.5%	8.5

Source: Maibach, H.I., and Feldmann, R.J., Systemic Absorption of Pesticides through the Skin of Man, in Task Group on Occupational Exposure to Pesticides, eds., *Occupational Exposure to Pesticides. Report to the Federal Working Group on Pest Management from the Task Group on Occupational Exposure to Pesticides*, Federal Working Group on Pest Management, Washington, 1974.
Note: ER, enhancement ratio (penetration of occluded chemical divided by penetration of nonoccluded chemical under otherwise identical conditions including dosing); Kow, octanol–water partition coefficients.

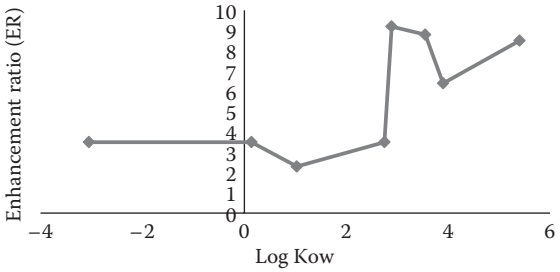

FIGURE 17.1 Log Kow of enhancement ratio (ER) of pesticides. This figure plots the ER of the various pesticides as a function of their log Kow values. Kow, octanol–water partition coefficient. (Modified from Maibach, H.I., and Feldmann, R.J., Systemic Absorption of Pesticides through the Skin of Man, in Task Group on Occupational Exposure to Pesticides, eds., *Occupational Exposure to Pesticides. Report to the Federal Working Group on Pest Management from the Task Group on Occupational Exposure to Pesticides*, Federal Working Group on Pest Management, Washington, 1974.)

then tracked the elimination of the compounds into urine. In the occlusive studies, after evaporation of the acetone vehicle, the site of application was covered with a plastic (Hill Top) chamber. In all cases, the application sites were washed after 24 h using a standardized procedure [35]. In the occlusive studies, the authors covered the administration site again with a new chamber after the washing. These studies reveal that occlusion significantly increased the percutaneous absorption of estradiol, testosterone, and progesterone but not that of hydrocortisone, which had the lowest octanol–water partition coefficient amongst the steroids used (Table 17.7 and Figure 17.2). Moreover, under both occlusion and nonocclusion, percutaneous absorption increased with increasing octanol–water partition coefficient for testosterone but declined for progesterone.

TABLE 17.6
Effect of Occlusion Duration on Penetration of Malathion

Duration (h)	Penetration (%)
0	9.6%
0.5	7.3%
1	12.7%
2	16.6%
4	24.2%
8	38.8%
24	62.8%

Source: Maibach, H.I., and Feldmann, R.J., Systemic Absorption of Pesticides through the Skin of Man, in Task Group on Occupational Exposure to Pesticides, eds., *Occupational Exposure to Pesticides. Report to the Federal Working Group on Pest Management from the Task Group on Occupational Exposure to Pesticides*, Federal Working Group on Pest Management, Washington, 1974.

TABLE 17.7
Occlusion's Effects on Percutaneous Absorption of Steroids in Man as a Function of Penetrant Octanol–Water Partition Coefficient

		% Applied Dose Absorbed (Mean ± SD)		
Compound	Log Kow	Nonoccluded	Occluded	ER
Hydrocortisone	1.61	2 ± 2	4 ± 2	2 ± 2
Estradiol	3.15	11 ± 5	27 ± 6	2.5 ± 1
Testosterone	3.32	13 ± 3	46 ± 15	3.5 ± 1
Progesterone	3.87	11 ± 6	33 ± 9	3 ± 2

Sources: Guy, R.H. et al., Kinetics of Drug Absorption Across Human Skin in Vivo, in Shroot, B., and Schaefer, H., eds., *Skin Pharmacokinetics*, Karger, Basel, 1987; Bucks, D., and Maibach, H.I., Occlusion Does Not Uniformly Enhance Penetration In Vivo, in Bronaugh, R.L., and Maibach, H.I., eds., *Percutaneous Absorption: Drugs, Cosmetics, Mechanisms, Methodology*, ed 3, Dekker, New York, 1999.

Note: The exposure period was 24 h at 4 μg/cm^2 prior to washing with soap and water. ER, enhancement ratio (penetration of occluded chemical divided by penetration of nonoccluded chemical under otherwise identical conditions including dosing); Kow, octanol–water partition coefficient; SD, standard deviation.

Bucks et al. [37] measured the percutaneous absorption of these same four steroids (hydrocortisone, estradiol, testosterone, and progesterone) in vivo in man under occluded and "protected" (i.e., covered but nonocclusive) conditions. Using the same methodology as Guy et al. [34], the 14C-labeled chemicals were applied in acetone to the ventral forearm of volunteers. After vehicle evaporation, the application sites were covered with a semirigid polypropylene chamber for 24 h; intact chambers were used as the occlusive condition, while the "protected" condition was created by boring several

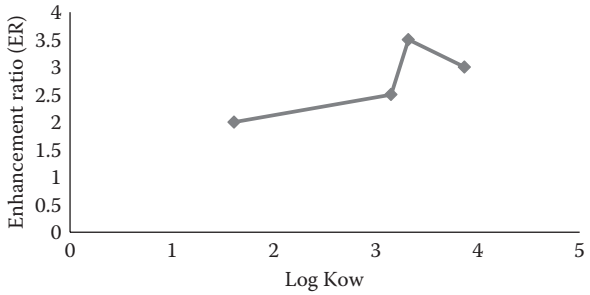

FIGURE 17.2 Log Kow of enhancement ratio (ER) of steroids. This figure plots the ER of the various steroids as a function of their log Kow values. Kow, octanol–water partition coefficient. (Modified from Guy, R.H. et al., Kinetics of Drug Absorption across Human Skin In Vivo, in Shroot, B., and Schaefer, H., eds., *Skin Pharmacokinetics*, Karger, Basel, 1987; Bucks, D., and Maibach, H.I., Occlusion Does Not Uniformly Enhance Penetration In Vivo, in Bronaugh, R.L., and Maibach, H.I., eds., *Percutaneous Absorption: Drugs, Cosmetics, Mechanisms, Methodology*, ed 3, Dekker, New York, 1999.)

small holes through the chamber. Urine was then collected for 7 days postapplication. In excellent agreement with the previous study by Guy et al. [34], steroid absorption increased with increasing lipophilicity up to a point, but the penetration of progesterone (the most hydrophobic of the steroids) did not continue the trend. With the exception of hydrocortisone, 24 h occlusion significantly increased ($P < .01$) percutaneous absorption of the steroids. From these studies, it seemed occlusion that enhanced the percutaneous absorption of the more liphophilic steroids but not that of hydrocortisone, the most water-soluble steroid.

Bucks et al. [37,38] then investigated the effect of occlusion on the in vivo percutaneous absorption of phenols. Nine 14C ring-labeled para-substituted phenols (4-aminophenol, 4-acetamidophenol, 4-propionylamidophenol, phenol, 4-cyanophenol, 4-nitrophenol, 4-iodophenol, 4-heptyloxyphenol, and 4-pentyloxyphenol) were applied in ethanol to the ventral forearm of male volunteers. After vehicle evaporation, the application site was covered with either an occlusive or a protective chamber. After 24 h, the chamber was removed, and the site was washed. The application site was then re-covered with a new chamber of the same type. Urine was collected for 7 days. On the seventh day, the second chamber was removed, the application site washed, and the upper layers of stratum corneum removed from the application site by tape stripping. These studies indicate that occlusion significantly increased ($P < .05$) the absorption of phenol, heptyloxyphenol, and pentyloxyphenol, but occlusion did not significantly increase the absorption of aminophenol, acetaminophen, propionylamidophenol, cyanophenol, nitrophenol, and iodophenol (Table 17.8 and Figure 17.3). The two compounds with the lowest octanol–water partition coefficient demonstrated the least enhancement in absorption under occlusion.

Afterwards, Bronaugh et al. [39] investigated the effect of occlusion on the percutaneous absorption of six additional volatile compounds (benzyl acetate, benzamide, benzoin, benzophenone, benzyl benzoate, and benzyl alcohol) in vivo in rhesus monkeys and humans for 24 h employing two occlusion methods, plastic wrap and glass chamber. In general, occlusion enhanced the absorption of these compounds (Table 17.9). However, differences in absorption were observed between the plastic wrap and glass chamber occlusive conditions. Benzoin and benzyl acetate absorption were lower under the plastic wrap condition compared to the nonocclusive conditions; the authors conjectured that this discrepancy may be due to compound sequestration by the plastic. Glass chamber occlusion resulted in greater absorption than the nonoccluded and plastic wrap occlusion conditions for all the compounds except benzyl benzoate and benzophenone. (Benzyl benzoate had greater absorption under plastic wrap occlusion than glass chamber occlusion, while benzophenone had the same magnitude increase in percent dose absorbed for both occlusive conditions.) The authors attempted to correlate the compounds' octanol–water partition coefficients with their occlusion-enhanced skin permeation, but surprisingly, no apparent trends were found. One explanation for this lack of correlation could be that the volatile chemicals evaporated prior to application of the occluding device, which can impact subsequent measures of penetration [40].

TABLE 17.8
Percutaneous Absorption of Phenols in Humans under Occluded and Protected Conditions

		% Dose Absorbed (Mean ± SD)		
Compound	**Log Kow**	**Protected[a]**	**Occluded[b]**	**ER**
Aminophenol	0.04	6 ± 3	8 ± 3	1.3 ± 0.8
Acetaminophen	0.32	4 ± 3	3 ± 2	0.75 ± 0.8
Propionylamidophenol	0.86	11 ± 7	19 ± 9	1.7 ± 1.4
Phenol	1.46	24 ± 6	34 ± 4	1.4 ± 0.4[c]
Cyanophenol	1.60	31 ± 16	46 ± 6	1.5 ± 0.8
Nitrophenol	1.91	38 ± 11	37 ± 18	0.97 ± 0.6
Iodophenol	2.91	24 ± 6	28 ± 6	1.2 ± 0.4
Heptyloxyphenol	3.16	23 ± 10	36 ± 9	1.6 ± 0.8[d]
Pentyloxyphenol	3.51	13 ± 4	29 ± 8	2.2 ± 0.9[c]

Sources: Bucks, D.A. et al., *J. Invest. Dermatol.*, 91, 1988; Bucks, D.A. et al., *Clin. Res.*, 35, 1987.

Note: Compounds were applied topically in a single dose (2–4 μg/cm^2) from 95% ethanol to the ventral forearm for 24 h. After 24 h, the site was washed with soap and water.

[a] Dose site was covered with a ventilated plastic chamber.

[b] Dose site was covered with an occlusive chamber.

[c] Significant difference at $P < .01$.

[d] Significant difference at $P < .05$.

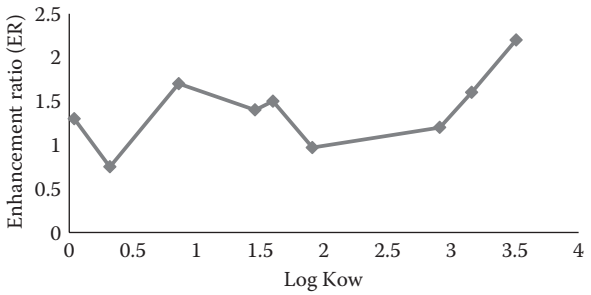

FIGURE 17.3 Log Kow of enhancement ratio (ER) of phenols. This figure plots the ER of the various phenols as a function of their log Kow values. Kow, octanol–water partition coefficient. (Modified from Bucks, D.A. et al., *J. Invest. Dermatol.*, 91, 1988; Bucks, D.A. et al., *Clin. Res.*, 35, 1987.)

Pellanda et al. [41] investigated the effect of preocclusion and postocclusion on the permeation of triamcinolone acetonide (TACA, log Kow = 2.53) into the stratum corneum. Their two experiments involved the forearms of 10 healthy volunteers. In experiment 1, they applied TACA in acetone to three sites per arm, with one arm being preoccluded for 16 h. In experiment 2, the same dose of TACA in acetone was applied on two sites per arm, with one arm being occluded after application till skin sampling. Then, stratum corneum samples were removed by tape stripping at 0.5, 4, and 24 h for experiment 1 and at 4 and 24 h for experiment 2. The amount of corneocytes adhering to the tape strips was quantified directly using a spectrophotometer, and the amount of TACA adhering to each tape was quantified using high-performance liquid chromatography (HPLC). They found that preocclusion produced no significant effect on TACA penetration into the stratum corneum, while occlusion after application enhanced TACA penetration significantly by a factor of 2.

DISCUSSION

Skin occlusion can increase the hydration of the stratum corneum by up to 50%, which can have substantial effects on the percutaneous absorption of penetrants by altering the partitioning between the chemical penetrant and the skin, swelling corneocytes, and promoting the uptake of water into the intercellular lipid domains [3–6]. Though occlusion is widely utilized to enhance the penetration of applied drugs in clinical practice, occlusion does not increase the percutaneous absorption of all chemicals; it is not well understood which chemical's occlusion enhances the penetration through skin [3,4]. Here, we focus on what effect occlusion has on the percutaneous absorption of compounds of varying lipophilicities/hydrophilicities.

First, occlusion enhances the percutaneous absorption of many but not all compounds. For example, Guy et al. [34] and Bucks et al. [37] found that when measuring occlusion's effects on the penetration of steroids, the most hydrophilic steroid of the ones being tested, hydrocortisone, did not demonstrate statistically significant enhanced penetration under occlusion. Moreover, Bucks et al. [38] also demonstrated that occlusion did not significantly enhance the penetration of many phenols being tested.

Second, occlusion seems to enhance the penetration of very lipophilic compounds more so than very hydrophilic compounds. Bucks and Maibach [36], Bucks et al. [37], and Guy et al. [34] showed that occlusion enhanced the penetration of the most lipophilic steroids (as measured by octanol–water partition coefficient) more so than the least lipophilic ones. In addition, Bucks and Maibach [36] and Bucks et al. [37,38] demonstrated that the phenols with the lowest octanol–water partition coefficients had the least enhancement in penetration under occlusion.

Third, though occlusion enhances the penetration of the most lipophilic compounds and often fails to enhance the

TABLE 17.9
Effect of Occlusion on Percutaneous Absorption of Benzyl Derivatives in Monkeys

		% Dose Absorbed		
Compound	**Log Kow**	**Nonoccluded**	**Plastic Wrap Occlusion**	**Glass Chamber Occlusion**
Benzamide	0.64	47 ± 14	85 ± 8 (ER = 1.8 ± 0.6)	73 ± 20 (ER = 1.6 ± 0.6)
Benzyl alcohol	0.87	32 ± 9	56 ± 29 (ER = 1.8 + 1)	80 ± 15 (ER = 2.5 ± 0.8)
Benzoin	1.35	49 ± 6	43 ± 12 (ER = 0.9 ± 0.3)	77 ± 4 (ER = 1.6 ± 0.2)
Benzyl acetate	1.96	35 ± 19	17 ± 5 (ER = 0.5 ± 0.3)	79 ± 15 (ER = 2.3 ± 1.3)
Benzophenone	3.18	44 ± 15	69 ± 12 (ER = 1.6 ± 0.6)	69 ± 10 (ER = 1.6 ± 0.6)
Benzyl benzoate	3.97	57 ± 21	71 ± 9 (ER = 1.2 ± 0.5)	65 ± 20 (ER = 1.1 ± 0.5)

Source: Bronaugh, R.L. et al., *Food. Chem. Toxicol.*, 28, 1990.

Note: ER, enhancement ratio (penetration of occluded chemical divided by penetration of nonoccluded chemical under otherwise identical conditions including dosing); Kow, octanol–water partition coefficients.

penetration of the least lipophilic steroids, a relationship between a compound's octanol–water partition coefficient and its occlusion-induced enhancement cannot be delineated. With regard to steroid penetration through skin, these studies showed that while a positive relationship exists between a penetrant's octanol–water partition coefficient and its occlusion-enhanced penetration, this relationship is not a linear one [34,37]. However, with regard to occlusion's effects on the penetration of phenols, no relationship was found between the octanol–water partition coefficient of the penetrant and the extent of its penetration under occlusion [36,38]. After investigating the effect of occlusion on the penetration of volatile compounds in vivo in rhesus monkeys, Bronaugh et al. [39] failed to correlate the penetrant's octanol–water partition coefficients with occlusion-enhanced skin permeation.

Finally, the general shape of the figures plotting the enhancement ratios (the ratio of occlusion-enhanced penetration over nonoccluded penetration) as a function of the logarithms of octanol–water partition coefficients suggest that the enhancement in penetration afforded by occlusion increases up to a point as the partition coefficients increase, but then, this enhancement declines as the partition coefficients further rise (see Figures 17.1 through 17.3). Occlusion may enhance the penetration of lipophilic compounds more so than hydrophilic ones due to the rich lipid composition of the stratum corneum, but as the partition coefficients further increase, permeation may be hindered for these very lipophilic compounds as the water content present in the epidermis, which occlusion only increases, assumes a larger role in limiting the permeation of liphophilic compounds.

Moreover, though the data regarding the effect of time on occlusion's effect on penetration are limited, it seems penetration increases with increasing duration of occlusion. However, the study that was presented only documented the effect of occlusion duration for one chemical, the lipophilic compound malathion. More experiments investigating the effect of occlusion duration on a range of chemicals, both liphophilic and hydrophilic, are needed.

In conclusion, occlusion does not universally enhance percutaneous penetration. Occlusion may enhance the penetration of the most lipophilic compounds but often fails to increase the penetration of compounds that are relatively hydrophilic. The in vivo studies reinforce the conclusions drawn from in vitro studies that partition coefficients cannot reliably predict the effect of occlusion on percutaneous penetration. It seems the degree of penetration enhancement provided by occlusion is compound specific and may be influenced by vehicle selection, temperature, humidity, and method of occlusion. As in many areas of skin biology, what seems to be a simple issue may, in fact, be complex—which seems to be the case for the effect of partition coefficient and occlusion on penetration. Taken together, these data provide some insights to aid the formulator especially interested in occlusive vehicles and should lead to experiments that will provide data on ingredients such as petrolatum, oils, and so forth, which are presumably not fully occlusive.

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18 Skin Ageprint
The Causative Factors

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INTRODUCTION

A rapid demographic change of pace has taken place in most populations over the past century. In Western affluent societies, a major shift occurred in the age profile of the population, with older people representing a progressively growing segment. Currently, the aging group of "baby boomers" exerts a major demographic impact. This is partly driven by the cultural push for both preventing aging and desperately trying to maintain a youthful appearance. The current demographic evolution therefore has deep social implications.

The aging process is a darling topic for both the media and medical community. As a result, any new purported treatment in this field is avidly watched by antiaging worshippers. Some middle-aged and even younger subjects show a craze for cosmetic dermatology when their once youthful skins exhibit early signs of wear and tear. This trend is no longer a typical womanly behavior because nowadays, men disdain an elderly demeanor. From time out of mind, few corrective breakthroughs and novel treatments fulfilled some of the appealing promises. In addition to new management advances, the forefront in the future scientific approach of skin aging will be focused on a better understanding of the relationships between cell biology, skin physiology, and the perceptible improvements in the ultimate clinical appearance [1].

Quandaries about aging are more complex and puzzling when skin has lost its ideal mechanical function. The dermoepidermal atrophy corresponds to the aspect of "transparent skin," also coined dermatoporosis [2]. Its clinical manifestations encompass morphologic evidence of fragility, including senile purpura, stellate "pseudoscars," and prominent skin atrophy. The functional alterations of skin fragility ensue following even minor trauma and present as skin lacerations, delayed wound healing, nonhealing atrophic ulcers, and subcutaneous bleeding leading to dissecting hematomas and large areas of tissue necrosis. The latter clinical signs bear prominent morbidity, bringing about hospitalization and surgical repair as a matter of urgency.

FROM GLOBAL TO MOLECULAR AGING AND BACK AGAIN

Any living organism is liable to aging. Such an evolution results from a multifaceted and dissimilar process in the variety of organisms. The limitation to any definition of aging lies in the diversity of life histories. Two distinct classifications of life histories have been offered. The first one distinguishes two groups of species. One group is characterized by a clear distinction between germinative cells and somatic cells. By contrast, the other group does not. The second classification makes out the semelparous species reproducing only once in their lifetime from the iteroparous species, which reproduce repeatedly.

Aging needs to be differently qualified when applied to species with other life histories. For instance, it is wrong to regard the postreproductive end life of semelparous species, which usually occurs in a highly determinate fashion, as being comparable with the more protracted process of senescence in iteroparous species. The correct models of human aging are clearly defined in iteroparous species exhibiting a distinct soma distinct from the germ line.

It is granted that human aging is a physiologic process corresponding to a progressive loss in homeostatic capacity of the body systems. This process ultimately increases the vulnerability to environmental threats and to certain diseases. Nobody escapes from aging. However, it is evident that aging progresses differently among individuals of the same age. In any given subject, senescence is heterogenous among organs and their constitutive tissues, cells, subcellular organelles, and molecules [3]. Each and every organ of the human body develops and ultimately fails at its own rate. Any stage of this process is referred to as its biologic age [4]. This systematic aging process occurs every place in the entire human body from the age of about 30–45 years (Table 18.1). To further blur the situation, regional variability of skin aging is expressed over the body. It is indeed obvious that at any time in adult life, the face, scalp, forearms, trunk, and other specific body sites show different manifestations of aging. In addition, scrutinizing skin aging shows a patchwork of aging severity at different tissue levels (epidermis, dermis, hypodermis, hair follicle) and further at the cellular level (keratinocyte, melanocyte, fibroblast, dermal dendrocyte, etc.).

Intracellular and extracellular biomolecules are involved differently by aging. Within each tissue system, molecular aging manifests itself as a progressive, nearly linear reduction in maximum function and reserve capacity [5]. Some aspects of aging are conveniently viewed as a predetermined

TABLE 18.1
Core Age Markers of Each of the Body Systems

Aging Type	Decline in	Average Age of Onset
Electropause	Electrical activity of brain waves	45
Biopause	Neurotransmitters	Dopamine 30, acetylcholine 40, GABA 50, serotonin 60
Pineal pause	Melatonin	20
Pituitary pause	Hormone feedback loops	30
Sensory pause	Touch, hearing, vision, taste, and smell sensitivity	40
Psychopause	Personality, health, and mood	30
Thyropause	Calcitonin and thyroid hormone levels	50
Parathyropause	Parathyroid hormone	50
Thymopause	Glandular size and immune system	40
Cardiopause/vasculopause	Ejection fraction and blood flow	50
Pulmonopause	Lung elasticity and function with increase in blood pressure	50
Adrenopause	DHEA	55
Nephropause	Erythropoietin level and creatinine clearance	40
Somatopause	Growth hormone	30
Gastropause	Nutrient absorption	40
Pancropause	Blood sugar level	40
Insulopause	Glucose tolerance	40
Andropause	Testosterone in men	45
Menopause	Estrogen, progesterone, and testosterone in women	40
Osteopause	Bone density	30
Dermopause	Collagen, vitamin D synthesis	35
Onchopause	Nail growth	40
Uropause	Bladder control	45
Genopause	DNA	40

Source: Braverman, E.R., *J. Eur. Anti-ageing Med.*, 1, 2005.

programmed process [6]. In addition, many of the age-associated physiologic decrements are thought to result in part from environmental insults, either acute or chronic. However, in some instances, such contention is supported by relatively little data. To add further difficulties, physical growth and senescence are both characterized by cumulative progression of interlocking biologic events. They are not always clearly distinguished because at some time in the organism's life, they possibly proceed as if they were in tandem.

Cellular Senescence in Perspective

The aging process brings about the termination of the replicative ability of cells as the individual becomes progressively older. Cells and organisms weaken their resistance to the onslaughts of the inimical environment. However, some cells and organisms are programmed to die in absence of assault from noxious environmental threats.

The age of any skin tissue is strongly reflected in the in vitro behavior of its cultured constitutive cells [7]. Replicative senescence of human cells is thus related to and perhaps caused by the exhaustion of their proliferative potential. According to the telomere hypothesis, aging somatic cells are deficient in telomerase activity maintaining the telomere repeats at the end of each cell replication. Hence, with each round of cell division, mortal cells loose some of their telomere repeats [8]. Since telomere length predicts the cell replicative capacity, it possibly provides a relevant biomarker for cellular aging [9].

Stress-induced premature senescence (SIPS) is thought to occur following a range of different individual sublethal stresses such as those induced by reactive oxygen species (ROS) [10,11]. This process results from the accumulation of DNA single-strand breaks induced by oxidative stress. According to the thermodynamic aging theory, the exposure of cells to sublethal stresses of various natures likely triggers SIPS, with possible bioenergetic modulations. A series of cells engaged in replicative senescence share common features with cells altered by SIPS, including morphologic changes, senescence-associated β-galactosidase activity, cell cycle regulation, gene expressions, and telomere shortening [10]. Thus, SIPS is presumably an important mechanism involved in the in vivo accumulation of senescent cells in the skin [12].

Cell senescence and cancer are closely related [13] by several biologic aspects including p53 mutations [8,14,15], telomere shortening [16], vitamin A depletion [17], and defects in intercellular communications [18]. At the clinical level, the age-related mottled subclinical melanoderma, even at an initial stage, appears to be a predictor for a carcinoma-prone condition [19–22].

Overall Skin Aging: 1, 2, or 7 Mechanisms?

Conceptually, human aging was viewed as one single chronologic process of progressive physiologic decline with age. Such a basic process exhibits multiple facets affecting differently the organs, tissues, and cells. This is particularly true where skin is concerned. Clearly, the genetically determined premature aging syndromes (progeria, Werner syndrome, etc.) form a group of rare disorders distinct from the physiologic aging conditions. They are not discussed in this chapter.

Over the past decades, the understanding of skin aging has considerably expanded, with a welcome emphasis on the distinction between intrinsic chronologic aging and photoaging (Table 18.2) resulting from chronic sun exposure [23,24]. According to this concept of skin aging, the changes observed in the cutaneous appearance reflected two main independent processes. First, the intrinsic aging changes in the skin are caused by the passage of time. This process is modulated by hereditary factors, along with inherent modifications in the tissue structure, physiology, and mechanobiology. Second, photoaging results from the cumulative exposure of the skin to ultraviolet (UV) light and other electromagnetic influences. Clinically, these two aging processes coexist, with intrinsic aging giving rise to smooth, dry-looking, pale, and finely wrinkled skin. By contrast, photoaging giving rise to coarse, roughened, and deeply wrinkled skin, accompanied by pigmentary changes such as solar lentigines and mottled subclinical melanoderma. These two different facets of skin aging are present in each individual on distinct skin areas. Commonly exposed area to sunlight, such as the face, neck, and dorsal forearms, differ from areas commonly shielded from the sun, for instance, buttocks and the inner aspect of the arms.

This duality concept in skin aging was challenged because it appeared as an oversimplification in clinical practice [25]. Thus, another classification into seven distinct types of skin aging was offered (Table 18.3). In this framework, the past history of the subject is emphasized. The important variables include the endocrine and overall metabolic status; past and present lifestyle; several environmental threats, including cumulative UV and infrared exposures; repeat mechanical solicitations by muscles; and external forces such as Earth gravity. Accordingly, global aging is considered to represent the cumulative or synergistic effects of specific features, each of them being independent from the others. Such a concept allows us to individualize or integrate typical processes including, among others, menopausal aging and smoking effects.

Increased awareness of the distinct age-associated physiologic changes in the skin allows for more effective and specific skin care regimens, as well as preventive measures and dermatologic treatment strategies in the elderly. Granting that the immutability of skin aging is possibly challenged [3,26], distinct factors of skin aging share some common mechanisms [27]. For instance, molecular mechanisms imply hyaluronate-CD44 pathways in the control and maintenance of epithelial growth, and the viscoelastic properties of the extracellular matrix (ECM) offer new opportunities for preventive intervention [2].

Environmental Aging and Photoaging

Environmental influences produce obvious alterations in the texture and quality of the skin, the major extrinsic insult being chronic exposure to UV radiations. The action spectrum of photodamage is not fully characterized. The cumulative effects from repeat exposures to suberythemal doses of UVB and UVA in human skin are involved in these processes [28]. The role of UVB in elastin promoter activation in photoaging is undisputable. In addition, UVA significantly contributes to long-term actinic damage. The spectral dependence for cumulative damages does not parallel the erythemal spectrum responsible for acute UV injury in human beings.

Both UVA and UVB initiate a number of cell responses, including ROS production within both dermal and epidermal cells. More specifically, cultures of human keratinocytes derived from donors of different ages and from paired sun-exposed and sun-shielded sites of older donors demonstrated that both chronologic aging and photoaging distinctly affect gene expressions. Chronologic aging alone strikingly

TABLE 18.2
Comparison of Intrinsic Aging and Photoaging

Feature	Intrinsic Aging	Photoaging
Clinical appearance	Smooth texture, unblemished surface	Nodular, leathery surface, sallow complexion, yellowish mottled pigmentation
	Fine wrinkles	Coarse wrinkles
	Some deepening of skin surface markings	Severe loss of elasticity
	Some loss of elasticity, redundant skin	
Epidermis	Thin and viable	Marked acanthosis, cellular atypia
Elastic tissue	Increased but almost normal	Tremendous increase, degenerates into amorphous mass
Collagen	Bundles thick, disoriented	Marked decrease of bundles and fibers
Glycosaminoglycans	Slightly decreased	Markedly increased
Reticular dermis	Thinner	Thickened, elastosis
	Fibroblasts decreased, inactive	Fibroblasts increased, hyperactive
	Mast cells decreased, no inflammation	Mast cells markedly increased, mixed inflammatory infiltrate
Papillary dermis	No Grenz zone	Solar elastosis with Grenz zone
Microvasculature	Moderate loss	Great loss, abnormal and telangiectatic

TABLE 18.3
Cutaneous Aging Types

Aging Type	Determinant Factor
Genetic	Genetic (premature aging syndromes, phototype-related, ethnic background)
Chronologic	Time
Actinic	Ultraviolet and infrared irradiations
Behavior	Tobacco, alcoholic abuse, drug addiction, facial expressions
Endocrine	Pregnancy, physiological and hormonal influences (ovaries, testes, thyroid)
Catabolic	Chronic intercurrent debilitating disease (infections, cancers), nutritional deficiencies
Gravitation	Earth gravity

Source: Piérard, G.E., *Dermatology*, 193, 1996.

increases the baseline expression of both the differentiation-associated gene SPR2 (small proline rich protein) and the interleukin (IL)-1 receptor antagonist gene. By contrast, it has relatively little effect on the UV inducibility of several other genes, including the proto-oncogenes c-myc and c-fos; the GADD 153, a gene inducible by growth arrest and DNA damage; and the IL-1α and IL-β genes. Photoaging differs by increasing the UV induction of c-fos, although it decreases the baseline expression of the differentiation-associated genes IL-1 and SPR2 [29,30]. The physiologic impact of photodamage occurs at variable pace on different skin structures. For instance, skin loosening and solar elastosis show clinical manifestations independently from the severity in subclinical mottled melanoderma [20].

Photoaging exerts profound effects on both the epidermis and dermis. The epidermis becomes atrophic compared to sun-shielded areas on the same individual, often with disordered keratinocyte maturation. Histologic features of photoaged skin are most apparent in the dermis, where the ECM shows marked alterations in its composition and organization [31]. Of note, fibroblasts are distinctly altered in the papillary and reticular dermis [32]. The collagen fiber scaffold of the dermal ECM is responsible for skin strength and resiliency and is intimately involved in the expression of photoaging. The major fibrillar components of the dermis are type I and III collagens. In photoaged human skin, precursors of both proteins are significantly reduced in the papillary dermis, and their reduction correlates with the clinical photoaging severity [33]. This reduction results from a combination of reduced procollagen biosynthesis and increased enzymatic breakdown by matrix metalloproteinases (MMPs) [11,34]. Collagen breakdown products within photoaged dermis negatively influence procollagen biosynthesis by fibroblasts [11,35].

Fibrillar collagen is closely associated with decorin, corresponding to a small chondroitin sulfate, proteolgycan. Its distribution closely mirrors that of type I collagen, regardless of the level of extrinsic aging [36]. Decorin allows interactions between fibrillar collagens and the microfibril-forming type VI collagen, which further interacts with type IV collagen, a major component of the basement membrane at the dermal–epidermis junction. Type VI collagen is likely to play an important physiologic role in the organization of the dermal ECM and is concentrated in the papillary dermis. It seems little affected by photoaging [37]. Type VII collagen was reported to be involved in the mechanism of wrinkle formation. It represents the major constituent of anchoring fibrils below the basement membrane providing cohesiveness between the epidermis and dermis. In photoaged skin, the number of anchoring fibrils is significantly reduced, thus increasing the potential fragility of photoaged skin [38].

The elastic fiber network supplies recoil and elasticity to the skin. The process of elastic fiber formation is under tight developmental control, with tropoelastin deposited on a preformed framework made of fibrillin-rich microfibrils. Mature elastic fibers are further encased in fibrillin, and they form a continuous network throughout the dermis. The elastic fiber network comprises (1) thick elastin-rich fibers within the reticular dermis, (2) a network of finer fibers with reduced elastin in the lower papillary dermis, and (3) cascades of discrete fibrillin-rich microfibrillar bundles, with only discrete elastin, in the upper papillary dermis, merging with the dermoepidermal junction. Fibrillin is both a product of dermal fibroblasts and keratinocytes.

The elastic fiber network is altered by considerable disruption in chronically photoaged skin. First, photoaged skin contains abundant amounts of dystrophic elastotic material in the reticular dermis [39], which is immunopositive for tropoelastin, fibrillin, lysozyme, elafin, and immunoglobulins [40] Versican, a large chondroitin sulfate proteoglycan, appears to be regulated along with dystrophic elastin deposits, resulting in a relative increase in photoaging. Although immunohistochemically identifiable fibrillin is present following actinic damage, the architecture and fibrillin-rich microfibrils are markedly altered. Minimally photoaged skin shows a similar marked loss of fibrillin-positive structures, implying that remodeling of the fibrillin-rich microfibrillar network is an early marker of photoaging [41].

Any structural change in the dermal ECM particularly affects skin viscoelasticity [42–44]. A cutaneous extrinsic aging score was derived from the difference between comparative photoexposed and photoprotected areas [45].

Ethnic and Phototype-Related Aging

People of darker skin color form the majority of the world population and Asian subjects comprise more than half of the total Earth population. The most obvious ethnic skin difference relates to skin color, which is dominated by the presence of melanin [46,47]. The photoprotection derived from this molecular polymer influences the rate of skin aging changes between the different ethnic groups. However, all ethnic groups are eventually subject to the photoaging process. Generally, Caucasians exhibit an earlier onset and both greater skin wrinkling and sagging signs than other skin

types. In general, increased pigmentary changes develop in melanotic skin, although East Asians living in Europe and North America have fewer pigment macules. Induction of hyperpigmention is thought to occur through signaling by protease-activated receptor-2, which, together with its activating protease, is increased in the epidermis of subjects with melanotic skin [48]. Changes in skin biophysical properties with age demonstrate that the more darkly pigmented subjects retain younger skin characteristics than the more lightly pigmented groups.

Endocrine Aging

Irrespective of age, most of the skin components are under the physiologic control of a number of endocrine and neuroendocrine factors (Table 18.4). As such, skin is recognized as a hormone-dependent organ [49–51]. Like any other system in the body, the aging process impairs every hormonal function, leading to deficiencies, which, in turn, potentially influence the aging machinery operative in the skin [51]. Quite distinct are the skin manifestations of some endocrinopathies, which possibly mimic or interfere with skin aging [49–51]. A few direct consequences of endocrine gland aging interfere with skin aging. They are mostly related to the decline in activity of the pituitary gland, adrenal glands, ovaries, and testes.

Some hormones and neuroendocrine mediators are synthesized by nerves, as well as by epithelial and dermal cells in the skin (Table 18.5). A number of environmental and intrinsic factors regulate the level of cutaneous neuroendocrine system activity. Solar radiation, temperature, humidity, as well as diverse chemicals and biologic xenobiotics represent important environmental factors. Some internal mechanisms affecting the neuroendocrine system of the skin are generated in reaction to environmental signals. They possibly result from local biologic rhythms as well as local or systemic disease processes [49].

TABLE 18.4
Neuroendocrine Receptors Active in the Skin

1. Adrenergic receptors
2. Androgen and estrogen receptors
3. Calcitonin gene-related peptide receptor (CGRP-R)
4. Cholinergic receptors
5. Corticotropin-releasing hormone and urocortin receptors (CRH-R)
6. Glucocorticoid and mineralocorticoid receptors
7. Glutamate receptors
8. Growth hormone receptor (GH-R)
9. Histamine receptors
10. Melanocortin receptors (MC-R)
11. Miscellaneous neuropeptide receptors
12. Miscellaneous receptors
13. Neurokinin receptors (NK-R)
14. Neutrophin receptors (NT-R)
15. Opioid receptors
16. Parathormone (PTH) and PTH-related protein (PTHrP) receptors
17. Serotonin receptors
18. Thyroid hormone receptors
19. Vasoactive intestinal peptide receptor (VIP-R)
20. Vitamin D receptor (VDR)

TABLE 18.5
Hormones and Neurotransmitters Produced by the Skin

1. Hypothalamic and pituitary hormones
2. Neuropeptides and neurotrophins
3. Neurotransmitters/neurohormones
4. Other steroid hormones
5. Parathormone-related protein
6. Sex steroid hormones
7. Thyroid hormones

The paradigm of deleterious hormonal effects is represented by the influence of corticosteroids on skin atrophy. Cushing syndrome and iatrogenic effects of topical and systemic corticotherapy are equally involved. Corticosteroids are known to regulate the expression of genes encoding collagens I, III, IV, and V; decorin; elastin; MMPs 1, 2, and 3; tenascin; and the tissue inhibitors of MMPs 1 and 2 [52]. However, the exact molecular mechanisms of skin atrophy induced by corticosteroids are not yet elucidated. The corticosteroid-induced atrophy is one of the most severe models of skin aging, corresponding to dermatoporosis [2].

Vitamin D is the major endocrine compound produced by skin. It corresponds to a regulator of the calcium metabolism, and it exerts other systemic effects as well. For example, there is epidemiologic evidence suggesting that sunlight deprivation with reduction in the circulating vitamin D_3 possibly results in increased incidence of carcinomas of the breast, colon, and prostate [53]. In addition, vitamin D_3 and its analogues modulate the biology of keratinocytes and melanocytes of the skin in vivo [54].

Growth hormone (GH) is secreted by the pituitary gland under the control of several hypothalamic and peripheral modulators exerting either positive or negative influences. The time-regulated balance among the modulating factors determines the pulsatile and circadian secretion of GH. Moreover, physiologic changes occurring in particular conditions (i.e., puberty, pregnancy, aging, severe acute illness) affect GH secretion. The peripheral effects of GH are mainly exerted through the insulin-like growth factor (IGF)-1, produced by the liver upon GH stimulation. The circulating IGF-1 is bioavailable and functionally active depending upon its binding with the IGF-binding proteins (IGF-BPs). Skin is a target for the GH–IGF system exerting obvious influences on the dermal and epidermal physiology [55]. GH, IGF-1, IGF-2, and IGF-BPs are present in the skin and are involved in its physiologic homeostasis. Thus, systemic as well as paracrine and/or autocrine cutaneous activity of the GH–IGF system contributes to skin homeostasis [55,56]. GH supplementation

induces skin changes, a part of which corresponds to some corrective effects on aging skin [57,58].

The progressive decline in dehydroepiandrosterone (DHEA) serum concentration with age possibly exerts some genomic effects [59] but has not demonstrated direct relevant effects on the skin. DHEA supplementation possibly increases sebum production.

Sex hormones exert a variety of biologic and immunologic effects in the skin [60–63]. Estrogen, alone or in combination with progesterone, prevents or reverses some aspects of skin atrophy, dryness, and wrinkles associated with both chronologic aging and photoaging. These hormones are expected to stimulate keratinocyte proliferation. In addition, estrogens downregulate apoptosis and thus help prevent epidermal atrophy. Furthermore, they enhance collagen synthesis. The combination of estrogens and progesterone reduces collagenolysis through the inhibition of MMP activity, thereby contributing to maintaining skin thickness. Estrogens might contribute to hold skin moisture by increasing the hyaluronic acid and versican amounts in the dermis. Progesterone increases sebum excretion.

Both the climacteric period following menopause and the andropause decade negatively affect the skin [60–62]. Hormone replacement therapy (HRT) during the climacteric period helps in limiting these changes [64–67]. However, there is a limitation because there seem to be both good and poor responders to the treatment [68]. The habit of smoking possibly interferes with the treatment result [69].

Deprivation and Catabolic Aging

The elderly often consume a substandard diet and have a deficiency in many of the nutrients thought to be essential to maintain health. Protein-containing foods such as meat and fish appear occasionally too expensive or troublesome to prepare for older individuals. Dietary faddism, confusional states, and forgetfulness are further responsible for inadequate diet. These conditions predispose to skin changes that often amplify the alterations induced by age-related hormone deficiency.

If insufficient fresh fruit and/or vegetables are eaten, vitamin C deficiency occurs, leading to scurvy. In this disorder, there is a defect in coagulation, and resulting in purpura, particularly in a punctate perifollicular pattern on the legs. In the elderly, iron deficiency is quite common, resulting in anemia, generalized pruritus, and diffuse hair loss.

Essential fatty acid and vitamin A deficiencies due to dietary faddism or deprivation in the elderly cause xerosis [70]. Many of the elderly are further deficient in zinc, which represents an important factor in preventing wound healing. Zinc supplementation, however, does not improve healing.

Clearly, some chronic debilitating conditions (infections like tuberculosis and AIDS, end-stage cancer, inflammatory gut diseases, etc.) are prone to provoke premature aging of the catabolic type. Chronic hemodialysis is another example of catabolic aging affecting the mechanical functions of skin [71,72].

Gravitation-Related Aging

Skin of any part of the body is subjected to intrinsic and extrinsic mechanical forces. Among them, Earth gravitation is important, as it influences skin sagging during aging. Any force generated by the skin or applied to it transduces information to cells that may, in turn, respond to it [73,74]. The effects of mechanobiology are particularly evidenced in fibroblasts, dermal dendrocytes, keratinocytes, and melanocytes [75–77]. Physical forces of gravity involve mechanotransduction in the skin [78] and affect cell tensegrity and the cell mechanosensitive ion channels. As a result, the structure of the dermal ECM is affected.

The Case of Wrinkles

There is evidence that wrinkles are not related to genuine skin microrelief [79–84]. In addition, the microanatomy of wrinkles is varied [79,80,85]. It depends on subtle changes in the structure of the superficial dermis, elastotic deposits in the upper reticular dermis, loosening of the hypodermal connective tissue strands, or, universally, on hypertrophic tethering of the dermis to the underlying facial muscles in facial frown lines [86]. The wrinkle severity rating [87] is influenced by the nature of the altered ECM. Similarly, the skin viscoelastic properties are under these influences [88–90].

Photoaged facial skin does not always present clinically with typical uniform wrinkling. In some individuals, usually those with fair skin phototype, smooth unwrinkled skin and telangiectasia predominate. These people appear to be more at risk of developing basal cell carcinomas (BCC) on sun-exposed facial skin [91,92]. By contrast, there is an apparent inverse relationship between the severity in facial wrinkling and the occurrence of facial BCCs [91]. It was suggested that melanoma risk was reduced by photoaging and smoking [93]. On a mechanistic standpoint, little is known regarding how these clinical outcomes occur in response to the same environmental stimulus, namely, sun exposure [94].

Smoking contributes to some wrinkling formation [95]. Elastic fiber alterations by ROS and repeat mechanical solicitations by some muscle contractions play a putative role in the formation of the smoker's wrinkles.

CONCLUSION

Each individual perceives a global appearance of skin aging. Aging is apparent at all levels of the physiology and anatomy of the body. Organs, tissues, cells, and molecules follow their own aging processes, which differ in their clinical relevance. Prevention and correction of skin aging benefit from targeting some of the specific underlying biologic processes.

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19 New Perspectives in the Control of the Skin Aging Process

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INTRODUCTION

The latest advancements in molecular biology have affected different fields in the medical sciences. As a result of this scientific revolution, new therapeutic possibilities arise from pharmacogenomics, considering the individual's personal needs as well as his or her response to personalized treatments. Based on its intrinsic complexity, aging is one of the most significant biological processes that can be favored by personalized therapies. Skin represents an exposed organ where the signs of the aging process become apparent, and it is composed of a barrier that must be transposed for the efficient action of topical treatments, as proposed by cosmetic products. Nanotechnology is another scientific improvement that can be considered to fight skin aging, favoring the absorption of specific components and its distribution among skin layers. This chapter evaluates possible applications of new technologies for skin aging control and perspectives that introduce cosmetic innovations in the era of molecular biology.

THE GENETIC REVOLUTION

We are experiencing a scientific revolution characterized by groundbreaking research in genetics and molecular biology, growing in volume and applicability, particularly in medical sciences. When Watson and Crick [1,2] discovered the spiral of life, DNA had already become the undisputed landmark of a genetic revolution that continues to expand. In 1985, Kary Mullis described the polymerase chain reaction (PCR) technique, which allowed replicating DNA sequences in vitro and increased the possibilities of accessing the information contained in the genetic code [3]. From point-by-point perspectives in studies related to gene structure, expression, and regulation, the research work in molecular biology progressed toward the development of global approaches, also known as "omics," which provide a better understanding of the workings of complex, ever-changing biological systems, such as the human being.

Among such comprehensive approaches, genomics was the first to gain prominent space in the scientific community with the Human Genome Project, which began in the mid-1980s based on the creation of new sequencing techniques. Originally, the project emerged as a joint public initiative of the United States Department of Energy (DoE) and the National Institutes of Health (NIH), which, in cooperation with international organizations, was intended to identify the gene positions in human DNA; determine the number and sequence of the nitrogen bases; store the resulting information in databases; and assess the ethical, legal, and social aspects that would surface with the new discoveries. Over approximately 15 years, this international public consortium was accompanied by Celera, a private American corporation that joined the race to decipher the human genome. When the project was completed, its significance had grown to such an extent that its results dominated the world scene with the simultaneous publication as the cover story in two important scientific journals in the world, *Nature* and *Science* [4,5].

Among its surprising results, the Human Genome Project led to some exciting conclusions. The first is that the human body has fewer genes than had been imagined. About 20,000 genes were identified, in contrast to previous estimates of about five times as many. Furthermore, genome studies conducted on other organisms showed that the number of genes in humans is not necessarily greater than the number in other species of animals such as cats and mice, which have 20,000 to 25,000 genes [6,7]. It therefore became apparent that DNA sequencing alone would not supply the answers for a deeper understanding of the source of the variability or complexity of all the biological processes in the living organisms. Subsequently, traditional genomics opened the door to an evolution that would eventually become known as functional genomics—an approach involving the assessment of molecules that result from the expression of genes in the DNA and possess biological functionalities, ensuring differentiation of the human species from other animals. With the ensuing technological progress and the appearance of new analytical platforms, the "omics" approach expanded to include the study of different biological molecules other than DNA alone: transcriptomics, to evaluate the set of transcripts or RNA molecules produced as a direct result of gene expression; proteomics, intended to study proteins; and metabolomics, intended to analyze different chemical processes involving metabolites. The identification of interindividual variability markers was yet another result generated by the Human Genome Project. The Human Genome Diversity Initiative was created in 2003 with the purpose of evaluating small distinctions in the DNA of different persons [8]. Also, as regards individualization, applying different global approach techniques based on the evaluation of biological molecules at different levels may help to explain the immense variety of phenotypes observed in man.

SKIN AND SKIN AGING IN THE ERA OF MOLECULAR BIOLOGY

Skin is a complex and dynamic system where some of the signs of the aging process become apparent. As the world population ages and individuals live a better life and consequently enjoy longer periods of activity and productivity, the aspect of their skin becomes increasingly important to make them feel safe and confident in their social life. Currently, skin treatment products are intended not only to indulge but also to provide new personal hygiene and health standards. The growing demand for skin care products represents an important indicator of the quest for dignified aging in today's society. With the completion of the Human Genome Project, which allowed deciphering a few pages of the "book of life," new prospects have opened for future generations to help them lead dignified, better lives.

Dermatology was also deeply affected by the scientific developments of the genetic revolution. In recent years, the number of scientific papers on the subject of gene expression applied to skin care has grown enormously. In addition, molecular biology is increasingly present in the research on skin aging, which, as a highly complex process, involves the simultaneous and continuous action of factors that trigger a progressive decrease in the homeostatic capacity of the skin. Such factors may be intrinsic (when determined by individual genetic constitution) or extrinsic (when caused by direct effects from environmental exposure and everyday habits) and lead to the appearance of clinical signs such as wrinkles and changes in skin elasticity, texture, and pigmentation, among others. External signs, in contrast, directly reflect internal and structural changes in skin tissue, including diminished blood flow in skin tissue; reduction in the thickness of the dermis and epidermis; disorganization in collagen and elastic fiber patterns; reduction of the activity of enzymes involved in posttranslational modification processes; formation of protein aggregates; changes in the deposition of glycosaminoglycans, which then tend to interact less with water molecules; and changes in the lipid content of the skin [9,10]. When viewed at the molecular level, skin aging affects the expression of genes and the production of specific proteins. Thus, considering the complexity of the skin aging process associated with the complexity of the skin tissue itself, it becomes clear why the studies now conducted in this area are being strongly supported by the application of technologies designed for the global evaluation of biological phenomena. The use of these technologies originated the term "skinomics," which refers to the comprehensive evaluation of biological molecules in conjunction with the assessment of the development and functionality of the skin [11].

DEVELOPMENT OF MORE EFFECTIVE SKIN AGING TREATMENTS AND FUTURE PROSPECTS

Along with the progress that molecular biology brought to studies related to the skin and its changes, it also offers advantages for the development of more effective treatments to fight skin aging. Cosmetic ingredients are becoming increasingly sophisticated and functional, and the current level of development of these components allows the production of products with actions and activities that could not have been predicted a short time ago. In addition, the evolution that came in the wake of the Genome Project makes it possible to offer customized products to target each individual's personal needs, as well as to optimize the formula according to the individual response to the selected treatment. This is pharmacogenomics, a new area of activity that sprang from these concepts and involves the application of technologies such as DNA sequencing and gene expression analysis, statistical techniques applied to research work, and tests related to pharmaceuticals or ingredients. This is a branch of pharmacology that deals with the effect of genetic variations on an individual's response to pharmaceutical products, thus correlating gene expressions or genetic polymorphisms with the efficacy of a treatment. A principle of pharmacogenomics is the development of the so-called customized medicine, whereby pharmaceutical products and their combinations are optimized in a unique composition for each individual [12]. This new perspective allows us to take individual variations into account for the identification of his or her needs, for the choice of active ingredients, and for monitoring the response of the individual to the treatment with the purpose of maximizing the action and effectiveness of the product. Again, these innovative technologies derived from the genetic revolution have increasingly favored the development and expert use of the concepts of pharmacogenomics applied to dermatology [13].

Reinforcing the importance of understanding skin aging as a multifactor, concomitant, and cumulative process driven by genetic heritage and environmental exposure, the evolution of skin aging should be treated as a completely individualized condition. In this case, the concepts of pharmacogenomics are fundamental and fully applicable since, in addition to the individual's genetic variations, all the variations resulting from his or her history or lifestyle should also be taken into account. Moreover, considering the individualization of this complex biological process, it is necessary to understand that the therapy chosen to treat signs of skin aging should also be designed in a customized fashion to be more effective.

The clinical signs of aging on the surface of the skin are known to result from an aggregate of internal processes that may be affected by aging, such as cell signaling, control of melanin production, control of oxidative stress, skin barrier composition as linked to functional hydration, control of inflammation processes, cell differentiation, vascularization, lipid metabolism, cell proliferation, extracellular matrix composition, and cell adhesion [10,14–27]. As all the abovecited biological processes are associated with specific genes, and as there is no need for the existence of mutations in the DNA sequence to affect the functions of these genes, the changes that affect the skin over the years may be attributed to a modulation of gene expression, which leads to changes in the production of biological molecules such as RNA or proteins. Several studies have demonstrated that the aging process

affects the expression of a considerable number of genes. In some instances, the change in gene expression may correlate with certain clinical signs, such as wrinkles, flabbiness, loss of skin hydration, and changes in vascularization or lipid metabolism. Aging, however, can influence the regulation of genes involved in processes that are undetectable in day-to-day clinical practice without the aid of laboratory testing, such as cell adhesion and the oxidative process, among others.

Another important consideration regarding the modulation of gene expression in skin aging is that this approach opens up a new therapeutic perspective: by applying appropriate substances, it is possible to predict the modulation of gene expression in the opposite direction from that which occurs with aging. This is yet another benefit provided by the evolution of functional genomics. Systems that have a natural plasticity may allow modulations to be stimulated, as opposed to changes or polymorphisms that affect the DNA and that, at this point in time, cannot be modified by simple, safe therapeutic interventions. In other words, since the production of RNA for a given gene changes with age, it would be possible to measure the level of transcription to find modulation alternatives or even to monitor the efficiency of a treatment after a certain period of time. Some such studies have been conducted by Grupo Boticário, which looks for innovative approaches to skin aging treatments. One proposal is the analysis of gene expression to generate a report showing the status of a specific patient in comparison with the population of his or her age bracket. This report, combined with a clinical evaluation, can serve as a basis for the prescription of a tailored product according to the needs of each patient (Figure 19.1). In addition, new tests or clinical evaluation may be conducted after a certain period to recheck the status and adjust the treatment for the best possible results, considering individual variability. Several studies were conducted for the identification of genes directly related to skin aging. The research was planned to cover the gaps in the scientific knowledge already available, including an expanded panel of women aged from 20 to 80 years and the application of the microarray technology with the simultaneous evaluation of 44,000 genetic sequences (Figure 19.2). Based on the results of gene expression, the genes strongly correlated with skin aging were selected and analyzed with respect to their capability of RNA expression modulation after the treatment with specific active ingredients.

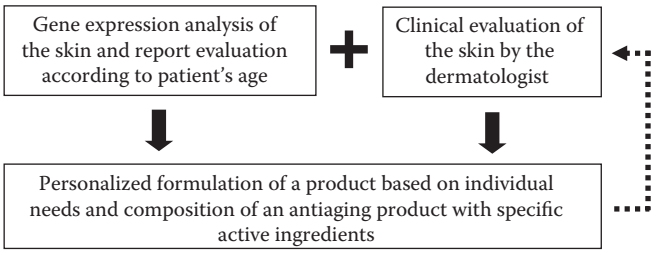

FIGURE 19.1 Proposed scheme for the formulation of a personalized antiaging product for skin treatment, based on combined gene expression analysis and clinical evaluation. The model considers repeated evaluation after some time of treatment for specific adjustments.

A primary function of skin is to protect the organism against environmental injury and the entry of foreign bodies or substances. To create this barrier, the skin is made of layers with different physicochemical characteristics, which constitutes a challenge for the delivery of substances in the treatment product. As previously discussed, the cells proliferate in the lower epidermis and then move upward to the upper layers to form different strata with specific properties and metabolic processes. There is, in addition, a complex composition in the dermis, which contains several types of cells and the extracellular matrix, among other elements. All these peculiarities contribute to make the skin a living, dynamic tissue containing elements that influence the percutaneous absorption of substances. The ingredients of the products applied to the skin respond to the laws of physics (solubility, partition coefficient, molecular weight, etc.) that affect the absorption process, in addition to the fact that the physical structure and chemical composition of the skin make it a barrier. To perform its function, an ingredient must cross lipophilic barriers in the horny layer to reach progressively more hydrophilic layers in both the epidermis and the dermis. The vehicle and the technology used in the product will have a substantial effect on its absorption and, consequently, on its action. Different formulation technologies may serve as supplementary tools to allow offering highly effective products for hydration, reduction of signs of aging, and protection of the skin against injury from daily exposure to external factors. One way of overcoming this challenge is to use nanotechnology with colloidal structures, which may permeate the intercellular space and release the

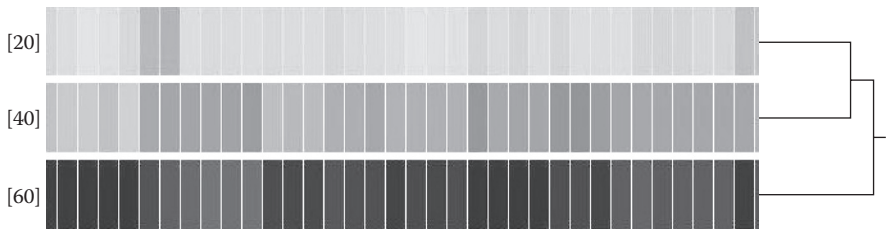

FIGURE 19.2 Example of hierarchic clustering for studying skin gene expression affected by aging. Different colors indicate differences in gene expression profile. The numbers at the left side represent approximated ages for different groups. The branches at the right side indicate the similarity between groups: The group of 20-year olds is more closely related to the 40-year olds than the 60-year olds. Based on the biological function and the expression level, specific genes can be selected for treatment with specific active ingredients.

active ingredients by affinity in the layers where the processes actually take place. Nanotechnology can be applied, based on the example of a deposited patent [28], to the composition of unique products formed by associating three nanoemulsions with distinct physicochemical properties, which are capable of delivering active ingredients to the targeted skin layers and potentiate the effects of the customized treatment even more. To minimize the risk of adverse reactions to the use of the product, the formulations can also contain ingredients that mimic the natural molecular composition of the skin. Different experiments were conducted to evaluate safety and efficacy of the treatment using a balanced formulation with the same proportion of each nanoemulsion (Table 19.1). For the evaluation of the efficacy of the skin treatment, analysis of gene expression after a period of 90 days with continuous use of the personalized product, combined with clinical supervising and image evaluation, was performed. The results showed significant improvements in the skin, including reduction in the number and intensity of wrinkles, improvement in skin elasticity, and a more homogeneous pigmentation. Also, those positive results were observed in patients of different ages.

This individualized treatment approach bears considerable market potential. If we examine the past, market evolution history started from commodities intended to fulfill all needs with similar products. It moved through the years to niche products, to reach specific markets were certain group of people had common needs. And now it is getting to search for niches within niches and so on to differentiate from the competitors. With this customization proposal, we may meet the needs of each person, and we can follow the reverse path since the niches are made up of individuals, as is the entire population; therefore, we foresee no limitation for the application of this system.

New studies and methodological approaches based on the development of molecular biology continue to appear and may contribute even further to the improvement of dermatological treatments, as in the case of the epigenetics concept or the evaluation of regulatory mechanisms based on noncodifying DNA sequences, which has been recently described in the publication of the ENCODE project [29]. Aging is a natural process, not a curable disease, and it can be effectively controlled with the available technologies engendered by the genetic revolution outlined in this chapter.

TABLE 19.1
Group of Analysis (In Vitro, Ex Vivo, and In Vivo) for Safety and Efficacy Evaluation of the Treatment with Triple Nanotechnology

Test Description	Results
In vitro cytotoxicity	Noncytotoxic
In vitro irritation test using reconstructed skin (epidermis and dermis)	Nonirritant
In vivo 48 h patch test for acute skin irritation (primary irritation)—55 volunteers aged 18 to 60 years	Nonirritant
In vivo 21-day cumulative skin irritation test—55 volunteers aged 18 to 60 years	Nonirritant
In vivo allergy test (21 days induction and challenge after 14 days)—55 volunteers aged 18 to 60 years	Hypoallergenic
In vivo 28-day patch test for comedogenicity—35 volunteers aged 35 to 60 years	Noncomedogenic
In vivo eye irritation test—35 volunteers aged 35 to 60 years	Noneye irritant
In vivo 21-day phototoxicity test—27 volunteers aged 18 to 60 years	Nonphototoxic
In vivo photoallergy test (21 days induction and challenge after 14 days)—27 volunteers aged 18 to 60 years	Nonphotoallergenic
Ex vivo 24 h skin stimulation and immunohistochemistry	Best preservation of collagen fibers in the dermis and more intense signals for the presence of beta-1 integrin in the epidermal basal layer
Ex vivo 24 h skin stimulation and immunofluorescence	More intense signals for the presence of filaggrin, fibronectin, elastin, and collagen 1 in the epidermis and/or dermis
Ex vivo 24 h skin stimulation and microarray (stimulation with triple nanotechnology or isolated active ingredients)—total of 216 microarrays with analysis of 44,000 genetic sequences	Reversion of the expression in 85% of the genes affected by aging
In vivo corneometry for skin hydration (only one application)—10 volunteers aged 21 to 47 years	Increase of 22.2% in skin hydration after 6 h and 7.4% after 24 h
In vivo 30-day Raman spectroscopy (application twice a day)—10 volunteers aged 40 to 50 years	Increase of 70% in the content of keratin, 38% in functional collagen (containing amide 1), and 6.4% in functional water (molecules associated to proteins); reduction of 50% in the content of natural moisturizing factors (NMFs) and 40% of melanin
In vivo 30-day stimulation and microarray of hair follicles from eyebrows—15 volunteers aged 40 to 50 years with analysis of 44,000 genetic sequences	Reversion of the expression in 68% of the genes affected by aging

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20 Skin Aging
Microrelief and Wrinkle Measurements

Urte Koop, Thorsten Bretschneider, Sven Clemann, and Sören Jaspers

INTRODUCTION

One of the downsides of aging is that we end up with dull, thin, sagging, rough skin with more and more wrinkles. Since appearance of the skin is an overt and discernible visual indicator of biological age, everyone wants to preserve one's healthy and youthful-looking skin, particularly on the face, as long as possible. So, understanding skin aging and consumer needs are key areas of focus in the cosmetics industry, as well as finding effective ways to treat and prevent rough skin and wrinkles: about 50% of all women in their 40s state that fine lines or first wrinkles are their most important skin problems, and nearly 70% of all women use an antiaging facial day care product to decelerate or postpone overt signs of skin aging (Internal Beiersdorf data from GfK Custom Research; Usage & Attitude Study Face Care & Cleansing, October 2010, based on 6050 women in 11 countries worldwide).

According to research, there are two distinct types of skin aging:

- *Intrinsic aging*: predominantly genetically predisposed, also known as the natural aging process. It is a continuous process normally beginning in our mid-20s with the signs of aging typically becoming evident decades later. Within the connective tissue of the skin, collagen production slows and elasticity is lost. Epidermal regeneration also slows down. The signs of intrinsic aging are fine wrinkles, thin and transparent skin, loss of underlying tissue, sagging skin, dry skin, graying hair, hair loss, unwanted hair, and thinning nail plates [1].
- *Extrinsic aging*: predominantly influenced by external, environmental factors, such as exposure to the sun's rays (photoaging), which breaks down collagen and impairs the synthesis of new collagen, repetitive facial expressions, gravity, sleeping positions, and smoking. The signs of extrinsic aging are age spots, spider veins, leathery skin, blotchy complexion, wrinkling, sagging skin including cheeks, actinic keratosis, eye bags, and graying skin tone [2].

Several well-designed neuropsychological studies have investigated the contribution of facial characteristics to the subjectively perceived age by raters. A major determining factor in these cases was skin topology, especially the manifestation of wrinkles [3,4]. In such studies, photographs of faces with skin surface topography cues removed were judged significantly younger and more attractive than their original (unmodified) counterparts, with modifications on the forehead and around the eyes showing the highest differences. In these areas, participants were able to detect at least a 20% visual change in skin surface topography [3].

Different causes and locations lead to a wide variety of wrinkle characteristics attended with partly confusing naming: static wrinkles, dynamic wrinkles, expression lines, fine lines, fine wrinkles, coarse wrinkles, crow's feet, furrows, laugh lines, smile lines, smoker lines, marionette lines, creases around mouth and chin, nasolabial folds, lip wrinkles, frown lines, labial lines, or nasolabial sulcus.

The quantitative determination of the skin's surface topology, both microstructures such as roughness and macrostructures such as wrinkles, is one of the most important and probably most frequently performed investigation in the field of cosmetics, and increasingly in dermatological research as well [5,6]. Quantification of topological skin parameters offers several distinct advantages: it allows standardization of assessments, improves the reproducibility of studies, allows interstudy and intrastudy comparisons, and often improves the statistical power of comparisons. Ideally, the output of quantification algorithms should yield numeric data sets that map human sense observations and experiences. Thus, quantification algorithms, especially those applied in dermatological or cosmetic science, often need to be validated against subjective assessments (e.g., by panelists). It has to be noticed that in some instances, especially when aesthetic aspects or aspects of attractiveness/preference are in the focus of the investigation, a quantitative assessment method may still be inferior to subjective panelist assessments or even not applicable.

Various instrumental techniques are available nowadays to evaluate changes in skin surface and measure antiwrinkle activity [5–8]. The following direct and indirect methods (Table 20.1) are commercially available in the market to measure the microstructures and macrostructures of the skin in a quantitative or semiquantitative sense. This list and the following descriptions are not meant to give a complete overview of suppliers but are rather focusing on the technical principles and limitations of established procedures. Addressing the complex mathematical functions underlying these procedures would be beyond the scope of this article.

TABLE 20.1
Direct and Indirect Methods to Measure the Microstructures and Macrostructures of the Skin

Assessment	Method	Comment
Direct	In vivo topometry	Mainly used for wrinkle measurement
Direct	Clinical live scoring	Used for wrinkle and roughness measurement
Indirect	Photo evaluation and analysis	Mainly used for wrinkle measurement
Indirect	Imprint analysis	Performed as scanning electron microscopy, topometry, shadow casting, profilometry, or transmission measurement of imprints; mainly used for roughness measurement

IN VIVO TOPOMETRY

The introduction of active image triangulation in conjunction with phase-shift techniques in skin topometry allows for fast, noninvasive measurement of the skin's surface in vivo. Active image triangulation systems are versatile tools to assess and quantify a variety of topological parameters of an investigated skin region, ranging from macroscopic structures of larger skin areas to microscopic structures on small areas [9–13].

Technical Background

The main attribute of in vivo topometry systems based on active image triangulation is the projection of a regular sinusoidal intensity (= fringe) pattern with a sophisticated digital projection device onto the surface of the skin at a certain angle of incidence. Height data for the structured surface are coded in the distorted intensity pattern recorded by means of a suitable video technique. A typical fringe projection picture on crow's feet (wrinkles that appear around the eye, classically starting at the outer corner of the eye) is shown in Figure 20.1. The topometry system itself consists of the projection unit and an image acquisition unit (Figure 20.2a), combined with a processing analysis unit. Evidently, a solid stand for the volunteer subjects with a highly reproducible positioning construction and sufficient degrees of freedom is necessary (Figure 20.2b). Measurement of skin topology involves (i) projecting the sinusoidal fringe pattern onto the object surface; (ii) recording the image of the fringe pattern that is phase modulated by the object height distribution; (iii) calculating the phase modulation by analyzing the recorded image with an appropriate analysis algorithm (e.g., Fourier analysis); and (iv) using a suitable phase unwrapping algorithm to get continuous phase distribution data proportional to the object height variations. The final data set may be used to construct detailed three-dimensional (3D) models of the investigated skin area. Examples of recalculated 3D models with gray-coded information about absolute height values are given in Figure 20.3. Depending on the algorithms used, the technique provides very satisfactory results from the point of view of accuracy, repeatability, and reproducibility [14]. Contemporary PRIMOS systems have an overlay feature that enables precise matching of photos taken at different visits.

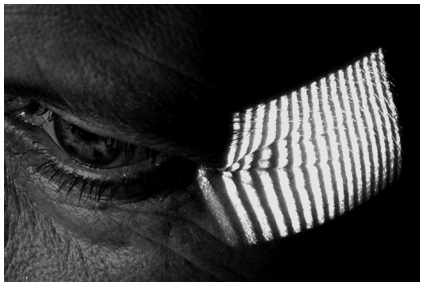

FIGURE 20.1 Distortion of the regular sinusoidal fringe pattern by the irregular surface of the skin. Presentation of crow's feet (wrinkles that appear around the eye, classically starting at the outer corner of the eye).

At present, the phase-shift rapid in vivo measurement of skin (PRIMOS) system (GFMesstechnik GmbH, Teltow/Berlin, Germany) is one of the most widespread in vivo systems for determining skin topology. This article will concentrate primarily on the technical background of and the possibilities offered by the various PRIMOS devices currently available. Other instruments are based on similar principles, such as the DermaTop (also known as FOITS) system (Eotech, Marcoussis, France) or SkinBio Technologies skin profile solution (SkinBioTechnologies, Cologne, Germany).

Principle of Active Image Triangulation: PRIMOS

The PRIMOS measuring head consists of a digital micro mirror device (DMD) used as the projection unit and a charge-coupled device (CCD) camera used as a recording unit. The angle between the optical axis of the projector lens and the optical axis of the camera lens is called the system's triangulation angle. Because of the limited dynamic range of the projector in conjunction with the spatially varying surface properties, like background intensity and reflection characteristics, it is not possible to encode each point of the surface unequivocally with only one projected intensity pattern. To avoid these matching problems, temporal phase-shift algorithms are used.

Technical Data and Handling

The most suitable devices at present for dermatological/cosmetic evaluations consist of a high-resolution projection unit and a state-of-the-art digital CCD camera. Most wrinkle measurement systems include fields of view measuring approximately 18 × 13 to 40 × 30 mm with a vertical resolution of about 2 μm. Four to eight phase shifts are typically

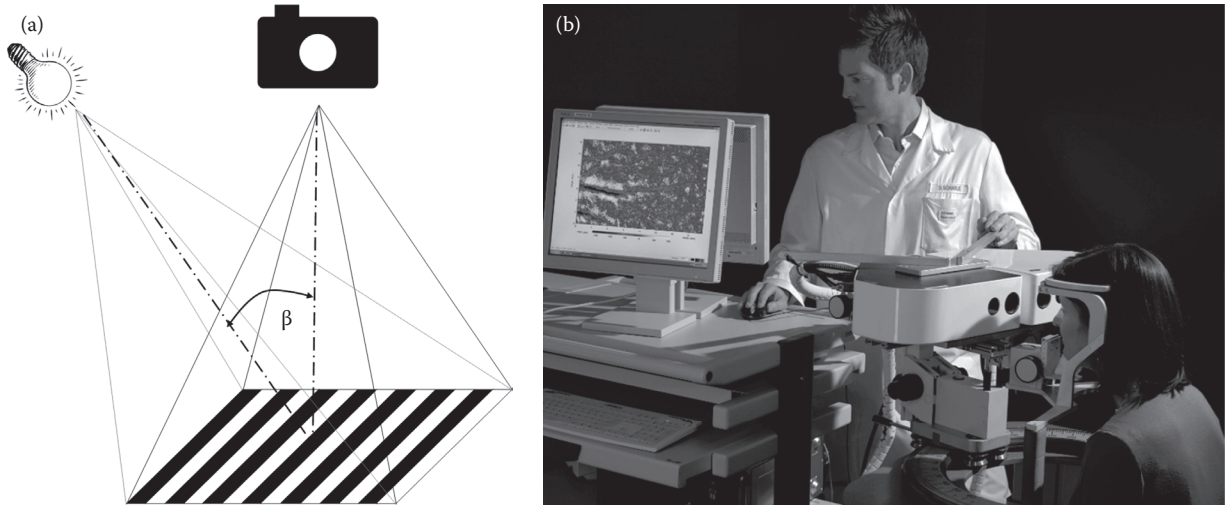

FIGURE 20.2 Setup of a typical PRIMOS system. (a) Schematic representation of computerized digital imaging assessment using fringe projection (interferometry) profilometry (β: triangulation angle). (b) Commercial PRIMOS system in operation.

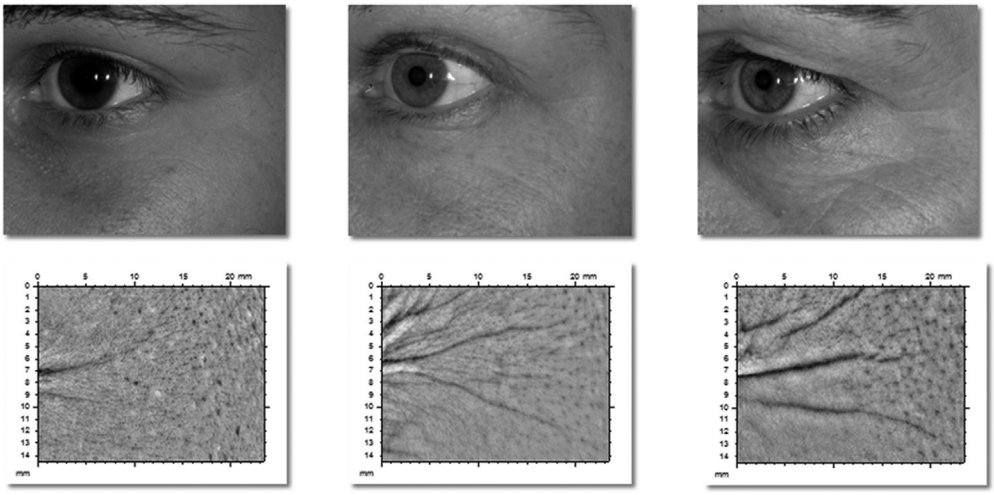

FIGURE 20.3 Three-dimensional display of crow's feet wrinkles processed using the PRIMOS system. High-resolution photographs and corresponding processed 3D displays of investigated skin area: three different wrinkle grades. Vertical height values are color-encoded.

performed during any given measurement, depending upon the desired quality. This results in typical measuring periods of approximately 70 to 140 ms. Today, systems are available that allow high-resolution overview measurements (i.e., full face) with the option of analyzing several sections in just one step (i.e., nasolabial folds and crow's feet).

Handling these systems necessitates a certain degree of accuracy and training. In this context, exact reidentification of the previously measured sites and the avoidance of any body movements are of particular importance to achieving optimum results. It is advisable to use tailor-made vibration-free stands with a highly reproducible positioning construction and numerous degrees of freedom for specific wrinkles. The PRIMOS system features a video overlay function and a focusing tool using projected central crosshairs and four corner marks, which need to be aligned to respective marks on the computer screen, which is quite helpful once the operator has had sufficient practice [15]. Furthermore, checking data quality online is highly recommended. Figure 20.4 shows black and white coded 3D displays before and after filtering: quality deficiencies such as motion blur or insufficient realignment are only visible in the filtered images. Filtering is applied to eliminate contributions from larger-scale body shape such as—in case of crow's feet—eye socket and temple. The choice of the appropriate filter is essential for the validity of the results. Figure 20.5a shows the effect of different filters with long-wave high pass filters, or more sophisticated, polynomial filters seem to be most appropriate (Figure 20.5b).

USEFUL PARAMETERS

ISO Standards

A panel of useful parameters has initially been developed for the characterization of roughness of technical surfaces. The

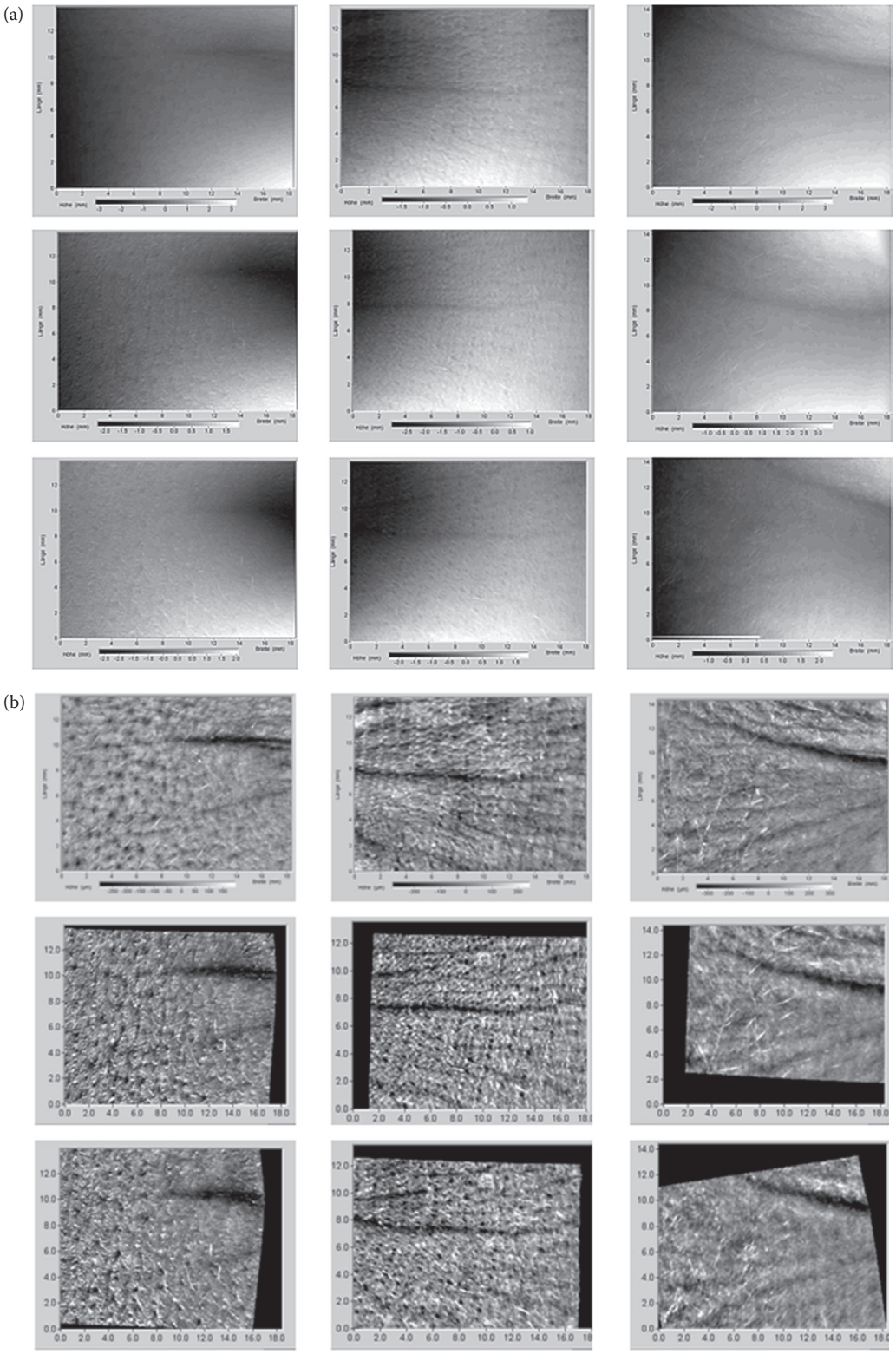

FIGURE 20.4 Examples of unfiltered (a) and filtered (b) 3D displays. Quality deficiencies such as motion blur or insufficient realignment are only visible in the filtered images: checking data quality online is highly recommended.

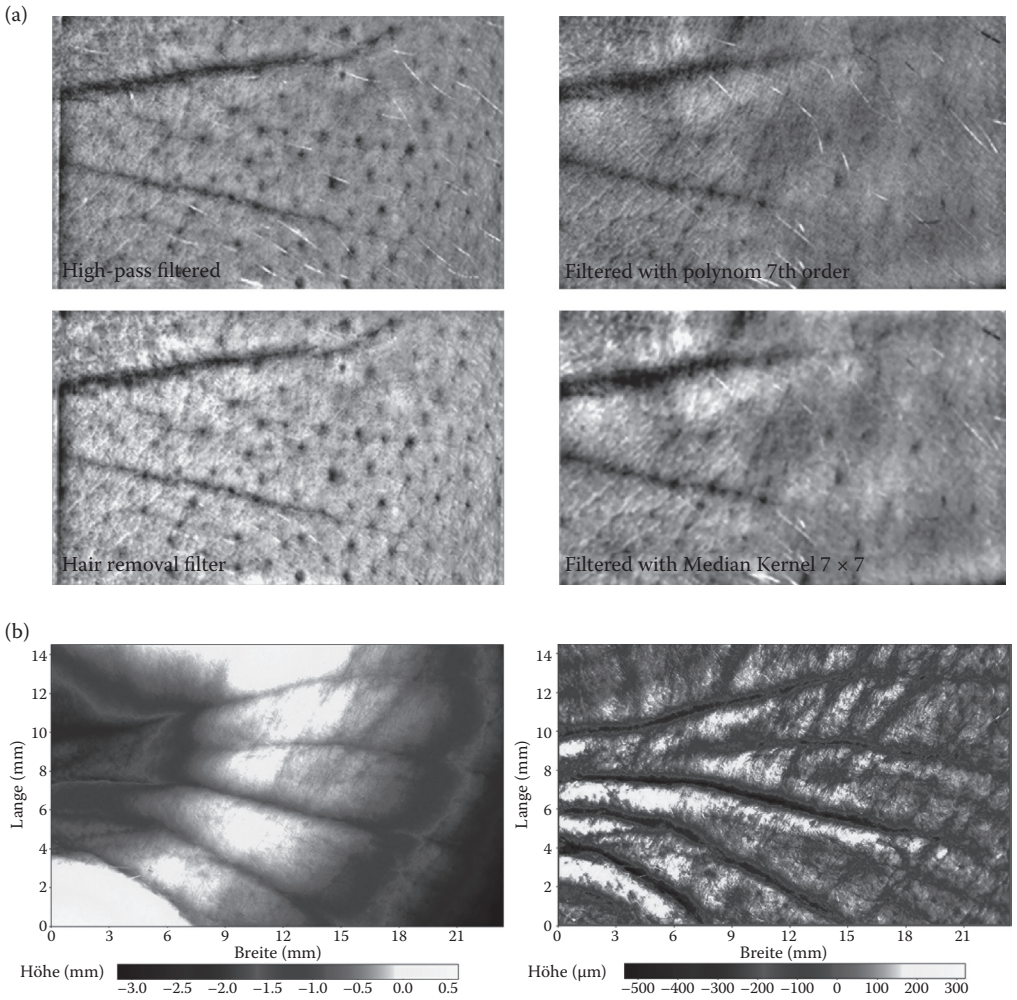

FIGURE 20.5 Examples of filtering effects. (a) To eliminate contributions from—in case of crow's feet—eye socket and temple, appropriate filtering is essential. Long-wave high pass filters or, more sophisticated, polynomial filter proved to be appropriate. (b) Original 3D display and polynomial filtered 3D display to visualize coarse and fine wrinkles in the periorbital region (crow's feet), microtopography, and pores.

measurement of surface roughness is defined by a series of international standards. These standards cover the characteristics of the measuring equipment and specify the mathematical definitions of the many parameters used today. The current basis for the use of ISO parameters in profilometry is ISO 4287: *Geometrical Product Specifications—Surface texture: Profile method—Terms, definitions and surface texture parameters* dated from 1997. These parameters may also be adopted for studies of skin, which is also a rough surface by nature.

According to ISO specifications, a surface is defined as the peripheral skin of a body that separates it from the surrounding medium. This surface incorporates structural deviations from a reference line, which are classified as form, waviness, and surface roughness. In order to separate the three elements, specific filters can be used. Either roughness or waviness filters can be selected for most measuring instruments. If a roughness filter is selected, waviness elements are removed leaving the roughness profile for evaluation (Figure 20.5a). If a waviness filter is selected, roughness elements are removed leaving the waviness profile for evaluation. There is no general fixed set point at which roughness becomes waviness or vice versa; according to the ISO, this depends on the size and nature of the application.

Selection of Roughness Parameters

A roughness value can either be calculated on a profile (R_a, R_q, and R_z according to ISO 4287) or on a surface (S_a, S_q, and S_z according to ISO/DIS 25178-2). The profile roughness parameters are more commonly used in cosmetic science, although the area roughness parameters may give more significant values. R_a, for example, is the arithmetic average of absolute height values and expresses the mean of the absolute values of the profile heights measured from a mean line averaged over the profile. In contrast, R_z is the average vertical distance between valleys and peaks across consecutive sampling lengths. Other profile roughness parameters express the maximum valley depth, maximum peak height, maximum peak to valley height, skewness, kurtosis, mean spacing

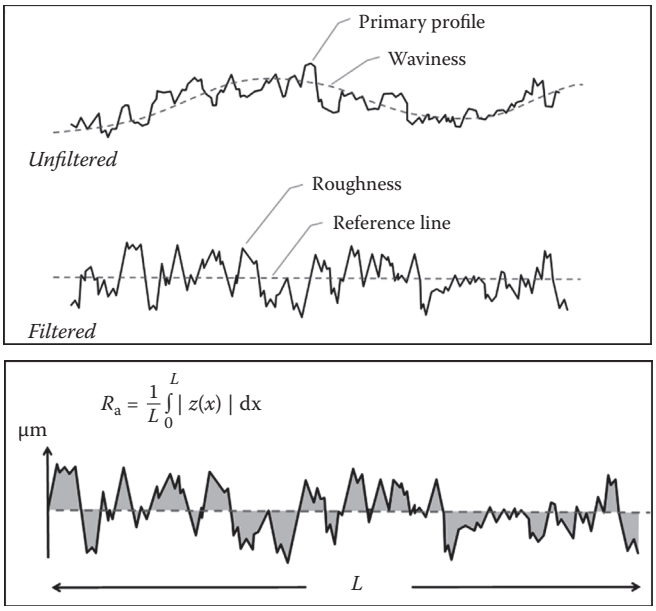

FIGURE 20.6 Example of R_a profile roughness values calculated from profile measurement. A profile filter separates the measured primary profile in the roughness profile and waviness. R_a is the arithmetic average of absolute height values and expresses the mean of the absolute values of the roughness profile heights measured from a mean line averaged over the profile.

between peaks, and many more. The real surface geometry is so complicated that a finite number of parameters cannot provide a full description. Since all individual parameters reduce the information in a profile to a single number, great care must be taken in applying and interpreting them. Although the literature is somehow contradictory about the suitability of the different profile parameters, the values R_a and R_z are commonly used in order to assess textural changes in body and facial skin [9,16–19]. Figure 20.6 gives an example of R_a values calculated from profile measurement.

Modern devices provide more than profile information; they take each single pixel of the measured surface into account (in most systems more than 600,000) and can extend the above profile roughness parameters into surface roughness parameters. Therefore, on behalf of the European Union, the 2D parameters normalized in the ISO 4287 standard were extrapolated into 3D [20]. Their handbook, *Development of Methods for the Characterization of Roughness in Three Dimensions*, provides the basis for a unified approach to three-dimensional surface finish assessment and was considered for the EN ISO 25178-2. It covers a range of issues related to 3D microtopography with an emphasis on standardization and interpretation. The most common parameter of surface roughness is S_a (corresponding to R_a in profile roughness).

Volume Parameters

Another approach, especially for assessing wrinkles such as crow's feet, is the calculation of wrinkle volume, which is often implemented in the standard analysis software of contemporary measuring devices. In the past, the users had to develop their own software tools detecting and analyzing wrinkles, for example, resulting from edge detection procedures in combination with suitable filter algorithms for separating roughness and wrinkle structures. Interactive determination of the volume parameter is still somewhat time-consuming, but the results are very precise and at the same time very meaningful.

Objective wrinkle analysis using a PRIMOS system is normally based on volume analysis and wrinkle depth analysis. Volume analysis represents the estimated volume (or occupied space) of skin cavities such as fine lines, wrinkles, or skin depressions, in a marked area below a reference plane. Volume analysis is conducted in a matched area using the 3D topographic models taken before and after treatments. For wrinkle depth analysis, a matched single line is marked on the 3D topographic models, across the same wrinkle before and after the treatment. Figure 20.7 gives an example of wrinkle analysis before and after treatment.

IMPRINT ANALYSIS

A still very common method of evaluating skin roughness involves the use of silicone imprints as used in dentistry. However, when the focus of an investigation is on macrostructures, making such imprints is both quite challenging and sometimes error-prone. There are different silicone materials and auxiliary materials such as location rings available to make silastic casts or imprints of the skin surface. Imprints can subsequently be analyzed by a variety of optical procedures to determine topological parameters such as scanning electron microscopy, transparency measurement, shadow casting methods, or fringe projection. The respective techniques are described below.

Fringe Projection

Imprints can also be measured with fringe-projection-based topometry. The basic principles are those already described above for the PRIMOS system. There are specialized fully automated robotic systems on the market for fringe projection analyses of imprints, such as the MicroCAD® system (GFMesstechnik GmbH, Teltow/Berlin, Germany). The most common parameters assessed with fringe projection-based topometry using imprints are roughness parameters such as R_q and S_q.

The following methods are less widespread and less practice-relevant.

Scanning Electron Microscopy

With high resolution and magnification up to 100,000×, scanning electron microscopy enables the three-dimensional observation of skin surface topography and ultrastructure [6]. To analyze skin surface topography, imprints or specially dissected skin biopsies can be measured. This method enables the observer to see the skin microtopography

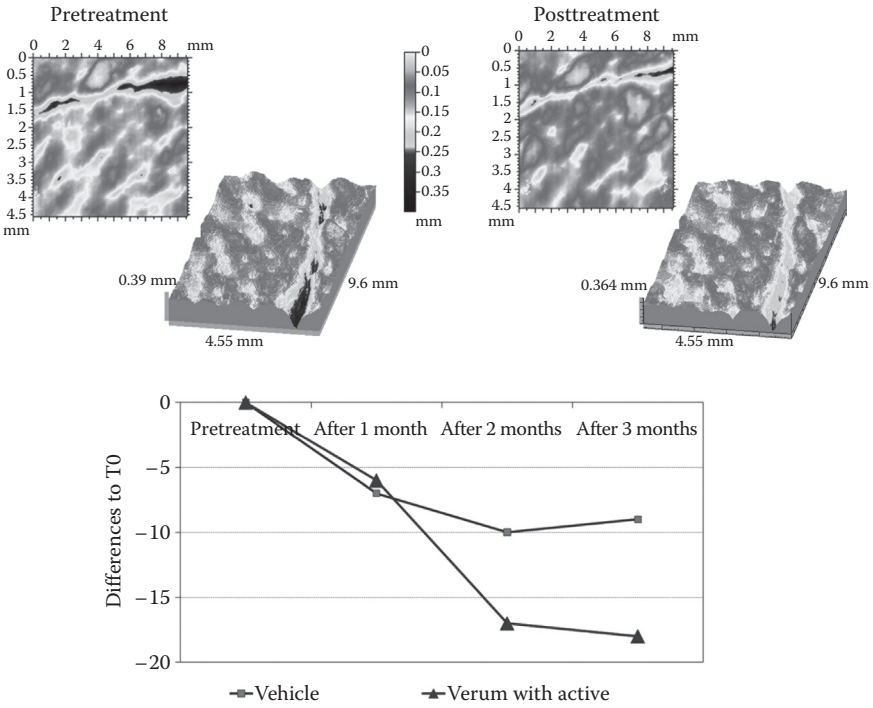

FIGURE 20.7 Wrinkle depth measurement using the PRIMOS system. For wrinkle volume analysis, a matched area is defined on the same wrinkle using the processed 3D displays before and after treatment. Wrinkle volume analysis: upper line represents volume change after vehicle treatment; lower line represents volume change after verum treatment.

as a pattern of flat fields with ridges and deep furrows in between, forming a characteristic triangular and polygonal network. Regularity of corneocytes, individual structure, and overall arrangement can be assessed. Disturbance of desquamation is accompanied by increasing exfoliation of corneocytes, and typical eczematous skin is characterized by widening of the furrows and vanishing of the regular polygonal structure. Scanning electron microscopy does not provide a metric quantification of the skin topography, for example, the depth and width of furrows, but an assessment of the ultrastructural quality of the skin as an overall view of different anatomic regions and different diseases. Typical examples of scanning electron microscopy skin images are depicted in Figure 20.8. The method is especially valuable for the qualitative analysis of skin microtopography but yields only limited quantitative data on macrotopographic structures such as wrinkles.

Transparency Measurement

Transparency measurement aims at measuring the skin roughness by light transmission of the imprints, which are formed out of a thin, special blue dyed two part silicone. The imprint is placed between a parallel light source and a b/w CMOS camera (640 × 480 pixels), where the light penetrates the imprint and is absorbed according to the thickness of the silicone material: the outgoing light is proportional to the incoming light, the thickness of the material, and the material constant. Light intensities can be recorded pixel-wise and processed to a 3D model of the investigated skin area.

Transparency measurement is often performed with Visiometer® devices such as the Visiometer 2000 (Courage+ Khazaka Electronic, Cologne, Germany). Within a short time, the standard roughness parameters and color 3D images are available and can be displayed quickly. Other displays of

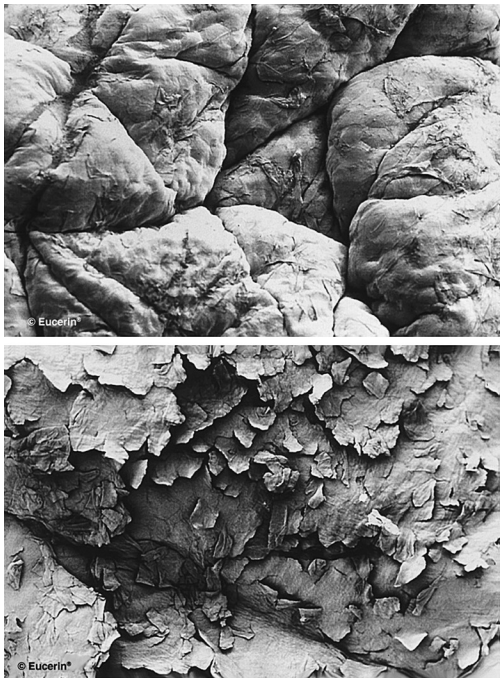

FIGURE 20.8 Examples of scanning electron microscopy data. Upper image normal skin and lower image dry, scaly skin.

the image (e.g., relief, false color, etc.) are possible. Special parameters have been created to describe the skin topography volume (mm^3) and percentage of unfolded surface.

Shadow Casting Method

This method is based on shadow casting and has been scientifically acknowledged and established for many years to analyze deeper lines and macro wrinkles such as crow's feet [5]. The imprint is illuminated uniformly with a defined light source mounted in a specified angle. The shadows visible on the imprint created by the oblique light are captured with a high-resolution camera, which is mounted vertically to the imprint. Software analyzes different characteristics of the wrinkle in length and depth (in mm) and a shape factor. A simple calibration tool enables the user to be sure of the accuracy of distance and depth (*x, y, z*). Special image tools as 3D and false color illustrate the output for marketing purpose. Shadow casting measurement is often performed with Visioline devices such as the Visioline VL650 (Courage+Khazaka Electronic, Cologne, Germany).

PHOTO DOCUMENTATION, EVALUATION, AND ANALYSIS

Clinical photography and corresponding evaluations can be regarded as a measuring device with broad applications. The specific setup has to be adapted to the objective of the study: resolution, magnification, illumination, and stands have to be chosen carefully and used in a standardized manner. Most applications using photo documentation have semiquantitative end points of evaluation. In these cases, subjective ranking and grading by panelists is the preferred method of analysis.

Taking Images

A key aspect of photo documentation and evaluation is the technique of taking images. Depending on the parameters to be analyzed, specialized equipment for illumination, filtering, recording, or image processing may become necessary to make target structures visible to the rater or analysis software. An in-depth discussion of all options to visualize target structures, to produce contrast, or to process images would be far beyond the scope of this article.

Figure 20.9 shows an example how the choice of light source can affect the information contained in a recorded image. Used carefully targeted, this can help to differentiate between structural changes and solely optical effects. Due to the fact that even smallest changes in illumination can cause a dramatically different impression, the operator has to focus on color calibration of each single part of the photographic setup and the repositing of the volunteers. Taylor-made stands and software features such as "overlay function" or "baseline viewer" are essential in order to get comparable images. Depending on further analysis, aspects such as esthetics, and the use of hair bands, caps have to be considered. For some evaluations, it is necessary that the volunteer respects restrictions during the study, but also the preconditioning such as avoiding permanent makeup and tattoos, dying or tweezing eyebrows, wearing colored eye lenses, changing hair color and style, and taking off jewelry and piercings. Choosing certain backgrounds and letting the volunteers wear standard cloaks might also be beneficial in order to avoid any kind of distraction. Close-up photography is often used to document microphotographic skin conditions (Figure 20.10).

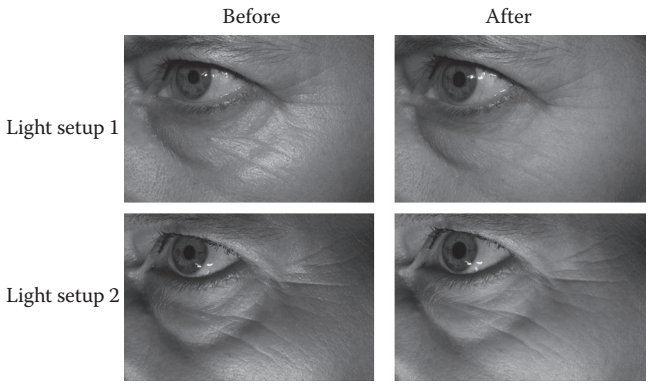

FIGURE 20.9 Effect of different illumination conditions on the visibility and impression of wrinkles. Upper line: frontally evenly illuminated, left: before, right: shortly after product treatment. Lower line: structured illumination and shadow effect, left: before, right: shortly after product treatment. Due to the fact that a pretest/posttest difference can only be seen in the frontally evenly illuminated setup, it can be concluded that the tested product had a purely optical effect on wrinkle appearance without altering the wrinkle structure.

Image Analysis

Photographs can be objectively evaluated with morphological image analysis software. Texture lines appear darker in the images, which can be used as a measure for height variation. Proprietary software development enables the investigators to reliably quantify the textural changes on the skin surface. As an example of applicable devices, the Visioscan® V98 (Courage+Khazaka Electronic, Cologne, Germany) is an easy-to-handle video-microscopic device consisting of a ring-shaped UV-A light and a high-resolution video camera. The captured digitized b/w images clearly show the structure of the skin and the level of dryness. In addition to the image processing function, special software permits the calculation of a variety of skin surface parameters with using the SELS® (Surface Evaluation of the Living Skin) software [21]. The gray-level distribution of the image is used to evaluate four clinical parameters as an index: smoothness (Sesm), roughness (Ser), scaliness (Sesc), and wrinkles (Sew). Figure 20.11 gives an example of automated roughness measurement using image analysis software designed for the analysis of recorded photographs. In this context, it has to be remarked that any kind of change in skin color or irregular pigmentation may alter the values significantly. This has to be considered

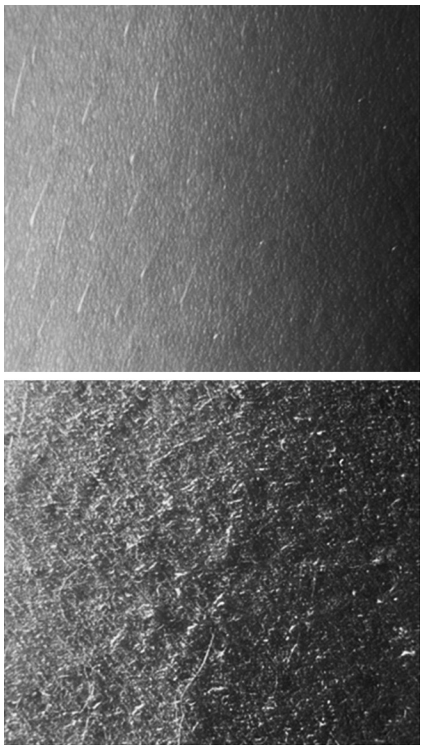

FIGURE 20.10 Photo documentation of skin roughness. Visible differences in skin roughness can be evaluated by blinded ranking or grading of the photographs.

when selecting volunteers, defining study rules and restrictions, and (re)locating test areas (Figure 20.11).

Ranking of Photographs

Unless objective quantitative measurements can be made using recorded images by appropriate image analysis tools, a common way to analyze photographic data sets is the ranking by panelists. Ranking of the photographs should be performed in a blinded manner with the help of specially designed network-compatible software. The program shows the pictures taken at the different points in time of each volunteer in a random way under observance of ICC color workflow instructions. The rater, who can be the volunteer himself, uninvolved layperson, or trained expert—depending on the objective of the study—sorts the images in line according to given criteria such as wrinkle intensity. The sorting then can be compared with the points in time on a statistical level.

Grading of Photographs

Grading of photographs should be done with the help of detailed descriptive or image scales or by trained and experienced graders. The documentation should ensure that previous grading scores are hidden from the grader's view.

It has to be noticed in this context that for cosmetic studies, subjective analysis by raters (ranking or grading of images) may be a valuable complement to objective quantitative

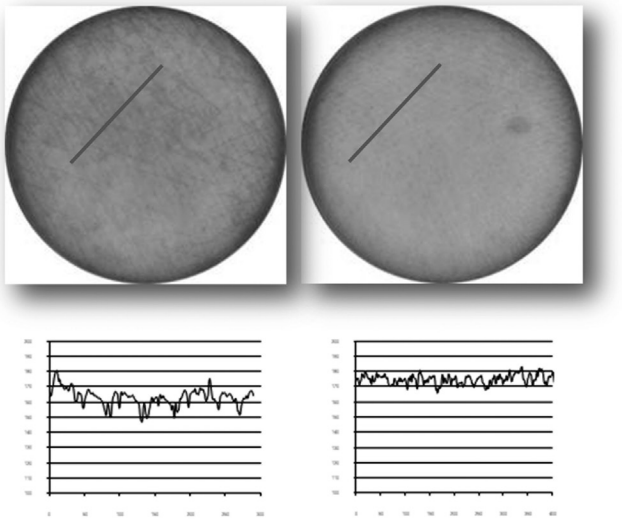

FIGURE 20.11 Quantification of roughness parameters using image analysis software. Morphological image analysis: texture lines appear darker in the images, which can be used as a measure for height variation.

measurements. This could be the case to complex end points such as attractiveness, youthful appearance, or any other neuropsychological parameter that can hardly be expressed by a numerical value.

CLINICAL LIVE SCORING

Tactile evaluation of skin roughness by trained experts with the help of descriptive scales such as defined for the assessment of dry skin (xerosis) and ichthyosis [22] or the severity of wrinkles [23] is a helpful tool to assess perceivable effects. Applicable criteria for the tactile evaluation of roughness are outlined in Table 20.2.

Concerning clinical wrinkle grading, three classifications are commonly used: Glogau's classification [24], which is often used by dermatologists; Fitzpatrick's classification [25], which refers only to the degree of wrinkling around the mouth and eyes; as well as the criteria outlined in the

TABLE 20.2
Applicable Criteria for Tactile Assessment of Roughness

Score	Roughness (Tactile Evaluation)
0 (absent)	Perfectly smooth and pliable
1 (slight)	Slightly irregular and scratchy on tangential tactile evaluation
2 (moderate)	Definitely irregular and scratchy and possibly slightly stiffened on vertical tactile evaluation
3 (severe)	Advanced irregularly and scratchy feeling associated with some stiffening
4 (extreme)	Gross irregularity and major disturbance of skin markings and definite stiffening

TABLE 20.3
Glogau Classification of Wrinkles

Classification	Represented by Age Class	Criteria
Mild	28–35 years	Few wrinkles, no keratoses
Moderate	35–50 years	Early wrinkling, sallow complexion with early actinic keratoses
Advanced	50–60 years	Persistent wrinkling, discoloration of the skin with telangiectases and actinic keratoses
Severe	65–70 years	Severe wrinkling, photoaging, gravitational and dynamic forces affecting skin, actinic keratoses with or without skin cancer

TABLE 20.4
Fitzpatrick Classification of Wrinkles

Classification	Criteria
Class I	Fine wrinkles
Class II	Fine-to-moderately deep wrinkles and moderate number of lines
Class III	Fine-to-deep wrinkles, numerous lines, and possibly redundant folds

AAD Guidelines of Care for Photoaging/Photodamage. Tables 20.3 and 20.4 give an overview of the Glogau and Fitzpatrick grading systems, respectively.

According to the AAD Guidelines of Care for Photoaging/Photodamage (Dermatology World Guidelines), wrinkle intensities can be discriminated as follows:

- *Fine wrinkles (lines)*: This parameter represents the visual assessment of the number and depth of superficial lines around the eye or mouth regions. Fine lines are most visible in the periocular, cheek, and mouth areas. Fine lines disappear when the skin of the surrounding area is stretched slightly.
- *Coarse wrinkles*: This parameter represents a visual assessment of the number and depth of deeper permanent lines and furrows around the eyes, forehead, and mouth regions. Wrinkles do not disappear when the skin is stretched slightly. Examples of coarse wrinkles include the frown lines and worry lines of the forehead, the laughter lines of the periocular area, and the smile and pucker lines of the cheeks and perioral areas.

In all cases of clinical wrinkle assessment, it might be meaningful to use image scales, possibly different scales for different kinds and location of wrinkles, for example, the COLIPA scale for crow's feet. To gain reliable results, the grader should be intensively trained, and the illumination and position of the volunteer should be standardized; in some cases, the use of magnification lenses might improve results.

CONCLUSION

Topographic features of the skin such as roughness or wrinkles can be quantitatively and semiquantitatively measured with different methodologies. Depending on the dimension of the structure of interest and the expected effect, the most appropriate method should be selected considering the respective strengths and pitfalls referred to the working principle of the active or product. Experimental design of studies is a complex subject. Scientifically valid results rely on knowledge and awareness of methodological and statistical principles in design and analysis of a study.

The fringe projection-based in vivo topometry is a very precise and reliable methodology in order to measure microstructures and macrostructures on different body sites, such as roughness and wrinkles, but also sagging cheeks, dimpled thighs, or eye bags. It should be considered that the setup (among others: field of view, resolution, parameter, stand) is to be adapted to each region of interest and that an experienced operator is essential to gain meaningful results. In future, new parameters and the improvement of quality control mechanisms, speed, and resolution will continue to be developed.

Imprints still have their rightful place in cosmetic investigations, especially in high-throughput roughness evaluation.

Photographic documentation and the diverse analysis options make this methodology extremely valuable, especially when focusing on visible result or actives and products having optical effects.

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21 Smoking and Skin Aging

Maral Rahvar

INTRODUCTION

In 2010, it was estimated that 19.3% (45.3 million) of the US adult population were smokers; among these, 78.2% (35.4 million) smoked every day and 21.8% (9.9 million) smoked some days. According to the same report, each year, 443,000 people die prematurely either from smoking or being a secondhand smoker, and 8.3 million live suffering from a severe disease because of smoking [1].

Based on a World Health Organization (WHO) report, presently 22.2% of the world's population over 15 years old are smokers, and every 6 seconds, one person dies because of smoking. So, tobacco use is still the number one cause of mortality and morbidity not only in the United States but also all over the world [2].

Skin aging is due to both intrinsic and extrinsic factors [3]. Intrinsic aging is the slow irreversible degeneration, which affects almost all of the body [4]. Intrinsic skin aging is mainly portrayed by wrinkling of the skin, cherry hemangiomas, and seborrheic keratoses [5]. The most distinguishable part of extrinsic skin aging is photoaging or photodamage caused by long-term exposure to solar ultraviolet (UV) light. A photodamaged skin is simply characterized by coarsely wrinkled skin, hyperpigmentation, and telangiectasia and is in association with malignant tumors [6–9].

The association of cigarette smoking and cardiovascular disease, lung cancer, and chronic obstructive pulmonary disease is well documented [10–12]. However, studies have shown the adverse effect of tobacco smoking on the integumentary system [13].

It is commonly believed that a youthful smooth facial appearance is associated with prolonged living. Today, multiple environmental factors are found in association with facial aging. Therefore, lifestyles that prevent facial skin aging are probably helpful in improving public health [14].

EPIDEMIOLOGY OF SKIN AGING

In 1856, in large series of British insurance examinations, it was noticed that smokers had a sallow complexion and markedly wrinkled skin [15]. In 1857, skin differences between smoking and nonsmoking British Army officers stationed in India were described [16].

In 1965, by evaluating 224 women aged 35–84 years, cigarette skin was described as a pale, thick skin with a grayish hue without local variation of pigmentation and wrinkles that were especially noticeable on cheeks. Because similar skin changes were observed in nonsmoking women over 70 years, these changes did not appear to be specific [17].

The effect of smoking on skin was noticed by Daniell as early as 1971. She studied the severity of wrinkles of 1104 subjects after adjusting age and outdoor sun exposure. Her results showed that premature wrinkling is an important symptom of smoker's skin [18].

Since then, several studies have assessed an association between smoking and skin aging. In 1981, characteristics of "smoking face" was described including prominent facial wrinkles, prominence of underlying bony contours, atopic skin, and plethoric, slightly orange purple or red complexion [19] (Table 21.1).

Later, Emster et al. [20] examined the association between pack-years of smoking and facial wrinkling in men and women. After age, average sun exposure, and body mass index were controlled, the estimated relative risk of moderate/severe wrinkling for current smokers compared to never smokers was 2.3 (95% CI = 1.2, 4.2) among men and 3.1 (95% CI = 1.6, 5.9) among women. Pack-years of smoking was positively associated with facial wrinkle score in women aged 40 to 69 years and in men aged 40 to 59 years. Moreover, in both groups, the increased risk of wrinkling was equivalent to about 1.4 years of aging.

A cross-sectional study of 83 subjects evaluated a relationship between tobacco smoking and UV radiation exposure in skin aging. They assessed sun exposure, pack-years of smoking history, and potential confounding variables by questionnaire. Facial wrinkles were quantified using this formula: Daniell score = –1.24 + 0.05 × age + 0.015 × pack-year + 0.158 × sun exposure. Logistic statistics analysis of data showed that age (odds ratio [OR] = 7.5, 95% CI = 1.72 – 19.87) and sun exposure (OR = 2.65, 95% CI = 1.0 – 7.0) independently contributed to facial wrinkle formation. When sun exposure (>2 h/day) and heavy smoking (35 pack-years) occurred together, the risk of developing wrinkles was 11.4 times higher than that of nonsmokers and those with less sun exposure (<2 h/day) at the same age [21].

In 2002, to objectively measure skin topography, the association between wrinkle formation and tobacco smoking was investigated in 63 volunteers by using a silicone rubber replica combined with computerized image processing. The replica analysis was used to study the changes in the surface furrows of the volar forearm in 63 volunteers. The results revealed that subjects with a smoking history of at least 35 pack-years had radically greater furrow depth (Rz) and variance (Rv) than nonsmokers ($p < 0.05$). However, the number

TABLE 21.1
Summary of Studies

Study (Year)	Study Design	Results	Ref.
Douglas model (1985)	Prospective survey	The association of smoker's face with current smoking (>10 years) was significant ($p < 0.001$) and remained after controlling confounding factors.	19
Ernster et al. (1995)	Cross-sectional	Estimated relative risk of moderate/severe wrinkling for smokers compared to nonsmokers was 2.3 (95% *CI = 1.2, 4.2) among men and 3.1 (95% CI = 1.6, 5.9) among women.	20
Yin et al. (2001)	Cross-sectional	Age (**OR = 7.5, 95% CI = 1.87 – 30.161), pack-years (OR = 5.8, 95% CI = 1.72 – 19.87), and sun exposure (OR = 2.65, 95% CI = 1.0 – 7.0) independently contributed to facial wrinkle formation. Excessive sun exposure (>2 h/day) and heavy smoking (35 pack-years) together made the risk for developing wrinkles 11.4 times higher than nonsmokers and those with less sun exposure (<2 h/day) at the same age.	21
Leung et al. (2002)	Cohort	In multiple logistic regression models, only age and daily cigarette consumption were significantly associated with skin aging. Smoking 20 cigarettes per day was equivalent in effect to almost 10 years of chronological aging.	28
Raduan et al. (2008)	Cross-sectional	The larger the tobacco load, the larger the amount of facial wrinkling, with an odds ratio of 3.92 in "heavy" smokers (>40 packs/year) in relation to nonsmokers.	24
Asakura et al. (2009)	Cross-sectional	After adjusting for age, smoking status and topical sun protection were significantly associated with skin condition among both men and women in this study population.	14
Jacobi et al. (2011)	-	Nicotine significantly accelerated wound healing as assessed by closure rate and histological score. The effects of nicotine were equal to bFGF and were mimicked by epibatidine and blocked by hexamethonium.	43

*CI: confidence interval, **OR: odds ratio.

of furrows in smokers was lower in subjects with a history of smoking compared to nonsmokers ($p < 0.05$) [22,23].

A cross-sectional study was done in 301 individuals (including 110 men and 191 women aged between 25 and 86 years) in a Brazilian population in 2008. To evaluate an association between tobagism and cutaneous aging, the investigators controlled other variables including solar exposure, age, skin phototype, sex, sunscreen use, alcohol consumption, coffee consumption, sports participation, body mass index, and history of relatives with precocious aging. Analysis revealed that age, chronic sun exposure, skin phototype, and tobacco load significantly contributed to the formation of facial wrinkles. Moreover, the larger the tobacco load, the larger the amount of facial skin wrinkling, with an odds ratio of 3.92 in heavy smokers (40 packs/year) in relation to nonsmokers. Moreover, heavy smokers (40 packs/year) who use larger amount of tobacco ended up with a higher number of wrinkles (odds ratio of 3.92) [24].

Asakura et al. [14] in another cross-sectional study attempted to characterize the condition of the skin (texture, hyperpigmentation, pores, and wrinkling) in a population of Japanese elders. They also tried to identify lifestyle factors associated to the mentioned visible signs of aging. Their findings support that after adjusting for age, smoking status and topical sun protection were significantly associated with signs of visible skin aging among both genders.

Twin studies are a special opportunity to minimize the effect of genetics. A voluntary cohort study, done on 65 twin pairs (130 individuals), has investigated the factors that affect skin aging. Photodamage scores among pairs of twins, whether MZ or DZ, were highly correlated ($p = 0.92$). Differences of photodamage between or among the level of other factors using the Kruskal–Wallis test found significant associations for quantity of cigarettes smoked ($p < 0.12$) [25]. Another study evaluated a unique twin pair who spent their first two decades of life together. They also had the same type of job at the same latitude, resulting in well-matched levels of significant sun exposure. But, their smoking history was absolutely different. The twin with 52.5 packs/year smoking history showed significant skin aging than the nonsmoking twin [26].

MOLECULAR EFFECT OF NICOTINE ON THE SKIN

The pathogenesis of smoking-associated skin aging is multifactorial [27]. The evidence suggests that smoking 20 cigarettes per day was equivalent in effect to almost 10 years of chronological aging [4,28].

Tobacco smoke is a mixture of at least 3800 compounds. The effect of all of them on connective tissue is not clear, but among them, nicotine has been investigated [29–31].

Acetylcholine has two different receptors: nicotinic (nAChR) and muscarinic (mAChR). Both of these receptors are presented in the skin. nAChR is a 290 kDa protein that consisted of a ring of five subunits. The function of the molecule depends on subunit composition. Nicotine works as an agonist of acetylcholine at almost all nAChR types [31].

Nicotine Effects on Keratinocytes

Keratinocytes can synthesize, secrete, and degrade acetylcholine [32]. A fresh mature keratinocyte from human

neonatal foreskin has about 35,400 binding sites for nAChR. Nicotine has different effects on keratinocytes, but it mainly enhances cell to cell adhesion and therefore has an inhibitory effect on keratinocyte migration. This mechanism can explain poor wound healing in smokers. Moreover, it is involved in keratinocyte differentiation by inducing Ca influx [33–35]. Acetylcholine through nicotinic channels can control the terminal stage of keratinocyte differentiation in the epidermis. This effect is mediated by expression of different genes and calcium influx and can result in squamatization [36]. Nicotine can also enhance apoptosis of keratinocytes. It is known that both cholinergic nicotinic agonists and muscarinic antagonists are necessary for this action [37].

Nicotine Effect on Fibroblasts

Specific nAChRs have been detected in human skin fibroblasts including α3, α5, α7, β2, and β4 nAChR. Nicotine causes amplification in cell-cycle regulators p21, cyclin D1, and Ki-67 and apoptosis regulators like Bcl-2 and caspase 3. It also increases gene expression of dermal matrix proteins collagen, metalloproteinase, and elastin. In α3 knockout mice, there are changes in fibroblast growth and function that are opposite to those observed in fibroblast treated with nicotine [38]. The skin of α7 knockout mice shows fewer amounts of the extracellular matrix proteins collagen 1 and elastin as well as metalloproteinase-1 [36]. Therefore, nicotine-induced alterations in physiologic control of the unfolding of the genetically determined program of growth and tissue-remodeling function of fibroblasts as well as alteration in the structure and function of fibroblast nAChRs could be responsible for the effect of tobacco products on extracellular matrix turnover in the skin [31].

Nicotine Effect on Blood Vessels

Smoking even one cigarette can cause vasoconstriction and decrease in blood flow of skin in anyone. Black et al. [39] showed that acute exposure of human skin vasculature to nicotine affects endothelial function by amplification of norepinephrine-induced skin vasoconstriction and impairment of endothelium-dependent skin vasorelaxation. But, habitual smokers tend to have a longer recovering phase, which may be evidence that microcirculation gets accustomed to smoke [40].

Nicotine Effect on Wound Healing

Nicotine has different roles in tissue remodeling and wound healing, but it mainly affects fibroblast function. As a vasoconstrictor agent, it can reduce the blood flow to the skin. Decrease in essential nutritional factors and ischemia result in delayed wound healing. Nicotine also increases platelet adhesiveness, which also adds to tissue ischemia. Moreover, nicotine decreases fibroblasts, red blood cells, and macrophage proliferation. In addition, it interferes with the second phase of wound healing by inhibiting keratinocyte migration and proliferation [35,41,42]. Jacobi et al. [43] have argued that nicotine could accelerate the wound healing through endothelial nAChRs and activation of angiogenesis.

Different studies indicate that cigarette smoking is closely associated with delayed wound healing. The probable underlying mechanism is nicotine interference with fibroblasts, red blood cells, and macrophage proliferation. Therefore, proper delivery of necessary substances to the healing area will be interrupted. Besides, in the presence of nicotine, platelet adhesiveness and blood viscosity increase and prostacyclin production decreases. These effects can cause clot production, hypoperfusion, and ischemia of the tissue. In addition, nicotine interferes with the second phase of wound healing by inhibiting keratinocyte migration and proliferation [41,44].

On the other hand, Jacobi et al. [43] argued that nicotine could accelerate wound healing in genetically diabetic mice through endothelial nAChRs and activation of angiogenesis.

CHANGES OF OXYGEN CONTENT AND TEMPERATURE BEFORE AND AFTER SMOKING

In a recent study, periorbital and periolar and oxygen content was measured before and after 30 minutes of smoking. The findings showed that there is a considerable increase in temperature after smoking. The oxyhemoglobin and partial pressure of oxygen decreased in both areas, but no changes in deoxyhemoglobin and partial pressure of carbon dioxide were detected. They believed that this mechanism could be an underlying cause of premature skin aging.

Carbon monoxide (CO) is one of the toxic gases of cigarette smoke. It quickly enters bloodstream and binds with hemoglobin to make carboxyhemoglobin (CoHb), which reduces oxygen supply to tissues [45].

NO is one of the thousands of chemicals released when a cigarette is lit. Exogenous NO can increase arterial stiffness, which might decrease blood flow [46].

Absorbed chemicals from the cigarette smoke can turn to reactive oxygen species (ROS) in the skin tissue. Excessive amount of ROS will interfere with enzymatic/gene pathway and cause multiple pathological disorders [47].

MOLECULAR BASIS OF TOBACCO-INDUCED SKIN AGING

Tobacco extract can directly impair collagen biosynthesis. Moreover, it can also cause collagen degradation through induction of matrix metalloproteinases (MMPs). Yin et al. [30] demonstrated that production of collagen precursors procollagen types I and III significantly decreased in supernatants of cultured fibroblasts treated with tobacco extract. Their experiment also showed that MMP-1 and MMP-3 were induced in a dose-dependent manner. But, tissue inhibitors of metalloproteinase remained unchanged. Therefore, the balance between these two substances was disturbed in favor of MMPs.

Matrix metalloproteinase (MMP-1) is a molecule that causes collagen and elastic fiber degradation. In 2001, Morita

et al. [48] identified higher levels of MMP-1 mRNA in a buttock dermal connective tissue of smokers.

In another in vivo study, tobacco smoke extract, applied topically and intracutaneously to male hairless mice, caused collagen damage, but intraperitoneal injection had no effect [49].

Transforming growth factor (TGF-β1) is a multifunctional cytokine, which regulates cell proliferation, differentiation, tissue remodeling, and repair [31]. In epidermis, TGF-β1 acts as a growth inhibitor by maintaining tissue homeostasis, but in dermis, it works like a potent growth factor by inducing the synthesis of extracellular matrix proteins. This molecule initiates a signaling pathway through a heterometric complex of type I/II TGF-β receptors, which finally activates signal transduction pathway [50–53].

In 2003, Yin et al. [30] also showed that tobacco smoke extract could induce the nonfunctional form of TGF-β in supernatants of cultured skin fibroblasts. Because of the lack of response to this nonfunctional form, TGF-β receptors will be downregulated, and this results in decreased synthesis of extracellular matrix proteins. They suggest that this could be evidence that tobacco smoke impairs collagen metabolism.

Tobacco and Aryl Hydrocarbon Pathway

Tobacco smoke is a considerable source of polycyclic hydrocarbons (PAHs). This class of carcinogen chemicals is implicated as a major cause of lung cancer. The pathological effect of PAHs is through the activation of a transcriptional pathway consisting of the aryl hydrocarbon receptor (AhR) and the AhR nuclear translocator (Arnt). AhR and Arnt belong to the transcription factor family, and their job basically involves regulation of development, hypoxia signaling, and circadian rhythms. These kinds of proteins usually stay in an inactive complex with accessory proteins inside the cytoplasm. After attaching PAH to Ahr, some of the accessory proteins detach from the complex, and Ahr translocates to the nucleus where it will dimerize with Arnt. The Ahr/Arnt molecule is able to activate the transcription of xenobiotic-metabolizing genes, which make proteins engage in growth control, cytokines, nuclear transcription, and regulation of extracellular matrix proteolysis. Because tobacco is a major source of PAH, it is suggested that the Arh pathway might have a role in the skin aging effect of tobacco [48,54–60].

The PAH 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and all trans retinoic acid both trigger Ahr pathway and increase MMP-1 in normal human keratinocytes [61]. Basically, MMP intensity is regulated through the level of gene activity. Therefore, to activate the transcriptional pathway, TCDD requires two activators protein-1 in the proximal promoter of the MMP gene. However, in vitro studies show that TCDD-induced MMP-1 expression in metastatic melanoma cells could increase MMP-1 activation and expression through sequences in the distal promoter region but without protein-1 requirement.

Participation of Ahr in tobacco smoke skin aging has been studied. Exposing human skin fibroblasts to hexane-soluble tobacco smoke extract causes MMP-1 mRNA induction significantly in addition to upregulation of the AhR-dependent gene, cytochrome P1B1 (CYP1B1) expression. Meanwhile, Ahr knockdown eliminates the increased transcription of CYP1A1/CYP1B1, triggered by the extract [24,48,62].

Tobacco smoke condensate contains more than 1000 different compounds; some of them are known to be phototoxic. Therefore, phototoxicity may be another possible mechanism by which tobacco smoke causes premature skin aging. Placzek et al. [61] evaluated a theory that the effect of UV radiation and cigarette smoke exposures on premature aging could be linked by phototoxic action of cigarette smoke. In a photohemolysis test, human erythrocytes were incubated with cigarette smoke condensate, followed by UV irradiation and measurement of exposure-dependent hemolysis. The result revealed that tobacco smoke has phototoxic properties elicited by radiation rich in both UVA and UVB. Hemolysis induced by UVA-rich radiation reached 100%, while radiation rich in UVB caused hemolysis up to 23% [61].

CONCLUSION

Cutaneous aging is a continuous process. Intrinsic aging is an inevitable alteration due to physiological changes in all body organs and systems [24]. Between environmental or extrinsic factors, tobacco smoking has been studied by several authors. The results of these investigations support the strong association of smoking with premature skin aging, developing wrinkles, marked alteration of skin structures, and composition of the epidermis [51].

The effect of tobacco smoking and premature skin aging provides a potential source of educational information to decrease the rate of smoking especially among youth and to encourage people who are more concerned about their appearance to stop smoking. Dermatologists can be very active in smoking prevention and cessation campaigns with counseling patients and providing them with information about the increased risk of facial wrinkling associated with cigarette smoking.

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22 Antioxidants

Claude Saliou, Stefan U. Weber, John K. Lodge, and Lester Packer

INTRODUCTION

Several decades after their introduction in the field of dermatology, antioxidants are still and more than ever a widely used class of ingredients in both topical applications and oral supplements for skin health. This chapter is intended to provide an overview of the current state of research on the use of antioxidants in cosmeceutical applications and will provide an overview of the different classes of antioxidants commonly used and the body of evidence supporting their use.

The skin is continuously exposed to both exogenous and endogenous oxidative conditions. Exogenous oxidative conditions arise from environmental pollutants such as smoke, smog, and solar ultraviolet (UV) radiations. Endogenous sources are a consequence of the body metabolic activity and diet. For example, mitochondrial respiration produces superoxide and hydrogen peroxide, while enzymes such as lipoxygenase, xanthine oxidase, and NADPH oxidase produce hydroperoxides and superoxide, respectively. In normal conditions, these oxidants are maintained in balance with a number of systemic antioxidants, whose functions are to scavenge reactive oxygen species preventing damage to macromolecules such as lipids, DNA, and proteins. The overall antioxidant protection is composed of both endogenous molecules, synthesized as part of metabolism, for example, GSH and uric acid, and essential vitamins that must be brought from the diet, for example, vitamins E and C. Enzymes, which decompose specific reactive oxygen species, for example, superoxide dismutase (against superoxide), catalase (against hydrogen peroxide), and the glutathione and thioredoxin peroxidases (against peroxides), further complement the endogenous antioxidant protection system. These various antioxidant systems provide protection in various intracellular and intercellular compartments. While a certain level of specific oxidants is required for cellular function, for example, nitric oxide and hydrogen peroxide playing a role in cell signaling [1], usually there is a tight balance between oxidants produced and antioxidant scavenging; however, under certain physiological or pathological conditions, the balance can be tipped in favor of the oxidants, a condition called oxidative stress. Potentially, oxidative stress can be caused either by an increase in the number of oxidants, for example, as a result of cigarette smoking or UV irradiation, or by a deficiency in one or more important antioxidants. This is of major concern since oxidative stress has been implicated in a number of skin conditions including skin cancer, photoaging and intrinsic aging, skin inflammation, and vitiligo [2].

Here, the most important classes of antioxidants, namely, vitamin E, vitamin C, thiols, coenzyme Q, and phenolics, will be introduced. Their intriguing cooperation as well as their role in signal transduction events will be discussed in view of their applications in skin health.

VITAMIN E

Vitamin E is the major lipophilic antioxidant in skin, and it is the most commonly used natural antioxidant in topical formulations. It is found in all layers of the skin, the dermis and epidermis, as well as in the stratum corneum (SC), and is believed to play an essential role in the protection of biomembranes against peroxidation, consequently stabilizing them [3].

Chemistry

Vitamin E is a family of eight naturally occurring isoforms: four tocopherols (α-, β-, γ-, δ-form) and four tocotrienols (α-, β-, γ-, δ-form) [4] (Figure 22.1). All forms consist of a chromanol nucleus that carries the redox-active phenolic hydroxyl group and a lipophilic phytyl tail. While tocopherols contain a fully saturated phytyl side chain with three chiral centers, the isoprenoid tail of the tocotrienols is polyunsaturated, making their side chain more rigid. The side chain is anchored in lipid membranes, while the nucleus is located at the lipid/aqueous interface.

In addition to the natural forms of tocopherols, many other nonnatural occurring derivatives of vitamin E are commonly used in dermatological and cosmetic applications, for instance, α-tocopherol esters such as α-tocopheryl acetate, α-tocopheryl phosphate, α-tocopheryl succinate, and ascorbate tocopheryl phosphate and ethers such as α-tocopherol ether acetic acid. Esters offer a greater stability in formulation. For this reason, α-tocopheryl acetate is the most commonly used vitamin E in cosmetic products [5].

Skin Occurrence and Status

Even though the radical scavenging activity of the different isoforms is essentially identical, their biological activity after oral administration differs dramatically [4]. This phenomenon can be explained by the existence of a liver α-tocopherol transfer protein that selectively incorporates RRR-α-tocopherol into VLDL, which leads to recirculation of the α-tocopherol pool, while this transfer protein does not recognize the other forms, which are therefore excreted

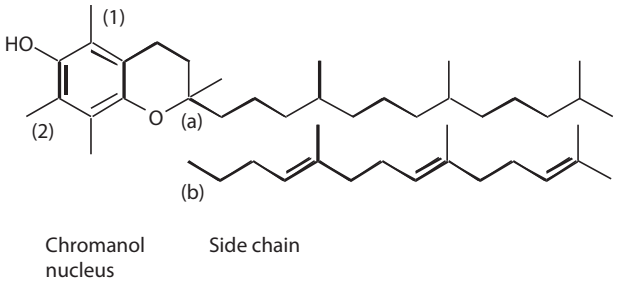

FIGURE 22.1 Naturally occurring forms of vitamin E. Tocopherols contain a saturated side chain (a), while the isoprenoid side chain of tocotrienols is polyunsaturated (b). The α-forms contain both methyl groups on the chromanol nucleus (1, 2), while the β-forms contain only methyl group (1), the γ-forms only (2), and the δ-forms none.

more rapidly [6]. Consequently, human plasma levels of α-tocopherol are higher than those of γ-tocopherol (20.5–22.9 μM vs. 1.74–3.24 μM, respectively) [7,8]. However, it is worth noting that γ-tocopherol is the most abundant vitamin E component of human diets in the United States [9], given its predominance in commonly consumed plant oils such as corn, soybean, and sesame [10].

In skin, as in the other human organs, α-tocopherol is the predominant form of vitamin E with 5–10 times higher concentrations than γ-tocopherol. Delivery of vitamin E to the SC occurs in two different modes. On the one hand, it is present in the membranes of differentiating keratinocytes and consequently moves up into the newly formed SC, which leads to a gradient-type distribution of α-tocopherol with decreasing concentrations toward the skin surface [11]. On the other hand, vitamin E is secreted by sebaceous glands and reaches the SC from the outside. After oral supplementation, vitamin E reaches the skin via sebaceous glands after a period of at least 2–3 weeks [12]. These findings give an additional argument that the sebaceous gland route is a significant delivery mechanism for vitamin E.

In sebaceous gland-rich regions like the face, this delivery mechanism is responsible for the enrichment of the outer SC with vitamin E [13]. Sebaceous gland activity is low in children and starts to increase during puberty to reach a plateau at the age of 19 years and remains relatively constant until it starts to decline in women starting in the cohort of 50–59 years of age and in men starting at 70 years of age [14]. Thus, children as well as women above 50 years and men above 70 years may have a comprised mechanism of vitamin E delivery to the skin surface. These populations may potentially benefit from topical vitamin E supplementation.

Various oxidative stressors, such as UV, ozone, or cigarette smoke, have been shown to deplete vitamin E, among other antioxidants. In the epidermis, a dose of at least four minimal erythemal doses (MEDs) of solar simulated UV radiation (SSUV) is needed to deplete α-tocopherol by about 70% [15], while doses as low as 0.75 MED diminish α-tocopherol in the human SC by 85% [11]. In contrast, γ-tocopherol is comparatively more resistant and requires 9 MEDs to reduce its SC concentration by 66% [11]. Mouse experiments have shown that a dose of 1 ppm × 2 h of ozone (O_3) depletes SC vitamin E [16]. Since this concentration of O_3 is higher than the naturally occurring levels of tropospheric O_3, the biological relevance of these findings for the skin of humans is not yet clear. Benzoyl peroxide is used for the treatment of acne. A single application of a 10% w/vol formulation of benzoyl peroxide almost completely depletes stratum corneum vitamin E in vivo [17].

Skin Applications and Benefits

α-Tocopherol is widely used as a cosmetic active ingredient in topical formulations. After topical application, it penetrates readily into skin [18]. Since the free form of vitamin E is quite unstable and light-sensitive (it absorbs in the UV-B range to yield a α-tocopheroxyl radical [19]), the active hydroxyl group is usually protected by esterification with acetate. This increases the stability but renders the compound redox inactive. If administered orally, vitamin E acetate is readily and quantitatively hydrolyzed in the intestines. There is, however, some controversy as to whether α-tocopherol acetate can be hydrolyzed in human skin. Chronic application of α-tocopherol acetate leads to an increase in free vitamin E in skin of both the rat [20] and the mouse [21], where it was recently shown that UV-B increases the hydrolysis of α-tocopherol acetate by induction of nonspecific esterases up to 10–30 folds [22]. While one study suggested that bioconversion of α-tocopherol acetate does not occur in human skin [23], significant hydrolysis was demonstrated in recent studies using a human epidermis–tissue culture model and in vivo [24]. A 0.15% formulation of vitamin E acetate increased the stratum corneum α-tocopherol content by far more than oral supplementation with 400 IU α-tocopherol could achieve [25]. A rinse-off application of vitamin E was also able to increase vitamin E [25,26].

The availability of the free form of tocopherols needs to be considered when analyzing their possible health benefits. The majority of studies have been carried out in animal models, while only limited data exist for human studies. Lipid peroxidation is inhibited after topical application of α-tocopherol [11]. Several studies indicate that topically applied α-tocopherol (more so than γ- and δ-tocopherol) inhibits UVB-induced DNA damage in a mouse model [27,28] and in keratinocyte cultures (with trolox, water-soluble tocopherol derivative) [29]. Protection against Langerhans cell depletion and immunosuppression by UV light was observed after topical application of α-tocopherol in a mouse model [30,31]. Several studies have demonstrated the potential of vitamin E in protecting against UV-induced skin carcinogenesis [32] and skin erythema or edema [33]. α-Tocopherol and its sorbate ester were studied in a mouse model of skin aging. Both antioxidants were found to be effective; sorbate even more so than α-tocopherol [34]. Systemic administration of vitamin E in humans (only in combination with vitamin C) increased the MED and reduced changes in skin blood flow after UV irradiation [35,36]. A rinse-off application of vitamin E was able to increase the vitamin E content in the barrier lipids. It

was also able to decrease the formation of squalene monohydroperoxide from squalene by low-dose UVA (8 J/cm^2) [26].

Yet several studies indicate that α-tocopherol acetate is not as effective as free vitamin E when applied topically. Inhibition of DNA mutation in mice was 5–10 times less effective [29]. Also, in a mouse model, unlike free vitamin E, the acetate form seemed to be ineffective [30]. In summary, even though some skin health benefits of vitamin E supplementation have been shown, there is still a need for controlled studies in humans under physiological conditions. So far, vitamin E was found to have anticarcinogenic, photoprotective, skin-stabilizing properties. This topic is reviewed in detail by Thiele et al. [37].

Dietary supplements for skin benefits have gain interests over the past decade. Some supporting evidence came from a couple of independent clinical studies (both were placebo-controlled) showing that a combination of vitamins E (670 mg–2 g/day) and C (2–3 g of vitamin C per day) increased the UV-induced erythema threshold and prevented sunburn [35,36]. Although effective and significant in protecting skin against photodamage, such dietary supplementation was not effective enough to replace the use of sunscreens.

In topical applications, the all-racemate mixture of α-tocopherol (also called *dl*-α-tocopherol) is most often used. Mixed tocopherols, containing a natural blend of tocopherols (predominantly RRR-γtocopherol, followed by RRR-α-tocopherol), are also available. See Table 22.1 for a list of other derivatives of vitamin E commonly found in cosmetics.

Recently, the tocotrienol forms of vitamin E have become a focus of interest, since they have been found to be more efficient antioxidants in some model systems than tocopherols [38]. Even if they are not bioavailable after oral supplementation, topical application circumvents the exclusion by α-TTP in the liver. In fact, free tocotrienols readily penetrate into mouse skin [18], and tocotrienyl acetate is hydrolyzed in skin homogenates and in murine skin in vivo [26]. Topical application of a tocotrienol-rich fraction has been demonstrated to protect mouse skin from UV- and O_3-induced oxidative stress [39,40].

TABLE 22.1
Illustrative List of Vitamin E or Vitamin E-Derived Ingredients Used in Cosmetics

Vitamin E or Derivatives (INCI Names)	Description
Tocopherol	Racemate version of α-tocopherol (*dl*-α-tocopherol), natural mixed tocopherols, etc.
Tocopheryl acetate	Ester with acetic acid
Tocopheryl linoleate	Ester with linoleic acid
Tocopheryl succinate	Ester with succinic acid
Tocopheryl phosphate	Ester with phosphoric acid
Ascorbyl tocopheryl acetate	Ester with acetic acid and ascorbate
Tocotrienols	Mixture of tocotrienol isomers

Benzoyl peroxide is used for the treatment of acne. During seven daily applications of a 10% w/vol formulation of benzoyl peroxide, endogenous SC vitamin E was progressively depleted. α-Tocotrienol (5% w/vol) supplementation for 7 days was able to significantly retain vitamin E in the stratum corneum. α-Tocotrienol supplementation significantly mitigated the BPO-induced lipid peroxidation. The transepidermal water loss was increased 1.9-fold by seven BPO applications, while there was no difference between α-tocotrienol treatment and controls [17]. In conclusion, tocotrienols bear a potential that yet remains to be explored.

The safety of vitamin E oral supplementation is still a topic of current discourse. However, α-tocopherol and other derivatives topically applied to the skin have been considered safe as used [5].

VITAMIN C

Chemistry

Ascorbic acid or vitamin C is one of the most important water-soluble antioxidants and present in high amounts in the skin. While most species are able to produce ascorbic acid, humans lack the enzymes necessary for its synthesis. Deficiency in ascorbic acid causes scurvy, a disease already described in the ancient writings of the Greeks [41]. Apart from the pure antioxidant function, ascorbic acid is an essential cofactor for different enzymes. The antioxidant capacity of vitamin C is related to its unique structure (Figure 22.2). Due to its pKa1 of 4.25, it is present as a monoanion at physiological pH, which can undergo a one-electron donation to form the ascorbyl radical with a delocalized electron and can be further oxidized to result in dehydroascorbic acid. Dehydroascorbic acid is relatively unstable and breaks down if it is not regenerated (see antioxidant network [42]—Figure 22.5). In vitro ascorbic acid can scavenge many types of radicals including the hydroxyl- ($^{\lambda}$OH), the superoxide- ($O_2^{-\lambda}$), and water-soluble peroxyl- (ROO$^{\lambda}$) radicals as well as other reactive oxygen species such as O_3, and quenches singlet oxygen ($^1O_2$). Due to their relative reduction potentials, ascorbate can reduce Fe(III) to Fe(II), which in turn can decompose hydrogen peroxide (H_2O_2) to the dangerous hydroxyl radical. Therefore, vitamin C can exert pro-oxidant effects in the presence of unbound iron (Fenton chemistry). In topical formulation, ascorbic acid is very unstable. The same reactions as described above occur. Light can generate fatty acyl peroxyl radicals, which will consume the ascorbic acid. Likewise, the presence of metals (iron or copper), even in trace amounts,

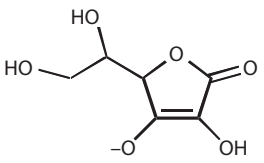

FIGURE 22.2 Structural formula of vitamin C as the monoanion ascorbate.

has the ability to oxidize ascorbic acid, rendering it to be the least useless. Formulators need to use particular care in their choice of ingredients, the process employed, and the packaging to avoid an early degradation of ascorbic acid.

Skin Occurrence and Status

In the skin, vitamin C is found in all layers. While vitamin E was found in relatively low abundance in the skin, total ascorbate (ascorbic acid and dehydroascorbic acid) levels in the epidermis and dermis are very high (7.6 and 1.3 μmol/g of tissue, respectively) [43]. In both epidermis and dermis, the concentrations of ascorbic acid and dehydroascorbic acid are roughly equal. This is distinct from the plasma, where the concentration of ascorbic acid is usually much greater than the dehydroascorbic one, perhaps altogether suggesting a greater oxidative challenge in the skin. Similar to vitamin E, vitamin C is detected in the stratum corneum, forming a gradient with decreasing concentrations from the deeper stratum corneum to the surface [44]. Vitamin C is rapidly depleted in the skin upon exposure to O_3 [44] and UV radiation [15].

Skin Applications

One of the earliest discoveries of vitamin C benefits in the skin was the observation that it stimulates collagen synthesis in dermal fibroblasts [45]. Vitamin C is not only a cofactor for lysyl and prolyl hydroxylases [46] but also stimulates type I and III collagen gene expression [47]. Also, vitamin C is essential in the formation of competent barrier lipids, including ceramides [48], in reconstructed human epidermis [49]. Vitamin C is particularly effective at regulating melanogenesis in vitro and in vivo [50,51]. Ascorbic acid and a few other derivatives (see Table 22.2) have gained "quasi-drug" status in Japan [52]. In contrast to many skin lighteners that work by inhibiting the tyrosinase enzyme activity, ascorbic acid appears to inhibit the oxidative conversation of DOPA into DOPA quinone, a reaction also mediated by tyrosinase [52,53]. 3-O-ethyl ascorbic acid, an ethyl ether derivative of ascorbic acid registered as a quasi-drug in Japan, works by preventing the polymerization of melanin monomers (i.e., 5,6-dihydroxyindole-2-carboxylic acid [DHICA] and its derivatives) [54,55] in UVA-induced immediate pigment darkening.

Several studies have investigated protective effects of vitamin C against oxidative stress. UV-B-induced immunosuppression, as a marker of damage to the immune system, was abrogated by topical application of vitamin C to murine skin [50,56]. UV-B-induced sunburn cell formation was mitigated by vitamin C in porcine skin [57]. While one study reported a protective effect for an intraperitoneal administration of vitamin C phosphate against UV-induced damage in mice [58], another study found no such effect in human skin [59]. However, in a 6-month double-blinded and randomized clinical study, topically applied ascorbic acid (5%) provided improvements against signs of photoaging [60]. Dietary supplementation with high doses of vitamin C in combination with vitamin E protected against UV-induced erythema in humans, raising the erythema threshold, while vitamin C alone did not [35,36]. Another study described protection against erythema, sunburn cell formation, and thymidine dimer formation in pigs by a combination of vitamin E (1%) and vitamin C (15%) [61]. In a keratinocyte cell culture system, vitamin C reduced UVB-induced DNA damage [29]. In mice, an anticarcinogenic effect of vitamin C was described [62]. However, no data regarding such benefits exist in humans. Long-term studies are needed to assess this benefit.

Since vitamin C is not very stable, it is difficult to incorporate it into topical formulations. Esterification with phosphoric acid or glycosylation is used to circumvent this limitation. The main vitamin C derivatives are listed in Table 22.2. In vitro experiments demonstrated that magnesium-ascorbyl-2-phosphate penetrates the murine skin barrier and is bioconverted into free ascorbate [63].

TABLE 22.2
Illustrative List of Vitamin C or Vitamin C-Derived Ingredients Used in Cosmetics

Vitamin C or Derivatives (INCI Names)	Description
Ascorbic acid	Free acid form
Ascorbyl glucoside	Glycosylated form of ascorbic acid—quasi-drug status in Japan
Magnesium ascorbyl phosphate	One of the most commonly used derivatives of vitamin C due to its stability in formulation—quasi-drug status in Japan
Sodium ascorbyl phosphate	Commonly used—quasi-drug status in Japan
Sodium ascorbate	Sodium salt of ascorbic acid
Ascorbyl palmitate	Ester with palmitic acid (to increase partitioning in oil phase)
Ascorbyl linoleate	Ester with linoleic acid (to increase partitioning in oil phase)
3-O-ethyl ascorbic acid	Quasi-drug status in Japan

THIOL ANTIOXIDANTS

The thiol class of organosulfur antioxidants shares an oxidizable sulfydryl (SH) group.

Glutathione (GSH) is a tripeptide (Figure 22.3a) whose SH group at the cysteine can be oxidized, forming a disulfide (GSSG) with another GSH. Physiologically in the skin, more than 90% of the GSH is in the reduced form. Similarly to vitamins C and E, human epidermis has a higher concentration of glutathione than the dermis (461 and 75 nmol/g of tissue, respectively) [43], which is an indication of the metabolic nature of the epidermis. Glutathione peroxidases use GSH as a substrate to reduce H_2O_2 and other water-soluble peroxides. The synthesis of GSH by the human cell is stimulated by N-acetyl-cysteine (NAC), which is hydrolyzed to cysteine intracellularly. Moreover, NAC acts as an antioxidant itself.

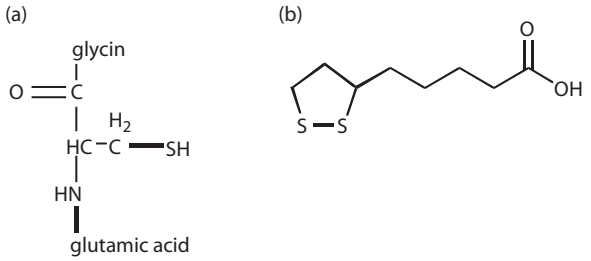

FIGURE 22.3 Chemical structures of thiols: (a) GSH consisting of glycin, cysteine, and glutamic acid; (b) lipoic acid as in its oxidized form as a disulfide.

For oral as well as topical application in mouse models, GSH-ethylesters and GSH-isopropylesters proved to be more efficient than free GSH. Oral supplementation decreased the formation of UV-induced tumors [63] and the formation of sunburn cells [64]. Topical treatment partially inhibited UV-induced immunosuppression [65]. NAC was able to reduce UVA-induced DNA damage in fibroblasts [66] and protected mice against UVB-induced immunosuppression after topical application [65] in a mode that did not involve de novo GSH synthesis [67].

Lipoic acid (1,2-dithiolane-3-pentanonic acid or thioctic acid [LA]) is a cofactor of multienzyme complexes in the decarboxylation of α-keto acids. Applied as the oxidized dithiol dehydrolipoic acid (DHLA), it is taken up by cells and is reduced by mitochondrial and cytosolic enzymes (NAD(P)H dependent). It thereby forms an efficient cycle, since it can in turn regenerate GSSG to GSH and stimulate the GSH synthesis by improving cysteine utilization [68]. Lipoic acid was demonstrated to penetrate into mouse skin [69], while oral supplementation of lipoic acid has actually been shown to have an anti-inflammatory effect in mice [70] and to prevent symptoms of vitamin E deficiency in vitamin E-deficient mice [71]. Both lipoic acid and NAC have been shown to inhibit UV-induced nuclear factor kappa B (NF-kB) [72], a central regulator of inflammation as well as skin photoaging [73].

Although not a thiol, sulforaphane, an isothiocyanate found in cruciferous vegetables such as broccoli and cabbage, protects murine skin against UV-induced inflammation and carcinogenesis [74].

Despite the benefits of thiols in skin, their use in skin applications has been somewhat limited due to their distinctive odor, limited cutaneous delivery, and poor solubility.

UBIQUINOLS AND DERIVATIVES

Ubiquinols and ubiquinones are lipophilic electron carriers used in the electron transport chain in the mitochondria membrane and resulting in ATP synthesis. Ubiquinols are also potent membrane antioxidants [75], capable of regenerating vitamin E.

Recently, idebenone, a ubiquinone derivative, was found to improve photoaging parameters, that is, reduced wrinkle score and overall photodamage appearance [76].

PHENOLICS

Phenolics are ubiquitous phytochemicals, also called secondary metabolites, usually utilized by plants to protect themselves from or respond to external aggressions. Phenolic acids, catechols, and resorcinols are the most common single-phenol-ring phenolics. Flavonoids (Figure 22.4), stilbenoids, chalcones, and tannins are the main polyphenols. Flavonoids are widely distributed plant pigments and tannins occurring in barks, roots, leaves, flowers, and fruits. Their roles in plants include photoprotection, pest defense, and contribution to the plant color. Our diet contains flavonoids, which can be found in a variety of foods from green vegetables to red wine.

In spite of the fact that flavonoids have been used in traditional medicine for several centuries, it was not until 1936 that their first biological activity, the vitamin C sparing action [77]. As a result, they received the name "vitamin P." Flavonoids, also referred to as plant polyphenols, have been recognized as potent antioxidants. Their free radical-scavenging and metal-chelating activities have been extensively studied. Nonetheless, given their polyphenolic structure (Figure 22.4), the electron- and hydrogen-donating abilities constitute the major feature of their antioxidant properties [78]. By opposition to the antioxidants previously described, flavonoids are not part of the endogenous antioxidant system but still interact with it through the antioxidant network (see below).

Among the applications found in traditional medicine, flavonoids account for anti-inflammatory, antiphlogistic, and wound healing functions. Their effect on skin inflammation has been thought, for a long time, to be limited to the inhibition of the activity of 5-lipoxygenase and cyclooxygenase. However, recent studies suggest a subtler mode of regulation of the inflammatory reaction by flavonoids. In fact, flavonoids such as silymarin, quercetin, genistein, and apigenin are effective inhibitors of NF-κB, a proinflammatory transcription factor, thereby reducing the transcription of proinflammatory genes and preventing inflammation [72,79,80].

Oral supplementation and topical application of green and black tea polyphenols show beneficial effects against UVR-induced skin carcinogenesis in mice [32,81,82]. In addition, these flavonoids as well as silymarin were found to prevent UVR-induced inflammation, ornithine

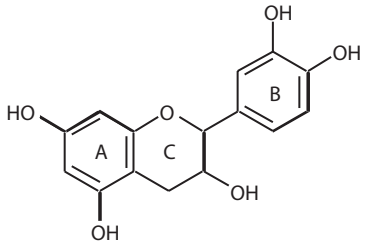

FIGURE 22.4 Chemical structure of catechin, a flavane, as an example of a flavonoid. Flavanes share a common base structure (rings A, B, C, one), which is hydroxylated in different patterns.

decarboxylase expression, and activity [83]; all of these events being potential contributors to carcinogenesis [84].

Procyanidins, also named condensed tannins, are flavonoids found in pine bark (Pycnogenol), grape seeds, and fruits for instance. By direct protein interaction, they were shown to protect collagen and elastin, two dermal matrix proteins, against their degradation [85]. Furthermore, some of these procyanidins exhibit a remarkable effect on follicle hair proliferation [86], thus extending the therapeutic applications of flavonoids to alopecia. Last, procyanidins from pine bark taken orally by human subjects were found to confer skin protection against UV, via inhibition of the NF-κB pathway [87]. Although the flavonoids are not part of our endogenous antioxidant defenses, they display a broad spectrum of properties particularly helpful in preventing UVR-caused deleterious effects in human skin.

Many other polyphenols have demonstrated benefits to the skin. These include green tea catechins [88], resveratrol, oat avenanthramides [89], and licorice chalcones (e.g., licochalcone) [90], all used to soothe irritated skin and prevent UV damage.

ANTIOXIDANT NETWORK

When antioxidants react with an oxidant, they are converted to a form that no longer functions as an antioxidant; it is said to be consumed. In order for the oxidized product to function again, it needs to be recycled to its native form. The antioxidant network describes the ability of the antioxidants to recycle and regenerate oxidized forms of each other, thereby providing extra levels of protection (Figure 22.5). Thus, the process is synergistic; the net antioxidant protection is always greater than the sum of the individual effects.

The major systemic antioxidants vitamin E, vitamin C, and glutathione are present in different cellular compartments, and all have the ability to interact with one another. Typically, the radicals formed on the antioxidants are more stable and longer-lived than the damaging radicals produced in vivo, which is mostly due to a delocalization of the unpaired electron. Thus, they have more chance to interact with each other and be reduced than to react with macromolecules. Vitamin E is the major chain-breaking antioxidant, protecting biological membranes from lipid peroxidation, which is a difficult task considering that the ratio of phospholipid molecules to vitamin E is ~1500:1. However, vitamin E is never depleted because it is constantly being recycled. When vitamin E becomes oxidized, a radical on vitamin E is formed (chromanoxyl radical). In the absence of networking antioxidants, this radical can either become pro-oxidant by abstracting hydrogen from lipids or react to form nonradical products (consumed). However, a number of antioxidants are known to be able to reduce the chromanoxyl radical and regenerate vitamin E [91]. These include vitamin C [42], ubiquinol, and glutathione (GSH) [92]. Vitamin C, the most abundant plasma antioxidant and first line of defense, can reduce the tocopheroxyl radical, forming the ascorbyl radical. Interactions between vitamins E and C have been demonstrated in various systems both in vivo (reviewed in reference [93]) and in vitro (reviewed in reference [94]). The ascorbyl radical is practically inert and oxidizes further to form dihydroascorbic acid. This can be reduced back to native vitamin C by GSH. This process is known to occur both chemically [95] and enzymatically [96] in both erythrocytes [97] and neutrophils induced by bacteria [98]; the latter may relate to a host defense mechanism. Glutathione is the major intracellular antioxidant. Oxidized GSSG is constantly recycled to GSH enzymatically by glutathione reductase, thus providing a constant pool of GSH. Glutathione recycling relies on NAD(P)H as the electron donor. Thus, metabolic pathways involved in energy production provide the ultimate electron donors for the antioxidant network. It is also known that GSH can directly recycle vitamin E [92,99], as well as ubiquinol [100], another lipophilic antioxidant that itself is recycled in mitochondria as part of the electron transport chain.

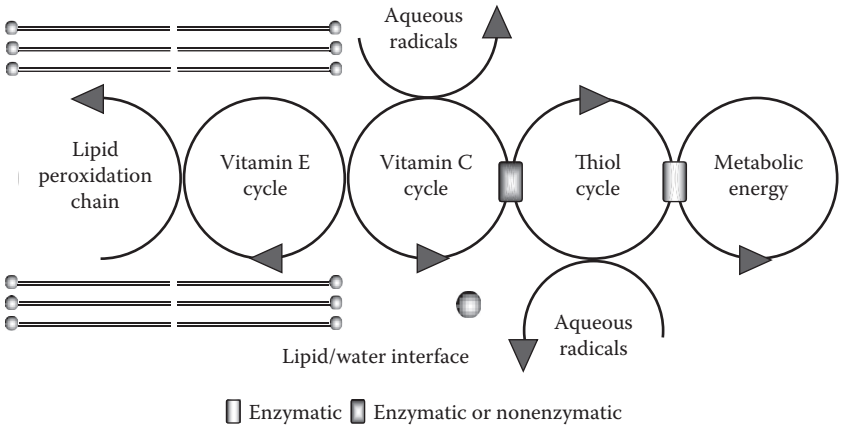

FIGURE 22.5 Schematics of the intertwined action of the antioxidant network. An ascorbate molecule can either recycle the vitamin E radical arising from breaking the lipid peroxidation chain or scavenge an aqueous radical. Glutathione can either regenerate ascorbate or scavenge a radical enzymatically. Glutathione itself then can be regenerated by the cellular metabolism.

Certain supplements are also known to contribute to the network by recycling antioxidants. Lipoic acid is a prime example since this potent antioxidant can recycle ascorbate, GSH, and ubiquinol in vitro (reviewed in reference [101]). Recently, it has been demonstrated that flavonoids may also play a networking role since they are also able to recycle the ascorbyl radical [102]. Thus, there exists a very organized defense system against free radical attack, which ultimately serves to protect and recycle antioxidants in various cellular compartments.

REGULATION OF GENE TRANSCRIPTION

The skin is the largest human organ and permanently exposed to a variety of stresses. Among these, oxidative insults such as ultraviolet radiation (UVR) and ozone exposure account for the etiology of many skin disorders. However, oxidative damage is not responsible for all biological effects engendered by these stressors in the skin. Indeed, UVR causes changes in the expression of genes encoding proinflammatory cytokines, growth factors, stress response proteins, oncoproteins, matrix metalloproteinases, etc. [103]. Although the immediate target(s) of UVR is(are) still unknown, certain kinases and transcription factors can be activated by UVR thereby increasing gene transcription [104]. One transcription factor, NF-κB, appears of particular interest for the skin since the lack of its inhibitory protein, IκBα, is associated with the development of a widespread dermatitis in knockout mice [105,106]. Furthermore, reactive oxygen species, such as those produced after UVR, are suspected to play an important role in the activation of NF-κB [107]. Consequently, antioxidants have been found to be among the most potent NF-κB inhibitors.

Vitamin E has specific effects on signaling events: Tocotrienols downregulate the 3-hydroxyl-3-methylglutaryl-coenzyme A (HMG CoA) reductase, a key enzyme of the mevalonate pathway that produces cholesterol [108]. Inhibition of side pathways of HMG-CoA-reductase is known to have shown anticarcinogenic effects. A-tocopherol is able to inhibit protein kinase C, an important factor of atherosclerosis [109]. Protein kinase C also regulates the activity of collagenase (MMP-1), an enzyme that degrades skin collagen. In cell culture models, α-tocopherol inhibits MMP-1 via inhibition of protein kinase C [110]. Recently, it was demonstrated that vitamin E inhibited the UV-induced expression of metalloelastase and thus may inhibit in the development of solar elastosis, the hallmark of sun-induced damage.

However, clinical studies are required in order to assess the effectiveness of these antioxidants, including the flavonoid silymarin, α-lipoic acid, and the glutathione precursor *N*-acetyl-*L*-cysteine, on skin inflammatory disorders. Using high-throughput procedures such as the cDNA arrays for instance [111], the evaluation of the antioxidants on the whole genome is henceforth possible. These studies will only confirm the hypothesis that antioxidants are responsible for a much broader action spectrum than their antioxidant functions per se and extend their role on more subtle regulatory mechanisms of gene expression. Such studies have already revealed interesting gene expression patterns following antioxidant supplementations that mimic an oxidative stress response (i.e., induction of antioxidant enzymes, protection mechanisms) without the oxidative damage. This suggests that antioxidants induce an adaptive response: increase the threshold of damage.

PERSPECTIVES

The general role of antioxidants in the protection against oxidative stress is now well established. In skin applications, antioxidants are a promising tool to mitigate oxidative injury. Even though a growing amount of literature deals with skin protection by antioxidants, properly controlled clinical studies are still needed to assess not only their role in the prevention of future damage but also their capacity to repair existing damage. Due to their high reactivity to counteract oxidants, antioxidants are also very unstable ingredients to use in topical formulations. Often, other antioxidants such as butylated hydroxytoluene (BHT) and metal chelators (phytic acid, lactoferrin, hyaluronic acid, citric acid, etc.) will be used to increase the stability of the antioxidant in the formula.

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23 UV Filters

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INTRODUCTION

The presence of ultraviolet (UV) filters in skin care and cosmetic products represents a key benefit that cosmetics can provide consumers. The hazards of UV light exposure are well known. The incidence of skin cancer is increasing at an alarming rate. More than 2 million people in the United States are developing over 3.5 million skin cancers every year [1]. UV-induced damage or photoaging accounts for most of the age-associated changes in skin appearance [2]. UV radiation damages the skin by both direct effects on DNA and indirectly on the skin's immune system [3].

In animal models, sunscreens prevent the formation of squamous cell carcinomas of the skin [4]. The regular use of sunscreens has been shown to reduce the number of actinic or precancerous keratoses [5] and solar elastosis [6]. Daily sunscreen use on the hands and face reduced the total incidence of squamous cell carcinoma in an Australian study [7]. The routine daily use of sunscreens may also reduce melanoma risk [8]. Double-blind photoaging studies show consistent improvement in the "untreated" control groups due in part to the use of sunscreens by all study subjects [9].

The cosmetic formulator has an expanding menu of active sunscreen ingredients for incorporation into a variety of cosmetic formulations. Selection is restricted by regulatory agencies in the country in which the final product is to be marketed. This chapter will concentrate on reviewing available UV filters.

DEFINITIONS

UV radiation (UVR) reaching the Earth's surface can be divided into UVB (290–320 nm) and UVA (320–400 nm). UVA can be further subdivided into UVA I (340–400 nm) or far UVA and UVA II (320–340 nm) or near UVA.

The sun protection factor (SPF) is defined as the dose of UVR required to produce 1 minimal erythema dose (MED) on protected skin after application of 2 mg/cm^2 of product divided by the UVR to produce 1 MED on unprotected skin. A "water-resistant" product maintains the SPF level after 40 or 80 min of water immersion. A "broad spectrum" or "full spectrum" sunscreen provides both UVB and UVA protection. Ideally, this includes both UVA I and UVA II coverage.

HISTORY

Acidified quinine sulfate was proposed for use as a chemical sunscreen in the 1890s [10]. At the beginning of the twentieth century, Unna found aesculin, a chestnut extract used in folk medicine for many years, to be more effective. Two UV filters, benzyl salicylate and benzyl cinnamate, were first incorporated into a commercially available sunscreen emulsion in the United States in 1928 [11]. In the early 1930s, phenyl salicylate (Salol) was used in an Australian product [12]. Aminobenzoic acid (PABA) was patented in 1943 leading to the development of PABA derivative UV filters. During World War II, red veterinary petrolatum (RVP) was used by the US military encouraging the development of further UV filters in the postwar period.

In the 1970s, increased interest in commercial sunscreen products led to refinements and consumer acceptance of these products over the next two decades. Facilitated by growing awareness as to the hazards of UVR, higher SPF products became the norm. Daily use consumer products containing UV filters, including moisturizers, color cosmetics, and even hair care products, have become more prevalent in the past decade. Concerns related to the adequacy of sunscreen protection for the prevention of melanoma and photoaging in the last decade has led to greater interest in broad spectrum sunscreen UV protection throughout the entire UVA range. New UV filters continue to be developed with antioxidants and UV repair stimulators being added to sunscreen products [13].

REGULATORY

United States

Sunscreen products in the United States are regulated by the FDA as over-the-counter drugs. The Final Monograph for Sunscreen Drug Products for Over-the-Counter Human Use (Federal Register 1999: 64: 27666-27693) established the conditions for safety, efficacy, and labeling of these products. A Proposed Amendment (Federal Register 2007: 72: 49070-49122) further elaborated on UVB (SPF) and UVA testing and labeling. As active ingredients in drug products, they are listed by their USAN Drug Name. The number of approved sunscreen ingredients is 16 (Table 23.1). All permitted UV filters can be used with any other permitted filters except avobenzone. The latter cannot be used with PABA, octyl dimethyl PABA, meradimate, and TiO_2. Maximum allowable concentrations are provided. Minimum concentration requirements were dropped providing that the concentration of each active ingredient was sufficient to contribute a minimum SPF of not less than 2 to a finished product. A sunscreen product must have a minimum SPF of not less than the number of

TABLE 23.1
FDA Sunscreen Final Monograph Ingredients

Drug Name	Max. Conc.%	Absorbance
Aminobenzoic acid	15	UVB
Avobenzone	3	UVAI
Cinoxate	3	UVB
Dioxybenzone	3	UVB, UVAII
	2	UVAII
Ecamsule[a]		
Ensulizole	4	UVB
Homosalate	15	UVB
Meradimate	5	UVAII
Octocrylene	10	UVB
Octinoxate	7.5	UVB
Octisalate	5	UVB
Oxybenzone	6	UVB, UVAII
Padimate O	8	UVB
Sulisobenzone	10	UVB, UVAII
Titanium dioxide	25	Physical
Trolamine salicylate	12	UVB
Zinc oxide	25	Physical

[a] Only available in patented products.

active sunscreen ingredients used in combination multiplied by 2. The FDA has also proposed that products with SPF values above 50 be limited to SPF 50+. The term "sunblock" is prohibited. The term "UVB" is to be included before the term "SPF" on the principal product display panel. Newer labeling requires the listing of UV filters not only as active ingredients but also their concentration in the product. A "water-resistant" product maintains the SPF level after 40 or 80 min of water immersion.

To address the inadequacies of UVA claims, a Final Rule (Federal Register 2011: 76:35620-35673) further elaborated on UVA testing. A "broad spectrum" sunscreen provides protection through the entire spectrum of both UVB and UVA as measured by the critical wavelength method. Using polymethacrylate plates coated with 0.75 mg/ml of sunscreen, the UV transmittance is recorded by spectrophotometer, and the absorbance curve is determined below which 90% of the total area under the UV absorbance curve resides. A broad spectrum sunscreen has a critical wavelength of equal to or greater than 370 nm.

Europe

In Europe, sunscreen products are classified as cosmetics, their function being to protect the skin from sunburn. The European Cosmetics Directive (EC No 1223/2009 30 November 2009) provides a definition and lists the UV filters that cosmetic products may contain. Table 23.2 lists UV filters that are fully permitted. As cosmetic products, cosmetic or INCI nomenclature is utilized as listed in the CTFA International Cosmetic Ingredient Dictionary. The European Union (EU) allows several ingredients not available in the United States (see discussion below). The EU includes titanium dioxide to the approved list. Microfine zinc oxide, as a nanomaterial, is not included in this list. Nanolabeling and approval are expected in the near future.

Commission directive 2006/647/EC classifies low to very high protection sunscreens with a minimum SPF of 6 to 50+. The Persistent Pigment Darkening (PPD) method is recommended for in vivo testing [14]. An in vitro method, the Critical Wavelength method [15], is to be utilized, in contrast with the Boots adaptation recommended by the US FDA. A critical wavelength of 370 nm or longer allows labeling for UVA protection.

The United Kingdom considers sunscreens as cosmetic products (The Cosmetic Products Safety Regulations 2008:1284) allowing the same UV filters as the EU. The Boots Star Rating is used to express a UVA protection rating displayed by a logo on the packaging.

Australia

Sunscreens in Australia are regulated as therapeutic goods. The current joint Australian and New Zealand standard is AS/NZS 2604:1998. Sunscreen products are classified as either primary or secondary depending on whether the primary function of the designated product is to protect from UVR as opposed to a product with a primary cosmetic purpose. SPF designations up to 50+ are now permitted. The terms "sunblock" and "waterproof" are not permitted. In general, Australian Approved Names (AANs) for allowed active sunscreen ingredients are the same as FDA drug nomenclature with a few differences.

Other Countries

Regulation of UV filters and sunscreen products varies significantly from country to country. Most non-EEC European countries follow the EEC directive. Other countries follow US trends with their own provisions. In Japan, sunscreens are classified as cosmetics. The Japan Cosmetic Industry Association provides self-regulated standards. The PPD in vivo method is required for UVA labeling. In Canada, sunscreens are regulated as drugs and generally follow US guidelines. Regulations for each individual country need to be consulted for selection of the various UV filters for incorporation into a sunscreen product to be marketed in a given jurisdiction.

MECHANISM OF ACTION

UV filters have been traditionally divided into chemical absorbers and physical blockers based on their mechanism of action. Chemical sunscreens are generally aromatic compounds conjugated with a carbonyl group [16]. These chemicals absorb high-intensity UV rays with excitation to a higher energy state. The energy lost results in conversion of the remaining energy into longer lower energy wavelengths with return to ground state. The evolution of modern chemical

TABLE 23.2
List of UV Filters Allowed in Cosmetic Products

	EEC Directive Annex VI		
Reference	**Substance**	**INCI Name**	**Maximum Authorized Concentration**
1	4-Aminobenzoic acid	PABA	5%
2	N,N,N-Trimethyl-4-(2-oxoborn-3-ylidenemethyl) anilinium methyl sulfate	Camphor benzylkonium methosulfate	6%
3	Homosalate (INN)	Homosalate	10%
4	Oxybenzone (INN)	Benzophenone-3	10%
6	2-Phenylbenzimidazole-5-sulfonic acid and its potassium, sodium, and triethanolamine salts	Phenylbenzyimidazole sulfonic acid	8% (expressed as acid)
7	3,3′-(1,4-Phenylenedimethylene)bis[7,7-dimethyl-2-oxo-bicyclo-(2,2,1)hept-1-ylmethanesulfonic acid] and its salts	Terephthalylidene dicamphor sulfonic acid	10% (expressed as acid)
8	1-(4-Tert-butylphenyl)-3-(4-methoxyphenyl) propane-1,3-dione	Butyl methoxydibenzoylmethane	5%
9	alpha-(2-Oxobron-3-ylidene)toluene-4-sulfonic acid and its salts	Benzylidene camphor sulfonic acid	10% (expressed as acid)
10	2-Cyano-3,3-diphenyl acrylic acid, 2-ethylnexyl ester	Octocrylene	10% (expressed as acid)
11	Polymer of N-(2 and 4)-[(2-oxoborn-3-ylidene)methyl] benzyl acrylamide	Polyacrylamidomethyl benzylidene camphor	6%
12	Octyl methoxycinnamate	Ethylhexyl methoxycinnamate	10%
13	Ethoxylated ethyl-4-aminobenzoate	PEG-35 PABA	10%
14	Isopentyl-4-methoxycinnamate	Isoamyl p-methoxycinnamate	10%
15	2,4,6-Trianilino-(p-carbo-2′-ethylhexyl-1′-oxy)-1,3,5-triazine	Octyl triazone	5%
16	Phenol,2-(2H-benzotriazol-2-yl)-4-methyl-6-(2-methyl-3-(1,3,3,3-tetramethyl-1-(trimethylsilyl)oxy)-disiloxanyl)propyl) (drometrizone trisiloxane)	Drometrizone trisiloxane	15%
17	Benzoic acid, 4,4-((6-(((1,1-dimethylethyl)amino)carbonyl) phenyl)amino)-1,3,5,triazine-2,4-diyl)diimino)bis-cbis (2-ethylhexyl)ester)	Diethylhexyl butamido triazone	10%
18	3-(4′-Methylbenzylidene)-d-t camphor	4-Methylbenzylidene camphor	2%
19	3-Benzylidene camphor	3-Benzylidene camphor	2%
20	2-Ethylhexyl salicylate	Octyl salicylate	5%
21	4-Dimethyl-amino-benzoate of ethyl-2-hexyl	Octyl dimethyl PABA	8%
22	2-Hydroxy-4-methoxybenzophenone-5-sulfonic acid and its sodium salt	Benzophenone-5	5% (of acid)
23	2,2′-Methylene-bis-6-(2H-benzotriaz0l-2yl)-4-(tetramethylbutyl)-1,1,3,3,-phenol	Bisoctyltriazol	10%
24	Monosodium salt of 2-2′-bis-(1,4-phenylene) 1H-benzimidazole-4,6-disulfonic acid	Bisamidazylate	10% (of acid)
25	(1,3,5)-Triazine-2,4-bis ((4-(2-ethyl-hexyloxyl)-2-hydroxy)-phenyl)-6-(4-methoxyphenyl)	Anisotriazine	10%
26	Dimethicodiethylbenzalmalonate	Polysilicone-15	10%
27	Titanium dioxide	Titanium dioxide	25%
28	Benzoic acid, 2-(4-(diethylamino)-2-hydrobenzoyl)-,hexylester	Dithylamino hydroxybenzoyl hexyl benzoate	10%

sunscreens represents a prototype study in the use of structure–activity relationships to design new active ingredients and has been well reviewed elsewhere [11].

Physical blockers reflect or scatter UVR. Newer micro-sized forms of physical blockers may also function in part by absorption [17]. Sometimes referred to as "nonchemical" sunscreens, they are more accurately designated as inorganic particulate sunscreen ingredients.

NOMENCLATURE

Sunscreen nomenclature can be quite confusing. They may be referred to by their chemical or trade name. In the United States, individual sunscreen ingredients are also assigned a drug name by the OTC Monograph. Annex VII of the EU may use either a drug or chemical name. Australia has its own approved list of names (AAN). Table 23.3 lists the most

TABLE 23.3
Sunscreen Nomenclature

CAS #	Drug Name (FDA)	INCI Name	Colipa #	EU Reference #	Trade Names	Solubility	Spectrum
150-13-0	Aminobenzoic acid	PABA	S 1	1	4-Aminobenzoic acid	Hydrophilic	UVB
70356-09-1	Avobenzone	Butyl methoxydibenzyl methane	S 66	8	Parsol 1789	Lipophilic	UVA I
104-28-9	Cinoxate	Cinoxate				Lipophilic	UVB
92761-26-7	Ecamzule	Terephthalylidene dicamphor sulfonic acid	S 71	7	Mexoryl SX	Hydrophilic	
27503-81-7	Ensulizole	Phenylbenzimidazole sulfonic acid	S 45	6	Eusolex 232, Neo Heliopan Hydro	Hydrophilic	UVB
118-56-9	Homosalate	Homosalate	S 12	3	Eusolex HMS	Lipophilic	UVB
134-09-8	Meradimate	Menthyl anthranilate			Dermoblock MA, Neo Heliopan, Type MA	Lipophilic	UVA II
6197-30-4	Octocrylene	Octocrylene	S 32	10	Escalol 597, Eusolex OCR, Uvinul N-539-50	Lipophilic	UVB
5466-77-3	Octyl methoxycinnamate	Octyl methoxycinnamate	S 28	12	Neo Heliopan AV, Parsol MCX, Eusolex 2292	Lipophilic	UVB
88122-99-0	Octyl triazone	Octyl triazone	S 69	15	Uvinul T-150	Lipophilic	UVB
118-60-5	Octisalate	Octyl salicylate	S 20	8	Escalol 587, Eusolex BS, Uvinul O-18	Lipophilic	UVB
131-57-7	Oxybenzone	Benzophenone-3	S 38	4	Eusolex 4360, Neo Heliopan, Uvinul M40	Lipophilic	UVB, UVA II
21245-02-03	Padimate O	Octyl dimethyl PABA	S 78	17	Escalol 507, Eusolex 6007	Lipophilic	UVB
4065-45-6	Sulisobenzone	Benzophenone-4	S 78	17	Escalol 577, Uvinul MS 40	Lipophilic	UVB, UVA II

commonly used names including their primary listing in the International Cosmetic Ingredient Dictionary (INCI designation) [18].

INDIVIDUAL UV FILTERS

Sunscreen ingredients may be considered by dividing them into larger overall classes by chemical structure. They may also be classified by their absorption spectrum. Although the lists of UV filters approved by the various regulatory agencies may seem quite extensive, fewer are used with any degree of frequency.

UVB

PABA and Its Derivatives

Aminobenzoic acid or PABA was one of the first chemical sunscreens to be widely available. Several problems limited its use. It is very water-soluble, was frequently used in alcoholic vehicles, stained clothing, and was associated with photodermatitis. Ester derivatives of PABA, mainly octyl dimethyl PABA or Padimate O, became more popular with greater compatibility in a variety of more substantive vehicles and a lower potential for staining or adverse reactions. Amyl dimethyl PABA and Glyceryl PABA (glyceryl aminobenzoate) are no longer used.

Padimate O or Octyl dimethyl PABA is a most potent UV absorber in the mid-UVB range. Because of problems with PABA formulations, marketers have emphasized the "PABA-free" claim. Although still used [19], it is sometimes confused with PABA limiting its use. The decline in the use of this PABA derivative along with the demand for higher SPF products led to the incorporation of multiple active ingredients in a single product to achieve the desired SPF.

Cinnamates

The next most potent UVB absorbers allowed by the FDA monograph, the cinnamates, have largely replaced PABA derivatives. Octinoxate or octyl methoxycinnamate is the most frequently used sunscreen ingredient [19]. Octyl or ethylhexyl methoxycinnamate is an order of magnitude less potent than Padimate O and requires additional UVB absorbers to achieve higher SPF levels in a final product. Cinoxate (Ethoxy-ethyl-p-methoxycinnamate) is less widely used. Isoamyl p-methoxycinnamate (EU no. 14) is available in Europe.

Salicylates

Salicylates are weaker UVB absorbers. They have a long history of use but were supplanted by the more efficient PABA and cinnamate derivatives. They are generally used to augment other UVB absorbers. With the trend to higher SPFs,

more octisalate or octyl salicylate (ethylhexyl salicylate) is being used followed by homosalate or homomenthyl salicylate. They do not readily penetrate the stratum corneum, are water-insoluble, and retain their effectiveness after exposure to water and perspiration. Both materials have the ability to solubilize oxybenzone and avobenzone. Trolamine or triethanolamine salicylate has good water solubility and has been used in hair products.

Camphor Derivatives

Not approved by the FDA for use in the United States, there are six camphor derivatives approved in Europe. 4-Methylbenzylidene camphor (EU no. 18) is the most widely used [19].

Octocrylene

2-Ethylhexyl-2-cyano-3,3 diphenylacrylate or octocrylene is chemically related to cinnamates. It can be used to boost SPF and improve water resistance in a given formulation. Octocrylene is photostable and can improve the photostability of other sunscreens. It is expensive and can present difficulties in formulation.

Phenylbenzimidazole Sulfonic Acid

Phenylbenzimidazole sulfonic acid or ensulizole is a water-soluble UVB absorber that can be utilized in the water phase of emulsion systems, in contrast to most oil-soluble sunscreen ingredients, allowing for a less greasy, more aesthetically pleasing formulation such as a daily use moisturizer containing sunscreen. Phenylbenzimidazole sulfonic acid boosts the SPF of organic and inorganic sunscreens. It can also be used in clear gels owing to its water solubility.

Triazones

Triazones have been designed with multiple chromophores and high molecular weights to diminish their penetration into the skin [16]. Octyl or ethylhexyl triazone (EHT) is a UVB filter available in Europe (EU no. 15). It is eligible to enter the FDA sunscreen monograph through the FDA Time and Extent Application (TEA) process [20], but not yet available. Diethylhexyl butamido triazone (DBT) (Methylene bis-benzotriazol tetramethylbutylphenol—EU no. 25) or Tinasorb S is a newer more efficient UVB filter with improved solubility over EHT [21]. An anisotriazine or bis-ethylhexyloxyphenol methoxyphenyl triazine (BEMT) or Tinasorb S (EU no. 25) is a new broadband filter that also provides UVA protection [13], as does Tinasorb M, methylene-bis-benzotriazolyl tetramethylbutyl phenol (MBBT), or bisoctyltriazol (EU no. 23). Both Tinasorb ingredients have also been submitted for the FDA approval through the TEA process.

UVA

Benzophenones

Although oxybenzone or benzophenone-3 absorbs most efficiently in the UVB range, absorption extends well into the UVA II range. It is used primarily as a UVA absorber, but boosts SPF values in combination with other UVB absorbers. Oxybenzone is supplied as a solid material and has poor solubility and a relatively low extinction coefficient. Sulisobenzone or benzophenone-4 is water-soluble, somewhat unstable, and used with less frequency. Toxicological reports related to issues including contact sensitivity and endocrine disruption [22] have resulted in COLIPA in the EU to requiring that benzophenone-3 be prominently listed on products.

Menthyl Anthranilate

Meradimate or menthyl anthranilate is a weak UVB filter and with absorption mainly in the near UVA portion of the spectrum. They are less effective than benzophenones in this range and are less widely used.

Butylmethoxydibenzoylmethane

Avobenzone or Parsol 1789 t provides strong absorption through a large portion of the UVA spectrum including the majority of the UVA I range with peak absorption at 360 nm. Photostability refers to the ability of a molecule to remain intact with irradiation. It is potentially a problem with all UV filters, but particularly with the use of avobenzone [23]. This effect may degrade other sunscreens in a formulation including octyl methoxycinnamate. Octocrylene and some of the newer sunscreens including BEMT stabilized avobenzone [24]. Non-UV filters such as diethylhexyl 2,6 naphthalate may also be used [20]. These molecules function as triplet–triplet quenchers [16]. Overall formulation with avobenzone is therefore particularly critical.

Terephthalylidene Dicamphor Sulfonic Acid

3,3′-(1,4-Phenylenedimethylene)bis[7,7-dimethyl-2-oxo-bicyclo-(2,2,1)hept-1-yl methanesulfonic acid (EU ref. No. 7) or Mexoryl SX is a UVA blocker more recently available in Europe with comparable [25] or superior efficacy to avobenzone [26]. As patents apply to the use of this ingredient, it is only found in certain proprietary formulations.

Physical Blockers

Some of the original sunblocks were opaque formulations reflecting or scattering UVR. Color cosmetics containing a variety of inorganic pigments function in this fashion. Titanium dioxide and zinc oxide are chemically inert and potentially protect through the full spectrum of UVR. They offer significant advantages. Poor cosmetic acceptance limited the widespread use of these two ingredients until micro-sized forms became available. By decreasing particle size of these materials to a microsize or ultrafine grade, it is less visible on the skin surface. Chemical treatment is also required to solubilize, disperse, and suspend these particles in formulations [16].

Micropigmentary sunblocks function differently than opaque sunblocks of pigmented color cosmetics by absorbing

and not simply reflecting or scattering UVR [17]. By varying and mixing particle sizes, differing levels of photoprotection are achieved throughout the UV spectrum. In addition to avobenzone, micropigmentary TiO^2 and ZnO offer the best available protection in the UVA II range.

These metal materials are photoreactive. Both TiO^2 and ZnO are semiconductors potentially absorbing light and generating reactive species [27]. These effects have been demonstrated in vitro [28]. Coating these materials reduces their photochemical reactivity. The in vivo relevance of these effects has not been demonstrated, and both materials have a long history of safe use. Physical blockers also have the significant advantage of lowered skin irritancy potential.

The EU has identified nanoscale TiO^2 and ZnO as needing further study with regard to health risks. The most significant concerns with nanomaterials relate to pulmonary inhalation and use in drug delivery. Skin absorption studies indicate that these materials do not appear to penetrate intact skin [29]. Overall, the benefits of these ingredients in sunscreens currently outweigh the risks [30].

Titanium Dioxide

TiO_2 was the first micropigment extensively used. Advantages include a broad spectrum of protection and inability to cause contact dermatitis. The use of rutile as opposed to anastase crystal forms of titanium dioxide lessens photoactivity. Newer materials are amphiphilic designed to be dispersed in both water and oil emulsion phases. Particle size and uniformity of dispersion are key to achieving SPF. Primary particle size may be 10–15 nm with secondary particle assembly to 100 nm. Particle size needs to be less than 200 nm to achieve transparency.

Despite advances in the technology and understanding of these materials, whitening remains a problem secondary to pigment residue. Adding other pigments simulating fleshtones may partially camouflage this effect. The net effect may be that the user is inclined to make a less heavy application of product effectively lowering SPF [31]. "Hybrid" formulations employing a combination of chemical absorbers with inorganic particulates may represent a practical compromise.

Zinc Oxide

Zinc oxide was added as an active sunscreen agent for the FDA OTC Sunscreen Monograph along with avobenzone. Reduced to a particle size of less than 200 nm, light scattering is minimized, and the particles appear transparent in thin films [32]. ZnO has a refractive index of 1.9, as opposed to 2.6 for TiO_2, and therefore causes less whitening than TiO_2. ZnO attenuates UVR more effectively than TiO_2 in the UVA I range [33] with a peak at 360 nm, as does photostabilized avobenzone [34]. Microfine TiO_2 at an equal concentration offers somewhat more protection in the UVB range. Fine particle ZnO is not approved as a sunscreen ingredient in the EU, but rather as a cosmetic colorant and general cosmetic ingredient.

FORMULATION

A detailed discussion of incorporating UV filters into various vehicles to achieve defined goals for efficacy and aesthetics is beyond the scope of this chapter and has been well reviewed elsewhere [35]. Briefly, the first step is to determine the type of product, SPF and UVA efficacy levels, aesthetics, and nonsunscreen claims desired. Sunscreen actives are chosen realizing that most products use multiple actives comprising up to 35% of the final formulation. Sunscreen ingredients are among the most expensive used in cosmetic formulation. Vehicle type determines which actives can be used based on the polarity and solubility characteristics of individual filters. The most commonly used sunscreen actives are oils significantly affecting aesthetics. Inorganic particulates such as ZnO and TiO_2 tend to make products feel dry and drag on application. Emulsions are the most popular vehicles to ideally incorporate active ingredients into both water and oil phases for greater efficiency. The final products rheological profile and polymer levels determine whether a uniform film coats the surface of insure efficacy. Water resistance needs to be considered [36]. Last, a photostable system needs to be designed.

ADVERSE REACTIONS—TOXICITY

In a longitudinal prospective study of 603 subjects applying daily either an SPF 15+ broad spectrum sunscreen containing octyl methoxycinnamate and avobenzone or a vehicle cream, 19% developed an adverse reaction [37]. Interestingly, the rates of reaction to both the active and vehicle creams were similar, emphasizing the importance of excipient ingredients in the vehicle. The majority of reactions were irritant in nature. Not surprisingly, a disproportionate 50% of the reacting subjects were atopic. Less than 10% of the reactions were allergic with none of the subject patch tested actually found to be allergic to an individual sunscreen ingredient.

Subjective irritation associated with burning or stinging without objective erythema from some organic UV filters [38] is the most frequent sensitivity complaint associated with sunscreen use. This is most frequently experienced in the eye area. Longer-lasting objective irritant contact dermatitis may be difficult to distinguish from true allergic contact dermatitis. In a postmarket evaluation of sunscreen sensitivity complaints in 57 patients, 20 of the patients had short-lasting symptoms, 26 long-lasting, and 11 mixed or borderline symptoms [39]. Half of the patients were patch and photopatch tested, and only three showed positive reactions to sunscreen ingredients.

Contact and photocontact sensitivity to individual sunscreen ingredients has been extensively reviewed [40]. Considering their widespread use, the number of documented allergic reactions is not high [41]. PABA and PABA esters accounted for many of the early reported reactions, but with a decrease in their use, an increase in reactions to

benzophenones was reported [42]. Skin reaction is probably higher with benzophenones than other UV filters [43]. Fragrances, preservatives, and other excipients account for a large number of the allergic reactions seen.

Virtually all sunscreen ingredients reported to cause contact allergy may be photoallergens. Although still relatively uncommon [44], sunscreen actives seem to have become the leading cause of photocontact allergic reactions [45]. Individuals with preexisting eczematous conditions have a significant predisposition to sensitization associated with their impaired cutaneous barrier. The majority of individuals who develop photocontact dermatitis to sunscreens are patients with photodermatides. Introduced in part to replace oxybenzone and photostability, Octocrylene has been reported with greater frequency as a photoallergen with increased use in the last decade [46].

Contact sensitivity is not an issue with the use of physical blockers. However, concerns related to dermal penetration of nanoparticles and other forms of toxicity continue to be raised. Studies examining the dermal penetration of nanoparticles TiO_2 and ZnO would indicate that they do not penetrate the skin and remain in the stratum corneum [29]. On balance, current research would favor the overall safety profile of these materials [30].

Some organic sunscreens show estrogenic activity in screening toxicological assays [47]. In vivo studies would question relevance to humans [48], but issues related to endocrine disruption with sunscreen ingredients continue to be discussed [49].

CONCLUSION

A limited menu of UV filters for incorporation into sunscreen products is available to the formulating chemist, depending on regulatory requirements in an individual country or jurisdiction. With the demand for higher SPFs, the trend has been to use more individual and a wider variety of agents in newer products. Recent research in sunscreen efficacy has emphasized the need for products protecting against the full UV spectrum with a limited number of available agents. Regulatory agencies are often slow to approve new ingredients. Rules governing the approval of new ingredients by the European Economic Community are more flexible.

Sunscreen efficacy remains very dependent on vehicle formulation. Solvents and emollients can have a profound effect on the strength of UV absorbance by the active ingredients and at which wavelengths they absorb [50]. Film formers and emulsifiers determine the uniformity and thickness of the film formed on the skin surface, which in turn determines SPF level, durability, and water resistance [51]. Last, product aesthetics play a large role in product acceptance, particularly with sunscreens being incorporated into daily use cosmetics. These constraints provide the sunscreen formulator with significant challenges in developing new and improved formulations.

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24 Sun Protection and Sunscreens

Bernard Gabard

INTRODUCTION

Visible sunlight is only a tiny part of the whole radiation spectrum emitted by the sun (400–700 nm). By increasing wavelength >700 nm, one enters the invisible radiation range called infrared (IRR), and by decreasing wavelength <400 nm, one enters the ultraviolet (UVR) domain. Overexposure to the sun's visible and invisible rays can be harmful to human skin. The damage can be immediate with effects appearing some hours later such as erythema and sunburn leading to cell and tissue degradation. The damage can also be long term (over years), and cumulative effects of prolonged exposure are now increasingly recognized to be the possible cause of degenerative changes in the skin such as premature wrinkling and skin cancers. Indeed, many skin changes that often are identified with aging actually result from damage by too much sun exposure [1–4].

DEFINITIONS

The shorthand notation for wavelength ranges in the UVR and IRR defined by the Commission Internationale de l'Éclairage (CIE) is closely related to the absorption depth of radiation in tissue. UVR from both sunlight and artificial sources is subdivided into three sections termed UVA, UVB, and UVC from the longer to shorter wavelengths: UVA from 400 to 320 nm, UVB from 320 to 290 nm, and UVC <290 nm. The UVA section is further divided in two subsections: UVA I (longer wavelengths 400 to 340 nm) and UVA II (shorter wavelengths 340 to 320 nm). The longer the wavelength and the higher the number, the deeper the UV penetrates the skin. The shorter the wavelength and the lower the number, the greater the energy level of the light and the more damage it can do. UVC, for example, is highly efficient in causing sunburn and could destroy the skin, but, fortunately, it is completely absorbed by ozone in the highest part of the earth's atmosphere. Sunlight's UVR at the surface of the earth is therefore constituted by variable proportions of UVB and UVA passing right through the atmosphere, even on a cloudy day. The variability is due to different factors such as latitude, height of the sun above the horizon (time of the day), altitude, atmospheric conditions, etc. As a rule, the amount of UVA reaching the earth's surface may be considered to be 10 to 30 times greater than that of UVB [1,2,4–6].

IRR is also subdivided in three sections termed IRA, IRB, and IRC, but from the shorter to the longer wavelengths: IRA (or near-IR) from 780 to 1400 nm, IRB from 1400 to 3000 nm, and IRC from 3000 nm to 1 mm. Contrary to UVR, the longer the wavelength and the higher the number, the less deep the IRR penetrates the tissue. Deep penetrating IRA does not cause any strong sensation of heat. But this is the case with the longer wavelengths IRB and particularly IRC, which, at sufficiently high intensities, may damage or even burn the skin [7].

BASICS ON PROTECTION

Ultraviolet Radiation

Skin Effects of UVR

It has long been known that UVB is the principal cause of acute sunburn and tanning as well as being immunosuppressive, mutagenic, and carcinogenic [1,3,4,6,8,10]. Meanwhile, the importance of the biological effects of UVA has been recognized [4,8–10]. UVA induces significant photobiological reactions, mostly of indirect nature and requiring the presence of oxygen such as immediate and delayed tanning reactions and new melanin formation. There is now considerable evidence that UVA definitively contributes to long-term degenerative changes of the skin, such as significant connective tissue damage (premature skin aging) and cancer formation, and may also contribute to UVB-induced carcinogenesis. UVR (UVA and UVB) plays a role in the pathogenesis of photosensitive diseases such as chronic actinic dermatitis, polymorphous light eruption, actinic prurigo, hydroa vacciniforme, and photoallergic or phototoxic drug reactions [1,4,6,10].

UV Filters, Sunscreens, and Photostability

Protection against the effects of UVR in the skin is achieved by specially designed molecules (i.e., UV filters) incorporated in suitable formulations (sunscreens) such as creams or lotions, oils, gels, sticks, etc. [3,11,12]. However, in view of the growing photobiological knowledge about the mechanisms of UVR-induced effects in the skin, this definition must now be revised to include new concepts. Besides providing a minimum sunburn (UVB) protection, modern sunscreens are now required to provide a significant protection against UVA-induced effects. The European Commission recommends that the minimum level of UVA protection (UVAPF) should be at least one-third of the sun protection factor (SPF), and the newest FDA and Australian regulations require sunscreens carrying a "broad spectrum" label [8,9,13]. Further, they may contain not only chemicals that absorb, reflect, or

scatter UVR but also chemicals that interfere with secondary reactions such as generation of free radicals and reactive oxygen species (ROS) in the skin, generation of inflammatory mediators, photoexcitation of different molecules, etc., and ultimately exert long-term protective effects on degenerative skin damage, sometimes called "secondary photoprotection" [3,6,10,14,15]. Consequently, non-UVR-absorbing, -reflecting, or -scattering molecules with a pharmacological action in the skin may also be incorporated in sunscreens, endowing the corresponding products with expanded properties [3,6,10–20].

UV filters used in cosmetic sunscreen formulations are roughly considered belonging to two groups [3,6,11,12]: organic molecules deliberately selected for their UVR-absorbing capacities (i.e., organic UV-filters) and particles that absorb, reflect, or scatter UVR. Particles may be inorganic (i.e., metal oxides) or organic (microfine polymeric molecules) [11,21,22].

Both classes aim at preventing UVR from striking the skin. By absorbing UVR, UV filters are transferred to an excited electronic state from which the energy may dissipate into molecular vibrations (organic molecules) and into heat via collisions with surrounding molecules. However, when an efficient dissipation of the absorbed energy is not possible via, for example, fluorescence, phosphorescence, heat, or internal conversion (isomerization) of an organic molecule, the UV filter may break and irreversibly change its chemical structure. The molecule is not photostable, and consequently, the performance of the sunscreen may be altered [11,23–25]. The method for determination of a UVA protection factor and compliance with "broad spectrum" labeling laid down in the newest regulations requires UVR irradiation of the sample to simulate in-use effect of sunlight and to take in account a possible photoinstability of the UV filters [9,13,26].

Infrared Radiation

Skin Effects of IRR

Interest in looking at the effects of IRR on the skin is growing, and the current understanding of the biological effects of IRR is evolving rapidly with vivid and sometimes controversial discussions, given IRA (near infrared) being currently the subject of detailed investigations [7,27–30]. IRR is inseparably linked to sunlight and perceived as heat, whereas IRA alone does not increase very much the temperature of the skin. In the skin, heat is implicated in erythema "ab igne" and elastotic degeneration, and may even be the consequence of modern way of living [31].

Chronic exposure to IRA is growing due to the increased popularity of outdoor activities combined with the lack of protection of sunscreens in the IRR range and increased use for wellness or therapeutic purposes including promotion of healing in acute and chronic wounds [7,29,30]. Recent publications, however, have reported controversial results concerning the biological effects of IRA in the skin. IRA seems to be involved in premature photoaging and also in photocarcinogenesis [7,28,29]. On a molecular basis, upregulation of endopeptidases in dermal fibroblasts (matrix metalloproteinases [MMP]), which are responsible for the degradation of the extracellular matrix, seems to be at the origin of these deleterious effects [32]. Induction of ROS is also involved [29,33,34]. On the other side, protective effect of IRA against UVB-induced changes have been reported [28,35], together with a lack of upregulation of MMP after IRA irradiation of dermal fibroblasts. The mechanisms of this protection are not completely elucidated and seem to implicate ferritin and/or heat shock proteins, although protection has also been observed without involvement of heat shock proteins [35–37]. A general view of these results points to temperature-induced protection against oxidative stress due to UV, a mechanism that could represent a natural process of cell protection acquired and preserved through evolution [35].

Protection against IRA

In the past, some sunscreens claimed to protect against IRR, but this has been rapidly abandoned due to the lack of a proper investigation method and the obvious lack of spectral absorption of UV filters in the IRR wavelength range. In addition, no irradiation devices were available at that time to allow UV-free IRR exposure [38]. However, given the fact that, similar to UVB or UVA radiation, IRA can cause skin damage and significantly contribute to photoaging of human skin, the question, nature, and mode of IRA protection should be thoroughly investigated. These investigations are now made possible by using standardized IRA light exposure and skin biopsies. They pointed out profound IRA effects on mitochondrial function and upregulation of MMP-1 in dermal fibroblasts, among others [32,38]. These effects may now be used to investigate protection against IRA exposure of the skin. In view of the importance of ROS-induction and of an oxidative stress response due to IRA exposure, topical or systemic application of antioxidants is currently being considered [14,29].

There are many unresolved issues about IRA exposure and skin, for example, exact influence of skin temperature [33,38]. Clearly, many more studies are presently needed about this issue, which is not explicitly addressed in the most recent regulatory monographs on sunscreens [8,9,13].

Visible Light

Skin Effects of Visible Light

Similarly to IRA, interest in investigating the effects of visible light on the skin is growing rapidly. Until now, the visible part of the sun spectra (400–700 nm) has received less attention in regard to its possible contribution to premature skin aging or skin cancer, despite broad use in the treatment of some skin diseases such atopic dermatitis, eczema, or antimicrobial photochemotherapy [34,39]. Recently, exposure of human skin to visible light was shown to generate considerable amounts of free radicals, originating from chromophores

different from the ones involved in free radical generation by UVB, UVA, or IRA light [34,38,39]. Dose-dependent increases in the production of inflammatory cytokines and MMP-1 were also measured [39]. It is likely that visible light also significantly contributes to the signs of premature aging in the skin.

Protection against Visible Light

Commercial sunscreens are designed to absorb and scatter wavelengths up to 380–400 nm. In the past, sunscreens containing ZnO and/or TiO_2 in nonmicronized form did scatter light in the visible range. For this reason, the skin appeared white, which was considered cosmetically unacceptable. Micronization of ZnO and TiO_2 particles improved the protection in the UVR range but simultaneously rendered the sunscreens transparent, thus eliminating protection against visible light. Therefore, in line with protection against IRA exposure, topical or systemic application of antioxidants is currently being advocated [34,39].

FREE RADICALS, FREE RADICAL SCAVENGERS, AND ANTIOXIDANTS

Photochemical reactions due to UVR, IRR, and visible light (i.e., sunlight) are inextricably coupled with the chemistry of free radicals. Globally, the free radical generation process may be considered as a "free radical generation spectrum" from low UVB (280 nm) up to end IRA (1600 nm) with multiple maxima and minima due to the different chromophores involved [29,34,38,39].

The role of reactive oxygen species (ROS) and, among these, of free radicals has now been recognized as a possible main cause of mainly extrinsic but also intrinsic skin aging [6,14,15,34,39–44]. On exposure of the skin to sunlight, suitable sensitizer compounds are electronically excited to the triplet state and transfer the excitation energy to generate singlet oxygen, which undergoes chemical reactions with molecules such as lipids, proteins, and nucleic acids. Inactivation of these ROS and of singlet oxygen depletes the skin of its natural endogenous antioxidant defenses, a situation termed "oxidative stress" [42–44]. Thus, incorporation of molecules being able to control ROS in sunscreens should be beneficial in terms of the so-called photo- or actinic damage of the sunlight-exposed skin [14,45].

Typical antioxidants used in sunscreens and other cosmetic products are vitamins such as vitamin C (water soluble) and vitamin E (lipid soluble), polyphenols, and carotenoids [15,29,46–49]. Polyphenols are naturally occurring chemicals derived from plants, fruits, and vegetables; such as onions (flavonols); cacao, grape seeds (proanthocyanidins); tea, apples, and red wine (flavonols and catechins); citrus fruits (flavanones); berries and cherries (anthocyanidins); and soy (isoflavones). They possess anti-inflammatory, immunomodulatory, and antioxidant properties [17,47]. They have been demonstrated to provide protective properties through topical application [14,17,48]. However, the precise mechanism of action of topically applied actives remain to be elucidated; there is a need to fully understand their effects at both cellular and molecular levels prior to supporting their therapeutic benefits as photoprotective agents [14,15].

Nutritional protection using targeted micronutrients with ROS scavenging activities is one further possibility of administering antioxidants. Several studies have already demonstrated that it is possible to modify some properties of the skin [49–52] or reactions to sunlight by administration of suitable nutritional complements [15,49–57]. Presently, nutritional protection against skin damage from sunlight is increasingly advocated to the general public, but its effectiveness is controversial in view of a natural diet including fruit and vegetables as a source of antioxidants [29,34]. Clearly, more research is needed.

SPECIFIC LEGISLATION CONCERNING SUNSCREENS

Sunscreens are subject to specific regulations in almost every country in the world. In particular, UV filters allowed to be used in sunscreens and their maximal concentrations are listed in specific regulatory documents issued by the authorities. In every country and notwithstanding a cosmetic status, sunscreens and the basis on which their efficacy is being claimed are viewed as important public health issues. For example, the European Commission has issued a recommendation on the efficacy of sunscreen products and the claims made relating thereto, which sets out claims that should not be made in relation to sunscreen products, precautions to be observed including application instructions, and the minimum efficacy standard for sunscreen products in order to ensure a high level of protection of public health [8]. The same concerns are addressed in other rules for sunscreens, such as those recently issued by the FDA and the Australian New Zealand authorities [9,58].

Safety requirements for registration of chemical UV filters are important and are based on a stringent risk/benefit assessment [11,58,59]. The safety dossier of a UV filter is evaluated and approved by national and international health authorities.

EVALUATION OF THE EFFICACY CLAIMS

Protection against Sunlight (UVB, UVA, Visible Light, and IRA)

Efficacy evaluation of sunscreens for UVR may now be considered almost similar over the world. Conduction of the measurements is now subject to ISO standards, which have been recently published and are accepted in a growing number of countries over the world. The following ISO standards apply to efficacy measurement of sunscreens:

- ISO 24442: in vivo UVA (published 2011)
- ISO 24443: in vitro UVA (published 2012)
- ISO 24444: in vivo SPF (published 2010)

ISO standards are accepted in the countries listed in Table 24.1.

TABLE 24.1
ISO Standard by Country

Countries	ISO 24444	ISO 24443
EU (28 countries)	Yes	Yes
Australia/New Zealand	Yes	Yes
USA	No	No
Canada	Under review	Under review
Mercosur	Yes	Yes
ASEAN	Yes	Yes
India	Yes	Yes
Japan	Yes	No; ISO 24442 in vivo compulsory
Korea	Yes	No; ISO 24442 in vivo compulsory

Source: Adapted from Dermatest Newsletter: Sunscreen News. June 2011 and December 2012. Available at http://www.dermatest.com.au/Newsletters/Dermatest%20News%20FDA%202011.pdf and http://www.dermatest.com.au/Newsletters/Dermatest%20News%20Dec%202012.pdf (accessed December 2012).

The newest Australian New Zealand sunscreen standard already incorporates ISO 24443 and ISO 24444. The most recent guideline from Cosmetics Europe (formerly COLIPA) for in vitro UVAPF and critical wavelength values determination is almost identical (with only minor differences) to ISO 24443. The final FDA sunscreen rules are aligned with the EU and other national guidelines.

Protective efficacy of a sunscreen against UVB is expressed as the SPF. The SPF is a number representing the ratio of the time required for a given irradiation to produce minimal perceptible erythema (MED: minimum erythemal dose, the UVR dose necessary to produce the minimal sunburn or minimal perceptible erythema 16 to 24 h after exposure) in sunscreen-protected skin to the time required in unprotected skin. All guidelines are now allowing SPFs to be claimed between a minimum of 2 (FDA), 4 (Australia New Zealand/ISO 24444), or 6 (EU) and up to a maximum of 50. Higher SPFs are labeled "50+" on the bottle.

Protection against UVA is measured with an in vitro procedure in some countries (see above) and with the in vivo persistent pigment darkening (PPD) method in some others (like Japan). The test method used by ISO 24443/Cosmetics Europe UVAPF guidelines is designed to correlate with the in vivo PPD method of in vivo UVAPF testing.

The determination methods are published, and most of these are freely available on the Internet [9,13,26,59–61]. The ISO 24443 standard addresses the photostability issue and incorporates a preirradiation step to the in vitro test method. The UVAPF determined after irradiation is the one used to calculate the ratio UVAPF/SPF, which should be at least 0.333.

There is presently no standard method published to evaluate efficacy claims concerning IRA and/or visible light. This results on one hand from the current debate concerning the question of IRR effects on the skin: should these effects be considered deleterious or not? On the other hand, defined criteria for measurements of IRA and visible light effects in the skin are missing. One possible solution could be the proposed "free radical PF" [62] or the "total sunlight PF" based on a biological end point essential for human life (vitamin D formation) [34]. This last approach takes in to account beneficial effects of sunlight on the skin such as maintenance of vitamin D status [10].

Water Resistance

Water resistance (WR) characterizes the property of a sunscreen to maintain its degree of protection under adverse conditions, such as repeated water immersion or sweating. Due to the outdoor use of sunscreens in conditions where water immersion is usual and abundant sweating may be encountered, water and sweat resistance is very important. Human testing is considered to be the most acceptable and definitive method for claiming WR, and all newest guidelines consider estimating the WR of a sunscreen.

WR tests have not attained the same level of harmonization as the measurement methods for efficacy testing. In the EU, the guideline for testing WR considers determining the SPF on dry skin as usual before immersion. The watering conditions are ensured by 2 × 20 min (40 min) immersion separated by a 15-min drying period without toweling. The SPF is then measured again after the immersion period, and the so-called "mean percentage water resistance retention" (%WRR) is calculated as the mean of the individual SPF ratios. A product may claim WR if the value for the 90% lower unilateral confidence limit of the %WRR is greater than or equal to 50% and the 95% confidence interval on SPF (dry skin) was within 17% of the mean SPF.

The new FDA guideline requires subjects staying immersed in water for 2 × 20 min separated by a 20-min drying period without toweling. This is repeated again for a total of 40 min of water immersion. This may be extended to 80 min by repeating the procedure. The measured SPF (after immersion) is then claimed on the label "Water resistant (40 minutes)" or "Water resistant (80 minutes)."

The requirements of the new Australia New Zealand guidelines for WR testing remain the same as in the old ones from 1998. The SPF of the sunscreen is determined after water immersion for not less than 40 min (2 × 20 min time periods separated by a 5-min rest period without toweling). If WR of greater than 40 min is to be tested, the schedule of alternating 20 min immersion/5 min rest is continued. The SPF measured after immersion determines the category classification of the sunscreen. Any claim of WR is to be qualified by a statement of the time for which the WR has been tested, up to the maximum claimed time.

All guidelines prohibit use of the claims "waterproof" and "sweat proof." Finally, one must be aware that an important difference remains concerning the labeling of the products: a water-resistant sunscreen in the United States or Australia is labeled with the SPF measured after water exposition, whereas in Europe, the SPF indicated is still the SPF measured on dry skin, with WR meaning a 50% allowed "discount" for postimmersion SPF [60].

CONCLUSION

The increasing awareness about the damaging effects of sunlight has led to a significant demand for more protection from sunscreens and to an enlargement of the concept of UVR protection toward "secondary photoprotection" and now "global photoprotection." "Global photoprotection" means the enlargement of this concept to visible and IRA wavelengths. At the time of writing, this remains a controversial issue as the exact role played by these wavelengths is not entirely clear concerning skin damage. Production of ROS and of free radicals in the skin is acknowledged to be one common consequence of exposure to sunlight, whereas other, more specific processes may also be activated by these wavelengths [38].

The demand for more protection is clearly reflected by the steady increase in the labeled SPF over the past years. Sunscreens with SPF >50 and good UVA protection are now available, which are claimed "50+." There are, however, still open issues about the minimum meaningful SPF that makes sense, given the fact that failure to use the sunscreen appropriately is a major concern, and a great educational effort is needed concerning amount of product applied and frequency of use [63–65]. This and recent investigations regarding a lifelong exposition to sunlight [66] have led some experts to advocate in supplement systemic sun protection through administration of micronutrient supplements. This is to consider within the frame of the enlargement of the traditional "sunscreen" concept to "global photoprotection" including visible and IRA wavelengths. This is still another controversial issue, notwithstanding the missing risk–benefit analysis for this kind of sun protection.

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25 Sun and After-Sun Products

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HISTORY BEHIND AFTER-SUN PRODUCTS

The fact that electromagnetic radiation has positive as well as negative effects was known already in ancient times. However, the science of photobiology started to develop only more recently [1]. It was at the beginning of the twentieth century when vigilance against significant sun exposure (a relic of the nineteenth century) started to erode and the roots of later attitudes toward sunbathing were already manifested. The first clinical observations associating long-term sun exposure not only with beneficial effects (phototherapy) but also with skin cancer were also reported during this time [2].

The role of sunlight as a major cause of skin cancer was increasingly mentioned in popular magazines in the 1940s and 1950s. However, rapid growth of the sunscreen and later of the after-sun industry has taken place, allowing sun tanning to still remain popular [3,4]. Sunburn treatment and after-sun products were already used at the end of the nineteenth century. Examples of such products are lactic acid with glycerine and rose water, hydrogen peroxide, iodine, lemon or cucumber juice, buttermilk baths, etc. [2].

AFTER-SUN RELEVANT EFFECTS OF SUNLIGHT ON THE SKIN

Sunlight is highly energetic. Upon interaction with the skin, sunlight can be reflected, scattered, or absorbed. In order to initiate a physical or chemical process, light needs to be absorbed by an atom or molecule. Human skin is an abundant source of numerous chromophores with strong absorption particularly in the UVB, UVA, and blue visible region; for example, porphyrin, bilirubin 2, pheomelanin, etc. [5].

Irradiation of the skin with ultraviolet radiation (UVR) is today known as a major cause of skin cancer and of local and systemic immunosuppression, and as a contributor of cutaneous photoaging [6]. Major changes concern all parts of the dermis and the dermal–epidermal junction [7,8].

UVR is proven to produce DNA damage directly and indirectly through oxidative stress. It provokes increased production of reactive oxygen species, leading to local inflammation and connective tissue degradation with an increase in collagen fragments, which further inhibit collagen and hyaluronan synthesis [8,9]. Increased production of endopeptidases, such as matrix metalloproteinases (MMPs), results in destruction of the extracellular matrix (ECM). Alterations of the ECM result in wrinkle formation with loss of moisture and elasticity, increased skin fragility, and impaired wound healing [7]. Furthermore, UVR induces synthesis of various neuropeptides (substance P, calcitonin gene-related peptide, propiomelanocortin, etc.) leading to immunosuppression and photoaging [10]. Finally, extensive UVR exposure leads to epidermal thickening, reduced skin barrier function, and breakdown of tissue homeostasis [5].

Sunlight affects human skin not only via UVR but also via IR radiation (IRR) and visible light and the combination of them [1,11]. IRR is perceived as heat. It has been observed that the temperature of human skin, measured inside the dermis, increases to 40°C–43°C within 15–20 min after exposition to direct summer midday sunlight [12]. Heat is one of the environmental factors that amplify the effects of UVR mentioned above reinforcing at the same time angiogenesis and dehydration [12]. The skin lipids may get thermally destroyed leading to further damage of the epidermal barrier. Additionally, thermal degradation of carbohydrates leads to modified osmolar environment and further dehydration. Even if the skin does not seem to have been affected by sun irradiation, increased transepidermal water loss (TEWL) is a fact and irritation of nerve endings as well [11,13] with the effects more pronounced on aged skin [14]. Other climatic conditions can reinforce dehydration. On the beach, wind is always blowing, accelerating water evaporation from the skin. Due to the cooling effect of the wind, heat perception is diminished or absent, and beachgoers may consequently be staying longer in the sun without any discomfort. With increased evaporation, the skin loses both moisture and the important water-soluble natural moisturizing substances too. In this respect, particular attention should be paid to elderly sunbathers. It is known that elderly skin is dry, exhibiting an overall dermal atrophy and reduced amounts of fibrillar collagens and elastic fibers. Thus, prolonged sunbathing may worsen the already present skin condition leading to accelerated photoaging [15].

Under the influence of the sun, the human body increases sweat production. The horny layers of the skin swell and loosen up facilitating penetration of the radiation into the epidermis. This may furthermore favor the effects described above [15].

Sunbathers do not immediately perceive most of the effects described above. Rather acute reactions to sunlight irradiation include erythema, edema followed by exfoliation, tanning, and epidermal thickening, depending on exposure dose. Erythema appears 2–4 h after irradiation, reaches its maximum by 24–48 h, and then gradually disappears [15].

Pain, in the form of tenderness to touch, is usually delayed for several hours after sun exposure [16].

The skin can manage the sunlight radiation stress only up to a limited grade of irradiation. Even suberythemal doses are influencing the skin–barrier function and structure with considerable ceramide decrease in stratum corneum intercellular lipid [11]. Sunscreen protection, although greatly limiting the effects of UVR in the skin, does not completely protect against DNA damage. Small damage may be immediately restored, but repair takes time. Therefore, a sufficient resting period, of at least 12 h, should be taken before going back into the sun [15].

SKIN CARE AFTER SUN EXPOSITION

Following intensive sun exposition, the skin needs appropriate care or treatment. Even after a sunbath without any signs of erythema, an appropriate skin care is recommended [11]. In case of sunburn, treatment is necessary [13,15].

As a first step after every sun exposure, the skin should be washed with lukewarm water to remove sweat and superficial mud particles. Thereafter, the skin should be treated in accordance with the general schema of skin care: cleanse, vitalize, and maintain the status of the skin.

For cleansing, surfactants showing little interaction with both skin lipids and skin proteins are suitable. Products of pH that is neutral for the skin, mildness enhancers, and moisturizing agents such as lipids, occlusives, and humectants minimize aggressive interactions with the epidermal barrier reducing thereby skin damage [17]. Recent clinical testing on leading facial cleansers shows that nonfoaming, emollient-based cleansers are extremely mild to the skin while still being efficacious [18].

In order to vitalize and maintain the healthy status of the skin, appropriate skin care is most important after sun exposition. Restoring the hydrolipidic skin barrier is mandatory [13]. Formulations containing moisturizers such as sodium lactate, urea, glycerine, panthenol, etc., are most suitable. Slightly occlusive water-in-oil formulations may further enhance the moisturizing effect by impairing TEWL [19,20]. However, they should be applied after first cooling and calming the sun-irritated skin with an indicated oil-in-water formula [15,21]. More recently, use of skin-like physiologic lipids or naturally occurring lipid complexes of precursor barrier lipids in the appropriate emulsion and molecular ratio has been shown to favorably influence skin repair, barrier function, and moisturization [22,23].

AFTER-SUN PREPARATIONS AND THEIR ACTIVE INGREDIENTS

Most after-sun formulations are emulsions (lotions, creams, sprays) or gels containing moisturizers and actives known for their anti-inflammatory and antioxidative effects. Only cosmetic, nondrug actives will be considered in this section. Thus, active ingredients are usually of plant origin such as azulene or bisabolol (from chamomile), glycyrrhizin (from the root of liquorice), Hamamelis distillate (witch hazel), or extracts from aloe vera or chamomile. Further, compounds such as allantoin, panthenol, menthol, jojoba, collagen, silk amino acids, unsaturated fatty acids, and fat soluble vitamins such as retinol (vitamin A) and vitamin E are also found [15]. Formulations containing vasoconstrictive and/or tanning or astringent agents (tannins) are used to alleviate heat and tension sensations due to erythema. However, owing to the potent astringent effect (protein precipitation), usage of such preparations on irregular pigmented skin or skin of elderly people is not recommended. New developments have led to preparations facilitating or supporting repair mechanisms [15]. Recent studies demonstrated the effect of specific molecular photoprotective agents. Prototype agents that antagonize, modulate, or reverse the chemistry of skin photodamage hold promise in delivering therapeutic benefits [24–26].

In general, the active components used in after-sun preparations can be divided into moisturizers, anti-inflammatory substances, antioxidants, and other ingredients.

Moisturizers

The name "moisturizer" is poorly defined and may be used to define a formula indented for application on the skin or the ingredient(s) incorporated in the formulation [20,27]. Moisturizing substances are classical, well-known cosmetic ingredients used "to reduce the signs and symptoms of dry, scaly skin" [27]. Certainly, effective moisturizers are important components of after-sun preparations for the reasons delineated previously. There is a huge amount of literature available on different moisturizers and their mechanisms of action; thus, the reader is referred to very recent pertinent monographs [28–30] and to Chapter 10 of this book.

Anti-inflammatory Substances

Ingredients such as dexpanthenol, azulene, glycyrrhetinic acid, bisabolol, and allantoin are incorporated into after-sun formulations to relieve erythema and its symptoms of pain, redness, and burning. For example, dexpanthenol was shown to alleviate dry, inflamed skin in experimental models of skin irritation due to repetitive washing. It improved stratum corneum hydration, stabilized the epidermal barrier function, and showed an anti-inflammatory effect [31–34], almost probably due to its key role in maintaining keratinocyte proliferation and differentiation [35,36]. The combination of skin hydrating and anti-inflammatory properties in the same molecule is of special interest for after-sun preparations.

Even so, more powerful are the tanning or astringent agents. They alleviate itch and possess a local anesthetic effect [15].

Hamamelis (Witch Hazel)

For a long time, Hamamelis (witch hazel) has been used in natural medicine. In the United States, Hamamelis is approved as an over-the-counter astringent in the external

analgesic (pain-relieving) and skin protecting categories. In Germany, Hamamelis extract containing preparations are approved among others for the treatment of minor skin injuries and local skin inflammation. Extracts from the leaves or from the bark, but also flower distillate, are mainly used. Main components of the distillate are tanning agents as well as flavonoids and essential oils. Gallotannine and proanthocyanidine act constrictively at the outer membranes of skin [37,38]. Flavonoids are known for their anti-inflammatory effects on the skin [39]. In several studies, the anti-inflammatory and erythema suppressing effect of Hamamelis has been demonstrated [33,40–42] even if lower than the one of hydrocortisone containing formulations [40]. In any case, the efficacy of the Hamamelis distillate lotions has been shown to be similar with an antihistamine gel containing 0.1% dimethindene maleate. The low toxicity of Hamamelis and the absence of known undesirable effects underline its favorable risk/benefit ratio thus supporting its usage in after-sun formulations.

Polyhydroxy Acids

Polyhydroxy acids (PHAs) are a special type of alpha hydroxyl acid (AHA) known to modulate keratinization, normalize stratum corneum exfoliation and thickness, and condition the barrier integrity due to the effects mediated by the alpha-hydroxy group. In addition, PHAs have been found to be less irritating to the skin than traditional AHAs, mainly due to their larger size [43]. For this reason, PHAs offer a distinct advantage over conventional AHAs (glycolic acid and lactic acid) when used on sensitive or irritated skin. Additionally to the fact that many PHAs are strong humectants, several PHAs including gluconolactone, lactobionic acid, and glucoheptonolactone have also been shown to function as antioxidants. Through chelating of oxidation-promoting metals, they may prove beneficial in protecting the skin from UVR-induced damage [44]. However, more information is still needed regarding the safety of their long-term use [45].

Cooling Compounds

Menthol and related "cooling agents" such as camphor, alcohol, and other compounds are widely used in cosmetics and particularly in after-sun products. The cooling and carminative actions of menthol have been repeatedly shown [46–49]. Green [47] demonstrated that menthol intensified paradoxical cutaneous sensations. In the study of Yosipovitch et al. [49], menthol had a subjective cooling effect lasting up to 70 min in most of the volunteers. However, it did not affect cold and heat pain threshold. Recently, Green and Schoen [50] found that dynamic contact can suppress steady-state cold sensations from menthol, proving the bimodal action of menthol. Lee et al. [51] observed that menthol's topical action is dependent on the body region and the body surface area.

Alcohol induces an immediate short cold sensation and lowers the threshold of cold sensation. This is mainly due to its rapid evaporation.

Thus, incorporation of cooling agents such as menthol or alcohol in after-sun preparations is meaningful, as they sooth and alleviate the sensations of warmth and tenseness of sun irritated skin, at least for a short time. This clearly improves their cosmetic acceptance.

Antioxidants

The cutaneous antioxidant defense system is complex, multilayered, and far from being completely understood. The human skin contains various antioxidants such as lipophilic ones (vitamin E with its active components tocopherol and tocotrienol, ubiquinones [coenzyme Q10], carotenoids, and vitamin A) as well as hydrophilic ones (vitamin C, uric acid, and glutathione) and enzymatic antioxidants (catalase) [52,53]. Being the most environmentally exposed skin layer, the stratum corneum may particularly benefit from an increased antioxidant capacity due to topical supplementation as shown in the example of vitamin E [54,55].

While efficacy of topical antioxidant application before UVR is demonstrated in several studies [44,56], the effect of these agents after irritation is less obvious [55,57]. Hence, use of antioxidants in after-sun preparations aims at replenishing the depleted antioxidant pool and/or at boosting or reinforcing the antioxidant defenses of the horny layer and not necessarily at a direct effect on sun-exposed skin. For this purpose, substances such as vitamins of the A, C, and E series, ubiquinones (coenzyme Q10), and/or plant polyphenols from green tea (catechins), vine (resveratrol), pomegranate (anthocyanidins and hydrolyzable tannins), or soybeans (genistein) are now incorporated in many after-sun preparations [58,59].

Green tea extract is particularly interesting in this respect because several studies are available regarding photoprotection and anti-inflammatory activities after topical application [60]. A protection against UVA has also been demonstrated [61–63]. The principal chemical constituents of green tea are polyphenols containing (-)-epigalocatechin (EGC), (-)-epicatechin (EC), (-) epicatechin-3-gallate (ECG), and (-) epigalocatechin-gallate (EGCG). Topical EGCG has been shown to reduce UV carcinogenesis in mice [64–67]. Furthermore, recent studies suggest that green tea polyphenols mediate probably almost all their UV-protective effects via induction of DNA repair [68].

Other Ingredients

Shea Butter

Shea butter may be incorporated in the lipid fraction of after-sun formulations. Shea butter contains a highly unsaponifiable fraction consisting of terpenic alcohols and sterols found almost exclusively as cinnamic acid esters. They give shea butter its known healing and anti-inflammatory properties [69], for example, the elimination of superficial irritation and erythema. The unsaponifiables also contain 5%–10% phytosterols, which are known to activate cellular growth stimulation. Moreover, shea butter contains the potent antioxidant butylated hydroxytoluene as well as various catechin compounds [70]. Shea butter is a nontoxic and nonirritating material. It has a highly unsaturated glyceridic fraction,

which makes it useful as a UV screen. It also contains linoleic acid [71].

Clinical studies were performed with shea butter showing protecting, regenerating, wound healing, and wrinkle reducing effects [72]. It has moisturizing, soothing, antiaging, and anti-inflammatory properties and contributes to an efficient release of active ingredients [72].

Olive Oil

Extra virgin olive oil (EVOO), which is obtained from whole fruit, is rich in phenolic compounds having potent anti-ROS activity (ROS = reactive oxygen species). Recently, it has been found that EVOO painted on mouse skin immediately after UVB radiation significantly retarded the onset and reduced the number of skin cancer and ROS-induced DNA damage. Interestingly, pretreatment with EVOO and pretreatment or posttreatment with regular olive oil neither retarded nor reduced skin cancer in UV-irradiated mice. Of the olive oil components tested, oleuropein has been found to reduce ROS-induced skin damage [73]. These results strongly suggest that topical use of olive oil after sun bathing in humans may prevent skin cancer formation by reducing ROS-induced DNA damage. Still, further human studies are needed in order to support recommendations in the public.

Molecular Photoprotective Agents

Based on the causative involvement of skin chromophores in skin photodamage, it is to be expected that molecular antagonism of photoexcited states offers a potential therapeutic opportunity for skin photoprotection. Reactivity-based approaches are widely followed for the design of photoprotective agents, including FDA-approved drugs, cosmeceuticals, and experimental therapeutics in preclinical development.

Two classes of reactivity-based agents for skin photoprotection can be distinguished based on their mechanism of action: *direct antagonists of photo-oxidative stress* (sunscreens, quenchers of photoexcited states, antioxidants, redox modulators, and glycation inhibitors) and skin photoadaptation inducers (nuclear factor erythroid 2-related factor 2 [Nrf2] activators, heat-shock response inducers, and metallothionein inducers) [26]. Of the first class, quenchers are worth mentioning more explicitly as many of them are allowed in cosmetics.

Quenchers are compounds capable of inactivating photoexcited states by direct chemical and/or physical interactions. Physical quenchers that can undergo repetitive cycles of excited state quenching without chemical depletion or the need of metabolic regeneration represent a very attractive class of compounds. Examples of such quenchers are vitamin E, ascorbate, L-proline, L-proline methylester, carotenoids (lycopene, lutein, zeaxanthin), the microbial osmporotectant ectoine, etc. [26]. Ectoine, which can be used as well as a strong moisturizer, is already included in various kinds of marketed products [74,75].

Two further molecular photoprotective concepts are worth mentioning.

Few studies have demonstrated that, when exogenously applied, the bacterial DNA incision repair enzyme T4 endonuclease V (T4N5) is able to protect human skin in vivo from UVR-induced DNA damage. Although neither erythemal response nor microscopic sunburn cell formation was influenced by T4N5 treatment, UVR-induced upregulation of interleukin 10 and tumor necrosis factor-alpha was prevented [25]. Interestingly, the liposomal lotion was applied after irradiation, thus as an after sun product would be. A further experiment in Xeroderma pigmentosum patients showed that the T4N5 enzyme clearly lowered the annualized rate of new lesions in these patients with defects in their DNA repair mechanisms. An interesting and perhaps fundamental finding was that the rate of new actinic keratoses and basal-cell carcinomas did not increase during the 6 months of follow-up after discontinuation of treatment [76]. More recently, DeBoyes et al. [77] found that T4N5 liposomal lotion was able to reduce effectively the number of actinic keratoses in 17 normal individuals with moderate-to-severe photodamaged skin. These observations may imply that T4N5 treatment reverses a fundamental and common source of these neoplasms and is not just a cosmetic treatment [78].

The second approach is similar to the first one but uses a different DNA repair enzyme. Photolyases are repair enzymes found in fish or prokaryotic organism and normally not present in human skin. They bind to cyclobutan–pyrimidine dimers after their formation due to UVR exposure. The complex enzyme plus dimer is activated by light in the range of 300 to 500 nm and the repair process gets induced. Here too, the photolyase from the blue algae *Anacystis nidulans* prepared from genetically engineered *Escherichia coli* was packed in liposomes and applied topically after irradiation. After a new irradiation with activating light of 365 nm, a reduction of 40% to 45% of the cyclobutan–pyrimidine dimers was noted. Similarly, a clear effect on immunosuppression and on the expression of the proinflammatory ICAM-1 was seen, which was clearly decreased by UVR, but the decrease was antagonized by topical enzyme treatment [24]. Furthermore, liposomes containing photolyase have been found to be able to prevent polymorphic light eruption, when applied immediately after UV exposure [79].

Topical application of molecular photoprotective agents is a very promising approach to skin cancer prevention and totally compatible with the principle of after-sun products. Even so, incorporation of them into sun protection products has also been considered, for example, with photolyase. Photolyase included in a sunscreen product contributed considerably to prevention of UVR-induced DNA damage and apoptosis in comparison to a traditional sunscreen alone [80].

CONCLUSIONS

Appropriate after-sun skin care is necessary in order to support the natural regeneration process of sun-irritated skin. Mild cleansing, cooling, moisturizing, and caring of the skin belong to the basis of a good after-sun treatment. In recent years, a lot of research has been successfully conducted

in order to find ways to inhibit or repair sun-induced skin damage and to prevent skin cancer. Of the investigated compounds, antioxidants and molecular photoprotective agents seem to be the most promising for future application in after-sun products. Although, ingredients with dual action showing UV filter properties as well as repair properties like, for example, caffeine or caffeine benzoate could certainly be a valuable alternative [81].

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26 Living Skin Equivalents and Skin Organ Culture

Preclinical Models for Cosmetic Efficacy Testing

Alain Mavon, Carine Jacques-Jamin, and Lucie Duracher

INTRODUCTION

The use of animals for research purposes and especially for cosmetic efficacy testing has been a sensitive matter for several decades. Regulatory agencies and political as well as scientific communities have put constant pressure to ban the use of animals for such purposes. In Europe, this has forced the Council of the European Union to approve a regulation that prohibits the performance of animal testing in the European Union for finished products, ingredients, or combinations of ingredients from March 2013. This implies that alternative methods require validation by European Center for the Validation of Alternative Methods (ECVAM). To date, only a few alternative testing methods have been validated for toxicological testing using reconstructed human skin models. Nevertheless, living skin equivalents, such as reconstructed human epidermis (RHE), full thickness (FT) skin, or skin organ culture (SOC) models, have already been adopted by cosmetic laboratories as alternatives to animal experimentation to test the efficacy of new ingredients or finished products. These models not only allow one to comply with the demands of regulatory authorities, animal welfare organizations, and consumers but also provide a means to improve and extend our knowledge on skin biology. Moreover, they have proven to be efficient, informative, and predictive tools for screening, bioavailability, and efficacy testing of active ingredients, as well as preclinical model, which is very informative in the design of clinical testing.

One of the main reasons for the development and the use of reconstituted human skin (RHS) models is their mimicking function of human skin in vivo, as they exhibit a fully differentiated epidermis, and the presence of the stratum corneum allows topical application of both aqueous and oily solutions as well as final formulations. Three-dimensional (3D) skin models are composed of either the epidermal compartment or both the epidermal and dermal compartments. Various cell types can be incorporated within each compartment, including melanocytes and Langerhans cells in the epidermis and fibroblasts and endothelial cells in the dermis. Because the use of RHS models requires a substantial amount of expertise, and considerable financial resources, and have some inherent limitations, such as an impaired barrier function, SOC models are being used as alternatives for cosmetic efficacy testing. These pig or human skin explants are easy to prepare, are simple to handle, and exhibit a good barrier function. One of the main limitations for the human SOC remains the sourcing as it involved an informed consent from the skin donor, as well as a great interindividual variability, which strongly limits the extensive validation.

In this paper, we first present a brief review of the tissue engineering of reconstructed skin models and SOC, followed by an overview of the commercially available ones. We then presented several examples of studies carried out to determine the benefits of active ingredient targeting some aging features as well as effects of UVB–UVA irradiation as an inducer of oxidative stress and photoaging. This overview is then followed by an in-depth focus on UV-induced damages and photoprotection assessed on both RHS and SOC. Finally, studies performed to monitor the pigmentation process in a pigmented skin model are also discussed.

The third part is dedicated to the barrier function and metabolic capacity of these models, with a focus on evaluation of cytochrome P450 (CYP) activity in a SOC. Finally, the last part is overviewing the progress on these skin models, some exhibiting skin pathology features, or the one used for clinical purpose to treat burn and chronic wounds. Finally, the paper ends by an emerging technology that will probably shift the production of RHS to the next level: the bioprinting.

In conclusion, these studies on cosmetic efficacy testing confirm that both RHS and SOC models represent complementary models with a genuine added value for cosmetic testing.

SKIN TISSUE ENGINEERING

New and stricter regulations have led researchers and companies to engineer in vitro tissue models to be used in the study of cutaneous biochemistry and physiology. An excellent guideline enumerates the processes used in developing

these cell culture models [1]. Briefly, the first in vitro reconstructed model, developed in 1979, was a dermal equivalent, Bell's model [2], composed of fibroblasts in a collagen lattice. Rheinwald and Green [3] fortuitment found that under certain culture conditions, a teratogenic cell line undergoes a maturation that mimics epidermal keratinization. Later, the major breakthrough was the culture of keratinocytes (KCs) at the air–liquid interface [4], leading to the formation of a multilayered and differentiated epidermis, a characteristic of all types of existing 3D skin models. This differentiation program provided the crucial stratum corneum (SC) layer, which constitutes a barrier function for reconstructed models. As cell culture undergoes constant evolution, various models were subsequently designed by differentiating KC cultures through different strategies and supports: inert filters [5–7], dermal substrate such as collagen matrices [8,9], lyophilized collagen-GAG membranes [10], de-epidermized dermis (DED) [11], and fibroblast-populated dermal substrates [12,13]. A recent review [14] is listing these models as well as overviewing cell components, supplement factors, and conditions of culture. Morphological studies [15,16] have shown that RHS models form a multilayered epithelium, displaying characteristic epidermal structure and expressing markers of epidermal differentiation. Ultrastructurally, keratohyalin granules, lamellar bodies, and lamellar structures filled with epidermal lipids are present in the stratum granulosum and SC. The SC layer is a cornified barrier for RHS models (Figure 26.1), composed of multiple lipid lamellae located in the intercellular spaces between keratinized cells, a corneocyte lipid envelope, and desmosomal structures. This barrier is associated with an epidermis calcium gradient, similar to that found in native human skin [17].

Table 26.1 lists the commercially available engineered skin models used for pharmacotoxicological trials. Reconstructed epidermis models are first used for irritation testing, and some have already been validated or are in the process of being validated for this use. Providers also offer pigmented epidermis as well as FT skin models, or long-term viability SOC. However, the fact that only a handful companies are capable of producing RHS in sufficiently large quantities demands for some careful consideration. For instance, in 2006, Episkin (l'Oréal group) acquired SkinEthic Laboratories. The resulting situation created a kind of monopoly situation in Europe [15]. However, since 2011, Mattek is offering RHS produced in Europe to European customers, which ease the delivery as well as limiting the previous monopoly situation. In addition, all models currently validated are either protected by patents and/or are being commercialized using proprietary tissue culture procedures; hence, their continued availability is dependent only and entirely on the corporate strategies of these companies and therefore cannot be guaranteed for the long-term future. In addition, although it is generally accepted that the reconstructed epidermal models currently available are all "comparable," intrinsic differences inherent to their proprietary manufacturing procedures have been causing reasonable concern in the scientific community: for instance, the test protocol transferability from one model to another is often limited and considered a bottleneck for method acceptance both at industry and regulatory levels.

Not only for regulatory toxicology testing but also in cosmetic efficacy testing, the use of these models requires great care and must include the appropriate controls and analysis of reliable and reproducible end points. Accordingly, multiple end point analysis (MEA) including tissue viability, morphology, and the release of proinflammatory mediators should be measured while determining the efficacy of a cosmetic product [18]. One of these end points is the MTT test [19], which measures either cellular viability and therefore the cytotoxic effects of external stress or a cosmetic formulation. For instance, we determine [20] that the release of lactate dehydrogenase (LDH) into the culture medium (Figure 26.2) is a more relevant end point than MTT assay to study irritation and solar-induced cellular damages in RHE and SOC models. In Figure 26.2, it should be noted that topical application of sunscreen reduced the release of LDH from UV-irradiated pig SOC and RHE.

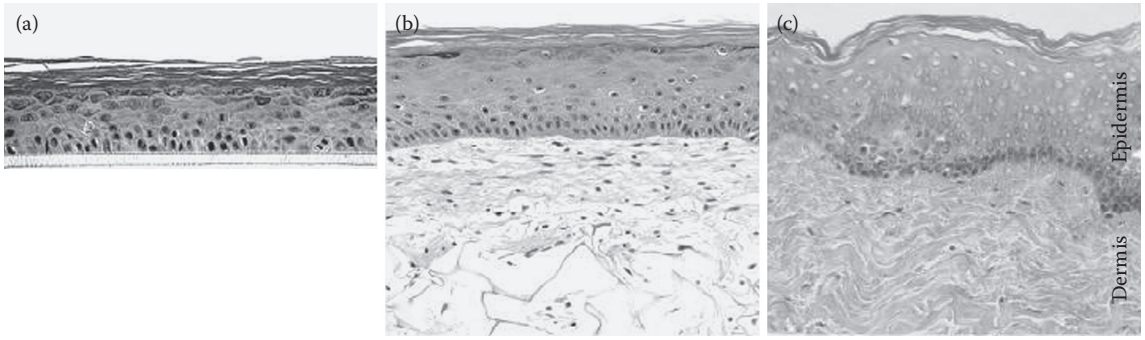

FIGURE 26.1 Histological section of different skin models. (a) Reconstructed human epidermis (SkinEthic Laboratories, Lyon, France [the skin models provided by other suppliers, listed in Table 26.1, would exhibit equivalent histological characteristics]); a polycarbonate membrane supports a stratified epidermis. (b) Full-thickness skin model (Phenion [the skin models provided by other suppliers, listed in Table 26.1, would exhibit equivalent histological characteristics]) with a stratified epidermis on a dermal. (c) Human SOC model from personal data.

TABLE 26.1
Commercially Available RHS and SOC Models

Company/Laboratory	Skin Models	Website
Bioprédic International, Rennes, France	Human skin organotypic culture Natskin	www.biopredic.com
CellSystems Biotechnologie Vertrieb GmbH Troisdorf, Germany	Reconstructed human epidermis EpiCS, 0.6 cm^2; *a model including melanocytes is under development*	www.cellsystems.de
Evocutis plc, Wetherby, United Kingdom	Full thickness skin (LabSkin), 4.5 cm^2; *available with colonization of skin microflora*	www.evocutis.com
Genoskin, Toulouse, France	Human skin organotypic culture (Nativeskin) 1.0 cm^2 Filaggrin-deficient reconstructed epidermis (FLGskin)	www.genoskin.com
MatTek Corp. Ashland, MA, USA	Reconstructed human epidermis (EpiDerm), 0.9 cm^2 Full-thickness skin (EpiDermFT), 1.2 cm^2 Reconstructed pigmented epidermis, (Melanoderm), 0.9 cm^2 Melanoma skin model, 0.6 cm^2 "Psoriasis" reconstructed tissue	www.mattek.com
Phenion GmbH & Co. KG (Henkel), Düsseldorf, Germany	Full-thickness skin Phenion FT, 1.3 cm^2	www.phenion.com
SkinEthic (L'Oréal), Lyon, France	Reconstructed human epidermis, 0.5/4 cm^2 and 0.11/0.33 cm^2 in 96/24-well plate Reconstructed pigmented epidermis, 0.5 cm^2 Reconstructed human epidermis on collagen matrix (Episkin), 0.38/1.07 cm^2	www.skinethic.com
Stratatech Corp. Madison, WI, USA	Full-thickness human skin StrataTest 0.6 cm^2/24 well plate	www.stratatechcorp.com
StratiCell, les Isnes, Belgium	Reconstructed human epidermis, EPI001 Reconstructed pigmented epidermis, MEL001 Customized skin model under request	www.straticell.com

Note: Technical specifications and ordering information about the skin models can be found in the respective website. Some of these companies also supply reconstructed epithelia models (buccal, vaginal, pulmonary, etc.).

APPLICATIONS OF SKIN MODELS IN COSMETIC EFFICACY TESTING

Below are reviewed several experiments carried out to determine the benefits of active ingredients targeting some aging features as well as effects of UVB–UVA irradiation as an inducer of oxidative stress and photoaging. This overview is then followed by an in-depth focus on UV-induced damages and photoprotection assessed on both RHS and SOC. Finally, studies performed to monitor the pigmentation process in a pigmented skin model are also discussed.

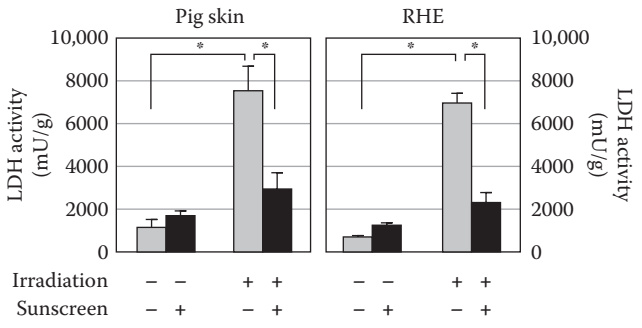

FIGURE 26.2 Cytotoxicity after solar irradiation in pigskin and RHE. Skin was pretreated with or without sunscreen and then exposed to solar-simulated radiation. Culture media were harvested 24 h later and tested for the presence of LDH released from the explants. LDH activity was measured by colorimetry using tetrazolium dye (mean ± SD, $n = 3$, $^*p < 0.01$).

Aging, Oxidative Stress, and Photoprotection

RHS offers a useful tool to determine the potential activity brought by active ingredients. Added in the culture medium, a jasmonic acid derivative was tested on RHE [21], and thanks to an increase in positive KI67 keratinocytes and epidermal thickness, the authors concluded that this ingredient could counteract signs of aging. Another study following a similar design confirmed that a Marine Complex [22] as a nutrient positively stimulated the RHS and can then be used to combat human skin aging in vivo. It should be noted that, for both experiments, the topical application of the ingredient was not tested, which therefore limits the validity of a potential in vivo efficacy highlighted in the conclusion.

Solar radiations are a major concern for human health since they have been recognized as an environmental human carcinogen, and via the oxidative stress, they also contribute to the photoaging process [23–25]. Today, sunfilters provide a good protection against the harmful effects of UVA and UVB radiations. The ban of animal testing in the cosmetic industry leads to the use of these alternative models to discover innovative strategies of skin photoprotection and to assess the photoprotective capacities

of a sunblock formulation. In vitro RHE has been used in the evaluation of ozone [26] or UVA [27] induced lipid hydroperoxides. Ex vivo pig and human SOC models have also been developed as a tool for use in investigating short-term UV-induced damage [28] and time course and spatial distribution of UV effects [29]. Such experiments have demonstrated that skin models are sensitive to UV and that topical application of various antioxidants could successfully be evaluated against UV-induced oxidative stress by reducing apoptotic response and DNA damages: combination of vitamins on a pig SOC model [30], topical application of genistein on a human SOC model [31], or epigallocathecinegallate [32] and salicylic acid [33] in RHS. In addition, UVA dermal alterations in a human SOC model and topical application of retinoids [34] induced the formation of newly synthesized collagen, suggesting dermal repair on this "photoaged" skin model. Moreover and although in vivo assays are essential for sunscreen testing (determination of SPF and PFA), in vitro techniques based on skin equivalents were developed and have shown to be very useful for these types of tests. The apoptotic response was assessed following UV irradiation with and without photoprotection in RHE [35], RHS [36], and pig SOC [37].

Focus on UV-Induced Damages and Photoprotection on RHE and SOC

This example [20,37] focuses on the biological response to UV irradiation through cytotoxicity, DNA damage, and apoptosis in both RHS and SOC models with and without photoprotection. RHEs were from SkinEthic Laboratories, and SOC is a short-term ex vivo skin culture model from domestic pig ears.

Skin samples were irradiated by using a Suntest apparatus and received a single solar-simulated radiation (SSR) dose up to 275 kJ m^2. This acute UV dose corresponds to about fivefold minimal erythemal doses (MEDs). The sunburn cells (SBCs) corresponding to apoptotic KCs following UVB exposure were easily detected by hematoxylin staining 24 h postirradiation in pigskin and RHE models. These dying cells were mainly located in the basal layers of the epidermis. Furthermore, the deleterious effects of SSR were associated to the formation of thymine dimers (Figure 26.3) and DNA fragmentation (Figure 26.4) in both the epidermal and dermal compartments in both tissue models, as, respectively, assayed by immunohistochemistry and TUNEL technique (TdT-mediated dUTP nick-end labeling). None of these

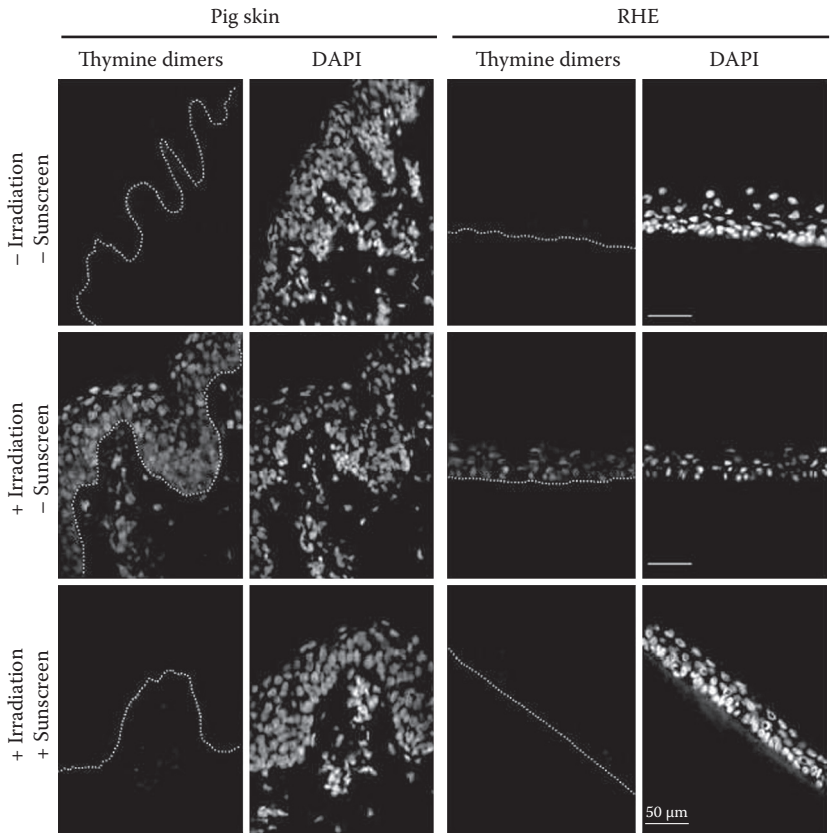

FIGURE 26.3 Thymine dimer formation after solar irradiation in pigskin and RHE. Skin was pretreated with or without sunscreen and then exposed to solar-simulated radiation. Twenty-four hours postirradiation, thymine dimers were identified by immunofluorescence. Note that topical application of sunscreen prevented the formation of DNA lesions in KC nuclei stained with DAPI. Dashed lines correspond to the dermal–epidermal junction and filter surface in pigskin and RHE, respectively.

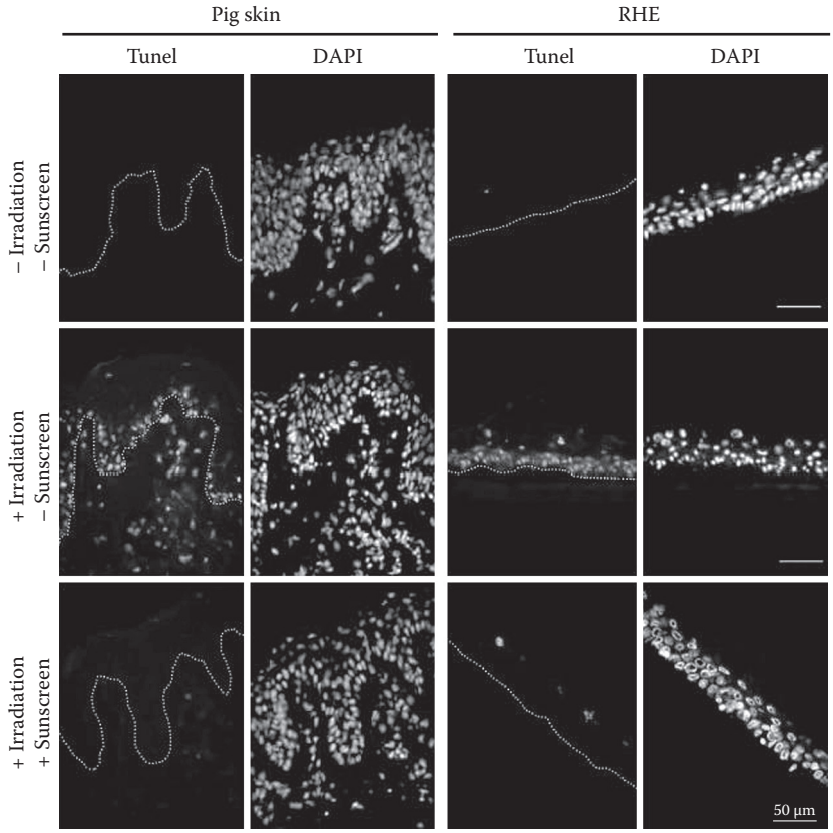

FIGURE 26.4 DNA strand breaks after solar irradiation in pigskin and RHE. Skin was pretreated with or without sunscreen and then exposed to solar-simulated radiation. Twenty-four hours postirradiation, DNA strand breaks were identified by TUNEL reaction using fluorescein-dUTP. Note that topical application of sunscreen prevented the formation of DNA lesions in both dermal fibroblast and KC nuclei stained with DAPI. Dashed lines correspond to the dermal–epidermal junction and filter surface in pigskin and RHE, respectively.

cellular responses were observed in nonirradiated skin. The DNA damage was clearly correlated to SSR-induced cytotoxicity since a significant level of lactate dehydrogenase (LDH) activity was recovered in the culture supernatant from the irradiated skin models (Figure 26.2). The SSR-induced apoptosis involved the upregulation of the p53 tumor suppressor and the activation of the caspase-3 protease. Interestingly, caspase-3 activation was detected mainly in the basal epidermis after irradiation in pig SOC. Thus, basal KCs might be more sensitive to UV exposure than suprabasal KCs and dermal fibroblasts.

To explore whether pig SOC and RHE models are suitable for investigating photoprotection, a broad-spectrum UVB + A sunscreen formulation was topically applied on skin samples at a dose of 2 mg cm^{-2}. The experiments showed that the sunfilter provides good photoprotection without affecting skin viability in both tissue models. Indeed, sunscreen application completely abrogated SBC and DNA damage formation (Figures 26.3 and 26.4), but also the LDH leakage and caspase-3 activation in irradiated models. These data are in agreement with previous results obtained in various skin models [28,29,35,36] and in clinical studies with sunscreen-treated volunteers [38–40].

Altogether, these results indicate that both pig SOC and RHE models are good surrogates to human tissue and that these 3D alternative models are relevant tools to both better understand SSR-induced phototoxicity and to evaluate sunscreen efficacy against UV-induced damages.

Pigmentation

UV light also stimulates skin pigmentation. Pigmented models have been developed as they provide an interesting alternative to animal testing for evaluating the regulation of mammalian pigmentation by melanogenic factors and for elucidating the mechanisms of action of these factors. Pigmented RHS are cocultures of both KCs and melanocytes. The integration of melanocytes from different ethnic regions results in a pigmented epidermis [41] or skin equivalent [42] reflecting Caucasian, Asian, and African-American skin phenotypes. Using these models, it has been demonstrated that the protease activated receptor-2 (PAR-2) pathway [43] regulates pigmentation via melanosome transfer, but only when KCs and melanocytes are in contact. Gibbs et al. [44] have shown that a complete program of melanogenesis occurs following UVB irradiation and supplementation with 3-isobutyl-1-methyl-xanthine: melanosome synthesis, melanosome transport to KCs, supranuclear capping of KC nuclei, and tanning of the epidermis. Dysfunctional pigmentation can also be assessed through the use of in vitro reconstructed models.

A xeroderma pigmentosa skin model was reconstructed to study genetic hyperphotosensitivity [45]. Recently, an RHS model was engineered with normal on nonsegmental nonlesional vitiligo cells [46] and tested under stressed conditions (H_2O_2). First results seem to support the melanocytorrhagic hypothesis of vitiligo.

Additionally, pigmented skin tissue models provide a useful tool in the comparison of the inhibitors (kojic acid, arbutin, and hydroquinone) and activators (α-MSH and dihydroxyphenylalanine) of melanogenesis [42] after repeated topical application or systemic delivery [46]. Pigmented skin tissue is also useful in the assessment of the antipigmentation effects of sunblocks.

Barrier Function and Metabolic Capacity

Despite a fully differentiated epidermis, morphological studies have established that there is a difference in the SC lipid organization, with a hexagonal lipid packing in RHS, whereas in native skin, it is orthorhombic [47]. This difference may account for a much higher permeability compared to excided human skin [48,49], with a 5- to 50-fold higher penetration rate observed in RHS models for most of the substances tested [49]. The development of an RHS with a barrier function similar to skin remains a difficult challenge, which does not seem achievable in the near future [48]. Accordingly, the latest Scientific Committee on Consumer Safety (SCCS) guidelines in dermal absorption of cosmetic ingredient stipulated that, "The use of cultured or reconstructed human skin models is under development and those systems are not yet advised for in vitro testing on the basis of their insufficient barrier function" [50].

Despite these limitations, the presence of the SC on skin equivalents makes possible to apply a great variety of active ingredients topically. In addition, having viable skin tissue, it gives the possibility to explore the skin biotransformation capabilities. Metabolism studies performed on 3D human reconstructs [51] show that most of 3D skin equivalents (RHE, FT skin, or SOC) are metabolically representative of human skin and therefore could be used as a good surrogate model for human skin. However, the enzymatic activities found differ from those of the authors. On the RHS, it is reported that these models exhibited several phase I and II enzymes [52,53], while another group reported that no CYP activity was detected in the epidermal and FT skin model [54] using substrates of reference. Esterase activity was confirmed on both SOC and RHS following topical application of prednicarbate and fluorescein diacetate [55]. We did not find any esterase activity using the well-known tocopherol acetate as a substrate on both RHE and SOC, while β-glucocerebrosidase activity was confirmed after topical application of another gluco-vitamin E conjugate, δ-tocopherol glucoside [56]. This experiment enabled us to demonstrate that RHE and human SOC models are complementary. Indeed, RHE can be used to confirm the metabolism and the efficacy of an active ingredient, as a result of its low barrier function, offering better bioavailability of the molecule being tested, while human SOC, having a more efficient barrier function, is more relevant as a preclinical model and/or in the optimization of an active ingredient concentration to be included in the formulation.

FOCUS ON SKIN CYP-DEPENDENT METABOLISM ON SOC [57]

The role of cutaneous biotransformation in the absorption process is still a matter of scientific debate [58]. Indeed, due to the difficulties in obtaining and maintaining viable skin, a few studies are available on true skin metabolism, and the majority of the skin delivery experiments are conducted with frozen skin with inactivated biotransformation enzymes. Reconstructed human skin models offer long-term viability but exhibit a weak barrier function, and as shown before, although CYPs are detected by expression profiling, low CYP activity is detected in the reconstructed models tested.

To overcome these limitations, studies were undertaken using a convenient SOC from domestic pig ears to characterize its metabolic capabilities. Pigskin, when sourced from a slaughterhouse, provides a valuable alternative owing to its close resemblance to human skin from both histological and physiological view points, and is recognized as an alternative model to animal testing. Skin viability was validated following UV irradiation [20] over a period of 72 h. Therefore, this pig-SOC model was used to explore both skin delivery and phase I and II enzyme activities. Three substrate models, 7-ethoxycoumarine, 7-EC [59], benzo(a)pyrene, BaP [60], and testosterone [61], were selected both according to their log $K_{o/w}$, which reflects increasing lipophilicity, and potential to be metabolized in the skin.

Extensive details for preparing the skin explants are described by Jacques et al. [59]. Briefly, SOC was developed from the ears of domestic pigs obtained from a local slaughterhouse. After cleaning and shaving, the skin was excised, dermatomed at a thickness of 500 μm, and punched into 28-mm diameter discs. Skin punches were seeded dermal side down in polycarbonate inserts and placed in a 6-well plate prefilled with 1.5 mL culture medium (DMEM) at 37°C in a 5% CO_2 air incubator.

To determine how and to what extent skin metabolism alters the skin delivery of xenobiotics, the diffusion of three compounds were studied on a pig ear skin model comparing frozen and fresh skin. For the three compounds, freezing the skin inactivated the biotransformation enzymes, and only the parent compound was recovered in the different skin compartments. The amount that diffused into the culture media, that is, receptor compartment, decreased according to the log $K_{o/w}$ of the compounds tested (Figure 26.5). However, for the less lipophilic compounds, no significant difference of the skin diffusion of 7-EC was observed in frozen or fresh skin. Due to its low molecular weight and log $K_{o/w}$ of 1.3, 7-EC diffused quickly through the skin and did not remain in contact with biotransformation enzymes. Therefore, the rate of metabolism was low, ranging from 17% at 10 nmol to 4% at 400 nmol of 7-EC [59]. For testosterone [61], the

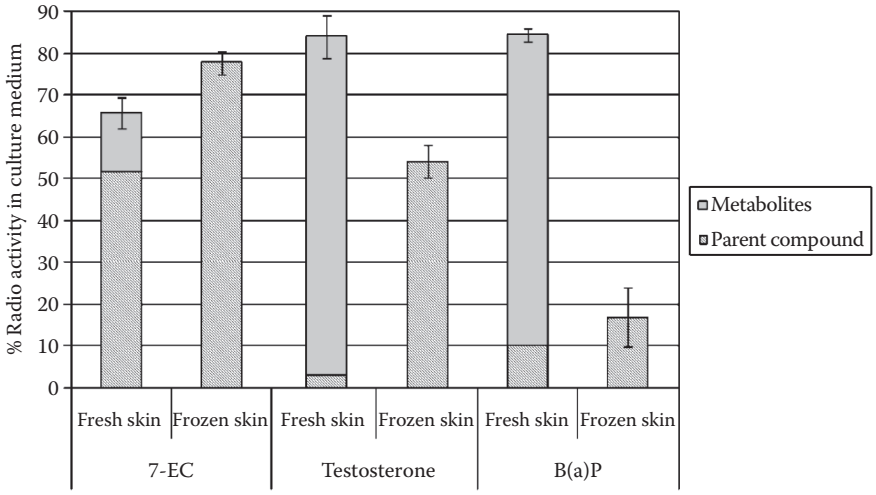

FIGURE 26.5 Extent of delivery of the parent compounds and metabolites through the skin (culture medium) on both frozen and fresh (viable) skin, dose-tested TBC. Data are expressed in percentage of the applied dose of radioactivity. Log $K_{o/w}$ of 7-EC, testosterone, and B(a)P is 1.3, 3.3, and 6.5, respectively.

percentage of radioactivity recovered in the culture media was significantly different with approximately 60% and 80% of the dose applied in frozen and fresh skin, respectively. In frozen skin, the 60% was the parent compound only. In fresh skin, only 3% was testosterone, together with approximately 78% of testosterone metabolites (see Figure 26.5). Regarding the most lipophilic molecule, BaP [60] penetrated easily into the lipidic layers of the stratum corneum but diffused in very small amounts through the hydrophilic epidermis and dermis. In frozen skin, after 72 h, only 4% of the applied radioactivity was found in the culture media. In fresh skin, about 30% of the radioactivity was detected in the culture media; 5% was the parent compound and the other 25% was BaP metabolites (see Figure 26.5). These are remarkable results, confirming that skin metabolism strongly alters the skin diffusion of lipophilic compounds.

According to the different metabolites identified from the three molecules tested, the following phase I enzymes were identified in the viable pig ear skin model: CYP1A1 and A2; CYP1B1; CYP2C; CYP3A; CYP19A; and 7-ethoxycoumarin-O-deethylase, also known as ECOD. CYP is mainly located in the epidermis. CYP1A1 is primarily localized in the basal cell layer of the epidermis, whereas CYP1B1 is localized in epidermal cells other than the basal cell layer. The phase II enzymes identified were glucuronyl-S-transferase; UGT 1A and 2B; and sulfo-transferase, SULT 1A, 1B, and 1E, also mainly localized in the epidermis.

Based on the findings, the viable pig ear skin model exhibits both a good barrier function and functional key phase I and phase II enzymes. Metabolism still occurs even after 72 h. The viable pig ear skin model represents a suitable alternative to human skin to study both the delivery and metabolism of lipophilic compounds in short-term experiments, either to test single topical applications or even to simulate short chronic applications.

The described studies give a better understanding of what happens to chemicals when they come into contact with skin. Accordingly, the stratum corneum acts as the first physical barrier, blocking the delivery of molecules having a molecular weight above 500 Da and most hydrophilic ones. The small lipophilic compounds are solubilized into the lipidic layers of the stratum corneum, thus creating a reservoir. According to the first Fick's law, a gradient of concentration appears, creating a driving force for slow delivery into the viable epidermis. Being in contact with the CYP enzymes, these compounds are therefore biotransformed into hydrophilic ones to ensure quick transfer into the bloodstream and then excreted. Accordingly, the skin is acting first as a barrier, via the stratum corneum; the skin is acting then as a peripheral liver. The SOC therefore represents a valid tool to explore both skin delivery and skin metabolism, where the RHS due to their weak barrier and a nonfully well-established CYP activity should be used with care.

PROGRESS ON SKIN MODELS

The dynamism in fundamental research, dermocosmetology, and the pharmaceutical industry has led to the evolution and complexification of reconstructed skin, which are more and more similar to physiological skin. It results into a wide range of models that will help researchers to elucidate physiological cutaneous mechanisms and also to better understand their aging or different pathological states. Hence, these models have applications in cosmetology as well as in clinical dermatology in the treatment of burn and chronic wounds. The following part is focusing [1] on a selection of recently developed models that would bring incremental benefits to the cosmetic research, [2] on an overview of the complex tissue engineered model dedicated to clinical purpose, and [3] onto an emerging approach to produce 3D skin reconstructs.

Improvement and Specialization of Skin Models

A key step to improve skin model culture conditions is to avoid serum in the media. This allows tissue culture to be carried out with a defined set of conditions as free as possible from confounding variables. Benefits are numerous: more consistent performance, precise evaluations of cellular function, increased growth and/or productivity, and better control over physiological responsiveness. Recently, it has been demonstrated that serum-free medium culture, on both a FT SOC with a validated viability of 72 h [62] and a reconstructed skin model over a period of 21 days [63], does not compromise the characteristics of the skin and therefore might be used for research purposes.

The key process that affects the skin is "aging." The listed models in Table 26.1 do not directly offer the possibility to explore this process, as none of them are mimicking the key features of aging such as increase in stiffness, loss of elasticity, or thinning of the skin. One of the causes of aging is the appearance of advanced glycosylation end products (AGEs). To understand consequences of glycation, a reconstructed system was developed with a "glycated" dermal compartment [64] showing consequences in the quality of the dermis as well as the epidermis. Another aspect of dermal aging was tested by cultivating aged in vitro papillary fibroblasts mimicking reticular fibroblasts, which are mostly represented in aged skin. The reconstructed skin equivalent generated showed a reduced terminal differentiation and fewer proliferating basal KCs, exhibiting a more "aged" phenotype [65].

Dry skin condition is also a key target for cosmetics. Genoskin [66] has developed the "FLG Skin," a commercially available filaggrin-deficient reconstructed epidermis (see Table 26.1) that aims to replicate key molecular events underlying abnormal epidermal barrier found in atopic dermatitis or dry skin. This model shows a reduction in keratohyalin granules compared to a normal RHE and a 50% diminution in filaggrin expression. Using siRNA targeting key genes involved in epidermal differentiation, an FT skin model has been designed to mimic autosomal recessive congenital ichthyosis [67] and could serve to test newly developed drugs. Psoriasis also affects KC differentiation, and several models have been developed. Mattek Corp. [68], using psoriatic fibroblasts, is selling an FT psoriatic tissue model (see Table 26.1) and academic laboratories–engineered skin model, one by using putrescine [69] to induce the psoriatic phenotype in the skin and a second one by cultivating uninvolved psoriatic cells [70]. These three models are exhibiting characteristics associated with psoriasis and could serve to study the biology of the psoriasis, accelerating the research of therapeutic candidates. Most of the previously mentioned skin models are composed of KCs, fibroblast, and melanocytes. However, the skin is an immune organ, displaying an elaborated enervated system. Immunoreactive reconstructed skin containing Langerhans cells has been reconstructed. The immune response of this RHS was demonstrated by a reduction in the number of Langerhans cells and by a modification in their dendritic morphology [71] after exposure to sensitizers or UV irradiation. Another immunocompetent reconstructed skin was developed, comprising epidermal Langerhans cells, dermal dendritic cells, and endothelial cells activated to acquire HLA-DR expression [72]. This model provides a complex environment integrating vascular components to study the differentiation of interstitial dendritic cells in the dermis. Also, a "neuronal" epidermal model was developed to evaluate the regeneration of sensory neurons on injured skin [73], which was mainly influenced by the extracellular matrix molecules, matrix-binding growth factors, and trophic factors.

Focus on Skin Models for Clinical Use

In addition to these "dermocosmetic" models, and their use in pharmacotoxicological trials, it is worth mentioning the amazing progress of tissue engineering to develop complex reconstructed skin models for clinical indications such as grafting ulcers, treatment of burn patients, and wound repair. Two reviews [74,75] describing the commercially available models as well as giving a short insight of those under development would provide an in-depth overview of this research. For instance, reference 74 provides an excellent classification of the models according to anatomical structure classification, as well as insights regarding their clinical performance.

Bioprinting and Skin Models

Bioprinting is an upcoming technology in the field of tissue engineering where cell-containing scaffolds are produced through layer-by-layer deposition process in order to create a 3D tissue-specific model. This technique allows the creation of a biological composite system by controlling the exact deposition of cells in a spatial control manner. A recent review [76] reports the 3D culture systems currently entering industrial R&D laboratories, as well as emphasizes the advantages and potential, but also challenges and hurdles of these novel developments. This technology was experimented to create a multilayered engineered skin tissue composite consisting of fibroblasts and KCs [77], and viable proliferation of each layer was observed. Another orgonamimetic skin model, containing fibroblasts, was produced from an extruded and printed hydrogel, mimicking the extra cellular matrix and cell compatibility [78].

These examples confirm the feasibility to tailor 3D skin models using an on-demand cell printing technique and the future potential applications in drug discovery and testing.

GENERAL CONCLUSION

A substantial amount of effort is being put into the development and validation of skin models, including reconstructed skin equivalents and SOC models. Even if most of these skin equivalents are not fully validated for toxicological purpose, they are extensively used to get a better understanding of the skin physiology, to model some skin pathology of aging process, or to simulate impact of external stress on skin conditions as well as to prove cosmetic product effectiveness,

and hence, they represent a genuine added value for the cosmetics industry. The increasing availability of complex reconstructed models offers a wide range of possibilities for use in the efficacy evaluation of cosmetic ingredients, in spite of limitations such as low barrier function. In addition, they should be provided preferentially by multiple and independent skin model suppliers as a guarantee of their availability to the cosmetic industry. The SOC models are less expensive than the in vitro reconstructed models, are easy to obtain, and possess good barrier function. Accordingly, the combination of RHE and SOC models currently provides a very useful and complementary test system and represents an essential step in the pharmacotoxicological trials needed in the development of cosmetic products, from screening on cellular models to final validation in clinical trials.

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27 Sensory Effects and Irritation
A Strong Relationship

Miranda A. Farage

INTRODUCTION

Most methods for testing the potential skin effects of consumer products rely on an objective evaluation of how the product affects the appearance of the skin, that is, erythema, as scored on a predefined numerical scale. In contrast, the end points that consumers typically describe are subjective in nature and are an evaluation of the sensations the product causes them to experience, that is, itchy, dry, rough, tight, etc. Even though these subjective sensory effects are the end points reported by the consumer and, therefore, arguably the most important, a few test methods incorporate a means to leverage these end points.

A few reports attempt to either quantitate sensory effects or to correlate sensory effects to the degree of irritation. In studies on soap and detergent bars, Simion et al. [1] determined that test subjects differentiated between products based on the sensations they felt during exaggerated arm-washing applications. In several test methods designed to simulate normal use conditions for detergent and personal cleansing products, the perceptions of the panelists are routinely recorded during the course of the study as an additional end point to consider when evaluating the data [2,3]. Sting tests have been used in evaluating some cosmetics and leave-on preparations [4,5].

This paper describes the incorporation of subjective sensory effects as end points of the behind-the-knee (BTK) test method developed as a means of evaluating the skin compatibility of consumer products [6–9]. The BTK measures both chemical irritation and the potential for mechanical irritation due to friction. In the course of these studies, we collected subjective sensory data from our panelists in parallel with the outward signs of irritation, that is, erythema, to determine if there may be value in attempting to quantitatively study sensory effects in order to learn more about the relationship between these sensations and irritant effects. We evaluated the results against the fundamental question: are differences in the irritation potential, as defined by objective scores of erythema, consistent with differences in the reported sensory effects?

Subjective sensory data were compared to more traditional end points: unaided visual scoring and enhanced visual scoring using polarized light. In the case of the products evaluated in the BTK, results of a descriptive analysis panel (DAP) were also considered. The DAP is used in the development of feminine care products to evaluate the intensity of six physical characteristics, including degree of plasticity, compression (loft), scratchiness, glide, cottony feel, and flexibility. We compared the results of the DAP to the results of the sensory effect analysis attempting to determine if certain physical characteristics of the products correlated to adverse sensations experienced by the BTK panelists.

METHODS

BTK Study Design

The test design and conduct of the BTK studies have been described in detail [6,9]. Each study consisted of 9–22 healthy, adult volunteers (male and female) who had signed an informed consent. For each study, the protocol was approved by the test facility's Institutional Review Board. Subjects were excluded from participation for certain skin abnormalities or health conditions that could adversely impact the test, as detailed in prior publications [6,9].

Test materials consisted of currently marketed products and products in development for the marketplace. These included standard feminine protection pads (products A, B, C, E, H, M, and N), a pantiliner (product P), a lotioned pad (product NL), intralabial pads (products IL2 and IL25), and tampons (products R and S). For application, a test material was placed horizontally and held in place behind the knee by an elastic knee band (Ace knee bandage) of the appropriate size, and removed by the panelists 30–60 min prior to returning to the laboratory for grading and/or reapplication of test materials. Exposures consisted of 6 h per day for five consecutive days.

BTK Study Scoring

Visual grading was conducted by an expert grader under a 100-W incandescent daylight blue bulb. Scoring was done using a scale of "0" to "4," where "0" indicates no apparent cutaneous involvement and "4" is moderate-to-severe, spreading erythema and/or edema [6,9]. The same grader was used throughout an experiment, and the grader was not aware of the treatment assignments.

Enhanced visual grading using polarized light has been described in detail by Farage [10]. Grading was conducted using a polarized light visualization system (Syris v600 Visualization

System, Syris Scientific, LLC, Gray, Maine, or syrisscientific.com) with separate scores recorded for parallel-polarizing illumination (surface mode) followed by cross-polarizing illumination (subsurface mode). Based on a communication with the manufacturer, the subsurface mode allows visualization of the site at a depth of 1 mm beneath the surface.

Panelists were asked to keep a daily diary of skin problems experienced at the test sites, as previously described [11,12]. They were asked if they experienced one or more of eight specific sensations including the sample rubbing against the skin, the sample sticking to the skin, chafing, burning, itching, pain, edema, or any other discomfort.

TABLE 27.1
Analyses for Differences between Samples in Mean Erythema and in Reported Adverse Sensory Effect in BTK Studies

			Results	
Study Number	**Samples Compared**		**Comparison of Mean Erythema Scores**	**Comparison of Reported Adverse Sensory Effects**
	a. Consistent Results from Nonaided Visual Scoring and Sensory Effects			
02017	Product N (d/i)[a]	Product NL (d/i)	Not significantly different	Not significantly different
03005	Product A (d/i)	Product A (w/c)[b]	Not significantly different	Not significantly different
03005-3	Product S (w/c)	Product R (w/c)	Not significantly different	Not significantly different
03005-3	Product S (d/i)	Product R (d/i)	Not significantly different	Not significantly different
03005-4a	Product GT (d/i)	Product M (d/i)	Not significantly different	Not significantly different
03005-4b	Product GT (d/i)	Product E (d/i)	Not significantly different	Not significantly different
03005-4c	Product GT (d/i)	Product H (d/i)	Not significantly different	Not significantly different
03005-9	Product A (d/i)	Product B (d/i)	Significantly different on days 3 and 4 Product A > Product B	Percentage of subjects experiencing burning is different on all days (1–4) Product A > Product B
	b. Inconsistent Results from Nonaided Visual Scoring and Sensory Effects			
02008	Product P (d/i)	Product IL25 (d/i)	Significantly different only on day 5 Product P > Product IL25	Not significantly different
02036	Product C (w/i)[c]	Product C (w/c)	Significantly different only on day 1 Product C (w/c) > Product C (w/i)	Not significantly different
03005	Product B (d/i)	Product B (w/c)	Significantly different on days 1, 3, and 5 Product B (w/c) > Product B (d/i)	Not significantly different
03005-3	Product S (w/c)	Product S (d/i)	Significantly different on days 2, 3, and 5 Product S (w/c) > Product S (d/i)	Not significantly different
03005-3	Product R (w/c)	Product R (d/i)	Significantly different on days 2, 3, and 5 Product R (w/c) > Product R (d/i)	Not significantly different
03005	Product A (d/i)	Product B (d/i)	Not significantly different	Percentage of subjects experiencing burning is different on day 1 Product A (d/i) > Product B (d/i)
03005	Product A (w/c)	Product B (w/c)	Not significantly different	Percentage of subjects experiencing pain and sticking is different on days 2 and 3, respectively Product A (w/c) > Product B (w/c)
02008	Product P (d/i)	Product IL2 (d/i)	Significantly different only on day 1 Product IL2 > Product P	Percentage of subjects experiencing itching is different on day 3 Product P > Product IL2

Note: In each study, panelists wore the test substances for 6 h daily for 4 days in close contact with the skin behind the knee. The two test areas (left knee and right knee) were randomly assigned one of the two test materials or conditions being compared in that particular study. Test sites were scored 30–60 min after removal of each product application. The mean erythema score for each treatment at each afternoon scoring was determined. The scores were compared using ANOVA/ANCOVA or, where model assumptions failed, CMH/stratified CMH tests. In addition, subjects kept daily diaries of sensations associated with each test site throughout the course of treatment. Panelists were specifically asked about the sample and knee brace rubbing against the skin or sticking to the skin, and about chafing, burning, itching, pain, or any other discomfort. Each individual complaint was tabulated, and the percentage of subjects who experienced that complaint was determined. The data were evaluated using McNemar's test.

[a] (d/i), dry sample on intact skin (standard protocol).

[b] (w/c), wet sample on skin compromised by tape stripping.

[c] (w/i), wet sample on intact skin.

DAP Study Design

The DAP uses 15 individuals specifically trained to evaluate six different physical characteristics of the products (degree of plasticity, scratchiness, glide and cottony feel of the topsheet, and compression and flexibility of the pad). These trained individuals evaluate products using their fingertips and assess the intensity of the various product characteristics based on an 8-point scale, with 0 = not at all, 4 = moderate, and 8 = extreme.

Statistical Analysis

Mean erythema scores (unaided and enhanced visual) were compared using analysis of variance/analysis of covariance (ANOVA/ANCOVA) or, where model assumptions failed, Cochran–Mentel–Haenszel (CMH)/stratified CMH tests. Subjective sensory results were evaluated for a higher occurrence of each individual skin problem with one treatment versus another using McNemar's test. Mean erythema scores were evaluated versus reported sensory effects using logistic regression, that is, the presence/absence of a particular skin complaint was regressed against the specific irritation scores.

Results in the DAP study were analyzed by ANOVA, and mean differences were tested for statistical significance using Duncan multiple comparison at the 90% confidence level.

All computations were carried out using PC SAS, version 9.2.

RESULTS

Sensory Effects and Unaided Visual Scores in the BTK

Sensory effects have been collected and analyzed statistically in nine BTK studies containing 16 comparisons between two products and/or sets of test conditions. In each experiment, the mean erythema scores for the test samples were compared to determine if any differences were apparent for the two products or test conditions. Separately, the number of reported sensory effects was compared for the two samples. Results are summarized in Table 27.1. In eight of the 16 individual product comparisons, the results were consistent for erythema and sensory responses (Table 27.1a). In seven of these cases, there were no differences in either the mean erythema scores or in the percentage of subjects reporting various sensory effects. In the remaining case, significant differences in the mean erythema scores appeared for at least one scoring time point, and the sample giving the higher mean score (product A) produced significantly more reports of sensory effects from the test group. Results were inconsistent in the remaining eight comparisons (Table 27.1b). In five comparisons, significant differences in the mean erythema scores appeared for at least one scoring time point, and no differences appeared for the reported sensory effects. In two comparisons (study #03005, product A vs. product B under two different test conditions), differences appeared in the reports of sensory effects that were not evident in the mean erythema scores. In one case, the reported sensory effects were in conflict with the mean erythema results (study #02008, product P vs. product IL2).

Regression analyses were conducted to determine if an increase in the severity of erythema scores correlated to an increase in reports of adverse sensory effects, regardless of the particular sample being tested. Results are summarized in Table 27.2. In all but one case (study #03005-3 under dry conditions on intact skin), there was an increase in the reports of adverse sensory effects as the severity of the erythema scores increased. Burning was the effect that was most

TABLE 27.2
Analysis for Correlation between Severity of Erythema Scores and Increased Reports of Sensory Effects in BTK Studies

Study Number	Test Conditions	Correlation of Sensory Effects with Severity of Erythema Scores
02017	d/i[a]	Reports of burning, pain, itching, and rubbing correlate to erythema score.
03005	d/i and w/c[b]	Reports of burning, pain, sticking, and "other" correlate to erythema score.
03005-3	w/c	Reports of burning, sticking, and chafing correlate to erythema score.
03005-3	d/i	No correlation.
03005-4a	d/i	Reports of burning correlate to erythema score.
03005-4b	d/i	Reports of burning, pain, and sticking correlate to erythema score.
03005-4c	d/i	Reports of pain, itching, rubbing, and "other" correlate to erythema score.
03005-9	d/i	Reports of burning, edema, and itching correlate to erythema score.
02008	d/i	Reports of burning, pain, and chafing correlate to erythema score.
02036	w/i[c] and w/c	Reports of burning and sticking correlate to erythema score.
02008	d/i	Reports of burning, pain, and chafing correlate to erythema score.

Note: Studies were conducted as described in the legend for Table 27.1. For each study, the erythema scores and reported sensory effects were considered for all test sites, regardless of the products or treatments being tested. Numerical erythema scores were then evaluated versus reported sensory effects using logistic regression.

[a] (d/i), dry sample on intact skin (standard protocol).

[b] (w/c), wet sample on skin compromised by tape stripping.

[c] (w/i), wet sample on intact skin.

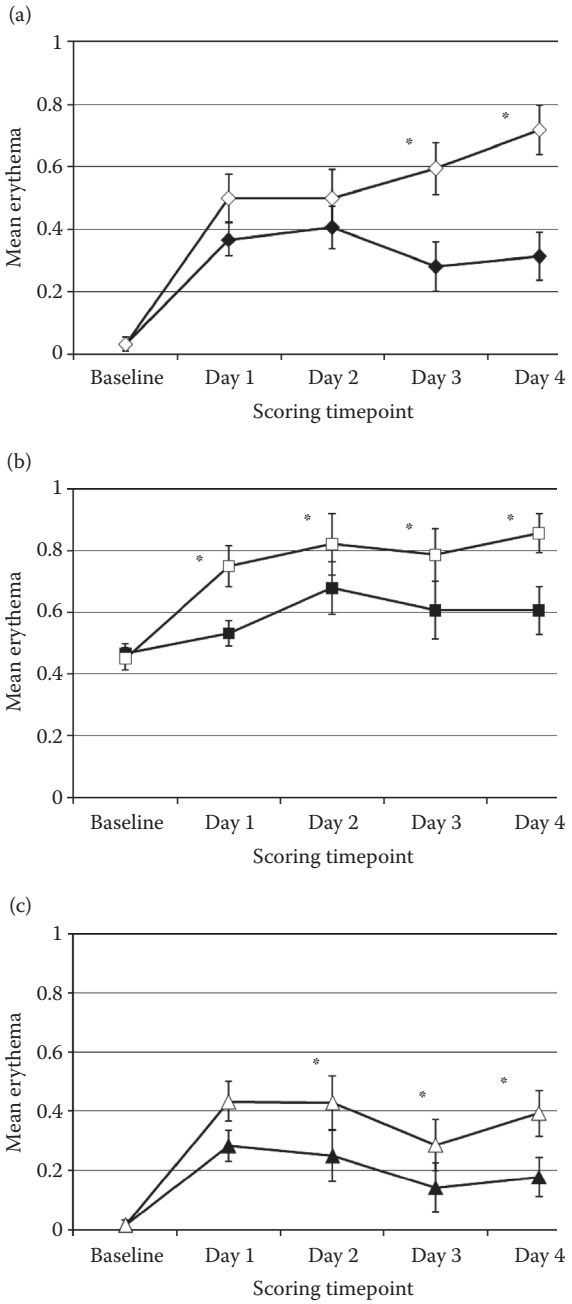

FIGURE 27.1 Standard visual and enhanced visual grading using two similar products in the BTK. Two feminine protection products (pads A and B) were evaluated in the BTK. Samples were applied for 6 h per day for four consecutive days (14–16 panelists per group). Scoring was conducted at baseline and the morning following each patch removal (days 1–4). The graph plots mean erythema (±S.E.) at each scoring timepoint. (a) Visual scores; (b) subsurface scores; (c) surface scores. Treatment comparisons were evaluated using the stratified CMH test (*significant difference between pad A and pad B, $p \leq 0.05$). Pad A = open symbols. Pad B = closed symbols.

commonly correlated to higher erythema scores (in seven cases), followed by pain (in five cases).

Sensory Effects and Polarized Light Enhanced Visual Scores in the BTK

Two feminine protection products were evaluated in the BTK using unaided and enhanced visual grading. Scores were recorded after each product application (6-h application followed by 18-h recovery period). As shown in Figure 27.1a, significant differences between the two products were apparent on day 3 (after three applications) using unaided visual scoring. Using polarized light-enhanced scoring, significant differences were observed after the first and second application (for subsurface and surface scoring, Figure 27.1b and c, respectively).

The number of panelists who experienced burning sensations at the test sites is shown in Figure 27.2. With every sample application, there were a significantly higher number of individuals reporting burning sensations with pad A compared to pad B. In addition, a significantly higher number of individuals reported pain with pad A during the third sample application and the sensation of the sample sticking to the skin during the second and third applications (data not shown).

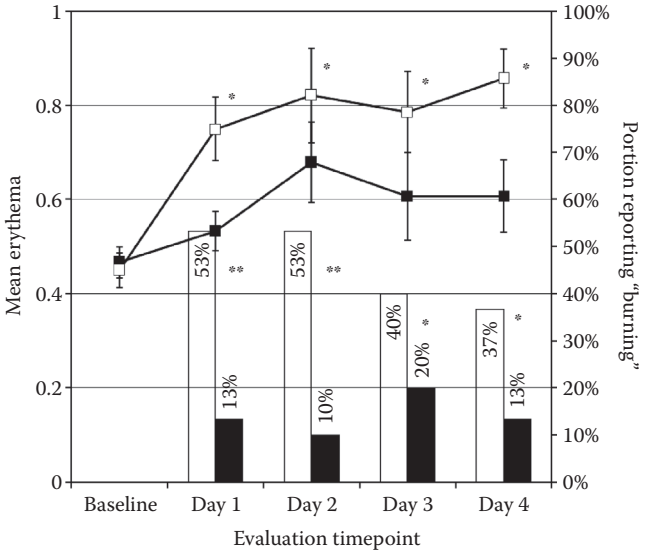

FIGURE 27.2 Reports of burning sensations in the BTK. In the BTK, each of the 30 panelists was asked to keep a daily diary of skin problems experienced at the test sites. Results were evaluated for a higher occurrence of each individual skin problem with one treatment versus another. The graph plots mean erythema (±S.E.) at each scoring timepoint (as shown in Figure 27.1) and the portion of the subject population reporting sensations of burning at the test sites during each patch application. Treatment comparisons for the sensory effect was evaluated using McNemar's test (**significant difference between pad A and pad B, $p < 0.001$; *significant difference between pad A and pad B, $p < 0.05$). Pad A = open symbols. Pad B = closed symbols.

TABLE 27.3
DAP Results for Products Tested in the BTK Test

	Mean Scores			
	Product A	**Product B**	**Product G[a]**	**Product E**
		Negative Attributes		
Plastic feel	6.8[b]	0.0	3.9[b]	0.3
Scratchiness	5.4[b]	1.5	3.5	5.6[b]
		Positive Attributes		
Glide	4.8	6.7[b]	2.6	5.5[b]
Cottony feel	0.0	6.3[b]	4.2[b]	3.4

Note: In the DAP, individuals specifically trained to evaluate the positive and negative physical characteristics of products grade these attributes on an 8-point scale, with 0 = not at all, 4 = moderate, and 8 = extreme. Mean differences were tested for statistical significance using Duncan multiple comparison.

[a] Topsheet tested.

[b] Higher at the 90% confidence level.

Sensory Effects and DAPs

DAPs, conducted in the course of normal product development activities, evaluate certain physical characteristics of the products. Results from DAPs on products that have also been tested in the BTK are shown in Table 27.3. In the comparison of products A and B, product B scored lower in the negative attributes and higher in the positive attributes. Results on the comparison of products G and E were mixed, with product G scoring higher on the negative attribute of plastic feel, while product E scoring higher on the negative attribute of scratchiness. Likewise, product E scored higher on the positive attribute of glide but lower on the positive attribute of cottony feel.

DISCUSSION

Unaided visual scoring of erythema has been used reliably for decades to detect skin irritation in a wide variety of test protocols on a large number of body sites. It requires no special equipment and is easily adaptable to large-scale testing, such as the type that is required to provide safety assurance for consumer products. Trained skin graders can accurately and reproducibly score test sites for erythema and dryness [13]. Several authors have demonstrated that trained graders can reliably detect evidence of irritation with equal or higher degrees of sensitivity to that of instrumental measures, as reviewed by Farage et al. [14].

Enhanced visual scoring using polarized light has been added to existing test protocols recently in an attempt to increase the ability to differentiate between very similar products without requiring other protocol modifications. When skin reactions are scored visually, the grader is seeing a combination of end points: the surface changes, which provides information about the shape and texture of the skin surface, and the subsurface changes, which provides information about internal components such as erythema, pigmentation, and the vasculature [15,16]. Use of polarized light sources can enable the observer to selectively examine either the surface or subsurface components. Authors have described the use of polarized light as an aid in visualizing various skin conditions, including irritation, acne vulgaris, rosacea, photoaging, lentigo simplex, and basal cell carcinoma [17–20].

In the BTK, we have observed that enhanced visual scoring, particularly the ability to evaluate subsurface reactions, results in earlier visualization of irritation reactions. Two similar test products exhibited a mean erythema that was significantly different at the first scoring timepoint with enhanced visual scoring (Figure 27.1b). With unaided visual scoring, the difference was not significant until day 3 (Figure 27.1a). In addition, enhanced visual scoring enables detection of physiological changes that are not apparent using standard visual scoring, that is, subclinical changes. Previously, we have reported that subjective consumer comments indicate that consumers can detect differences in skin effects caused by the use of two similar products. For example, consumers have consistently indicated that pad B is seen as less irritating than pad A in "real use" situations; however, most test protocols repeatedly fail to differentiate between these two products [7]. Sensory effects in the BTK have been shown to be consistent with consumer comments, and reliably differentiate between pad A and pad B [12]. This current investigation confirms that sensory effects correlate with visual scoring in the BTK and enable the differentiation between two very similar products (Figures 27.1 and 27.2).

Sensory effects can provide an additional end point for irritant effects, particularly for mild products that produce no or little visible effects. Although we were not always able to reliably differentiate between the samples in the BTK using solely sensory effects, we did observe that an increase in the erythema score is correlated with a higher percentage of panelists reporting adverse sensory effects, as shown in Table 27.2. This indicates that, even among volunteer panelists who are not specifically trained in observing and reporting sensory effects, the increased reports of sensory

TABLE 27.4
Qualitative Summary Comparison of Objective and Subjective End Points in the BTK Test

			Topsheet Attributes in DAP			
			Negative Attributes		Positive Attributes	
Product Comparisons	Comparison of Mean Erythema Scores	Adverse Sensory Effects	Plastic Feel	Scratchy	Glide	Cottony Feel
Product A vs. Product B	Product A > Product B in 8 of 10 studies[a]	A > B[b]	A > B	A > B	B > A	B > A
Product GT vs. Product E[c]	Product GT = Product E in 1 study	GT = E	G > E	E > G	E > G	G > E

Note: Mean erythema scores and adverse sensory effect data were taken from Table 27.1. In addition, several other studies were included in which products A and B were compared (data not shown). DAP results were taken from Table 27.3.

[a] Seven studies were conducted on complete products A and B, and three were conducted on the product topsheets.

[b] Sensory effects collected in one study on complete products.

[c] Topsheet for product GT tested in DAP.

effects reliably reflect an increase in irritation, as measured by scoring erythema. This correlation may be improved by modifying the manner in which data on sensory effects are collected. Currently, panelists are given eight different terms to describe an unpleasant sensation, including rubbing, sticking, chafing, burning, itching, pain, or "other." By providing eight different choices, and treating these as separate end points, we may have inadvertently designed the experiments in a way that undermines our ability to detect trends. Statistical differences for any one of the eight choices may be extremely difficult to achieve with a panel of only 15–18 individuals. Modifying the sensory diaries to reduce the number of choices that panelists can choose to describe any unpleasant sensation may improve our abilities to discriminate based on sensory effects.

Collecting sensory responses in tests designed to evaluate overall skin compatibility had the potential to provide valuable information on the overall acceptability of a product that can aid in the development process. In the textile industry, physical attributes of fibers and fabrics determine the overall comfort of apparel. The sensory signals associated with clothing comfort have been grouped into four categories of sensations [21], including tactile (prickly, tickling, rough, itchy, etc.), moisture (clammy, wet, sticky, etc.), pressure (snug, loose, heavy), and thermal (cold, hot). There is considerable overlap between these groupings and the descriptive terms used in the DAP. As shown in Table 27.3, specifically trained individuals who conducted evaluations in the DAP were able to clearly differentiate between products A and B for four key product attributes (two positive and two negative). In contrast, products G and E produced mixed results, resulting in no clear conclusion on which product would be superior overall.

The end points used in the DAP are described differently than the sensory effect descriptors used in the BTK; however, the same product attributes are being evaluated. This is illustrated in Table 27.4, which provides a qualitative summary comparing the results on products or topsheets tested in the BTK and in the DAP. As mentioned above, in most BTK tests (8 of 10 studies), product A produced significantly higher mean erythema scores than product B. In the one BTK study where sensory effects were collected, adverse sensations were reported significantly more often with product A than product B. This is consistent with the positive and negative product attributes identified in the DAP, where product A had significantly higher scores for the negative attributes and significantly lower scores for the positive attributes. When products G and E are compared in the BTK, they are similar with regard to both mean erythema scores and adverse sensory effects. Likewise, these products could not be clearly differentiated in the DAP, where each product scored higher in one of two positive attributes and in one of two negative attributes.

For consumer products intended for contact with the skin, manufacturers have long recognized that certain product attributes associated with the "feel" of the product are important for product acceptability. These same attributes may also contribute to overall skin compatibility. Identifying these attributes, and the right descriptors for those attributes, is a logical next step.

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28 Silicones
A Key Ingredient in Cosmetic and Toiletry Formulations

Isabelle Van Reeth

UNIQUE MATERIALS

Silicone is a generic name for many classes of organosilicone polymers that consist of an inorganic siloxane (Si–O) backbone with pendant organic groups (usually methyl). It is this structure that gives silicones their unique combination of properties and, in particular, their surface properties (Figure 28.1). The starting material for silicone is silicon metal. While silicone itself does not exist in nature, silicon is derived from quartz (SiO_2). Silicones are obtained in a three-step synthesis—chlorosilane synthesis, chlorosilane hydrolysis, and polymerization and polycondensation—resulting in an infinite assortment of silicone fluids, elastomers, gels, and resins [1].

SILOXANE BACKBONE

The prime role of the siloxane backbone is to present the available methyl groups to their best advantage and it does this by virtue of its unique flexibility. In most hydrocarbons, the bond angles are very rigid, and steric packing considerations often prevent the available methyls from adopting lowest surface energy orientations. In silicones, the Si–O bond length is significantly longer and the Si–O–Si bond angle flatter than comparable C–C and C–O bonds resulting in a very low barrier to rotation and making the polymer chains very flexible. This flexibility makes many orientations possible and provides "free space" to accommodate different sized substituents or to allow easy diffusion of gaseous molecules: a property useful in the formation of "breathable" films. Coupled with the low intermolecular forces between methyl groups, this flexibility also has a profound effect on the bulk as well as the surface properties of silicones, seen in the small variation of physical parameters with temperature and molecular weight, the low freezing and pour points of fluids, the high compressibility, and the retention of liquid nature to unusually high molecular weights. It also makes a number of structural and compositional variations possible, resulting in many families of silicones, including linear and cyclic structures, a wide range of molecular weights, and varying degrees of branching or cross-linking.

Additionally, the siloxane bond is exceptionally strong providing the polymer with a high degree of thermal and oxidative stability and ensuring stability in formulation [2–4].

PENDANT ORGANIC GROUPS

The key function of the organic (methyl) groups is to provide the intrinsic surface activity of the silicones. The order of increasing surface energy for single carbon-based groups is –CF3 > –CF2– > –CH3 > –CH2–. Liquid surface tension measurements show that, as expected, the order of increasing surface activity is hydrocarbon–silicone–fluorocarbon. Interfacial tension measurements against water, however, show the order of increasing interfacial activity to be fluorocarbon–hydrocarbon–silicone. Silicones do not fit the simple pattern that a reduction in surface energy means an increase in hydrophobicity and interfacial tension because of their backbone flexibility, which allows them to adopt various orientations at different interfaces. The interfacial tension of silicone is also independent of chain length indicating high molecular chain freedom. In addition, critical surface tensions of wetting values for silicones have been found to be higher than their liquid surface tension values, meaning that they are able to spread over their own absorbed film. This has an advantage in achieving complete, uniform surface coverage; facilitates the efficient spreading of other materials; and results in smooth, lubricating films.

In addition, due to the organic groups, the solubility parameters of silicones are significantly lower than those of water and many organic materials making them useful in forming barriers to wash off, improving wear and increasing the substantivity of formulations.

The introduction of functional groups such as phenyl, alkyl, polyether amino, etc., onto the backbone expands the properties and benefits of silicones further [2–4].

KEY INGREDIENTS IN THE COSMETICS AND TOILETRIES INDUSTRY

Silicones were first used in the cosmetics and toiletries industry in the 1950s, when low levels of medium-viscosity dimethicone (polydimethylsiloxane [PDMS]) were used to prevent the whitening effect, characteristic of soap-based skin lotions. It was not until the 1970s, when formulators were concerned about the use of chlorofluorocarbons in aerosols, that silicones were considered more seriously as possible ingredients for cosmetic formulations, and their unique

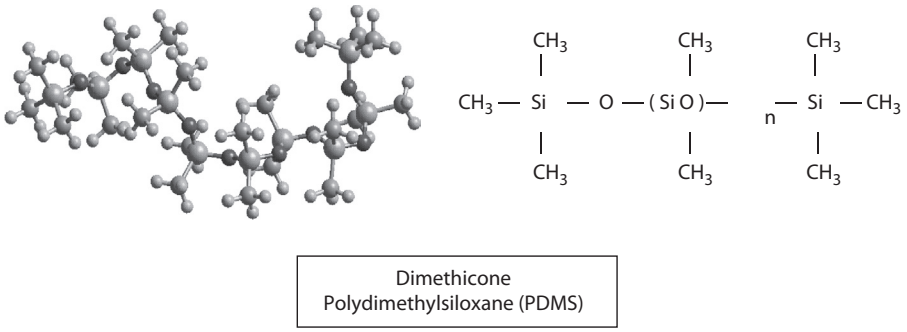


FIGURE 28.1 Unique chemical structure of silicones.

properties began to be recognized. Since then, the use of silicones has expanded rapidly to virtually all segments, and today, more than 50% of all new products being introduced into both US and European markets contain silicone, with many different types being used [5].

There are five main families of silicones that are used in the cosmetics and toiletries industry today:

1. Volatile siloxanes (cyclosiloxanes, ring structure) and low molecular weight linear PDMSs. They can be used as such or blended with each other to adjust the volatility. They are good solvents and serve as carriers for high molecular weight silicones that would otherwise be very difficult to handle. In addition, they have very low heats of vaporization compared to water or ethanol giving them a noncooling feel when drying. Cyclomethicones are classified as nonvolatile organic compounds (non-VOCs) in the United States (Figure 28.2).
2. Dimethicones (PDMSs) are linear structures with molecular weight ranging from 700 to more than 100,000. The nonvolatile dimethicones exist as fluids with viscosities of 5.0 mm^2/s up to gums. They are one of the most widely utilized silicone materials in cosmetic and personal care. They exhibit unique physical properties that render them effective for conditioning and protecting hair and skin and improving sensory attributes of formulations [6]. Dimethicone emulsions make handling of higher molecular weight fluids easier. Specialized emulsion polymer technologies allow the production of ultrahigh molecular weight linear PDMS emulsions with an internal dynamic viscosity superior to 100 million mm^2/s.
3. Silicone blends consist of dimethiconol or dimethicone gums or silicone resins (highly cross-linked silicones known under the following INCI names: trimethylsiloxysilicates, polymethylsilsesquioxanes, or polypropylsilsesquioxanes) dispersed in lower molecular weight dimethicones, cyclomethicones, and organic solvents such isododecane. The solid resins are available as neat powders or flakes. They have been developed to improve ease of formulation and compatibility of high molecular weight gums or

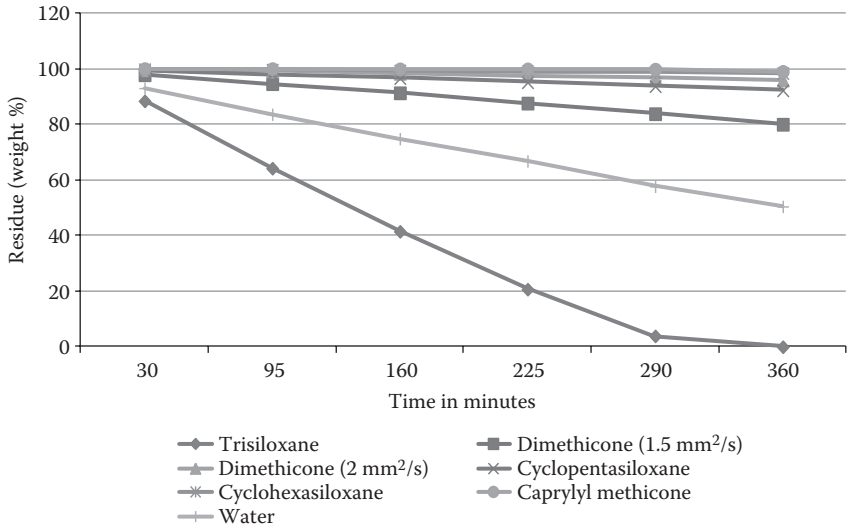


FIGURE 28.2 Comparison of volatility for volatile methylsiloxanes (1 g, room temperature, 20°C–25°C). When taking into consideration the skin temperature (32°C) and the ability of silicone to spread very easily, the volatility profile will be different. For example, in the case of cyclopentasiloxane, no residue is left on the skin after 15 min as measured with sebumeter equipment on the panelist forearm.

resins. Silicone resins are primarily used for their substantivity [7] and nontransfer properties [8].

4. Dimethicone and vinyldimethicone cross polymers or blends are silicone elastomers that are cross-linked to different degrees, resulting in different product forms. They exist as free flowing powders, pure or coated with particles, as powder suspensions, or as elastomeric silicone gels that are swollen with solvent (usually volatile silicone). The introduction of different functionality into such products is also possible, increasing their compatibility with organic materials or allowing the incorporation of water [9]. They are used as rheology modifiers in skin care and antiperspirant products, providing a dry, powdery feel to formulations [10].
5. Functional silicones:
   a. Dimethicone copolyols (silicone polyethers) are fluids or waxes where some of the methyl groups along the siloxane backbone have been replaced with polyoxyethylene or polyoxypropylene groups. The addition of polyoxyethylene substituents increases the hydrophilicity of silicones. Polyoxypropylene substituents are used to balance out this hydrophilicity by increasing the hydrophobic characteristics of the copolymer. Some of these silicone polyethers are also very good water-in-silicone emulsifiers [11]. The addition of alkyl chains results in a material able to emulsify water into low to medium polarity oils.

      Among these polyether modified silicones, silicone carbinol fluid (Bis-hydroxyethoxypropyl dimethicone) [12] is a very versatile material with good compatibility with organic materials that can act as a wetting agent to stably disperse pigments and actives, while also providing moisturization.
   b. Phenyl trimethicones are fluids where some of the methyl groups have been replaced by phenyl groups. The phenyl groups increase the refractive index, increasing the potential for shine, and improve compatibility with organic materials.
   c. Amodimethicones or trimethylsilylamodimethicones are fluids where some of the methyl groups have been replaced by secondary and primary amine groups. The polar amine groups have a profound effect on the deposition properties of the silicone, giving it an affinity for negatively charged surfaces, such as the proteinaceous surface of the hair. Emulsions of these fluids are commonly used. In the last 5 years, the offering of amino silicone derivatives has been increased with materials such as amino silicone quats (silicone quaternium-16) and amino silicone elastomer (silicone quaternium-16/glycidoxy dimethicone cross-polymer).
   d. Alkyl dimethicones are fluids or waxes where some of the methyl groups have been replaced by alkyl groups. This results in a family of silicone–hydrocarbon hybrids with possibilities for variations in viscosities, softening temperatures and rheological characteristics. They have increased compatibility with organic materials.
   e. Silicone acrylate copolymers (acrylates/polytrimethylsiloxymetacrylates copolymer). These film-forming copolymers can be delivered from both silicone and organic solvents and provide enhanced durability, wash-off resistance, and improved aesthetics coupled with easy formulation for skin care and color cosmetic formulations [13].

SKIN CARE, SUN CARE, AND DECORATIVE PRODUCTS

Skin Feel/Emolliency

The main reason that silicones are used in all types of skin care products is because of their sensory properties. Silicones give an entirely different feel and different solubility characteristic, extending the bench chemist's formulating possibilities [14]. Studies on the emollient properties of various materials have shown that silicones have high spread ability when compared to organic emollients, attributed to their low surface tension [15]. When mixed with vegetable oils, they improve the spread ability of the oils and decrease the greasy, heavy skin feel by lowering surface tension [16].

Volatile silicones are used for transient effects giving slight lubricity, a light texture, fast spreading, and good distribution of the product on application, while leaving no residual effects. They are often included in formulations to remove the greasy or oily feel of hydrocarbon-based emollients and are the basis for "oil-free" type claims [17].

An alky modified volatile silicone, caprylyl methicone, was found to decrease the film residue and the greasiness and tackiness of vegetable oils when compared to the pure oil as measured by paired sensory comparison [18]. They are used in light products for daily use such as facial cleansers, day creams, or liquid foundations.

Higher molecular weight silicones such as dimethicone and dimethiconol are used to give a more lubricious, longer lasting effect in richer, more nourishing skin treatment products such as night creams or after-sun products [19]. Silicone elastomers have a particular skin feel that has been described using terms such as "smooth," "velvety," and "powdery," and their skin feel can be further modified by the type of solvents used [10]. Thanks to their ability to incorporate water within their gel network, the so-called hydrophilic elastomers allow combination of the above sensory parameters with a refreshing feel, extending further the possibilities for new textures and feel [9].

Silicones are also noncomedogenic/nonacnegenic unlike many occlusive, lipophilic fatty emollients, which can promote comedone/acne formation on the skin [20].

Substantivity (Long-lasting/Durability)

High molecular weight dimethicone or cyclomethicone (and) dimethiconol blends and silicone resins form water-resistant films on the skin, which can help prolong the effects of skin care, sun care, or decorative products. Figure 28.3 compares the wash off resistance of different types of silicones using Fourier transform infrared spectroscopy with attenuated total reflectance applied on the volar forearm of panelists [21].

The use of silicones to improve the substantivity of other ingredients in cosmetic and toiletry formulations has been demonstrated in sun care products. The addition of alkylmethysiloxanes to a water-in-oil (w/o) sun care formulation containing organic sunscreens reduced the amount of sunscreens washed away using an in vitro method [22].

Volatile silicones are the basis for long-lasting/nontransfer decorative products, especially lipsticks. They are used to disperse waxes and pigments, improve application, and impart a pleasant skin feel, often replacing nonvolatile hydrocarbon oils. When they evaporate, a uniform film of waxes and pigments remains, which is resistant to transfer and wear [23].

Silicone acrylate copolymers and silicone resins have the ability to form solid nonocclusive films that resist wash-off but also reduce the transfer. This film-forming property is useful in sunscreens [24] and color cosmetics such as lipsticks, foundations, and eyeliners. By combining hard resin (trimethysiloxysilicate) and soft resins (polypropylsilsesquioxane), the flexibility of the final film can be optimized resulting in improved wearing comfort while maintaining the nontransfer performance [8].

Resin wax (alkyldimethylsilyl polypropylsilsesquioxane) is a hybrid material with both silicone resin and silicone wax properties, bringing texture to anhydrous systems and creams while improving the nontransfer performance especially in lipsticks [25].

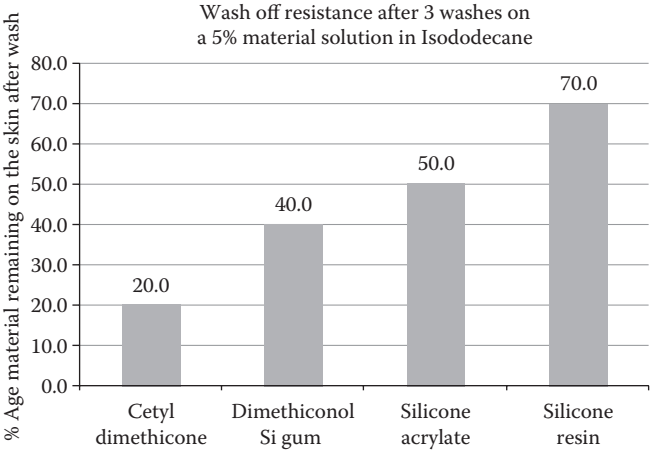


FIGURE 28.3 Wash-off resistance of different silicones.

Permeability/Controlled Moisturization/ Protection against Dehydration

Due to the flexibility of the Si–O–Si backbone, the majority of silicones are permeable to water vapor, producing "breathable" films. This is an important parameter for cleansing products and color cosmetics to avoid clogging pores. The presence of an alkyl group in the chain, however, reduces this permeability, resulting in semipermeable silicones, for example, cetyl dimethicone, or occlusive material similar to petrolatum, for example, C30-45 alkyl methicone (Table 28.1) [8,25,26].

Enhanced Efficacy

Apart from improving the feel and long-lasting benefits of skin care products, silicones can also enhance the efficacy of other ingredients in the formulation. Studies carried out on sun care products have shown that the alkyldimethicones can enhance the SPF of products containing either organic or inorganic sunscreens. A 2% addition level of stearyl dimethicone into an oil-in-water silicone containing 11% of organic sunscreens resulted in an in vivo SPF of 49.7, an SPF/UVB ratio of 4.5, and thus demonstrating high efficiency [24]. For inorganic sunscreens, a 100% increase in in vitro SPF was seen with an oil-in-water system containing 2 wt% cetyl dimethicone and a 75% increase in the SPF for a w/o system containing C30-45 alkyl methicone [22].

In antiaging formulations, the addition of silicone elastomer powders, at a level of 4%, has been shown to provide wrinkle masking benefits in addition to unique skin feel [27].

By combining silicone elastomers and silicone emulsifiers, it is possible to formulate highly stable glycerin in silicone systems, allowing the stabilization of as much as 10% vitamin C while maintaining an acceptable sensory profile [11].

Protection

Combining their film-forming properties and their low surface tension resulting in water repellency, silicones can be good ingredients for the formulation of barrier creams.

TABLE 28.1
Permeability of Different Materials Payne Cup Method

Material	Water Vapor Permeability (g/m²/h)
Volatile silicone	156
Silicone gum	148.6
PDMS	107
Mineral oil	98
Silicone resin	87
Silicone resin wax	51
C18 Alkylmethylsiloxane	37
C30 Alkylmethylsiloxane	2
Petrolatum	0

Dimethicone is listed in the FDA Monograph for Skin Protectant Drug Products for OTC Human Use in the United States. Due to their hydrophobicity, silicones are used in protective hand creams to provide a water-resistant barrier against water-borne contaminants. As early as the 1950s, an ointment containing 30% dimethicone was demonstrated to be effective in the treatment of patients with various kinds of dermatological disease, especially in conditions caused or aggravated by water-soluble or oil-soluble irritants [28].

Cleansing

The excellent spreading characteristics, dry non-greasy/oily feel, and good solvency of volatile silicones make them ideal for use in skin cleansers to help lift and remove dirt without stinging. They can be used alone or in combination with ingredients such as mineral oil. Silicone emulsifiers allow low viscosity silicone fluids to be present in the continuous phase. The incorporation of polar ingredients such as water, glycerin, etc., allows formulation of rinseable foaming facial washes [29].

Volatile alkyl silicones (caprylyl methicone) were demonstrated to have superior cleansing properties over cyclopentasiloxane and isohexadecane, ingredients often used in biphasic makeup removers [18].

Powdered silicone elastomers have the capability of absorbing lipophilic materials such as sebum from the skin, making them very useful for greasy skin application [27].

Water-soluble dimethicone copolyols have shown benefits in foaming facial washes. They provide creamy, dense foam, and improve foam volume. In liquid body cleansing products such as foam baths, shower gels, and liquid soaps, they can improve foaming and foam stabilization. They have also been recognized as additives that reduce eye and skin irritation from anionic surfactants [30].

Emulsions of ultrahigh molecular weight linear silicones can be perceived on the skin at very low levels due to their very low sensory threshold [31].

Rheology Modification/Structural Integrity (Sticks)

Aside from improving the aesthetics of formulations, silicones can also act as rheology modifiers. This is particularly applicable to w/o or water-in-silicone type systems. One such silicone rheology modifier is the C30-45 alkyl methicone where 149% and 93% increases in emulsion viscosity have been observed for water-in-silicone and w/o emulsions, respectively, with 2 wt% of the wax [32]. Resin wax (alkyldimethylsilyl polypropylsilsesquioxane) also acts as a rheology/texture modifier for w/o systems but especially in lipsticks where high levels can be used while maintaining very good payout and improving nontransfer performance [25]. Rheology modification using 2–4 wt% stearyl dimethicone is believed to be part of the reason for the SPF enhancement of sun care products containing organic sunscreens [33]. Silicone elastomers can be used to modify the rheology of skin care and antiperspirant formulations. Such elastomers have the capacity to absorb large amounts of solvents such as cyclomethicone or low viscosity dimethicone without exhibiting any syneresis. It is this property that allows them to successfully thicken formulations. The ability of elastomers to significantly modify the rheology of a formulation combined with their unique powdery feel has led to their use in antiperspirant products [10].

Formulating Flexibility

Silicones can be used in all types of skin care products ranging from simple oil-in-water gels or emulsions to water-in-silicone and w/o emulsions, from crystal clear to white in color. Silicones containing phenyl, alkyl, and carbinol functionalities have improved compatibility with organic materials, including organic sunscreens such as ethyl hexyl methoxycinnamate, making their use quite straightforward even if heating might be required due to the presence of waxes. The grafting of polyether functionalities on the silicone backbone results in total water solubility, allowing clear aqueous formulations with the benefits of silicones [30]. Silicone emulsifiers increase this flexibility further. They allow silicones to be present in the continuous phase as well as to incorporate polar ingredients such as water, glycerin, etc. Matching the refractive index of the water phase with the oil phase in such emulsions makes the formulation of clear gels possible [34], and adjusting the phase ratio determines the product form from lotions to gels. This technology is the basis for the clear antiperspirant gels seen on the market today. It is also possible to make nonaqueous emulsions using silicones to deliver hydrophilic ingredients or those that are sensitive to hydrolysis. In addition, the benefits of w/o systems such as good sensory profiles, improved wash-off resistance, and excellent moisturization have been demonstrated. Silicone emulsifiers offer versatility for low or high shear systems, as well as cold processing, presenting new opportunities for cost-effective and highly innovative skin care and underarm products [11]. Silicone elastomers have also been modified to increase their formulation flexibility by the incorporation of polypropylene glycols within the elastomer network. The incorporation of polypropylene glycols results in a significant increase in compatibility with organic materials such as esters and organic sunscreens. Furthermore, the introduction of a polar functionality such as PEG-12 allows the incorporation of up to 75% of water, potentially containing water-soluble actives. High levels of glycerin can also be incorporated, impacting positively the moisturizing potential of the final formulation [9].

HAIR CARE PRODUCTS

Hair Conditioning/Improved Combing

Various types of silicone are used to give different degrees of hair conditioning. Dimethicone copolyols provide light conditioning due to their solubility in water and low level of substantivity. They can also help reduce eye irritation associated

with shampoos and similar products that contain anionic surfactants [30]. Higher molecular weight dimethicones/dimethiconols or trimethylsilylamodimethicones/amodimethicones provide a higher level of conditioning due to their insolubility in water and greater substantivity. The latter have an affinity for negatively charged surfaces such as the proteinaceous surface of the hair that contributes to their substantivity [7].

Evaluation of the average detangling times of dimethiconol (gum), amodimethicone, and dimethicone (high viscosity fluid) emulsions in two-in-one shampoo formulations indicates that they all show significant improvement over the untreated control tress [35].

Synergistic effects have been observed between quaternary polymers commonly used in shampoos for conditioning and silicone polymers, resulting in improved conditioning performances [36].

Similar evaluation of silicones in conditioners indicates that dimethicone emulsions provide good conditioning effect in rinse-off products. Combinations of silicones such as cyclomethicone, silicone blends, and phenyl trimethicone are the basis for anhydrous leave-in conditioners, sometimes referred to as "cuticle coat" products [37].

Sensory Enhancement

As in skin care, silicones impart a soft, smooth feel to the hair. Sensory evaluations of cuticle coat formulations consisting entirely of blends of silicone have shown that, in addition to ease of combing, they improve spreadability, silkiness and softness, gloss, and perceived repair of split ends compared to the control [37].

By adding 2%–3% of an emulsion containing a blend of high molecular weight dimethicone and amodimethicone (60% active) to a shampoo or a conditioner, improvement in

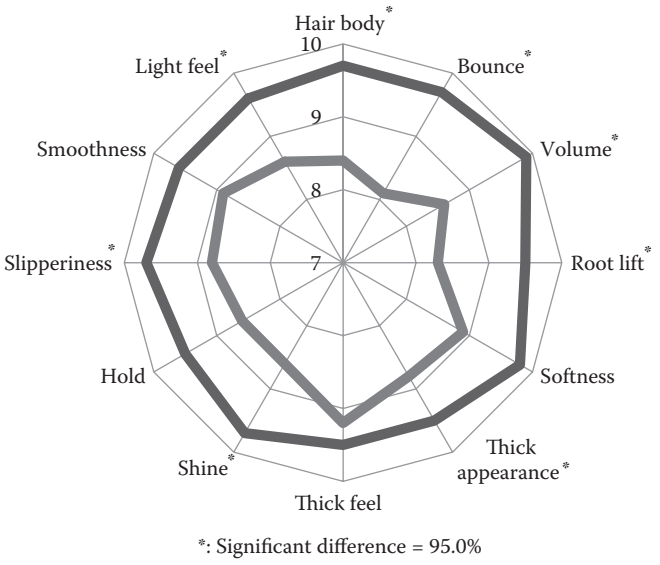
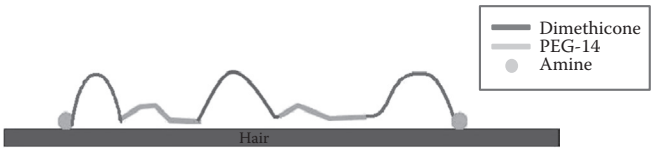


FIGURE 28.4 Half head test results with conditioner containing ABn amino polyether silicone polymer.



Silicone provides premium softness
Polyether (EO-based) imparts hydrophilicity
Amine for anchoring to hair

FIGURE 28.5 Potential mechanism of action of the ABn amino glycol silicone polymer.

the hair moisturization perception (defined as an improvement of combing, slipperiness, and flexibility of dry hair) can be perceived. New types of amino glycol silicone polymer with ABn structure (INCI:Bis-diisopropanolamino-PG-propyl dimethicone/Bis-isobutyl PEG-14 copolymer) delivered from an emulsion also provide this hair moisturization perception from both shampoos and conditioners while maintaining very good conditioning performance.

Performance was demonstrated using half head testing from a conditioner using 1% of the polymer. This is illustrated in Figures 28.4 and 28.5.

Foam Boosting

Dimethicone copolyols can be used to boost the foaming properties of shampoos as well as provide a light conditioning effect [38].

Reduced Flyaway

Tests comparing hair care compositions containing dimethicone copolyols show an improvement in static control. Sensory evaluation has also shown a reduction of flyaway hair with dimethicone emulsions [36].

Improved Shine

Silicones, in particular, phenyl trimethicone and amino silicones, are recognized for their ability to enhance hair shine and gloss along with adding softness, manageability, and smoothness to the abraded hair cuticle [36,39].

Natural-Look Fixatives

Because of their low surface tension, silicones spread easily to help fixative products distribute evenly on the surface of hair, improving their effectiveness. They are also used in conjunction with or as a replacement for organic plasticizers. Organic materials tend to be hydrophilic, which can diminish the holding power of a resin. In contrast, the hydrophobic nature of silicones helps repel water so there is less opportunity to reduce the resin's holding properties. The use of dimethicone copolyol as a resin plasticizer can also help give hair a more natural look [35].

Longer Lasting Permanent Wave and Coloring Products

Silicones, such as trimethylsilylamodimethicone, can be used to provide a more durable conditioning effect and a longer lasting permanent wave. Pre-treatment containing silicone blends help prevent hair damage during the harsh perming process. In hair color products, blends of volatile and nonvolatile silicone (cyclomethicone and trimethylsilylamodimethicone) can be used to seal the hair cuticle and hold color. The volatile silicone evaporates, leaving behind a smooth, uniform film on the surface of the hair [40].

Amino-functional silicones can decrease the degradation of hair color due to the combination of UV exposure and washing and provide conditioning benefits such as ultra shine, improved drying time, moisturization, and a smooth and light feel without negatively impacting hair body and volume [41].

Evaluation of rinse-off conditioners indicates that silicones show excellent potential to provide color lock properties in permanent hair color products.

Hair Strengthening

Amino functional silicones leave a medium-weight film deposited primarily at the open edges of the cuticle, resulting in improved hair strength. Two possible mechanisms could explain this effect [42]:

1. The film may protect the hair cuticle during elongation.
2. The film may assist in sealing the hair cuticle, helping prevent moisture penetration into and out of the hair cortex, maintaining an optimal moisture level for hair strength.

Hair Repair

All hair has some degree of damage due to various causes including brushing, styling, coloring, and perming. Environmental conditions such as UV exposure result in hair damage: frizz, dryness, breakage/split ends, lack of body, or even static and unmanageability.

By treating damaged hair swatches with leave-in conditioner containing 2% PDMS derivatives such as ultrahigh molecular weight PDMS (INCI: divinyldimethicone/dimethicone copolymer), a quaternized amino silicone (silicone quaternium-16) and an amino silicone elastomer (silicone quaternium-16/glycidoxy dimethicone cross-polymer), the following parameters were positively impacted:

- Glide and smoothness were restored.
- The number of hair tangles was decreased.
- The raspy feel of dry hair was reduced.
- The visual signs of damaged hair were reduced, leading to improved beauty and appearance.

These results were achieved when the silicones were delivered from an emulsion, important for ease of formulation. Additionally, using a repeated combing test, 52% and 78% improvement in hair resistance to breakage was measured with the ultrahigh molecular weight PDMS or the amino silicone elastomer emulsion [43].

Optical microscopy demonstrated an improved deposition on the damaged zones of the cuticle when hair fibers are treated with leave-in conditioner containing the above PDMS derivatives. Wettability of untreated hair versus silicone-treated hair has also been studied, showing that hair hydrophobicity can be recovered. It is thought that PDMS derivatives are helping to form a deposit especially on damaged fiber areas. Cuticles are lifted back while damaged zones of cuticle ends are more particularly conditioned. By improving fiber surface smoothness and slipperiness, and by decreasing fiber-to-fiber friction, the use of these siloxane derivatives leads to repair of damaged hair fibers, decreasing the tactile and visual perception of dryness [44].

ANTIPERSPIRANT AND DEODORANT PRODUCTS

In addition to the benefits that silicones bring to skin care products such as improved feel, delivery of actives, low residue, formulating flexibility, etc., the following advantages are seen in antiperspirant and deodorant formulations.

Antiwhitening

Dimethicones and phenyl trimethicone have been shown to reduce/mask the whitening effect caused by antiperspirant salts by allowing refractive index matching [45].

Improved Spray Characteristics

Low levels of cyclomethicone and dimethiconol have been demonstrated to reduce the spray width, height, and particle size of antiperspirant pump spray and aerosol formulations, leading to a more directional spray with low mist and dust [45,46]. The silicone blend may also contribute to the substantivity of the antiperspirant active and lubricate the spray valve to prevent clogging.

Noncooling

The heat of vaporization of volatile silicones such as cyclomethicone is much lower than that of water or ethanol, which means that much less energy is required for them to evaporate. This leads to a noncooling effect in formulation [45].

CONCLUSION

The multifunctional benefits of silicones make them invaluable ingredients in cosmetic and toiletry formulations. Today, the choice is highly leveraged, giving the bench chemist flexibility to combine different materials resulting in advanced performance. When navigating the increasingly complex

global regulatory situation, silicones can provide a valuable path to move quickly to market.

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29 Tribology of Skin

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INTRODUCTION

Tribology is the science of friction and studies the friction properties and characteristics between surfaces. Because the skin is a surface, tribological studies can offer insight into its characteristics and how these characteristics alter with changes in age, health, and hydration [1]. They can also track changes induced by exogenous moisturizers and chemicals [1]. Two tribological parameters that have been studied in human skin include a direct one, the friction coefficient, and an indirect one, the electrical impedance, which are investigated by using noninvasive methods to quantitatively assess the skin surface.

COEFFICIENT OF FRICTION

The coefficient of friction is measured by moving a surface tangentially to the skin while they are in contact. The perpendicular force between the two surfaces is defined as the normal force (N), and the friction force (F_f) is the tangential force that opposes relative motion between the two surfaces. The coefficient of friction (μ) is defined as the ratio of the friction force to the normal force according to Amontons' law:

$$\mu = \frac{F_f}{N}$$

The friction coefficient is described in two forms: the static friction coefficient (μ_s) and the dynamic friction coefficient (μ_d). The static friction coefficient refers to the friction that builds up prior to *initiation* of relative movement. The dynamic friction coefficient refers to the friction that exists while two forces are moving relative to one another. Most work has focused on the dynamic coefficient of friction, although some have studied the static coefficient of friction.

Classically, by Amontons' law, the dynamic friction coefficient remains constant irrespective of the perpendicular force or the velocity of the relative movement. Several studies have assessed whether this law holds true for human skin. Although Naylor [2] concluded Amontons' law to be true for skin, other studies [3–6] determined that the skin deviates from it. El-Shimi [4] and Comaish and Bottoms [3] reasoned that the viscoelastic nature of human skin lead to nonlinear deformation of the skin with reduced normal loads. In addition, the presence or absence of hydration on the skin can change the friction coefficient with velocity [2,4,7].

Overall, two methodologies have been devised to measure the friction coefficient on skin. Either the probe is moved across the skin in a linear fashion [3] or a rotating probe is placed in contact with the skin surface [8,9].

Because friction measurements depend on the interaction of two surfaces, the shape, size, material, and the texture of the probe will affect friction coefficient measurements. Table 29.1 outlines various probes that have been used. More accurate results are possible when the normal force and the velocity are controlled, and the presence of repetitive springs [9] or motorized apparatuses with feedback control of the normal force and the velocity [6] allows for greater control during measurements (Table 29.1).

Previously measured values for the friction coefficient are outlined in Table 29.2. Special mention should be made for friction measurements of the fingers, palms, and soles because these parts of the body are more often in contact with another surface. Since common activities such as gripping handrails, gripping car steering wheels, or holding a cup involve the static friction coefficient more than the dynamic friction coefficient, it may be more relevant to measure static friction coefficients at these sites. Some authors have studied the dynamic friction coefficient of the palm [10,11], while other authors have studied the static friction coefficient of the palms and fingers [12–14]. Increased sweating in the hands and feet [3], and increased roughness (due to the presence of epidermal ridges [11]), may contribute to increased friction coefficients.

ELECTRICAL IMPEDANCE

In tissue, electrical impedance describes a measure of resistance to current flow and extends the concept of resistance to current flow in circuits [22]. Electrical impedance measured at one position at an anatomical site gives unipolar impedance, whereas the electrical impedance measured at two different positions of an anatomical site yields bipolar impedance. The electrical impedance is useful in measuring skin moisture content and hydration [23,24].

Electrical impedance can be used to assess skin hydration in normal skin [23,24], diseases such as atopic dermatitis [25,26], and after interventions that increase or decrease the moisture content [20,26]. Seasonal variations for different skin sites must be taken into consideration along with interindividual variability when interpreting results [27]. Electrical impedance has been suggested as a possible screening tool against skin neoplasms [28], but more clinical studies will be needed to assess its utility.

TABLE 29.1
Characteristics of Friction Coefficient Measurement Methodology

Author	Probe Size and Shape	Probe Material	Motion of Test Apparatus	Maintenance of Normal Load
Asserin et al. [15]	Sphere	Ruby	Linear	Balloon; static weights
Comaish and Bottoms [3]	Annular ring	Teflon, nylon, polyethylene, wool	Linear	Static weights
Cua et al. [10]	Disc	Teflon	Rotational	Spring load
Egawa et al. [16]	Square	Piano wire	Linear	Computer controlled
El-Shimi [4]	Hemisphere	Stainless steel (rough), stainless steel (smooth)	Rotational	Static weights
Elsner et al. [17]	Disc	Teflon	Rotational	Spring load
Highley et al. [8]	Disc	Nylon	Rotational	Spring load
Johnson et al. [18]	Lens	Glass	Linear, reciprocating	Static weights
Koudine et al. [5]	Hemisphere lens	Glass	Linear	Static weights; balance beam
Li et al. [12]	Planar surface	Sandstone, slate, granite	Linear	Supplied by the participant
Naylor [2]	Sphere	Polyethylene	Linear, reciprocating	Static weights
O'Meara and Smith [13,14]	Cylindrical "grabrail"	Chrome, stainless steel, powder-coated steel, textured aluminum, knurled steel	Linear	Supplied by the participant
Prall [19]	Disc	Glass	Rotational	Spring load
Sivamani et al. [6]	Sphere	Stainless steel	Linear	Computer controlled
Sivamani et al. [20]	Cylinder	Copper	Linear	Computer controlled
Zhang and Mak [11]	Annular ring	Teflon	Rotational	Spring balance

FACTORS THAT INFLUENCE TRIBOLOGICAL MEASUREMENTS

Normal Load

Wolfram provided a theoretical analysis where he shows that the dynamic friction of the skin should relate to the normal load as [29]:

$$\mu \propto N^{-(1/3)}$$

where N is the applied load normal to the skin. Koudine et al. [5] found this dependence to hold experimentally:

$$\mu \propto N^{-0.28}$$

and this was confirmed by the analysis of Sivamani et al. [6]:

$$\mu \propto N^{-0.32}$$

The normal pressure that is applied to the skin surface influences electrical impedance. In cervical tissue, it has been shown that pressure alters the impedance measurement [30]. Therefore, control of the normal force is essential in obtaining reproducible results.

Probes

The probe material and geometry influence the value of the friction coefficient. Several studies have evaluated the role of the probe: El-Shimi [4] studied probe roughness, Comaish and Bottoms [3] probe roughness and material, Zhang and Mak [11] studied probe material, Li et al. [12] studied various rock surfaces under static friction coefficient conditions, and O'Meara and Smith [13,14] studied static friction coefficient conditions for handrails of various materials.

Smoother probes resulted in higher friction coefficient measurements [3,4]. This was shown for several probe materials and designs including a stainless steel probe [4], a sheeted nylon probe [3], and a knitted nylon probe [3]. Smoother probes were postulated to form more contact points with the skin and have greater skin contact area than the rougher probe leading to higher friction coefficient values [4]. Zhang and Mak [11] tested several different probe materials and found that silicone probes resulted in the highest friction measurement. They proposed that probe materials such as silicone that have higher adhesion forces will have higher friction coefficients than another comparable material. Li et al. [12] found that sandstone produced a higher static friction coefficient than granite and slate. Smooth surfaces produce higher friction coefficients for dry or watery (nonsoapy) palm skin, whereas textured surfaces, like knurled steel or textured aluminum, produced higher friction coefficients against soapy palm skin [13].

Moisture

The local skin moisture is a multifaceted phenomenon influenced by multiple factors including age, anatomical site, ambient humidity, and chemical exposures. Hydration studies have correlated increases and decreases in skin hydration with the changes in the friction coefficient [7]. These studies reveal that drier skin has lowered friction while hydrated skin has an increased amount of friction [2–4,6,8,18–21,31–34]. However,

TABLE 29.2
Friction Coefficient Measurements

Author	μ
Asserin et al. [15]	0.7
Comaish and Bottoms [3]	0.2 (Teflon)
	0.45 (nylon)
	0.3 (polyethylene)
	0.4 (wool)
Cua et al. [10]	0.34 (forehead)
	0.26 (ventral forearm)
	0.21 (palm)
	0.12 (abdomen)
	0.25 (upper back)
El-Shimi [4]	0.2–0.4 (stainless steel rough)
	0.3–0.6 (stainless steel smooth)
Elsner et al. [17]	0.48 (forearm)
	0.66 (vulva)
Egawa et al. [16]	0.2–0.3
Gerhardt et al. [21]	Dry: 0.41 ± 0.04 (men) and 0.42 ± 0.03 (women)
	Moist: 0.56 ± 0.06 (men) and 0.66 ± 0.11 (women)
Highley et al. [8]	0.2–0.3
Johnson et al. [18]	0.3–0.4
Koudine et al. [5]	0.24 (dorsal forearm)
	0.64 (ventral forearm)
Li et al. [12]	2.48–3.25 (rock types)
	3.00 (no-chalk on hands)
	2.47 (chalk on hands)
Naylor [2]	0.5–0.6
O'Meara and Smith [13]	1.44–1.91 (dry palm-active grip)
	1.10–1.92 (wet palm-active grip)
	0.34–0.64 (soapy palm-active grip)
O'Meara and Smith [14]	0.78–1.39 (dry palm-no grip)
	0.90–1.09 (wet palm-no grip)
	0.14–0.34 (soapy palm-no grip)
Prall [19]	0.4
Sivamani et al. [6]	0.33–0.55
Sivamani et al. [20]	0.45–0.65
Zhang and Mak [11]	0.40–0.62 (anatomical site)
	0.37–0.61 (probe material)

the skin response is more complex because lower friction coefficeints were noted in very wet skin [35]. Most studies have focused on a zone of hydration where the skin has been moistened without a "slippery" layer of water on the skin: this increased friction coefficient lasts only for minutes before the skin returns to its "normal" state [4,6,8,33]. Moisture is believed to change the topography of the skin and increase surface contact area [21,36], resulting in a higher friction coefficient [21,29]. On the other hand, reduced local moisture content is expected to reduce the friction coefficient. For example, isopropyl alcohol-induced drying of skin lowered the friction coefficient, and a lowered friction coefficient was also found in studies involving subjects with clinically dry skin [4,6,32]. The agreement between the experimentally induced dry skin and clinical dry skin is expected [37].

Electrical changes in the skin have also been utilized to measure hydration [38]. Skin with more hydration is more permissive of electrical flow and has a lowered resistance when compared to dry skin. Decreases in hydration have been detected with electrical measurement in clinical conditions that have a loss of hydration, like psoriasis [24] or atopic dermatitis [25,26,32].

Anatomic Region, Age, Gender, and Race

A few differences have been found with regard to gender [10,20,31] or race [20,39], although one study found that moisture-induced friction coefficient increases were higher among women [21]. Age-related studies have been debatable and may depend on the anatomical site that is used for comparisons.

The friction coefficient varies with anatomical site. Manuskiatti et al. [39] studied skin roughness and found significant differences in skin roughness at various anatomical sites. Zhang and Mak [11] found the coefficient of friction to differ from 0.40 on the leg to 0.62 on the palm. Cua et al. [10,31] found that friction coefficients varied from 0.12 on the abdomen to 0.34 on the forehead and postulated that this was due to differing sebum and hydration levels in the skin of different areas of the body. Elsner et al. [17] measured the vulvar friction coefficient at 0.66, whereas the forearm friction coefficient was 0.48. Differences in environmental influences (i.e., sun exposure) and hydration may account for this. Sivamani et al. [20] found the friction coefficient to differ along the volar forearm with the proximal forearm to be more hydrated and therefore have a higher friction coefficient than the distal forearm. Normally, the proximal volar forearm tends to be covered by the arm when the elbow is flexed, and this can lead to less water loss and increased hydration than the distal volar forearm. Electrical impedance varies with anatomical site as well [25], although it was not found to vary along the volar forearm in areas where the friction coefficient varied [20].

With respect to age, cumulative sunlight exposure can increase collagen cross-linking and possibly alter skin friction. Cua et al. [31] found no significant age-related changes at various parts of the body including areas that were exposed to the sun. However, Elsner et al. [17] found a higher volar forearm friction coefficient in younger subjects but found no differences in the vulvar friction coefficient. They postulate that light-protected vulvar skin does not undergo photoaging and no resulting change in the friction [17]. However, Sivamani et al. [20] and Egawa et al. [16] found that the volar forearm did not change with age. This may be a reflection of variation in sun exposure among study populations and variations in the measurement sensitivity and methodologies.

Gender- and ethnicity-related friction studies have shown a few differences in the friction coefficient. Cua et al. [10,31] and Sivamani et al. [20] found no significant friction differences between the genders. However, one study noted a difference in the moisture-induced increase to the friction coefficient [21], although the mechanism and reason for this difference are not clear. Manuskiatti et al. [39] found no

differences in roughness and scaliness between black and white skin, and Sivamani et al. [20] found no differences in the friction coefficient or electrical impedance among subjects of Caucasian, African-American, Asian, or Hispanic/Latino descent or in their responses to treatments. Overall, race does not appear to influence the friction coefficient, and gender differences may exist in the presence of moisture but this remains to be further studied.

Emollients

Of interest to the cosmetic/moisturizer and lubricant industries is how the application of topical chemicals can influence the skin surface properties. Previous studies have investigated the effects of powder [3,4,12], oils [3,4,8,33], and skin creams/moisturizers [19,33] and how temperature alters the friction coefficient after emollient application [40].

Qualitative descriptions of skin include roughness, greasiness, and moisturization [29,41]. Previous reports have described these subjective, qualitative descriptions in a quantitative fashion through tribological measurements. Nacht et al. [33] found a linear correlation between perceived greasiness and the friction coefficient. Prall [19] found a quantitative correlation for skin smoothness by taking into account the friction coefficient, skin topography, and hardness into the analysis. Sivamani et al. [20] described an amplitude/mean measurement parameter that differentiates levels of greasiness between different emollient agents. When this marker is used in conjunction with electrical impedance, occlusive and directly hydrating agents could be differentiated. Occlusive agents, like petrolatum, lowered amplitude/mean measurements and increased the electrical impedance. On the other hand, directly hydrating agents, such as glycerin, increased amplitude/mean and increased the electrical impedance [20]. When used in conjunction, these two parameters may aid in comparatively assessing lubricants, emollients, and moisturizers.

The friction coefficient decreases after the application of powder [3,4], although the probe material may influence whether a change in the friction coefficient is detected after powder application [3]. Of note, wetted talc powder caused an increase in the measured friction [3]. Li et al. [12] also studied the effect of magnesium carbonate, or "chalk," in the static friction coefficient regime and found that the application of "chalk" decreased the static friction coefficient. This contradicts with the popular use of "chalk" to increase friction in rock climbing. Li et al. [12] suggests that the "chalk" can be useful in drying the climber's hands before the climb, but suggested that all traces of the chalk be removed before the actual climb. Other studies have found that particulates increase the friction coefficient, but these studies were done with two nonskin surfaces [42]. In light of the traditional use of powders to increase friction, such as the use of chalk for rock climbing, further studies evaluating the role of powders, the role of moisture, and the role of powder quantity are needed.

The friction coefficient decreases after application of oils and oil-based lubricants [4,8,33]. However, it has been noted that after the initial decrease in friction, the oils eventually elevate the skin's friction coefficient [8,33].

The friction coefficient increases with the addition of emollients and creams in a similar fashion to water [19,33]. However, the effects of the creams lasted for hours while the water effects lasted for about 5–20 min [6,19,33]. Hills et al. [40] also studied emollients, but they examined how different emollients compared against one another and how changes in temperature changed the friction coefficient. At a lower temperature (18°C), most emollients resulted in an increased friction coefficient compared to emollients at a higher temperature (45°C).

The skin friction is altered in three general ways when exposed to emollients/moisturizers [29,33]:

1. *A large, immediate increase in the friction coefficient with a slow decrease in the friction coefficient.* These agents act by immediately hydrating the skin through some aqueous means to give an immediate increase in friction.
2. *An initial decrease in the friction coefficient that is followed by an overall increase in the friction coefficient.* These agents are fairly greasy and cause an immediate decrease in the friction coefficient. The eventual rise in the friction coefficient is probably due to an occlusive effect that prevents water loss from the skin, thereby increasing the hydration of the skin.
3. *A small, immediate increase in the friction coefficient that then continues to increase.* These agents act by a combination of mechanisms seen in the previous two cases. These lubricants/moisturizers have ingredients and agents that both hydrate through aqueous mechanisms, and prevent water loss through occlusive mechanisms.

Soap

O'Meara and Smith [13,14] showed that the application of soapy water to the palmar surfaces decreased the friction coefficient 2–9 fold from the dry palm static friction coefficients. They also show that textured surfaces (e.g., knurled steel) produce higher friction coefficients against soapy skin than smooth surfaces (e.g., chrome) and suggest that textured materials may be more appropriate as handrails in showering areas [13].

CONCLUSION

The friction coefficient and the electrical impedance are tribological studies that can be used to quantitatively assess the skin. It can be utilized to understand how skin differs on various anatomical sites, between different clinical conditions, and under various environmental and chemical exposures.

The test apparatus design is critical. Skin tribology spans several important areas including the design of clothing, cosmetics, moisturizers, sporting equipment, and safety equipment (such as handrails).

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30 Skin Wettability and Friction

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INTRODUCTION

Hydrophobicity of soft tissue surfaces in the human body, including those of the human oral cavity, has been described for decades as playing an important role in many biological processes, like cellular adhesion [1], contact inhibition, elasticity [2], tissue membrane functions, intracellular structures [3], and adhesion of infectious microorganisms [4]. Generally, tissues with absorption and exchange functions, or when needed, lubrication, tend to be more hydrophilic. On the other hand, tissues requiring protection against pathogenic microorganisms or acids tend to be hydrophobic [5].

From a fundamental point of view, wetting is an important phenomenon because of its diverse applicability in everyday life. Friction and lubrication are intimately coupled to wettability.

In this chapter, we will first cover human skin wettability by showing the effects of some treatments and applications on wettability parameters. Second, the skin friction coefficient (μ) will be investigated through the effect of the surfaces hydrophobic/hydrophilic balance (Ho/Hi).

HUMAN SKIN WETTABILITY

Wetting refers to the contact between a solid surface and a liquid; it depends on intermolecular interactions. The degree of surface wetting is evaluated through the measurement of the contact angle. The smaller the contact angle manifests, the better the wetting of the surface. When the contact angle $\theta = 0°$, the surface wets completely; the opposite corresponds to $\theta = 180°$ (dewetting), and partial wetting refers to θ ranging from 0° to 180° (Figure 30.1).

Theory

Contact Angle and Superficial Energy

Young's equation (Equation 30.1) [6] relates to the surface tension between the liquid–vapor (γ_{LV}), the solid–vapor (γ_{SV}), the solid–liquid (γ_{SL}), and the free surface energy by contact angle (θ). The general form of this equation for the solid–liquid–air system is (Figure 30.2)

$$\gamma_{LV} \cos\theta = \gamma_{SV} - \gamma_{SL} - \pi_e \qquad (30.1)$$

where π_e (external pressure) = 0 for low energy solids [7].

Critical Surface Tension (γ_c) and Ho/Hi

Critical Surface Tension γ_c

The definition of γ_c is based on an empirical relationship between the cosine of the contact angle and the surface tension of a series of homologous liquid (Figure 30.3) [8]:

$$\cos\theta = 1 - b(\gamma_{liquid} - \gamma_c) \qquad (30.2)$$

where γ_{liquid} is the liquid surface tension (mJ/m²). Note that a reduction of γ_c means an increase in the surface hydrophobia.

Hydrophobic/Hydrophilic Balance

For decades, the surface hydrophobicity has been reported to play an important role in many biological processes, such as cellular adhesion, contact inhibition, elasticity, functionality of tissue membranes, functioning of intracellular structures, and adhesion of infectious microorganisms [9].

The skin hydrophobia balance (Ho/Hi) is quantified by a relationship between γ_c and the water surface tension [10]:

$$Hi = \gamma_c / \gamma_{H_2O} \qquad (30.3)$$

where Hi is the surface hydrophilia and Ho is the surface hydrophobia.

This parameter is expressed by the ratio of its critical surface tension γ_c to the water surface tension γ_{H_2O} normalized by the latter.

Free Surface Energy (γ_s)

Free skin energy is a topical parameter that determines most of the surface properties such as adsorption, wetting, adhesion, etc. The γ_s of the solids cannot be measured directly because of the very weak mobility of the molecular atoms. It is necessary to resort to indirect methods such as the study of the interactions between a solid and a liquid. γ_s is derived from the measurement of the contact angle of pure liquids, with known surface tension parameters.

Several approaches are mentioned in the literature; the two most commonly used for the skin are described below:

1. *Geometric mean approach* [11]: γ_s (mJ/m²) proportional to the intermolecular energy is the sum of the dispersion component γ_s^d and the polar component γ_s^p.
2. *Acid–base approach* [12,13]: γ_s can be expressed as the sum of Lifshitz–van der Waals γ_s^{LW} and acid–base

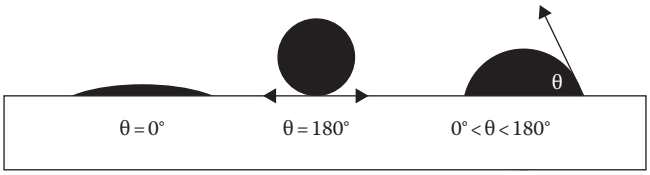

FIGURE 30.1 Solid wettability. θ = 0: total wetting; θ = 180°: nonwetting = dewetting; 0° < θ < 180°: partial wetting.

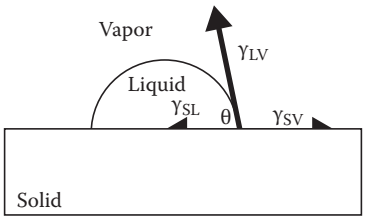

FIGURE 30.2 Equilibrium of a liquid drop on a solid surface: contact angle and surface energy.

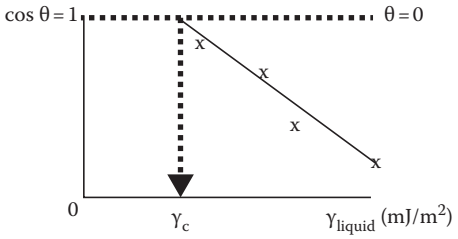

FIGURE 30.3 Critical surface tension γ_c: total wetting condition ($\gamma_{liquid} \leq \gamma_c$).

γ_s^{AB} components: $\gamma_s = \gamma_s^{LW} + \gamma_s^{AB}$. The acid–base components can be expressed as $\gamma_s^{AB} = 2(\gamma_s^+ \cdot \gamma_s^-)^{1/2}$; the γ_s^+ and γ_s^- components indicate, respectively, the electron-acceptor and the electron-donor components.

Contact Angle measurement

For the visualization and the measurement of the contact angle, we developed a tool especially designed to measure wettability in vivo (Figure 30.4). This tool is based on the use of a mirror directed at a 45° angle to the skin (profile drop method).

The advancing contact angle of test liquids, that is, the maximum value of the contact angle when the drop is inflated without the line of contact moving, is measured on the skin surface. A drop of test liquid is deposited on the skin surface using a microsyringe and inflated up to a final drop volume of 5 µl.

The drop's image is recorded using a video camera (CDD-Iris, Sony, France) connected to a computer and mounted on a microscope (Wild Heerbrugg M650, Switzerland), with a magnification of 16×, fitted with a slanted mirror. After visualization and storage of the drop profile, the contact angle is measured using a program that can determine θ from the tangents of both sides of the drop. The influence of roughness and skin temperature on the contact angle is treated in the literature [14–16]. The temperature effect in the liquid in contact with the skin is minimized with the nature of the deposit (advancing contact angle) and with the very short time of deposit (15–20 s).

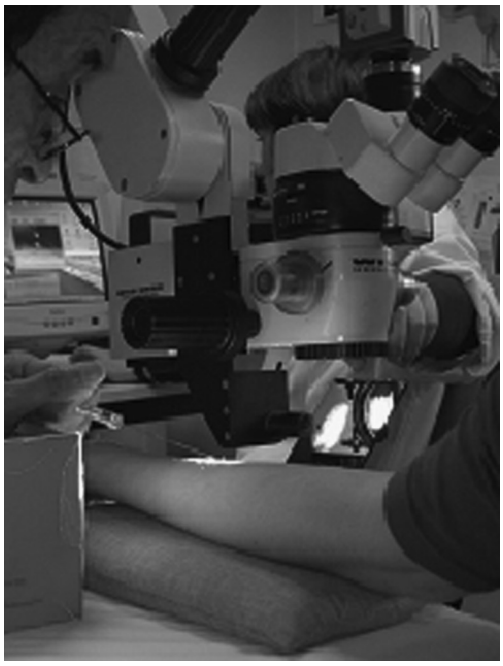

FIGURE 30.4 Contact angle visualization and measurement: measurements are based on the use of a mirror directed 45° to the skin; "profile drop method."

Data Analyses

Water Contact Angle θ_w

Skin

Water is an important factor for normal skin function. When the water content decreases, the skin becomes dry, itchy, and uncomfortable (Table 30.1). The spreading degree of a water drop on the skin surface is an indication of its hydrophobic (Ho) or hydrophilic (Hi) properties.

On the skin, water spreads differently. On the volar forearm, a poor sebum site, water forms a semihydrophobic contact angle θ_w (=80°–91°) [16–19]. On the forehead, a rich sebum site, water spreads out widely θ_w (= 57°–73°) [20–22]. A study of ten different sites (Figure 30.5) [20] confirmed that the skin poor in sebaceous lipids is a hydrophobic surface (θ_w = 91°–102°). In contrast, the sites rich in sebum are more hydrophilic (θ_w = 60°–85°). Fotoh et al. [21] showed that the forehead skin wettability is significantly different ($p < 0.05$) between Black people (African or Caribbean) (θ_w = 71°) and mixed races (African or Caribbean) (θ_w = 67°) or Caucasians (θ_w = 67°).

In a recent study, it was shown that the contact angle measured on the forehead of 60 children (aged 7–11 years) was higher than in adults (θ_w = 87°). It is worth noticing that the

TABLE 30.1
Human Skin Wettability

	Volar Forearm		Forehead	
	Untreated	**Degreasing "Ether"**	**Untreated**	**Degreasing "Ether"**
θ_w	80° [14]; 84° [15] 88° [7]; 91° [16]	92° [15]; 101° [7]	57°–73° [19]; 60° [17] 67°–71° [18]	84°[7]
γ_c	26 [20]; 26.8 [21] 27 [22]; 27.5 [23,24] 30.6 [15]	21.6 [24]	33.2	22.4
Ho	62% [10]	70% [10]	54%	69%
γ_s	38.5 [7,10]	32.4 [7]	42–46 [19]	34.5 [7]

Note: θ_w: water contact angle; Ho: surface hydrophobicity; γ_c: critical surface tension (in mJ/m^2); γ_s: surface free energy (in mJ/m^2).

sebum level measured on these children was particularly low (17 µg/cm^2) [23,24].

Hair

Virgin hair shows a mean thickness of 1.1 nm. The outermost layer of virgin hair surface is made up of fatty acid, primarily 18-methyleicosanoic acid (18-MEA), which strongly contributes to the hydrophobicity (θ_w = 103°) and lubricity of virgin hair [25]. Due to its hydrophobicity, its surface is free of any water film, and therefore, film thickness on the surface is measured as very low. Damaged hair, however, is slightly hydrophilic due to the removal of the fatty acid layer during damaging processes (θ_w = 50°–80°) [25].

Nail

In vivo, the nail is a hydrophilic surface with θ_w = 67° (Figure 30.6a). No significant difference was found in relation to ethnicity (France, China, Iran, and Morocco) or sex [26].

Critical Surface Energy γ_c and Ho/Hi

The skin hydrophobia increases while the critical surface tension (γ_c) decreases. As in the case of θ_w, the critical surface

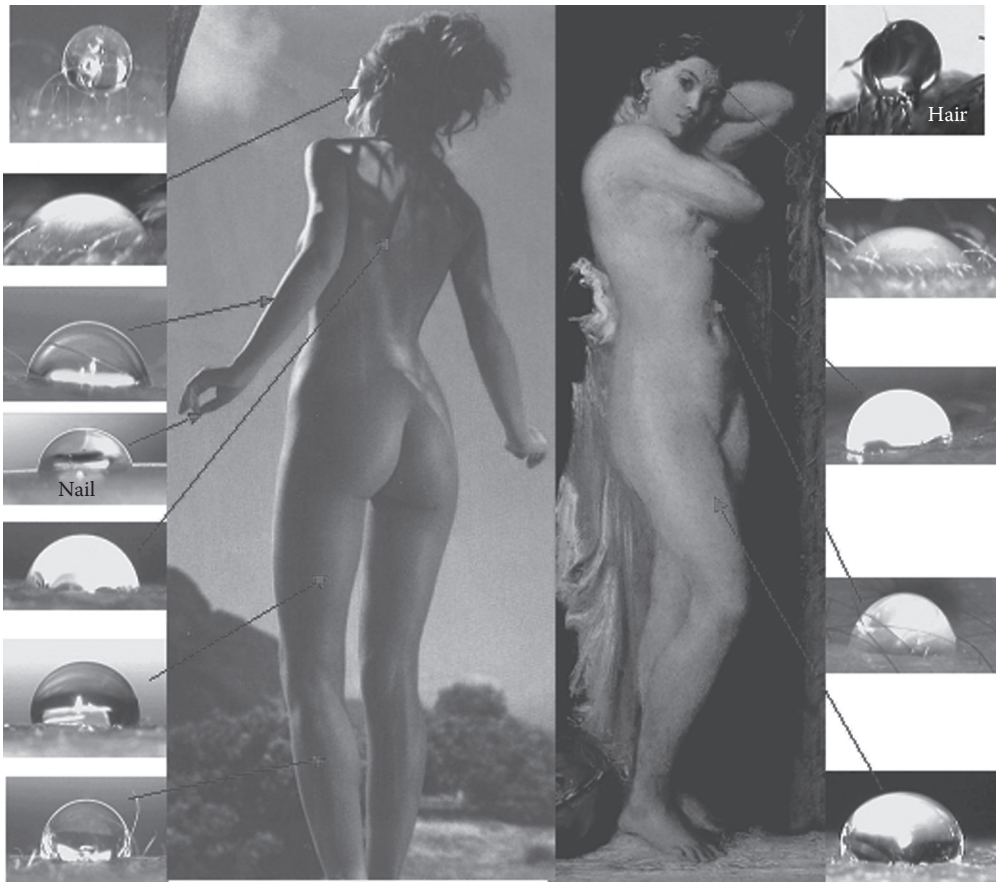

FIGURE 30.5 Human skin wettability. Effect of sebum on hydrophobic/hydrophilic balance.

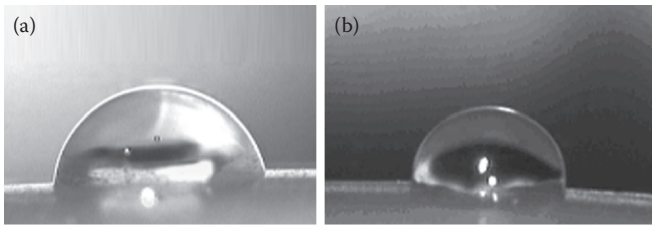

FIGURE 30.6 (a) Water contact angle of the nail; (b) degreasing effect.

tension (γ_c) values show that in the presence of sebum, the skin is less hydrophobic than in its absence. On the forearm, γ_c = 26–27.5 mJ/m^2 [27–31], and on the forehead, a rich sebum site, γ_c increases (33.2 mJ/m²) [31] indicating an increase in skin wettability. According to Equation 30.3, the hydrophobia Ho percentage of the forearm is between 62% and 64%. The presence of sebum on the forehead reduces the skin hydrophobia to 54%.

Surface Free Energy (γ_s)

The surface wettability increases while γ_s increases. The value measured on the forearm is approximately 38.5 mJ/m^2 [10,16,22]. On this poor sebum surface, the bulk of γ_s came from the Lifshitz–van der Waals component γ_s^{LW}. The forearm hydrophobicity, at baseline, as found from contact angle measurement, was related to the low levels of polar components and, consequently, this area should be referred to as a weak monopolar basic surface (γ_s^- = 4.2 mJ/m^2) [22]. On the forehead, a rich sebum area, the surface free energy was higher than on the forearm (42 and 46 mJ/m^2) based on the skin type (oily, normal, dry) [22]. The γ_s^{LW} component value was the same as the forearm, and the main difference came from the γ_s^- (26 mJ/m^2), a component that was considerably higher ($p < 0.001$) [22].

Effects of Some Treatments

Skin hydrophobia increases while θ_w increases and γ_c and γ_s decrease (Table 30.1).

Degreasing and Washing

Degreasing with organic solvents or washing with soap and water increases greatly skin hydrophobia. This effect is noted by the increase in water contact angle θ_w (+10° to 15°) and by the reduction of the critical surface energy γ_c and free surface energy γ_s. The initial skin hydrophilia of the forehead was found 2 h after degreasing, which is the time required for the reconstitution of the current level of sebum [22]. A similar effect is observed on the nails θ_w = 89° (+22°) (Figure 30.6b) [26].

Application of Moisturizers (Cream, Thermal Water)

Our skin needs an adequate daily fluid intake, firstly in order to replenish the stock of water in the dermis (risk of dehydration with loss of skin elasticity, skin fold) and secondly to renew the hydrolipidic film essential for the appearance and for the barrier function of the epidermis. Applying a moisturizer for a week on the face of 60 children significantly decreased θ_w (–10°) indicating an increase in skin hydration (increased by +15 arbitrary units measured by Corneometer Courage and Khazaka [23]).

The application of moisturizers increases the critical surface energy (γ_c) and the free surface energy γ_s. The application of thermal water reduces the skin hydrophobia (decrease of θ_w: –10°). This effect disappears 30 min after application [31].

Other Applications

Nutritional supplementation provides comprehensive care of the skin envelope of the healthy individual together with well-being. The effect of a nutritional supplement on skin dryness in postmenopausal women has been shown with the decrease in the contact angle, which was initially hydrophobic [32].

In another study, measurements have shown that the presence of mucus layer on pork tongue makes its surface significantly more hydrophilic (more wettable). This effect is shown by the decrease in the angle θ_w by –27° and the increase of energy γ_s by +11 mJ/m^2 [33].

Discussion

Studies on the skin wettability clearly show the role of the hydrolipidic layer on the skin hydrophobia. The suppression or the alteration of this layer increases the skin hydrophobia. This capacity of the cutaneous lipids, which increases skin wettability, was ascribed to the free fatty acids, especially to those composing the sebum. Skin wetting by lipids was found to increase with the amount of squalene and paraffin in sebum [34].

The in vivo quantification of physicochemical parameters analyzed after wetting the nails has potential value in the field of research. These studies are increasingly in the core attention of researchers because of the practical interest of investigations on the penetration of drugs and transungual barrier function. The development and selection of films, including antifungal treatments (varnish), depend on the knowledge of these parameters.

HUMAN SKIN FRICTION COEFFICIENT

The frictional behavior of the skin with different materials plays a critical role in its sensory perception. The friction is extremely important in our perception of cosmetic application such as antiaging cream and moisturizers [35]. Consumer exposure to a wide range of cosmetic products is limited to dermal contact. When touching an object, a contact is formed between our skin and the object; the tribological properties of such a contact influence how an object is perceived. Sensory perception is an important factor in the decision-making process of consumers [36].

The friction coefficient is the measurement of the level of sliding between two surfaces. The initial force to start the slide is called the dynamic friction coefficient, while

the force necessary to continue this same slide is called the kinetic friction coefficient. A high friction coefficient represents a weak sliding, while a low friction coefficient indicates a favorite sliding.

The review of the published literature on skin friction shows a wide range of measured values of μ (Table 30.2). These differences indicate that the assessment of the friction coefficient of the skin is a highly complex problem. It involves skin elasticity, skin anisotropy, microtopography, anisotropy of the skin relief, variation in testing conditions, and individual differences, in measuring techniques. The latter point can be divided into two types of designs for the test apparatus. One design incorporates linear motion, wherein a probe is pressed onto the surface and dragged across the skin in a straight line. The other design is rotational and consists of a probe pressed onto and rotated against the skin surface. The friction coefficient does not vary significantly with gender but varies considerably among the anatomical regions of the body [41,43,44]; the age effect was also measured [42–44]. The friction coefficient is influenced by load [42,44,49,50]; however, it is increased due to water application [39,42,45]. On the other hand, the application of petrolatum and glycerine on the forearm and hand decreases the friction coefficient immediately, and this effect lasts for at least 1 h after application [50]. The application of isopropyl alcohol [44] and washing with soap [47] dry the skin and decrease its friction coefficient. The finger has a friction coefficient μ ranging from 0.27 to 0.70 and varying among individuals due to different states of skin hydration [46]. Recently, our group [21] showed a significant difference ($p < 0.05$) of μ measured on the forehead depending on the ethnic affiliation. In 2004 [19], we showed the influence of the hydrophobic and hydrophilic characteristics of sliding and slider surfaces on μ. In this study, the wettability parameters for six surfaces (volar forearm, Teflon®, silicone impression material [Silflo®], vinyl polysiloxane impression material resin, steel, and glass) were measured, and their influences were compared to the friction coefficient μ.

The tribometer (Figure 30.7) used in this study was developed and validated [19,21,33,42] to characterize the friction properties between the skin in vivo and different sliding surfaces. A sliding ball of 10-mm diameter was pressed on the ventral forearm with a constant normal load (F_N) of 0.1 N and then moved at a constant velocity of 0.5 mm s^{-1}. In order to maintain surfaces as flat as possible, a short sliding distance of 10–15 mm was selected.

TABLE 30.2
Human Skin Friction Coefficient (μ)—Literature Data

Author	Sliding Material	Motion of Test	μ	Ref.
Comaish et al.	Teflon(1); nylon(2); polyethylene(3); wood(4)	Linear	0.2(1)–0.45(2)–0.3(3)–0.4(4) forearm	[37]
Kenins	Different wool fabrics	Linear	0.32–0.48: dry skin 0.48–1.23: wet skin (forearm, finger)	[38]
El-Shimi	Steel (rough[a], smooth[b])	Rotational	0.2–0.4[a] 0.3–0.6[b] (volar forearm)	[39]
Highley et al.	Nylon	Rotational	0.19–0.28 (volar forearm)	[40]
Cua et al.	Teflon	Rotational	0.34 (forehead) 0.26 (volar forearm) 0.21 (palm), 0.12 (abdomen) 0.25 (upper back)	[41]
Asserin et al.	Ruby	Linear	0.7 (volar forearm)	[42]
Elkhyat et al.	Teflon(1); steel(2); glass(3)	Linear	0.18(1)–0.42(2)–0.74(3) (volar forearm)	[19]
Elsner et al.	Teflon	Rotational	0.48 (volar forearm) 0.66 (vulva)	[43]
Sivamani et al.	Steel	Linear	0.56 (normal skin: dorsal finger) 0.50 (isopropyl alcohol exposure: dorsal finger) 0.2 (normal skin in vitro) 0.3 (water exposed skin in vitro)	[44]
Sivamani et al.	Steel	Linear	0.4–0.6 (volar forearm)	[45]
Derler et al.	Textile sample	Linear	0.27–0.7 (finger)	[46]
Egawa et al.	Finger print		0.4 (volar forearm)	[47]
Lodén et al.	Steel	Rotational	0.55 (dorsum of the hand) 1.1 (lower back) 0.65 (volar forearm) respectively in atopic skin 0.4–0.65–0.55	[48]
Fotoh et al.	Steel	Linear	0.7–0.9 (forehead)	[21]

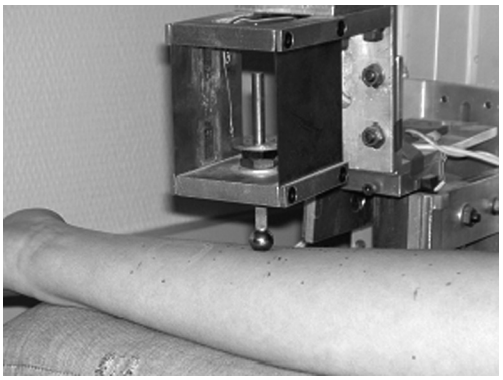

FIGURE 30.7 Linear tribometer for in vivo friction coefficient measurement.

In this study, we showed that when the skin is rubbed against a hydrophobic surface such as Teflon, the friction coefficient μ is lower than when rubbed against a hydrophilic surface such as glass or steel (hydrophobic surfaces = lowest friction coefficient).

DISCUSSION

Frictional properties of the human skin depend on either the skin itself, its texture, suppleness or smoothness, and dryness or oiliness [48], or its interaction with external surfaces and the outside environment [51].

In this chapter, we saw the role of the skin hydrophobia in the skin friction coefficient. The larger hydrophobicity of the abdomen (see Figure 30.5) explains its lower friction coefficient compared to the forehead measured by Cua et al. [41]. Water application decreases the skin hydrophobia and consequently increases its friction coefficient measured by Egawa et al. [47] and Sivamani et al. [45]. The decrease in μ after degreasing (isopropyl alcohol) [44] or after washing with soap and water [47] is quite normal; indeed, these treatments increase the skin hydrophobia (Table 30.1). The skin hydrophobia increased with age, while the atopic skin has a lower μ reported in the literature [42,48].

The role of the surface lipids has been suggested as one possible factor contributing to the frictional properties of the skin, and the correlation between μ and the skin lipid content has been evaluated: Cua et al. [52] showed that the skin lipid content plays a role in the frictional properties of the skin. Moreover, in the skin, the friction resistance depends on hydrophilic and lipophilic elements present on the cutaneous surface.

Fotoh et al. [21] assumed that the hydrophilic/hydrophobic balance of the cutaneous hydrolipidic film is different between the different ethnic groups studied. Black women could have a decreased skin friction coefficient as well as an increased cutaneous hydrophobicity compared to mixed-race and Caucasian women.

GENERAL CONCLUSION

The exploitation of these parameters should allow one to classify the types of skin according to their affinity with water, which is of major importance in biology and in cosmetology. These data should also be used to conduct the formulation process in order to discriminate emulsions that do not spread properly on the skin.

Investigation of skin frictional properties is relevant to several research areas, such as skin physiology, skin care products, textile industry, human friction-dependent activities, and skin friction-induced injuries [51]. The skin friction forms an integral part of tactile perception and plays an important role in the objective evaluation of consumer-perceptible skin attributes [53,54].

Studies of physicochemical parameters of the skin after wetting bring new information on the interactions of formulation/skin. These parameters are fundamental tools to orientate formulation processes, and their analysis is used to evaluate certain activities or medicated cosmetics. The knowledge of the physicochemical parameters of wetting the skin surface can provide useful information in the fields of hygiene, cosmetics, and topical medications.

The interest of this technique has also been demonstrated to predict the water resistance of ultraviolet-protecting products in vivo [55] or to quantify changes that occur at the near surface region of the stratum corneum [56].

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31 Principles and Mechanisms of Skin Irritation

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INTRODUCTION

In contrast to allergic contact dermatitis (ACD), irritant contact dermatitis (ICD) is the result of unspecified damage attributable to contact with chemical substances that cause an inflammatory reaction of the skin [1] and individual susceptibility [2]. The clinical appearance of ICD is extremely variable. It is determined by the type of irritant and a dose–effect relationship [3]. The clinical morphology of acute ICD as one side of the spectrum is characterized by erythema, edema, vesicles that may coalesce, bullae, and oozing. Necrosis and ulceration can be seen with corrosive materials. Clinical appearance of chronic ICD is dominated by redness, lichenification, excoriations, scaling, and hyperkeratosis.

Any site of skin may be affected. Most frequently, the hands as human "tools" come into extensive contact with irritants, whereas most adverse reactions to cosmetics occur in the face because of the particular sensitivity of this skin region. Airborne ICD develops in uncovered skin areas, mostly in the face and especially the periorbital region after exposure to volatile irritants or vapor [4–6].

Despite their different pathogenesis, ACD and ICD, particularly chronic conditions, show a remarkable similarity with respect to clinical appearance, histopathology [7,8], and immunohistology [9,10]. Therefore, ICD can be regarded as an exclusion diagnosis after negative patch testing. The histological pattern of chronic ICD is characterized by hyperkeratosis and parakeratosis, spongiosis, exocytosis, moderate to marked acanthosis, and mononuclear perivascular infiltrates with increased mitotic activity [11,12].

MOLECULAR MECHANISMS OF SKIN IRRITANCY

As mentioned, striking clinical similarities exist between ICD and ACD, and even extensive immunostaining of biopsies does not allow discrimination between the two types of dermatitis [10]. Nevertheless, the underlying pathophysiological mechanisms are thought to be substantially different [13]. Attempts to differentiate the types of contact dermatitis with new methods are constantly underway. Recently, in vivo reflectance confocal microscopy has been suggested as an adjunctive tool in contact dermatitis diagnosis [14]. With this technique, features of ACD and ICD that include spongiosis, exocytosis, vesicle formation, and blood vessel dilatation can be visualized. Hallmarks of ICD are stratum corneum disruption, epidermal necrosis, and hyperproliferation, whereas ACD is supposed to present more typically with vesicle formation [14]. However, these findings probably relate to acute dermatitis, whereas chronic allergic and irritant dermatitis can be expected to be undistinguishable by in vivo reflectance confocal microscopy just as they are by light microscopy.

In contrast to ACD, ICD lacks hapten-specific T lymphocytes. The pathogenic pathway in the acute phases of ICD starts with the penetration of the irritant into the barrier, either activation or mild damage of keratinocytes, and release of mediators of inflammation with unspecific T-cell activation [15]. In a recently published study of Meller et al. [16], it was demonstrated that chemokine responses are helpful characteristics to distinguish the chemical-induced allergic from the irritant skin inflammation. They found that allergic and irritant skin responses have distinct molecular expression profiles. Chemokine genes predominantly regulated by T-cell effector cytokines demonstrated differential upregulation in hapten-specific skin inflammation. C-X-C chemokine receptor 3 (CXCR3) ligands, such as Chemokine (c-x-c motif) ligand (CXCL)9 and CXCL10, were selectively induced during hapten-specific but not irritant-induced skin inflammation. It was demonstrated that effector cytokines released by a small number of activated hapten-specific memory T cells stimulate gene expression of a large number of surrounding resident cells, leading to the production of a discriminative chemokine signature. In contrast, the absence of antigen-specific T-cell activation in irritant skin responses results in only negligible amounts of T-cell-derived effector cytokines.

Epidermal keratinocytes play a crucial role in the inflammation of ICD; they can be induced to produce several cytokines and provoke a dose-dependent leukocyte attraction [17]. In responseto the impairment of the stratum corneum barrier with a direct toxic effect on keratinocytes [14,18,19], preformed interleukin (IL)-1α is released. It stimulates other keratinocytes and fibroblasts to produce more IL-1α, IL-1β, IL-6 IL-8, and tumor necrosis factor (TNF)-α [13,20–23]. The cytokine-induced cascade leads to vasodilatation in the dermis and cellular infiltrate in the epidermis [13,24,25]. But keratinocytes also produce anti-inflammatory cytokines to counteract these inflammatory processes. The IL-1 receptor antagonist (IL-1RA) blocks IL-1 activity by competitive binding to the IL-1 receptor without triggering a signal cascade. IL-10 is another anti-inflammatory cytokine [20,23].

The upregulation of certain adhesion molecules like α6 integrin or contact dermatitis 36 is independent from the stimulus and not cytokine induced [18,26]. A number of agents and cytokines themselves are capable of mediating cytokine production in keratinocytes. IL-1 and TNF-α play a role as inflammatory cytokines; IL-8 and IP-10 are known to act as chemotaxins; and IL-6, IL-7, IL-15, granulocyte-macrophage colony-stimulating factor (GM-CSF), and TGF-α can promote growth. Other cytokines, such as IL-10, IL-12, and IL-18, are known to regulate humoral versus cellular immunity [27]. It is still controversial whether the cytokine profile induced by irritants differs from that induced by allergens [28–31]. In irritant reactions, TNF-α, IL-6, IL-lβ, and IL-2 have been reported to be increased [32,33]. De Jongh et al. [34] recently investigated stratum corneum cytokines and skin irritation responses after single and repetitive exposures to sodium lauryl sulfate (SLS). They found an IL-1α decrease of 30% after repeated exposure, while IL-1RA increased tenfold, and IL-8 increased fourfold. Baseline IL-1RA and IL-8 values after single exposure were predictors of transepidermal water loss (TEWL) and erythema. Their results suggest that subjects with higher baseline stratum corneum levels of IL-1RA and IL-8 have a stronger response to skin irritation and that baseline levels of these cytokines can serve as indicators of skin irritability [34].

In subliminal contact with irritants, barrier function of the stratum corneum and not the keratinocyte is the main target of the insulting stimulus. Damage of the lipid barrier of the stratum corneum is associated with loss of cohesion of corneocytes and desquamation with increase in TEWL. This is one triggering stimulus for lipid synthesis, and it promotes barrier restoration [35]. Nevertheless, recent studies show that the concept of TEWL increase after SLS being directly related to a delipidizing effect of surfactants on the stratum corneum cannot be kept up without limitation. Moreover, SLS exposure for 24 h causes damage in the deeper nucleated cells of the epidermis, leaving the lamellar arrangements of lipids intact [36].

However, lipids of the stratum corneum play an important role for barrier function. Proksch et al. [37,38] observed an increase in skin lipid synthesis after acute irritation with acetone treatment. Heinemann et al. [39] observed an upregulation of the production of ceramide 1 in response to repeated irritation with SLS, thus suggesting that this upregulation might play a major role in the development of a hardening phenomenon. The hardening effect is understood as the adaptation of skin to repeated exogenous irritative noxes clinically resulting in a stabilized skin state in spite of ongoing irritant exposure.

The stratum corneum influences epidermal proliferation after contact with irritants by increasing the mitotic activity of basal keratinocytes and, in this way, enhancing the epidermal turnover [40,41]. Disruption of the stratum corneum can stimulate cytokine production itself and, in this way, promote the inflammatory skin reaction, as shown by Wood et al. [42]. They found an increase in TNF-α, various interleukins, and GM-CSF.

It has been shown that chemically different irritants induce differences in the response in the epidermis during the first 24 h with respect to cytokine expression, indicating different "starting points" for the inflammatory response that results in the same irritant response clinically after 48 h. Nonanionic acid, but not SLS, induced an increase in messenger RNA (mRNA) expression for IL-6, whereas mRNA expression for GM-CSF was increased after SLS [43]. Forsey et al. [44] saw a proliferation of keratinocytes after 48 h of exposure and apoptosis of keratinocytes after 24 and 48 h of exposure to SLS. In contrast, nonanionic acid decreased keratinocyte proliferation after 24 h of exposure and epidermal cell apoptosis after only 6 h of exposure.

Other interesting details for understanding the molecular mechanisms of skin irritation have been contributed by Ma et al. [45]. They investigated the role of metallothioneins (MTs) in SLS-induced skin irritation in knockout mice with MT genes I and II (MT[–/–]) and demonstrated that MT (–/–) mice showed a much higher degree of skin inflammation than MT (þ/þ) mice did. With this result, they suggested that MT I and II genes presumably play an important role in skin irritation.

FACTORS PREDISPOSING TO CUTANEOUS IRRITATION

The skin of different individuals differs in susceptibility to irritation in a remarkable manner, and a number of individual factors influencing development of irritant dermatitis that have been identified include age, genetic background, anatomical region exposed, and preexisting skin disease.

Although experimental studies did not support sex differences in irritant reactivity [46,47], females turned out to be at risk in some epidemiological studies [48,49]. Presumably increased exposure to irritants at home, caring for children younger than 4 years, lack of a dishwashing machine [50], and preference for high-risk occupations contribute to the higher incidence of ICD in females [47].

The most established individual risk factor in several epidemiological studies concerning irritant hand dermatitis is atopic skin diathesis [48,51–54]. On the other hand, experimental studies concerning the reactivity of atopics and nonatopics to standard irritants have given contradictory results [55,56], and, as shown in a Swedish study, about 25% of the atopics in extreme-risk occupations, such as hairdressers and nursing assistants, did not develop hand eczema [57]. Age is also related to irritant susceptibility insofar as irritant reactivity declines with increasing age. This is true not only for acute but also for cumulative irritant dermatitis [58,59]. Fair skin, especially skin type I, is supposed to be the most reactive to all types of irritants, and black skin is the most resistant [60–63].

Clinical manifestation of ICD is influenced by type and concentration of irritant, solubility, vehicle, and length of exposure [64] as well as temperature and mechanical stress. Pathogenesis of ICD is complex and may be related to a combination of different types of irritants as well as to different

types of irritation. Sequential ("tandem") exposure to different irritants often occurs in the workplace and modifies the cutaneous response, in contrast to repeated exposure to each irritant alone, indicating a potential aggravating effect of the combination of chemically different irritants [65,66]. In several studies, the synergistic or additive effect on skin response of irritants in combination was investigated [67]. It has been demonstrated that the repeated sequential application of occlusion (with gloves, water, or SLS) and mechanical irritation enhances the effect on barrier disruption caused by single application [68]. It was also described that concurrent application of an anionic detergent and a mild acidic irritant can lead to disruption of the barrier function, which, although not additive, is still considerable. The combined application of SLS and mild acids (ascorbic and acidic acid) did not prevent SLS-induced irritation. NaOH in low concentrations may also act as a potent irritant, but its effect is not enhanced by SLS [69]. Contact with substances that are potentially barrier disruptive, especially in combination with other irritants, boosts the susceptibility for ICD. In contrast, exposure to low concentrations of organic fruit acids either alone or in combination with SLS did not significantly contribute to the development of ICD or increase susceptibility to SLS-induced irritation [70].

Changes in climatic conditions are known to influence barrier function and to induce ICD [71–76] or to aggravate preexisting skin irritation. Sequential treatment with airflow and SLS led to an impairment of barrier function and irritation stronger than caused by SLS alone [76]. Similar effects might occur under low-humidity conditions, which are known to desiccate skin, such as during the winter months [73,74,77,78].

EPIDEMIOLOGY

Population-based data on the incidence and prevalence of ICD are rare. The figures on the incidence of ICD vary considerably, depending on the study population. Most data stem from studies about occupational hand dermatoses.

Coenraads and Smit [79] reviewed international prevalence studies for eczema attributable to all causes conducted with general populations in different countries (England, the Netherlands, Norway, Sweden, the United States) and found point prevalence rates of 1.7% to 6.3% and 1 to 3-year-period prevalence rates of 6.2% to 10.6%.

An extensive study by Meding [48] on hand eczema in Gothenburg, Sweden, included 20,000 individuals randomly selected from the population register. She estimated a 1-year period prevalence of hand eczema of 11% attributable to all causes, and a point prevalence of 5.4%. ICD contributed to 35% of the cases, whereas 22% were diagnosed as atopic hand dermatitis and 19% as ACD. In a multicenter epidemiological study on contact dermatitis in Italy by GIRDCA (Gruppo Italiano Ricerca Dermatiti da Contatto e Ambientali), 42,839 patients with contact dermatitis underwent patch testing. In accordance with the findings of Meding, nonoccupational as well as occupational ICD affected women at a higher percentage compared with males [48,49]. In Heidelberg, Germany, a retrospective study of 190 cases of hand dermatitis revealed 27% as ICD, 15.8% as ACD, the majority (40%) as being of atopic origin, and 10% miscellaneous diagnoses [80]. Even still higher rates of ICD were found by Soder et al. [81] in cleaning and kitchen employees. One hundred and sixty-eight (79.2%) of 212 participants suffered from hand dermatitis, and ICD, at 46.2% (n = 98), was the predominant diagnosis. A Danish study on occupational hand eczema revealed rates of 61.9% for ICD and 21.2% for ACD [82]. The proportion of occupational ICD was similar for males and females (59.7% and 63.1%, respectively), even though females were overrepresented in wet occupations [83]. In accordance with these findings, a retrospective epidemiological study of occupational skin disease in Singapore over a 2-year period also demonstrated that ICD is more common than ACD: ICD made up 62.4% of all cases of occupational contact dermatitis, and ACD constituted 37.6% [84].

Interesting findings result from investigations of the severity of irritant hand dermatitis 5 years after initial diagnosis [85]. Fifty percent of 124 ICD cases still had medium and 32% had severe hand dermatitis, demonstrating that irritant hand dermatitis is chronic in duration. Skoet et al. [83] found a mean disease duration of 4.4 years for males and 4.9 years for females.

Reports on adverse reactions to cosmetics, including those with only subjective perceptions without morphological signs, are more frequent than assumed. In a questionnaire carried out in Thuringia, eastern Germany, as many as 36% of 208 persons reported adverse cutaneous reactions against cosmetics, 75% of them being female [86]. Adverse reactions to cosmetics and hygiene products occur predominantly in females [87]. Clinical examinations have revealed that the majority of self-reported reactions are of irritant type [88,89]. Most untoward reactions caused by cosmetics occur on the face, including the periorbital area [90].

In a study by Broeckx et al. [91], 5.9% of a test population of 5202 patients with possible contact dermatitis had adverse reactions to cosmetics. Patch testing classified only 1.46% as irritant reactions, whereas 3.0% could be classified as ACD. More than 50% of the cases of irritation were attributable to soaps and shampoos. In Sweden, the top-ranking products causing adverse effects, as reported by the Swedish Medical Products Agency, were moisturizers, hair care products, and nail products [87]. In a Danish population survey with persons aged 19 to 80 years asked for self-diagnosed dermatitis, the reported 1-year prevalence of skin symptoms on the face (acne excluded) was 14%. Of those who reported skin symptoms on the face, 33% also reported hypersensitivity to cosmetics [92].

In other studies, the incidence of cosmetic intolerance varied between 2.0% and 8.3%, depending on the test population [90,93,94]. In a large multicenter prospective study on reactions caused by cosmetics, Eiermann et al. [95] found irritancy to account for only 16% of 487 cases of contact dermatitis caused by cosmetics. Of 8093 patients tested for contact dermatitis, 487 cases (6%) were diagnosed as contact dermatitis

caused by cosmetics. Since most consumers just stop using cosmetics and hygiene products when experiencing mild irritant or adverse reactions and seldom consult a physician, it can be assumed that the prevalence of mild irritant reactions to cosmetic products is still underestimated [92,96].

The symptoms of discomfort such as stinging, burning, and itching noticed by many persons following product applications are summarized in the term "sensitive skin." Only little epidemiological evidence exists with respect to its prevalence. In 2001, Willis et al. [97] published an epidemiological study in the United Kingdom to assess the prevalence of sensitive skin in the population and to examine possible factors that may be associated with sensitive skin. They found that sensitive skin is a common phenomenon, with about 50% of women and 40% of men regarding themselves as having a sensitive skin. Ten percent of women and 5.8% of men described themselves as having very sensitive skin. Jourdain et al. [98] reported that 52% of women aged between 18 and 45 years agreed with the following statement: "I have a sensitive facial skin." Approximately 30% of the total population strongly agreed with this statement.

CLINICAL TYPES OF ICD

According to the highly variable clinical picture, several different forms of ICD have been defined. The following types of irritation have been described [15,99]:

- Acute ICD
- Delayed acute ICD
- Irritant reaction
- Cumulative ICD
- Traumiterative ICD
- Exsiccation eczematid
- Traumatic ICD
- Pustular and acneiform ICD
- Nonerythematous
- Sensory irritation

Acute ICD

Acute ICD is caused by contact with a potent irritant. Substances that cause necrosis are called corrosive and include acids and alkaline solutions. Contact is often accidental at the workplace. Cosmetics are unlikely to cause this type of ICD because they do not contain primary irritants in sufficient concentrations.

Symptoms and clinical signs of acute ICD develop with a short delay of minutes to hours after exposure, depending on the type of irritant, concentration, and intensity of contact. Characteristically, the reaction quickly reaches its peak and then starts to heal; this is called "decrescendo phenomenon." Symptoms include burning rather than itching, stinging, and soreness of the skin and are accompanied by clinical signs such as erythema, edema, bullae, and even necrosis. Lesions are usually restricted to the area that came into contact, and sharply demarcated borders are an important sign of acute ICD. Nevertheless, clinical appearance of acute ICD can be highly variable and sometimes may even be indistinguishable from the allergic type. In particular, combination of ICD and ACD can be troublesome. Prognosis of acute ICD is good if irritant contact is avoided.

Delayed Acute ICD

For some chemicals, such as anthralin, it is typical to produce a delayed acute ICD. Visible inflammation is not seen until 8 to 24 h or more after exposure [100]. Clinical picture and symptoms are similar to acute ICD. Other substances that cause delayed acute ICD include dithranol, tretinoin, and benzalkonium chloride. Irritation to tretinoin can develop after a few days and result in a mild to fiery redness followed by desquamation or large flakes of stratum corneum accompanied by burning rather than itching. Irritant patch-test reactions to benzalkonium chloride may be papular and increase with time, thus resembling allergic patch-test reactions [101]. Tetraethylene glycol diacrylate caused delayed skin irritation after 12 to 36 h in several workers in a plant manufacturing acrylated chemicals [102].

Irritant Reaction

Irritants may produce cutaneous reactions that do not meet the clinical definition of "dermatitis." An irritant reaction is therefore a subclinical form of irritant dermatitis and is characterized by a monomorphic rather than polymorphic picture. This may include one or more of the following clinical signs: dryness, scaling, redness, vesicles, pustules, and erosions [103]. Irritant reactions often occur after intense water contact and in individuals exposed to wet work, such as hairdressers or metal workers, particularly during their first months of training. It often starts under rings worn on the finger or in the interdigital area and may spread over the dorsum of the fingers and to the hands and forearms. Frequently, the condition heals spontaneously, resulting in hardening of the skin, but it can progress to cumulative ICD in some cases.

Cumulative ICD

Cumulative ICD is the most common type of ICD [99]. In contrast to acute ICD, which can be caused by single contact with a potent irritant, cumulative ICD is the result of multiple subthreshold damage to the skin when time is too short for restoration of skin–barrier function [104]. Clinical symptoms develop after the damage has exceeded a certain manifestation threshold, which is individually determined and can vary within one individual at different times. Typically, cumulative ICD is linked to exposure of several weak irritants and water contact rather than to repeated exposure to a single potent irritant. Because the link between exposure and disease is often not obvious to the patient, diagnosis may be considerably delayed, and it is important to rule out an allergic cause. Symptoms include itching and pain caused by cracking of the hyperkeratotic skin. The clinical picture is

dominated by dryness, erythema, lichenification, hyperkeratosis, and chapping. Xerotic dermatitis is the most frequent type of cumulative toxic dermatitis [105]. Vesicles are less frequent in comparison to allergic and atopic types [48]; however, diagnosis is often complicated by the combination of irritation and atopy, irritation and allergy, or even all three. Lesions are less sharply demarcated in contrast to acute ICD.

Prognosis of chronic cumulative ICD is rather doubtful [47,83,85,106–108]. Some investigators suggest that the repair capacity of the skin may enter a self-perpetuating cycle [104].

Traumiterative ICD

The term "traumiterative ICD" has often been used similarly to cumulative ICD in the past [99,103]. Clinically, the two types are very similar as well. According to Malten and den Arend [3], traumiterative ICD is a result of too-early repetition of just one type of load, whereas cumulative ICD results from too-early repetition of different types of exposures.

Exsiccation Eczematid

Exsiccation eczematid is a subtype of ICD that mainly develops on the extremities. It is often attributable to frequent bathing and showering as well as extensive use of soaps and cleansing products. It often affects elderly people with low sebum levels of the stratum corneum. Low humidity during the winter months and failure to remoisturize the skin contribute to the condition. The clinical picture is typical, with dryness, ichthyosiform scaling, and fissuring. Patients often suffer from intense itching.

Traumatic ICD

Traumatic ICD may develop after acute skin traumas such as bums, lacerations, and acute ICD. The skin does not heal as expected, but ICD with erythema, vesicles and/or papulovesicles, and scaling appears. The clinical course of this rare type of ICD resembles that of nummular dermatitis [99].

Pustular and Acneiform ICD

Pustular and acneiform ICD may result from contact with irritants such as mineral oils, tars, greases, some metals, croton oil, and naphthalenes. Pustules are sterile and transient. The syndrome must be considered in conditions in which acneiform lesions develop outside typical acne age and locations. Patients with seborrhea, macroporous skin, and prior acne vulgaris are predisposed along with atopics.

Nonerythematous ICD

Nonerythematous ICD is an early subclinical stage of skin irritation that lacks visible inflammation but is characterized by changes in the function of the stratum corneum that can be measured by noninvasive bioengineering techniques [99,109].

Sensory Irritation

Sensory irritation is characterized by subjective symptoms without morphological changes. Predisposed individuals complain of stinging, burning, tightness, itching, or even painful sensations that occur immediately or minutes/hours after contact. Those individuals with hyperreactive skin often report adverse reactions to cosmetic products, with most reactions occurring on the face. Fisher defined the term "status cosmeticus," which describes a condition in patients who try a lot of cosmetics and complain of being unable to tolerate any of them [110,111]. Lactic acid serves as a model irritant for diagnosis of so-called stingers when it is applied in a 5% aqueous solution on the nasolabial fold after induction of sweating in a sauna [111]. Other chemicals that cause immediate-type stinging after seconds or minutes include chloroform and methanol (1:1) and 95% ethanol. A number of substances that have been systematically studied by Frosch and Kligman [111] may also cause delayed-type stinging [112]. Several investigators tried to determine parameters that characterize those individuals with "sensitive skin," a term that still lacks a unique definition [113,114]. It could be shown that individuals who were identified as having sensitive skin by their own assessment have altered baseline biophysical parameters, showing decreased capacitance values, increased TEWL, and higher pH values accompanied by lower sebum levels [114–116]. Possible explanations for hyperirritability (other than diminished barrier function) that have been discussed are heightened neurosensory input attributable to altered nerve endings, more neurotransmitter release, unique central information processing or slower neurotransmitter removal, enhanced immune responsiveness, and increased sweat glands [113,117,118]. It is not clear whether having sensitive skin is an acquired or inherited condition; most probably, it can be both. As in other forms of ICD, seasonal variability in stinging with a tendency to more intense responses during winter has been observed [119]. Detailed recommendations for formulation of skin care products for sensitive skin have been given by Draelos [113]. Recent reviews on experimental studies on the nature of sensitive skin and on host factors were published by Kligman et al. [120] and Farage et al. [116] in 2006.

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32 Mechanism of Skin Irritation by Surfactants and Anti-Irritants* for Surfactant-Based Products

C.T. Jackson, Marc Paye, and Howard I. Maibach

INTRODUCTION

Each day, our skin is in contact with a multitude of aggressions that we need to minimize. This can be done by decreasing the intrinsic irritation potential of the insult, by placing an additional barrier between the irritant and our skin or by changing our behavior. Chemical irritants are usually the best-known irritants that inflame our skin, but physical, biological, and environmental factors are also important causes of irritation (Table 32.1).

In some cases, several irritant categories may act simultaneously on the skin to potentiate their effect. For instance, scrubbing products involve a mechanical stress of the skin by rubbing the skin with solid particles and a chemical stress by the surfactants used to formulate the vehicle. With so many types of potential irritants, it is obvious that skin irritation can be induced through different pathways.

SURFACTANTS

Surfactants: A Good Model to Investigate Skin Irritation

Surfactants are frequently used as a model to investigate skin irritation and the effect of anti-irritants for three main reasons.

Surfactants Are a Major Cause of Skin Irritation

As a result of their detergent and foaming properties, surfactants find broad use in many domestic products that contact the skin (Table 32.2). Furthermore, many subjects take several showers/baths a day for cleansing as well as for relaxation and pleasure.

It is Quite Easy to Obtain Very Well-Standardized Surfactants to Work with

In the scientific literature sodium lauryl sulfate (SLS) is regularly used as the "gold" standard to induce skin irritation [18] for several reasons:

- SLS is classified as a skin irritant, Xi-R38 [19].
- SLS can be obtained in a very pure form, which allows different laboratories to work on the same material.
- SLS can be easily formulated in various vehicles.
- Allergic reactions to SLS are not common, although a few cases have been reported [20].
- The level of induced irritation can be controlled by adjusting the concentration [21,22].
- Any skin damage is rapidly reversible.

Unlike Other Irritants, Surfactants May Induce Irritation through Several Pathways

Due to their structure and physicochemical properties, surfactants interact with various targets of the skin: constitutive and functional proteins, intercellular or cell membrane lipids, and living cells.

Surfactant Behavior in Solution: Their Physicochemistry Properties

Surfactants are amphiphilic molecules, meaning that they contain two opposing parts: hydrophilic (water-loving) and hydrophobic (water-hating). When dissolved in water, the former is readily hydrated, while the latter avoids water. As a surfactant is added to water, it concentrates as independent molecules (called monomers) at the air/water interface, with the hydrophobic part trying to avoid the water environment. At a certain concentration, called the critical micellar concentration (CMC), the surfactant can no longer concentrate at the surface and goes into the bulk of the solution. In order to avoid contact with water, the hydrophobic part of the surfactant molecules tends to aggregate together into larger particles called "micelles" [23] (Figure 32.1). However, the hydrophilic part of the surfactant, either by repulsive forces between similar charges (for anionic or cationic surfactants) or by trying to interact better with water (all surfactant types), tends to work to disaggregate the micelles. Based on these attractive and repulsive forces, micelles are dynamic structures that continuously form and disrupt to define an overall relative proportion of monomers and of micelles in the bulk. As a consequence, any system that is able to stabilize the micelles or able to facilitate the incorporation of free monomers into the micelles will reduce the relative proportion of monomers in the solution [24].

* The term "anti-irritant" is currently used to express a reduction of the irritation potential; it never means a total suppression of skin irritation.

TABLE 32.1
Examples of Potential Skin Irritants

Chemicals
Surfactants [1], solvents [2,3], acids and alkalis [3], dessicants [4], concentrated salt solutions [5], alcohol [6], oils [7], water in wet work conditions [8]
Environmental Conditions
Extreme weather conditions (very warm, very cold, dry atmosphere) [9], ultraviolet radiation or pollution [10]
Physicals
Abrasives [11], occlusion [12], needles [13], burns [14], rubbing [15]
Biological Factors
Some enzymes or combination of enzymes [16], some plants [17]

TABLE 32.2
Surfactant-Containing Products

Cosmetics and Toiletries
Body cleansing liquids (shower gels, facial cleansers, liquid soaps, foam baths)
Body cleansing solids (soap bars, syndet bars, combars)
Shampoos
Shaving products
Toothpastes
Household Products
All-purpose cleaners
Window cleaners
Hand dishwashing liquids
Automatic dishwashing products
Fabric detergents
Fabric softeners

MECHANISM OF INTERACTION BETWEEN SURFACTANTS AND THE SKIN

When surfactants come into contact with the skin, they can interact with it in different ways [25]:

- By binding to the surface proteins of the skin
- By denaturing skin surface proteins
- By solubilizing or disorganizing the intercellular lipids of the skin
- By penetrating through the lipidic barrier
- By interacting with the living cells

All these interactions may lead to irritation. Whatever the mechanism of interaction between the surfactant and the skin, the free monomeric form will be the key driver to initiate irritation, and a disruption of the skin barrier will be one of the first signs of irritation.

Alteration of the Skin Barrier: An Early Predictor of Irritation

SLS's effect on skin barrier disruption in mice expressing a constitutively active signal transducer and activator of transcription 6 (STAT6) in T cells was examined; specifically, researchers compared normal mice to mice with abnormal immune function that predisposes them to atopic dermatitis [26]. In the study, Th2 cytokines were noted for their ability to regulate skin barrier function and for their potential as an influencing variable for the development of atopic dermatitis, which is a chronic inflammatory disease that leads to severe itching. The authors used mice that expressed a constitutively active signal transducer and activator of transcription 6; they noted that these mice have increased Th2 cells and a predisposition to allergic irritation [26].

SLS is a common ingredient found in various cosmetic products; moreover, it is an anionic detergent known to

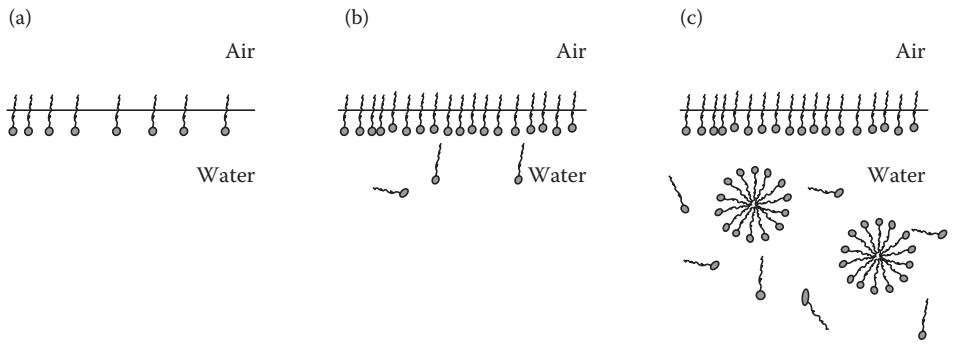

FIGURE 32.1 Surfactant behavior in solution. (a) Surfactant molecules in aqueous solution concentrate at the air–water interface with the hydrophobic part oriented toward the air side. (b) When the concentration of surfactant increases, the interface saturates of surfactant molecules that penetrate into the solution. (c) To minimize their interaction with water, the hydrophobic parts of the surfactants interact together and form micelles in solution. The micelles are unstable structures that form and disaggregate to establish a balance between monomers and micelles in the solution.

activate a prolonged skin barrier disruption that can last up to a week following a single-day-long exposure, as shown in the mice model [26]. The stratum corneum (SC) is a complex that contains corneocytes and intracellular lipids, which together form a structure that provides most of the epidermal permeability barrier. The rate at which water moves from the viable skin through the SC and into the external surroundings, known as transepidermal water loss (TEWL), is used as a measurement of the integrity of the SC, whereby an increase in TEWL is indicative of skin barrier water dysfunction. This study found that TEWL was increased in the STAT6VT mice, while in the control mice, this appeared less pronounced. Overall, SLS-treated STAT6VT mice had an increase in TEWL, indicating skin barrier dysfunction, as compared to SLS-treated control mice [26]. The species difference between mice and humans should provide an avenue to further study water loss related to SLS as an irritant in people experiencing atopic dermatitis.

Skin barrier damage can occur as a result of the overlap of mild chemical and physical factors that individuals are exposed to on a daily basis [27]. Yan-yu et al. [27] examined irritant contact dermatitis (ICD); specifically, a comparison of skin barrier damage severity resulting from minor chemical, physical, and mechanical stimuli versus damage due to sequential irritant dermatitis produced by an occlusive patch test (PT) with 0.5% SLS was studied. In order to generate the chemical, physical, and mechanical exposure experienced daily, an open PT with 1% SLS, a 10× TAP (tape stripping test), and ultraviolet B (UVB) irradiation were used.

As a way to assess skin barrier damage induced by minor chemical, physical, and mechanical stimuli, and the influence that these irritants had on the severity of irritant dermatitis, a PT with 0.5% SLS was applied to the skin after exposure to irritants for a period of 5 days [27]. With the exception of one participant, the level of irritancy after the PT with 0.5% SLS on day 6 and was significantly greater on skin pretreated with SLS (open PT with 1% SLS) or by tape stripping (10× TAP) than on untreated skin. These results demonstrate the influence that previous irritants have on the skin. Exposure to prior mild irritants increases both the incidence and the degree of irritant dermatitis induced by the 0.5% SLS PT. Moreover, the authors noted that both pretreatments functioned to impair overall skin barrier function, to increase the incidence and severity of ICD stimulated by the 0.5% SLS PT, as well as to slow inflammation healing.

SC damage allows an increase in dermal absorption of materials placed on human skin [28,29]; detergent exposure may influence skin barrier function. Using a simple in vitro model, researchers examined the effect of detergents on skin permeability to water and nickel [28]. Anionic, cationic, and nonionic detergents were the three main classes of detergents used in this study; 12 of the detergents under investigation caused a statistically significant increase in diffusion of water, nickel, or both, during a 66 h period. Although the increase in penetration of water, nickel, or both was recorded, the researchers found no difference in this effect between nonionic and anionic detergents [28]. The primary importance of this study was to show how an in vitro method can be a desirable experimental tool to gather helpful information regarding the skin barrier function of human skin; specifically, the researchers noted how the in vitro approach can be a successful way in which to explore in vivo mechanisms [28].

Interaction of Surfactants with Skin Proteins

Binding of surfactant to isolated SC, the most external layer of the skin, saturates at or near the CMC [30], which is consistent with the fact that only monomers of surfactants can adsorb to the proteins of the skin [31]. After binding to the proteins, surfactants cause the proteins to denature, leading to a swelling of the SC [32]. Rhein et al. [33] investigated the swelling of isolated SC when exposed to various single surfactant solutions and showed that the swelling was dependent on concentration and time up to the CMC before leveling off. The authors interpreted their results as support for a single interaction between the surfactant monomer and skin proteins.

Denaturation of functional proteins and especially enzymes have multiple consequences, such as impaired desquamation process, impaired maturation of lipids and proteins in the epidermis, impaired defense system against free radicals and enhancement of oxidative stress [34–35], and so forth.

However, even if the interaction of surfactants with skin proteins is related to the CMC of the surfactants or surfactant mixtures, above the CMC, there is no more a direct relationship. It is proposed that above the CMC, the affinity of individual surfactants for skin proteins plays the critical role in skin irritation [36].

Interaction of Surfactants with the Intercellular Skin Lipids

The protective lipidic barrier of the skin is composed of highly organized lipid layers located between the cells of the SEC. In order to disorganize these lipids and alter the skin barrier function, surfactants have to integrate into the lipidic layers that are mostly hydrophobic. Because of their small size, monomers of surfactants can easily reach the intercellular lipids and disturb the skin barrier function, making such an effect dependent on the relative proportion of monomers in solution. However, it has been recently shown [37] that micelles formed from sodium dodecyl sulfate only have a hydrodynamic radius size that is compatible with partial penetration into the SEC and should thus be capable of interacting with the intercellular lipids. This would partly explain why increasing the concentration of single SLS surfactant solutions above the CMC leads to increased irritation. For other surfactant types, micelles thus have to release their monomers to interact with the lipidic barrier. The dose-related level of irritation caused by such surfactants above their CMC [38] should thus be related to another mechanism.

Interaction of Surfactants with Living Cells

Once the lipidic barrier has been disrupted or weakened, monomers of surfactants can reach the living part of the epidermis and interact with the keratinocytes and Langerhans cells, leading to the following:

- A lysis of the cells in the case of severe irritants and the release of chemical mediators into the intercellular space
- An alteration of the cellular membrane and passive diffusion of chemical mediators from the cytoplasm into the intercellular space
- A stimulation of the cells with subsequent active release of chemical mediators into the intercellular space or synthesis of new mediators

Whatever the pathway, these mediators will initiate a multitude of reactions at the site of irritation, such as a stimulation of cell proliferation, stimulation of neighboring cells to produce additional mediators, vasodilatation of blood capillaries in the dermoepidermal papillae, and attraction of blood cells. Many different chemical mediators will also be upregulated at the site of irritation such as interleukin-1 alpha and beta; interleukins-2, 6, 8, and 10; granulocyte-macrophage colony-stimulating factors (GM-CSFs), tumor necrosis factor alpha (TNF-alpha), interferon-gamma, and others.

Interaction of Surfactants with Neuroreceptors

In persons with sensitive skin, initial contact with some surfactants results in sensory irritation characterized by stinging, itching, or a burning sensation. Such an early signal of irritation was exploited a long time ago with the development of the so-called "lactic acid stinging test" [39] to detect subjects with the "highest level of skin sensitivity" in the face.

This type of sensory irritation occurs when thin, unmyelinated, chemically sensitive type-C nociceptors are activated and transmit a depolarizing signal via the dorsal root ganglia in the spinal cord to the brain, where the sensation is appreciated [40]. These receptors are extensively distributed through the dermis and the epidermis, allowing excitation even by faint stimuli. For a more intense irritant, a retro signal can be transmitted from the dorsal root ganglia up to the inflammation site and contribute with the inflammatory pathway to the erythematous reaction.

ANTI-IRRITANTS FOR SURFACTANT-BASED PRODUCTS

Fortunately, nowadays, many systems have been developed to minimize the risks of intolerance to cosmetics or surfactant-based products. This is extremely important because of the increased use of toiletry products. They must be as mild as possible to the skin. Not only are the mildest ingredients used, but also, finished hygiene products have to contain one or more anti-irritant systems.

Anti-Irritation by Using Only Mild Surfactants

The first approach to develop a surfactant-based product that is mild to skin is to carefully select the mildest surfactants. Nonionic surfactants are generally considered as the mildest and are typical ingredients in body cleansing products for babies, for sensitive-skin subjects, or for face cleansing products. However, several anionic surfactants are also extremely respectful of the skin condition and are often introduced in the same categories of products. These are, for instance, highly ethoxylated (at least 5-EO) alkyl sulfates, sulfosuccinate esters, sarcosinates, fatty acid-protein condensate, alkyl phosphate ester, alkyl glutamate, taurates, and others. Amphoteric surfactants are rarely used alone but rather, as secondary surfactant; thus, their intrinsic irritation potential has no real meaning. Cationic surfactants are essentially used for their antibacterial properties rather than their detergent properties and are often described in the literature as the most irritating surfactants. However, like anionic surfactants, it is also possible to find very mild cationic surfactants. Due to their low usage, the cationic surfactants will not be discussed in this chapter.

Anti-Irritation by an Appropriate Combination of Surfactants

The best counterirritants for surfactants are other surfactants. Several authors have clearly demonstrated such a positive interaction between various surfactants in vitro [33,41] and in vivo [36,42,43], with diluted [33,41,42] or with highly concentrated solutions [36,43]. Amphoteric surfactants are well known to decrease the irritation potential of anionic surfactants [44], but nonionic surfactants can display the same effect when used at a sufficiently high concentration. More surprisingly, certain anionic surfactants can reduce the irritation potential of another anionic surfactant, instead of cumulating their effects [43].

How Can Secondary Surfactants Reduce the Irritation Potential of Primary Surfactants?: The Principle of Surfactant Antagonism

Skin being a complex organ with different potential targets for surfactants, several mechanisms may occur to explain the reduced irritation observed by mixed surfactant systems as compared to single surfactant solutions.

Overall Mechanism for All Targets

Based on the fact that mainly monomers irritate the skin and that there is an equilibrium in solution between micelles and monomers, any factor able to stabilize the micelles, and hence decrease the relative proportion of monomers, plays a major role in reducing irritation [24]. This is the case for secondary surfactants added to the system as explained in Figure 32.2 but also for other kinds of macromolecules like proteins or other polymers [45].

As shown in Figure 32.2, any kind of secondary surfactant is able to stabilize the micelles and reduce the relative amount of irritant monomers in the solution.

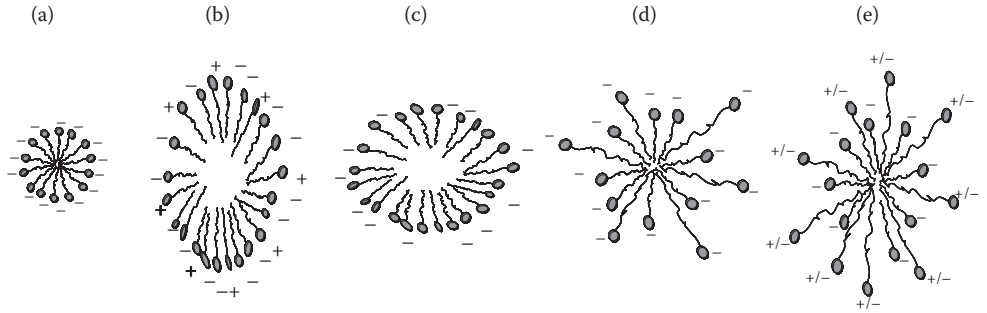

FIGURE 32.2 Mixed micelles of surfactants in solution. (a) In aqueous solution, monomers of surfactants tend to aggregate by their hydrophobic tail to escape water and form micelles. When micelles are formed of only one single type of surfactant (e.g., anionic surfactants), electrostatic repulsion forces tend to disrupt the micelles that are not stable. (b) By adding to the solution cationic surfactants, these latter incorporate into the micelles, increase the size of the micelles, modify their form, and introduce attractive charges between the positive and negative polar heads of the surfactants to stabilize the micelles. (c) When adding nonionic surfactants to solution A, uncharged surfactant heads incorporate into the micelles and increase the size of the micelles as well as the distance between the anionic polar heads, which reduces the repulsive forces between the monomers and leads to a stabilization and change of form of the micelles. (d) When a second type of anionic surfactant is incorporated to solution A, the hydrophobic tail being different from the primary surfactant tail, the distance between the anionic surfactant heads is higher; repulsive forces decrease; and the micelles are more stable, larger, and of a different form. (e) When amphoteric surfactants are added to the solution of anionic surfactants, their behavior depends on the electronic charge of the surfactant (positive at a pH below the lowest pKa of the surfactant [case b], negative at a pH higher than the highest pKa of the surfactant [case d], and both positive and negative at a pH between the lowest and highest pKa of the surfactant [case e]). In case (e), at all pH, the micelles will be stabilized as compared to situation (a). However, the balance between attractive and repulsive charges in the micelles will depend on the pH of the solution and will determine how stable are the micelles.

Furthermore, in the case of mixed micelles of types B, C, or E (Figure 32.2), the overall electrical charge density at the surface of the micelles is lowered. This effect allows the micelles to be less repulsive to surfactant monomers in the surrounding bulk, thus allowing the monomers to more easily incorporate into an ever-expanding micelle. This mechanism explains the reduction in the amount of free monomers in the solution.

Additional Mechanism for Interactions with Surface Proteins

The affinity of the monomers for proteins may also displace the monomer–micelle equilibrium. This affinity depends on the intrinsic properties of the surfactant (e.g., ionic charge, tertiary structure, hydrophobic domains, carbon chain length, level of ethoxylation, etc.); on the mobility of the monomer in the solution (which is related to the size of the monomer); and on the availability of the protein's binding sites. This latter parameter is mainly significant in the case of concentrated solutions of surfactant mixtures because their monomers compete for binding sites on the surface proteins as well as with interactions within the micelle. The amount of available monomers that will bind to the protein will thus be decreased, and the irritation potential of the mixture lowered. Such a decrease in the binding of the anionic surfactants to the skin surface has been demonstrated by attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FTIR) in the presence of a secondary surfactant of any type [46].

Similarly, proteins or polymers added to a surfactant solution may also compete for the same binding sites as the surfactant monomers at the surface of the skin and are often counterirritants for the surfactants [46].

Additional Mechanism for Interactions with Intercellular Skin Lipids

Micelles formed from a single surfactant type are smaller than micelles formed from several surfactant types. While the former category may have a size allowing them to partially penetrate through the SC (e.g., the case for SLS [37]), the latter should be sterically hindered from penetrating into the skin and interacting with the intercellular lipids [47]. This effect results in a partial protection of the skin barrier function when adding a secondary surfactant to the primary one.

Scientific Cases of Reduced Irritation in Surfactant Mixtures

Many peer-reviewed scientific publications have reported that mixtures of surfactants are less irritating than expected based on the sum of the irritation potential of each species taken separately. Several review papers by Goldemberg and Safrin [48], Coldemberg [50], Effendy and Maibach [50], Paye and Pierard [51], and Paye [52,53] have illustrated examples of antagonisms between surfactants. The following section aims at giving additional concrete examples grouped by the type of interaction between the skin and the surfactant investigated by the author.

Interaction of Surfactant with Proteins In Vitro *or* In Vivo

- Ohbu et al. [54] evaluated the protein denaturation properties of surfactants using circular dichroism and demonstrated that the sodium dodecyl sulfate (SDS)-induced denaturation of bovine serum albumin (BSA) was counteracted by dodecyltrimethylammonium chloride or by *N*,*N*′-dimethyldodecylaminoxide.

- Dominguez et al. [44], using human callus as a skin model, demonstrated a considerable inhibition of adsorption of SLS on the callus when alkyl amido betaine (AAB) was present in the same solution. They deduced from their data that the two individual surfactants was more irritating than any of the combinations tested. They explained their data by a stabilization of the micelles of mixed surfactants and, hence, a reduction of bioavailable monomers.
- Miyazawa et al. [55] showed in vitro that mixed surfactants reduced protein denaturation compared to the single surfactant solutions. Again, this was explained on the basis of the reduced level of free surfactant monomers in the mixed surfactant solution as compared to single surfactant solutions.
- Blake-Haskins et al. [56] showed, using an in vitro protein denaturation assay (collagen swelling), that the addition of an amphoteric surfactant to an anionic surfactant reduced the denaturation potential of the anionic surfactant.
- Paye and Jacobs [46], using ATR-FTIR, demonstrated, by a study on human volunteers, that the binding of anionic surfactant (SLS and linear alkyl benzene sulfonate [LAS]) to skin surface proteins was significantly reduced when amphoteric or nonionic surfactants were added in the solution. This study illustrated the competition between the two types of monomers for the binding sites on skin surface proteins.
- Tadenuma et al. [57] showed that alcohol ethoxylate (AE) added to SLS resulted in less denatured BSA. The higher the concentration of AE for a fixed concentration of SLS, the greater the inhibitory effect of AE on SLS-induced protein denaturation. By measuring the adsorption isotherms of SLS onto agarose-immobilized BSA in presence and absence of AE, the authors correlated reduced protein denaturation by AE with a dramatic reduction of binding of SLS to BSA due to the adsorption of AE onto the protein.
- Paye et al. [58], using commercial surfactants (as provided by manufacturers) and in the exact proportions as in standard commercialized laundry detergents, demonstrated that the protein denaturation potential (using the in vitro zein test) of the mixtures of surfactants was, in all cases, lower than expected based on the cumulative protein denaturation effect measured for the surfactants separately.

Interaction of Surfactant with Lipids or Membranes

- Charaf and Hart [59] investigated in vitro the interaction of surfactants with membranes and demonstrated that the addition of lauryl ether sulfosuccinate to a given concentration of SLS decreased the aggressiveness of the latter surfactant for the membrane.
- Garcia et al. [24] demonstrated that mixtures of surfactants diffused less through a membrane than the same surfactants tested separately. This observation was interpreted by the fact that micelles were too big entities to penetrate through the membrane and that the relative proportion of monomers was lowered in the mixed solutions.
- Kawasaki et al. [60], using the electron paramagnetic resonance (EPR) technique, demonstrated an increased fluidity, indicating disordering, of the SC intercellular lipid structure after application of a solution of SLS, most likely due to an intercalation of SLS monomers into the intercellular lipids' organization. The addition of sodium lauryl glutamate (SLG), another anionic surfactant, to SLS inhibited the fluidization of the intercellular lipids induced by SLS.
- Moore et al. [47], using dynamic light scattering measurements, determined the size of the hydrodynamic radius for SDS micelles that was compatible with partial penetration inside the SC, while the size of the mixed micelles from SDS and dodecyl hexa(ethylene oxide) (C12E6) was higher and hindered the penetration of the mixed micelles inside the SC. They confirmed their hypothesis by measuring the hydrodynamic radius of surfactants evaluated in skin penetration studies and showed that the addition of C12E6 to the SDS solutions was found to decrease the amount of SDS penetrating into the epidermis. They attributed this decreased penetration to two causes: A decrease in the concentration of SDS monomers due to a stabilization of the micelles and a decreased penetration of the mixed micelles due to an increase in their steric size.

Interaction of Surfactants with Living Cells In Vitro

- Earl et al. [61] showed in a three-dimensional cell culture model of human skin that equal mixtures of SDS and *N*,*N*-dimethyl-*N*-dodecylaminobetaine have reduced cytotoxicity potential compared to their single applications at the same concentrations. Their observation correlated well with the results of a 4 h human patch study in which the same single surfactants were tested versus the surfactant combinations at high concentrations.
- Benassi et al. [62], using cell culture models, demonstrated that the cytotoxic effect of SLS was reduced when it associated with different tensides such as cocamidopropyl betaine, polysorbate-20 and polysorbate-80. They compared their results to previous data showing that the barrier damage caused by SLS in vivo was lower when SLS was used in combination with other tensides because they are able to reduce the critical micelle concentration of SLS.

Interaction of Diluted Surfactant Solutions with Skin In Vivo

- Rhein et al. [63] used in vivo skin irritation studies (21-day cumulative irritation test) to show that the addition of (C12–C14) alkyl, 7-ethoxy sulfate (AEOS-7EO) to a constant dose of SLS resulted in a significant reduction of erythema, hence producing a milder system.
- Marti [64], using four in vitro and two in vivo models for skin and mucous membrane irritation prediction, showed that the irritation potential of sodium lauryl ether sulfate used as a primary surfactant could be significantly decreased by adding cocamidopropyl betaine, coco amphocarboxypropylate, or protein fatty acid condensate a as secondary tenside for mildness synergy in shampoo formulations.
- Zehnder et al. [65] evaluated the effect of sodium laureth carboxylate with two different levels of ethoxylation (5 and 13 ethoxylations) for their effectiveness in reducing the irritation potential of SLS in a 5-day human PT. Both carboxylates were counterirritants to SLS as shown by clinical examinations, measurements of superficial blood flow, skin barrier alterations, and skin electrical conductance.
- Lee et al. [42], in a 24 h PT, showed that adding SLG to a solution of SLS decreased the irritation potential of the latter.
- Teglia and Secchi [66], using a 3-week arm-soaking test on human volunteers, showed that the amphoteric surfactant, cocamidopropyl betaine, had a similar anti-irritant effect to wheat protein when added to a solution of SLS. Both the wheat protein and cocamidopropyl betaine protected the skin against alteration of the skin barrier and subsequent irritation.
- Teglia and Secchi [45] reported that using SLS, sodium laureth sulfate (SLES), and olefin sulfonate as primary surfactants, and obtaining some new formulations by mixing these primary surfactants with four different auxiliary surfactants and protein hydrolysates, reduced the damage to the SC.
- Paye and Cartiaux [67] showed in a short-term PT on human volunteers that alkyl betaine (amphoteric surfactant) and AE (nonionic surfactant) reduced the alteration of the SC caused by SLS or by LAS (anionic surfactants).
- McFadden et al. [68] mentioned in one of their publications that they had run an unpublished clinical study demonstrating that the direct addition of benzalkonium chloride (BC) to a solution of SDS reduced the irritant inflammatory response of the volunteers to SDS. They explained their observation through a stabilization of the mixed micelles by BC.
- Vilaplana et al. [69] emphasized the importance of the physicochemical behavior of surfactants in solution as a way of minimizing their irritant properties. In a 48 h PT on human volunteers, the authors showed that the addition of disodium cocoyl glutamate or of sodium Polyethylene Glycol (PEG)-4 lauramide carboxylate to a solution of SLS produced a significant reduction in the TEWL, skin color reflectance, and laser Doppler velocimetry even though there was a two-time increase in the total surfactant concentration.

Interaction of Concentrated Surfactant Preparations with Skin In Vivo

- Dillarstone and Paye [43], using the 4 h human PT with concentrated surfactant systems, demonstrated that the addition of 10% of cocoamidopropyl betaine, ethanolamide, SLES, or AE, to a solution of 20% SLS or of LAS decreased, in all cases, the level of erythema induced by the anionic surfactant alone, even though the overall concentration of surfactant in the mixture was increased. Even more, a solution with 20% LAS + 10% SLES + 10% AE (total concentration of 40%) was found to be less irritating than a solution of only 20% LAS.
- Hall-Manning et al. [36], using the 4 h human PT, investigated the interaction between highly concentrated anionic and amphoteric surfactants and showed that the irritant effect on skin of the mixtures (20% of SDS + 20% of dimethyl dodecyl amido betaine) was significantly lower than the effect of the anionic surfactant (at 20%) tested alone. The authors pointed out the correlation between the reduced irritation and the reduced CMC for the mixture of surfactants. However, at such a high surfactant concentration, they also attributed the lowered irritation potential to a reduced affinity of the individual surfactants to the skin proteins.

Anti-Irritation by Polymers or Proteins/Peptides

The counterirritant capability of polymers or proteins on surfactants has been reported in the literature for a long time [65,70–72]. The mechanism by which polymers and proteins function is similar to the one described above for surfactant mixtures. They incorporate into the micelles and thus decrease the relative amount of free monomers in solution. Their skin substantivity can also involve blocking of binding sites at the surface of the skin, thus making them nonaccessible to surfactants.

Polymers or proteins differ in their ability to interact with the skin surface and to be incorporated into the micelles. The following parameters should be considered when selecting a polymer/protein:

- Better interaction with the micelles correlates with increased hydrophobicity [70].

- Better substantivity to the skin correlates with higher hydrophobicity when the polymer is quaternized or is cationic or when the net charge or the size of the polymer/protein increases [71,72].

As stated above, more hydrophobic and/or larger polymers/proteins are much more effective to depress the skin irritation potential of surfactants.

However, in the literature, the anti-irritant effect of proteins/polymers on surfactants has been demonstrated mostly in single surfactant solutions and at a high polymer/surfactant ratios not always compatible with other properties of the finished product. When they are formulated into finished products already optimized for skin compatibility through an appropriate combination of surfactants, most polymers or proteins do not bring any further mildness benefit to the product.

Anti-Irritation by Refattening Agents

One of the negative effects of surfactants on skin is the alteration of its lipid barrier. This can be easily assessed by measuring the TEWL [60,73], which increases with an impairment of the barrier. Using refattening ingredients or skin barrier repairing ingredients in surfactant-based products can reduce the disruption of the barrier function if those ingredients are appropriately delivered to the skin surface. Such ingredients are often the basis for the barrier cream effect of creams (lotions) topically applied before or after contact with an irritant. Some of these ingredients can also be formulated into a surfactant system and act directly as anti-irritants in the mixture. The occlusive effect they bring at the surface of the skin delays the water loss and maintains the skin in a less dehydrated state. Furthermore, they can progressively form an additional barrier protecting the skin against the surfactants in repetitive product application conditions. Several types of refattening ingredients are available and can be formulated in surfactant systems. Among these are ethoxylated monoglycerides, diglycerides, and triglycerides; fatty alcohols and ethoxylated fatty alcohols; fatty acid esters; lanolin derivatives; are silicone derivatives. A few products containing a high percentage of oil also exist and can possibly be added to surfactant systems to serve in a barrier protection role.

Anti-Inflammatory Effect

Anti-inflammatory ingredients are not specific for surfactant-induced irritation, and most of them are used in pharmacology rather than in cosmetology. Due to the complexity of the inflammatory process, several families of anti-inflammatory ingredients have been developed such as glucocorticoids; nonsteroidal anti-inflammatory drugs (tacrolimus, cyclosporin, rapamycin, ascomycin, and leflunomide); flavonoids; essential oils; and alpha-bisabolol [74–76]. In order to be effective, such ingredients must be delivered to the skin in a bioavailable form and in a sufficient amount.

In a study examining topical anti-inflammatory drugs and emollients in the treatment of eczema, researchers examined the behavior of topically applied corticosteroid, tacrolimus, and emollient on SEC lipids and barrier parameters [77]. Damage of the skin barrier may be caused by a variety of everyday chemical, mechanical, and physical stimuli [27]. Although topical corticosteroids have been used to treat eczematous diseases, in which skin barrier impairment is considered as an important/prime contributor, such a treatment might result in further skin barrier damage [30]. The SC is comprised of the skin barrier; this complex is principally influenced by the layer's lipid and protein composition; corticosteroid treatment may cause a lessening in lamellar body count and, therefore, a decrease in lipids [77]. Due to a lack of research on the effect of emollients on the SC lipid composition of human skin, the objective was to examine the influence of topical betamethasone and tacrolimus on skin barrier function; the authors explored this effect/these effects structurally regarding lipids as well as functionally via susceptibility to irritant challenge. They found a statistically significant difference between ceramide/cholesterol ratio for betamethasone versus emollient-treated skin; a higher ceramide/cholesterol ratio for betamethasone compared to emollient-treated skin was noted [77]. Moreover, betamethasone leaned toward a higher ceramide/cholesterol ratio than did the untreated control area. TEWL values were in agreement with the positive barrier hypothesis from the ceramide/cholesterol ratio, regarding values after SLS irritation [77]. In comparison with the emollient, the authors recorded that tacrolimus topical treatment produced a significantly greater ceramide/cholesterol ratio. Overall, results demonstrated that betamethasone and tacrolimus had a positive influence on the ratio of ceramide to cholesterol versus the emollient treatment; moreover, betamethasone and tacrolimus active ingredients lessened SLS irritant response.

The case of essential oils, flavonoids, and alpha-bisabolol is discussed in more details in other sections of this handbook.

Antioxidants

In biological systems, antioxidant processes have a protective role against oxidative stress through three different mechanisms:

- By scavenging the early prooxidant species
- By preventing the initiation or the propagation of the free-radical reactions
- By returning oxidized groups to their reduced state

In dermatology and cosmetology applications, antioxidants belong to a relatively new field of investigation and interest. Some of the most important antioxidants with known applications are vitamin E, vitamin C, thiols, and flavonoids. Their mechanism of action in the antioxidant process is reviewed by Saliou et al. (Chapter 22). In surfactant-based products, antioxidants are only occasionally used to reduce the skin irritation potential of the product

[78]. However, several cutaneous enzymes are involved in the protection of the skin against free radicals and reactive oxygen species (ROS). Such enzymes are partly denatured once surfactants penetrate the skin, and the natural defense mechanisms of the skin may then become overwhelmed, leading to an oxidative stress situation. Any supplementation of the skin with scavenging systems to, for example, combat surfactant irritation could result in a reduced irritation response.

Anti-Sensory Irritation

Although much less discussed than the clinical irritation, which is characterized by observable or functional alterations, subjective irritation also exists. It does not have great interest for dermatologists, but for cosmetologists, it can be the reason consumers like or reject their product.

Three different categories of sensory signals of irritation have been identified, briefly,

- Stinging, burning, itching signals
- Dryness, tightness perception preceding clinical signs of irritation
- Peculiar "irritated-skin" perceived signals unrelated with a true irritation process

These types of irritant signals will require different "anti-irritant" systems.

Anti-Irritants for Stinging, Burning, and Itching Sensations

Strontium salts have been demonstrated to be effective and selective anti-irritants for chemically induced sensory irritation associated with stinging, burning, or itching manifestations [40]. Strontium salts (nitrate or chloride) are claimed to be especially indicated for subjects with sensitive facial skin and prone to stinging sensations [40,79]. The interest of strontium salts, as described by Hahn [40], is that they are very specific and selective inhibitors of the sensory signals of irritation, without suppressing other receptors (such as temperature, tactile, pressure, etc.).

Several controlled clinical studies [40,80] were run to show that strontium nitrate or chloride, at concentrations from 5% to 20%, effectively suppressed or reduced sensory irritation caused by chemical or biological irritants over a wide range of pHs, from 0.6 to 12. In tests, the strontium salts were included in the solution with the irritant, or before or after the application of the irritant, as shown in Table 32.3.

Although not tested in surfactant systems, strontium salts may play a similar beneficial effect on surfactant-induced sensory irritation, mainly in certain classes of sensitive-skin subjects.

As described within the mechanism of sensory irritation, it has been observed in some studies that, on top of reducing the sensorial signs of irritation, strontium salts could also decrease the level of erythematous reactions generated by the irritant.

TABLE 32.3
Clinical Tests Support the Anti-Sensory Irritant Potential of Strontium Salts

Irritant	Test Site	Timing of Application[a]
Lactic acid, 7.5%, pH 1.9 (solution)	Face	Mixed, pre or post
Lactic acid, 15%, pH 3.0 (solution)	Face	Mixed
Glycolic acid, 70%, pH 0.6 (peeling solution)	Arm	Mixed
Capryloyl salicylic acid, 1% (exfoliant cream)	Cheek	Mixed
Ascorbic acid, 30%, pH 1.7 (solution)	Face	Mixed
Aluminum chloride, 20% (antiperspirant preparation)	Axilla	Pre
Aluminum/zirconium salt, 25% (antiperspirant solution)	Arm	Mixed
Calcium thioglycolate, pH 9–12 (depilatory lotion)	Leg	Post
Histamine (intradermal injection, 100 μg)	Forearm	Pre

Source: Frosch, P.J., and Kligman, A., *J, Soc. Cosmet. Chem.*, 47, 1977.

[a] "Pre" means that strontium salts were applied to skin prior to the irritant, "post" means that the salts were applied after skin had been irritated by the irritant, and "mixed" means that strontium salts were included in the preparation with the irritant.

Although several hypothesis have been communicated to explain the mechanism of action of strontium salts [40,81], the mode of action still remains unclear. Below are some thoughts on this matter:

- Because the action of strontium salts is quite immediate after application, it is assumed that they act directly on the type-C nociceptor and suppress the neuronal depolarization that normally transmits the sensory signal to the brain.
- By their analogy to calcium, strontium salts could use calcium channels to induce the release of neurotransmitters in synapsis or could antagonize the usual calcium-induced depolarization.
- It is also not impossible that strontium salts could directly influence keratinocytes or inflammatory cells and regulate the release of some cytokines.

Anti-Irritants for Dryness/Tightness Perception

Tightness and dryness perception are usually the earliest warning signs detected by highly receptive subjects using products that are not irritating with one single use but that can become slightly irritating or skin drying after multiple

exposures. These signs are generally followed, if the product is not discontinued, by the progressive development of clinical signs of intolerance such as scaling, flaking, or even erythema [82].

This kind of subclinical irritation is essentially observed for surfactant-based products, and the anti-irritant systems described for surfactant-induced irritation should thus be employed. Additionally, topical skin rehydrating preparations can also be effective in some cases to decrease the dryness/tightness perception.

Anti-Irritants for Negative Sensory Skin Feel

Negative subjective sensory signals that are translated as "irritated skin" by the consumers while totally independent of irritation can be addressed in two ways:

1. If these signals are induced by the surfactant-based product, the surfactant system should be reformulated. Indeed, each surfactant is associated with a specific perception to the skin such slipperiness, smoothness (perception of a mild product), or, at the extreme, roughness and drag (perception of an irritant product). A good combination of surfactants can provide the desired skin feel and signal.
2. Skin-feel additives may be added to the product to deliver smoothness, silkiness, hydrated feel, and so forth associated with a "nonirritated" skin signal. A review of the skin-feel additives has been made by Zocchi [83].

Effect of Divalent Cations on Skin Irritation

Magnesium Is Not an Anti-Irritant for Surfactants

Magnesium is frequently described as a depressor of skin irritation. Such a false idea is essentially arising from in vitro data based on protein denaturation tests. In those tests, the more a surfactant solution denatures a protein, the more it is predicted to be an irritant to the skin; magnesium clearly depresses surfactant-induced protein denaturation in vitro [84]. However, when well-controlled in vivo tests are performed to investigate the effect of magnesium directly on human volunteers, it is confirmed that magnesium does not decrease the skin irritation potential of surfactants or surfactant-based products [85]. The in vivo studies included both acute irritation by occlusive PTs and chronic irritation by repetitive short-term applications of the products. The study compared sodium and magnesium salts of surfactants (e.g., magnesium and SLS) in single solutions or incorporated into finished products, and they investigated the effect of adding magnesium sulfate to a solution of surfactant.

Some preliminary studies with calcium showed a behavior similar to magnesium (personal data), with an inhibition of protein denaturation in vitro and no reduction of irritation in vivo.

Zinc Salts May Be Potent Anti-Irritants for Surfactants

Zinc is a key co-element in more than 200 enzymatic reactions that happen in the skin and is, as such, of critical importance to the skin [86]. A few publications have shown the beneficial protective effect of topically applied zinc oxide on skin irritation [87] and as a mediator of oxidative stress [88]. Zinc oxide has also been incorporated into skin-protective aerosol compositions to protect a baby's bottom from erythema [89] and in surfactant-based liquid products to exert a substantial anti-irritant effect on skin [90]. Other zinc salts have been incorporated in leave-on products (gels, creams, lotions, or ointments) to reduce or prevent dermal or mucosal irritation [91].

Rigano et al. [92] have shown that zinc salts of coceth sulfate were very mild to the skin and that zinc salts of lauryl ether sulfate were milder than their sodium counterpart.

In view of the many situations in skin where zinc plays an essential role, the exact mechanism by which the zinc element exerts its beneficial effect on skin irritation has still, however, not been elucidated.

CONCLUSION

This chapter describes how surfactants interact with the skin and briefly reviews several systems by which it is now possible to control the skin irritation potential of surfactant-based products. This can be done

- Through a modification of surfactant behavior in solution
- Through a modification of their interaction with the skin surface
- Through a protection of the skin surface via ingredients (e.g., lipids, proteins, etc.) delivered from the solution
- Through a control of their subjective perception by the consumer using strontium salts or skin-feel agents

These anti-irritant systems, combined with a selection of mild surfactants, allow the cosmetic formulator to design very mild hygiene products.

Other anti-irritant systems also exist for leave-on cosmetics and in pharmacology, such as antioxidants and anti-inflammatory ingredients. They are still not commonly used in surfactant-based products, but, if correctly delivered to the skin during the use of the product, they could provide a new field of research for improving the tolerance of cleansing products.

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33 In Vivo Irritation

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INTRODUCTION

Irritant Dermatitis

Skin irritation is a localized nonimmunologically mediated inflammatory process. It may manifest objectively with skin changes such as erythema, edema, and vesiculation or subjectively with complaints of burning, stinging or itching, with no detectable visible or microscopic changes. Several forms of objective irritation exist (Table 33.1). *Acute irritant dermatitis* may follow a single, usually accidental, exposure to a potent irritant and generally heals soon after exposure. An *irritant reaction* may be seen in individuals more extensively exposed, for example, in hairdressers and wet work-performing employees, who are regularly exposed to irritants. Repeated irritant reactions may develop into a contact dermatitis, which generally has a good prognosis. Other forms of irritant dermatitis include *delayed acute irritant contact dermatitis*, which occurs when there is a delay between exposure and inflammation, and *cumulative irritant dermatitis*, which is the most common form of irritant contact dermatitis. Following exposure, an acute irritant dermatitis is not seen, but invisible skin changes occur, which eventually lead to an irritant dermatitis when exposure reaches a threshold point. This may follow days, weeks, or years from exposure [1]. These various forms require specialized models to predict their occurrence following exposure to specific products.

Need for Models

Prevention of skin irritation is important both to the consumer who will suffer from it and to industry, which needs a licensable and marketable product. Accurate prediction of the irritation potential of industrial, pharmaceutical, and cosmetic materials is therefore necessary for consumer health and safety, and for product development.

In the United States, the Food and Drug Administration (FDA) is responsible for assuring that cosmetics are safe and properly labeled, through enforcement, the Federal Food, Drug, and Cosmetic Act 2010 (FD&C Act 2010), related statutes, and regulations promulgated under these laws. Under the FD&C Act, it is not a requirement to use animals in testing cosmetics for safety, nor does the act subject cosmetics to FDA premarket approval. It is the responsibility of the manufacturer to substantiate the safety of both ingredients and finished cosmetic products prior to marketing [2]. In Europe, the European Commission (EC) foresees and enforces regulatory framework for product testing [3].

Presently, there are emphases for using alternative (nonanimal) methods for cosmetic testing. Main regulatory bodies such as the FDA [2,4] and the EC [5,6] have produced reports, guidance, and relevant resources on this. The FDA joined with the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) and its supporting center, the National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM), to coordinate the development, validation, acceptance, and harmonization of alternative toxicological test methods in the United States. Their focus is to find alternatives that may improve toxicity characterization; increase savings in time and cost; and even refine, reduce, or replace animal use [2]. Similarly, the EC set out a regulatory framework ("The Cosmetics Directive") with the aim of phasing out animal testing through a *testing ban** and *marketing ban*.† The marketing ban for cosmetic ingredients tested on animals is due to be fully enforced in 2013 [7]; however, in most recent reports, experts noted that there were significant contributions to reduce, refine, and partially replace animal testing, and they concluded that considerable scientific challenges have to be overcome before a full replacement of animal tests will be possible [8,9]. Until alternative models can substitute, in vivo models provide a means by which a cosmetic can be tested on living skin, at various sites, and under conditions that should closely mimic the intended human use.

Many aspects of irritation have been described, ranging from the visible erythema and edema to molecular mediators such as interleukins and prostaglandins. Therefore, a variety of in vivo and in vitro approaches to experimental assay are possible. However, no model assays inflammation in its entirety. Each model is limited by our ability to interpret and extrapolate the features of inflammation to the desired context. Therefore, predicting human responses based on data from nonhuman models requires particular care.

Various human experimental models have been proposed, providing irritant data for the relevant species. Human models allow the substance to be tested in the manner that the

* Testing ban: a prohibition to test finished cosmetic products and cosmetic ingredients on animals. The testing ban on finished cosmetic products has applied since September 11, 2004, and the testing ban on ingredients or combinations of ingredients has applied since March 11, 2009.

† Marketing ban: a prohibition to market in the European Community finished cosmetic products and ingredients included in cosmetic products that were tested on animals. The marketing ban has applied since March 11, 2009, for all human health effects, with the exception of repeated-dose toxicity, reproductive toxicity, and toxicokinetics.

TABLE 33.1
Classification of Irritant Dermatitis

Classification	Features	Clinical Picture
Acute irritant dermatitis	Single exposure	Reaction usually restricted to exposed area, appears within minutes
	Strong irritant	Erythema, edema, blisters, bullae, pustules, later eschar formation
	Individual predisposition considered	Symptoms include burning, stinging, and pain
	Generally unimportant	Possible secondary infection
		Good prognosis
Irritant reaction	Follows repeated acute skin irritation	Repeated irritant reactions may develop into contact dermatitis
	Often occupational—hairdressers, wet workers	Good prognosis
Cumulative irritant dermatitis	Repeated exposure required	Initially, subject may experience stinging or burning
	Initial exposures cause *invisible* damage	Eventually, erythema, edema, or scaling appears
	Exposure may be weeks, months, or years until dermatitis develops	Variable prognosis
	Individual variation is seen	
Delayed acute irritant	Latent period of 12–24 h between	Clinically similar to acute irritant dermatitis
Contact dermatitis	Exposure and dermatitis	Good prognosis
Subclinical irritation	Irritation detectable by bioengineering methods prior to development of irritant dermatitis	
Subjective irritation	Subject complains of irritant symptoms with no clinically visible irritation	Perceived burning, stinging, or itching
Traumatic irritant dermatitis	Follows acute skin trauma, for example, burn or laceration	Incomplete healing, followed by erythema, vesicles, vesicopapules and scaling; may later resemble nummular (coin-shaped) dermatitis
Pustular and acneiform dermatitis	Caused by metals, oils, greases, tar, asphalt, chlorinated naphthalenes, polyhalogenated	Develops over weeks to months
		Variable prognosis naphthalenes, cosmetics
Friction dermatitis	Caused by friction trauma	Sometimes seen on hands and knees

general public will use it: For example, wash testing (see below) attempts to mimic the consumer's use of soaps and other surfactants. Also, humans are able to provide subjective data on the degree of irritation caused by the product. However, human studies are also limited by pitfalls in interpretation and, of course, by the fear of applying new substances to human skin before their irritant potential has been evaluated.

ANIMAL MODELS

Draize Rabbit Models

The Draize model [10] and its modifications are commonly used to assay skin irritation using albino rabbits. Various governmental agencies have adopted these methods as standard test procedure. The procedure adopted in the US Federal Hazardous Substance Act (FHSA) is described in Tables 33.2 and 33.3 [11–13]. Table 33.4 compares this method with some other modifications of the Draize model.

Draize utilized the above scoring system to calculate the Primary Irritation Index (PII). This is calculated by averaging the erythema scores and also averaging the edema scores of all sites (abraded and nonabraded). These two averages are then added together to give the PII value. A value of <2 was considered nonirritating, 2–5 mildly irritating, and >5 severely irritating. A value of 5 defines an irritant by Consumer Product Safety Commission (CPSC) standards. Subsequent laboratory and clinical experience has demonstrated that the value judgments (i.e., nonirritating, mildly irritating, severely irritating) proposed in 1944 require

TABLE 33.2
Draize–FHSA Model

Number of animals	6 albino rabbits (clipped)
Test sites	2 × 1 in.^{2} sites on dorsum
	One site intact, the other abraded, for example, with hypodermic needle
Test materials	Applied undiluted to both test sites
	Liquids: 0.5 mL
	Solids/semisolids: 0.5 g
Occlusion	1 in.^{2} surgical gauze over each test site
	Rubberized cloth over entire trunk
Occlusion period	24 h
Assessment	24 and 72 h
	Visual scoring system

TABLE 33.3
Draize–FHSA Scoring System

	Score
Erythema and Eschar Formation	
No erythema	0
Very slight erythema (barely perceptible)	1
Well-defined erythema	2
Moderate to severe erythema	3
Severe erythema (beet redness) to slight eschar formation (injuries in depth)	4
Edema Formation	
No edema	0
Very slight edema (barely perceptible)	1
Slight edema (edges of area well defined by definite raising)	2
Moderate edema (raised >1 mm)	3
Severe edema (raised >1 mm and extending beyond the area of exposure)	4

Source: Patrick, E., and Maibach, H.I., Comparison of the time course, dose response and mediators of chemically induced skin irritation in three species, in Frosch, P.J., editor, *Current Topics in Contact Dermatitis*, Springer, New York, 1989.

clinical judgment and perspective and should not be viewed in an absolute sense. Many materials irritating to the rabbit may be well tolerated by human skin.

Although the Draize scoring system does not include vesiculation, ulceration, and severe eschar formation, all of the Draize-type tests are used to evaluate corrosion as well as irritation. When severe and potentially irreversible reactions occur, the test sites are further observed on days 7 and 14, or later if necessary.

Modifications to the Draize assay have attempted to improve its prediction of human experience. The model is criticized for inadequately differentiating between mild and moderate irritants. However, it serves well in hazard identification, often overpredicting the severity of human skin reactions [13]. Therefore, Draize assays continue to be recommended by regulatory bodies for drugs and industrial chemicals.

Cumulative Irritation Assays

Several assays study the effects of cumulative exposure to a potential irritant. Justice et al. [14] administered seven applications of surfactant solutions at 10 min intervals to the clipped dorsum of albino mice. The test site was occluded with a rubber dam to prevent evaporation, and the skin was examined microscopically for epidermal erosion.

Frosch et al. [15] described the guinea pig repeat irritation test (RIT) to evaluate protective creams against the chemical irritants sodium lauryl sulfate (SLS), sodium hydroxide (NaOH), and toluene. The irritants were applied daily for 2 weeks to shaved back skin of young guinea pigs. Barrier creams were applied to the test animals 2 h prior to and immediately after exposure to the irritant. Control animals were treated with the irritant only. Erythema was measured visually and by bioengineering methods: laser Doppler flowmetry and transepidermal water loss (TEWL). One barrier cream was effective against SLS and toluene, while the other tested was not. In a follow-up study, another allegedly protective cream failed to inhibit irritation caused by SLS and toluene and exaggerated irritation to NaOH, contrary to its recommended use [16]. The RIT is proposed as an animal model to test the efficacy of barrier creams and is further proposed as a human version, described below.

Repeat application patch tests have been developed to rank the irritant potential of products. Putative irritants are applied to the same site for 3–21 days, under occlusion. The degree of occlusion influences percutaneous penetration, which may, in turn, influence the sensitivity of the

TABLE 33.4
Examples of Modified Draize Irritation Method

	Draize	FHSA	DOT	FIFRA	OECD
No. of animals	3	6	6	6	6
Abrasion/intact	Both	Both	Intact	2 of each	Intact
Dose liquids	0.5 mL undiluted	0.5 mL undiluted	0.5 mL	0.5 mL undiluted	0.5 mL
Dose solids in solvent	0.5 g	0.5 g moistened	0.5 g moistened	0.5 g	0.5 g
Exposure period (h)	24	24	4	4	4
Examination (h)	24, 72	24, 72	4, 48	0.5, 1, 24, 48, 72	0.5, 1, 24, 48, 72
Removal of test materials	Not specified	Not specified	Skin washed	Skin wiped	Skin washed
Excluded from testing	—	—	—	Toxic materials pH ≤ 2 or ≥ 11.5	Toxic materials pH ≤ 2 or ≥ 11.5

Source: Patrick, E., and Maibach, H.I., Comparison of the time course, dose response and mediators of chemically induced skin irritation in three species, in Frosch, P.J., editor, *Current Topics in Contact Dermatitis*, Springer, New York, 1989.

Note: DOT, Department of Transportation; FHSA, Federal Hazardous Substance Act; FIFRA, Federal Insecticide, Fungicide and Rodenticide Act; OECD, Organization for Economic Cooperation and Development.

test. Patches used vary from Draize-type gauze dressings to metal chambers. Therefore, a reference irritant material is often included in the test to facilitate interpretation of the results. Various animal species have also been used, such as the guinea pig and the rabbit [17,18]. Wahlberg [18] measured skin fold thickness with Harpenden calipers to assess the edema-producing capacity of chemicals in guinea pigs. This model demonstrated clear dose–response relationships and discriminating power, except for acids and alkalis, where no change in skin fold thickness was found.

Open application assays are also used for repeat irritation testing. Marzulli and Maibach [19] described a cumulative irritation assay in rabbits that utilizes open applications and control reference compounds. The test substances are applied 16 times over a 3-week period, and the results are measured with a visual score for erythema and skin thickness measurements. These two parameters correlated highly. A significant correlation was also demonstrated between the scores of 60 test substances in the rabbit and in man, suggesting that the rabbit assay is a powerful predictive model.

Anderson et al. [20] utilized an open application procedure in guinea pigs to rank weak irritants. A baseline response to SLS solution was obtained after three applications per day for 3 days to a 1 cm^2 test area. This baseline is used to compare other irritants, of which trichloroethane was the most irritant, similar to 2% SLS. Histology demonstrated a mononuclear dermal inflammatory response.

Immersion Assay

The guinea pig immersion assay was developed to assess the irritant potential of aqueous surfactant-based solutions but might be extended to other occupational settings such as aqueous cutting fluids. Restrained guinea pigs are immersed in the test solution, while maintaining their head above water. The possibility of systemic absorption of a lethal dose restricts the study to products of limited toxic potential. Therefore, the test concentration is usually limited to 10%.

Ten guinea pigs are immersed in a 40°C solution for 4 h daily, for 3 days. A comparison group is immersed in a reference solution. Twenty-four hours after the final immersion, the animals' flanks are shaved and evaluated for erythema, edema, and fissures [21–24]. Gupta et al. [25] concomitantly tested the dermatotoxic effects of detergents in guinea pigs and humans, utilizing the immersion test and the patch test, respectively. Epidermal erosion and a 40%–60% increase in the histamine content of the guinea pig skin was found, in addition to a positive patch test reaction in seven of out eight subjects.

Mouse Ear Model

Uttley and Van Abbe [26] applied undiluted shampoos to one ear of mice daily for 4 days, visually quantifying the degree of inflammation as vessel dilatation, erythema, and edema. Patrick and Maibach [27] measured ear thickness to quantify the inflammatory response to surfactant-based products and other chemicals. This allowed quantification of dose-response relationships and comparison of chemicals. Inoue et al. [28] used this model to compare the mechanism of mustard oil-induced skin inflammation to the mechanism of capsaicin-induced inflammation. Mice were pretreated with various receptor antagonists, such as 5-hydroxytryptamine receptor 2A (5-HT_2), H_1, and tachykinin antagonists, demonstrating that the tachykinin neurokinin receptor 1 (NK1) receptor was an important mediator of inflammation induced by mustard oil. The mouse models provide simplicity and objective measurements. Relevance for man requires elucidation.

Other Methods

Several other assays of skin irritation have been suggested. Humphrey [29] quantified the amount of Evans Blue dye recovered from rat skin following exposure to skin irritants. Trush et al. [30] utilized myeloperoxidase in polymorphonuclear leukocytes as a biomarker for cutaneous inflammation.

HUMAN MODELS

Human models for skin irritation testing are species relevant, thereby eliminating the precarious extrapolation of animal and in vitro data to the human setting. As the required test area is small, several products or concentrations can be tested simultaneously and compared. Inclusion of a reference irritant substance facilitates interpretation of the irritant potential of the test substances. Prior animal or in vitro studies, depending on model relevance and regulatory issues, can be utilized to exclude particularly toxic substances or concentrations before human exposure.

Single-Application Patch Testing

The National Academy of Sciences (NAS) [31] outlined a single-application patch test procedure determining skin irritation in humans. Occlusive patches may be applied to the intrascapular region of the back or the volar surface of the forearms, utilizing a relatively nonocclusive tape for new or volatile materials. More occlusive tapes or chambers generally increase the severity of the responses. A reference material is included in each battery of patches.

The exposure time may vary to suit the study. NAS suggests a 4 h exposure period, although it may be desirable to test new or volatile materials for 30 min to 1 h. Studies longer than 24 h have been performed. Skin responses are evaluated 30 min to 1 h after removal of the patch, using the animal Draize scale (Table 33.2) or similar. Kligman and Wooding [32] described statistical analysis on test data to calculate the IT50 (time to produce irritation in 50% of the subjects) and the ID50 (dose required to produce irritation in 50% of the subjects after a 24 h exposure).

Robinson et al. [33] suggested a 4 h patch test as an alternative to animal testing. Assessing erythema by visual scoring, they tested a variety of irritants on Caucasians and Asians. A relative ranking of irritancy was obtained, utilizing 20%

SLS as a benchmark. Taking this model further, McFadden et al. [34] investigated the threshold of skin irritation in the six different skin types. Again using SLS as a benchmark, they defined the skin irritant threshold as the lowest concentration of SLS that would produce skin irritation under the 4 h occluded patch conditions. They found no significant difference in irritation between the skin types.

Cumulative Irritation Testing

Lanmnan et al. [35] and Phillips et al. [17] described a cumulative irritation assay that has become known as the "21-day" cumulative irritation assay. The purpose of the test was to screen new formulas prior to marketing. A l in. square of Webril was saturated with liquid or 0.5 g of viscous substances and applied to the surface of the pad to be applied to the skin. The patch was applied to the upper back and sealed with occlusive tape. The patch is removed after 24 h and then reapplied after examination of the test site. This is repeated for 21 days, and the IT50 can then be calculated. Note that the interpretation of the data in best done by comparing the data to an internal standard—for which human clinical experience exists.

Modifications have been made to this method. The chamber scarification test (see below) was developed to predict the effect of repeated applications of a potential irritant to damaged skin, rather than healthy skin. The cumulative patch test described above failed to predict adverse reactions to skin damaged by acne or shaving or sensitive areas such as the face [36].

Wigger-Alberti et al. [37] compared two cumulative models, testing skin reaction to metalworking fluids (MWFs). Irritation was assessed by visual scoring, TEWL, and chromametry. In the first method, MWFs were applied with Finn Chambers on the volunteers' midback, removed after 1 day of exposure, and reapplied for a further 2 days. In the second method, cumulative irritant contact dermatitis was induced using a repetitive irritation test for 2 weeks (omitting weekends) for 6 h per day. The 3-day model was preferred because of its shorter duration and better discrimination of irritancy. For low-irritancy materials in which discrimination is not defined with visual and palpatory scores, bioengineering methods (i.e., TEWL) may be helpful.

Chamber Scarification Test

This test was developed [38,39] to test the irritant potential of products on damaged skin. Six to eight l mm sites on the volar forearm were scratched eight times with a 30-gauge needle, without causing bleeding. Four scratches were parallel, and the other four were perpendicular to these. Duhring chambers, containing 0.1 g of test material (ointments, creams, or powders), were then placed over the test sites. For liquids, a fitted pad saturated (0.l mL) may be used. Chambers containing fresh materials are reapplied daily for 3 days. The sites are evaluated by visual scoring 30 min after removal of the final set of chambers. A scarification index may be calculated if both normal and scarified skin is tested, to reflect the relative degree of irritation between compromised and intact skin: this is the score of scarified sites divided by the score of intact sites. However, the relationship of this assay to routine use of substances on damaged skin remains to be established. Another compromised skin model, the arm immersion model of compromised skin, is described in the immersion tests section below.

The Soap Chamber Test

Frosch and Kligman [40] proposed a model to compare the potential of bar soaps to cause "chapping." Standard patch testing was able to predict erythema but unable to predict the dryness, flaking, and fissuring seen clinically. In this method, Duhring chambers fitted with Webril pads were used to apply 0.1 mL of an 8% soap solution to the human forearm. The chambers were secured with porous tape and applied for 24 h on day 1. On days 2–5, fresh patches were applied for 6 h. The skin was examined daily before patch application and on day 8, the final study day. No patches were applied after day 5. Applications were discontinued if severe erythema was noted at any point. Reactions were scored on a visual scale of erythema, scaling, and fissures. This test correlated well with skin washing procedures but tended to overpredict the irritancy of some substances [41].

Immersion Tests

These tests of soaps and detergents were developed in order to improve irritancy prediction by mimicking consumer use. Kooyman and Snyder [42] described a method in which soap solutions of up to 3% were prepared in troughs. The temperature was maintained at 105°F while subjects immersed one hand and forearm in each trough, comparing different products (or concentrations). The exposure period ranged from 10 to 15 min, three times each day for 5 days, or until irritation was observed in both arms. The antecubital fossa was the first site to demonstrate irritation, followed by the hands [14,42]. Therefore, antecubital wash tests (see below) and hand immersion assays were developed [13].

Clarys et al. [43] used a 30 min/4-day immersion protocol to investigate the effects of temperature and also anionic character on the degree of irritation caused by detergents. The irritation was quantified by assessment of the stratum corneum (SC) barrier function (TEWL), skin redness (a* color parameter), and skin dryness (capacitance method). While both detergents tested significantly affected the integrity of the skin, higher anionic content and temperature, respectively, increased the irritant response.

Allenby et al. [44] described the arm immersion model of compromised skin, which is designed to test the irritant or allergic potential of substances on damaged skin. Such skin may demonstrate an increased response, which may be negligible or undetectable in normal skin. The test subject immersed one forearm in a solution of 0.5% sodium dodecyl sulfate for 10 min, twice daily, until the degree of erythema

reached 1 to 1+ on a visual scale. This degree of damage corresponded to a morning's wet domestic work. Patch tests of various irritants were applied to the dorsal and volar aspects of both the pretreated and untreated forearms and also to the back. Each irritant produced a greater degree of reaction on the compromised skin.

Wash Tests

Hannuksela and Hannuksela [45] compared the irritant effects of a detergent in use testing and patch testing. In this study of atopic and nonatopic medical students, each subject washed the outer aspect of one forearm with liquid detergent for 1 min, twice daily for 1 week. Concurrently, a 48 h chamber patch test of five concentrations of the same detergent was performed on the upper back. The irritant response was quantified by bioengineering techniques: TEWL, electrical capacitance, and skin blood flow. In the wash test, atopics and nonatopics developed irritant contact dermatitis equally, whereas atopics reacted more readily to the detergent in chamber tests. The disadvantage of the chamber test is that, under occlusion, the detergent can cause stronger irritation than it would in normal use [46]. Although the wash test simulates normal use of the product being tested, its drawback is a lack of standard guidelines for performing the test. Charbonnier et al. [47] included squamometry in their analysis of a handwashing model of subclinical irritant dermatitis with SLS solutions. Squamometry demonstrated a significant difference between 0.1% and 0.75% SLS solutions, whereas visual, subjective, capacitance, TEWL and chromametry methods were unable to make the distinction. The authors suggest squamometry as an adjunct to the other bioengineering methods.

Frosch et al. [15] described an antecubital washing test to evaluate toilet soaps, utilizing two washing procedures per day. Simple visual scoring of the reaction (erythema and edema) allows products to be compared. This comparison can be in terms of average score or number of washes required to produce an effect.

Assessing Protective Barriers

Zhai et al. [48] proposed a model to evaluate skin-protective materials. Ten subjects were exposed to the irritants SLS and ammonium hydroxide (in urea), and Rhus allergen. The occluded test sites were on each forearm, with one control site on each. The irritant response was assessed visually using a 10-point scale, which included vesiculation and maceration unlike standard Draize scales. The scores were statistically analyzed for nonparametric data of the barrier creams studied; paraffin wax in cetyl alcohol was found to be the most effective in preventing irritation.

Wigger-Alberti and Elsner [49] investigated the potential of petrolatum to prevent epidermal barrier disruption induced by various irritants in a repetitive irritation test; they assessed its potential as a standard reference product. White petrolatum was applied to the backs of 20 human subjects who were exposed to SLS, NaOH, toluene, and lactic acid. Irritation was assessed by TEWL and colorimetry in addition to visual scoring. It was concluded that petrolatum was an effective barrier cream against SLS, NaOH, and lactic acid, and moderately effective against toluene.

Frosch et al. [15] adapted the guinea pig RIT described above for use in humans. Two barrier creams were evaluated for their ability to prevent irritation to SLS. In this repetitive model, the irritant was applied to the ventral forearm, using a glass cup, for 30 min daily for 2 weeks. One arm of each subject was pretreated with a barrier cream. As in the animal model, erythema was assessed by visual scoring, laser Doppler flowmetry, and TEWL. Skin color was also measured by colorimetry (L and a* color values). The barrier cream decreased skin irritation to SLS, the most differentiating parameter being TEWL and the least differentiating being colorimetry.

Behind-the-Knee Test

Farage developed a method called Behind-the-Knee (BTK) test to include other irritant factors [50–52]. This method not only demonstrates the potential of chemical irritation from substrates/products but also illustrates that mechanical irritation could contribute to the overall irritation potential. In this test protocol, samples are applied to the back of the knee using an elastic band, allowing panelists to carry on with their daily activities. Movements during these activities help generate friction between the test sample and the skin. This adds the element of mechanical irritation to patch testing. The main advantage of this method is that two products can be tested on the same panelist at the same point in time. Compared to other standard patch tests, BTK testing consistently showed higher irritation levels with reproducible results. Although this test was developed to test catamenial products, the author suggests that it has potential for evaluating textiles, facial tissues, baby and adult diapers, and laundry products, because mechanical irritation may contribute to the overall irritation potentials [53].

BIOENGINEERING METHODS IN MODEL DEVELOPMENT

Many of the models described above do not employ the modern bioengineering techniques available, and therefore, data based on these models may be imprecise. Despite the skill of investigation, subjective assessment of erythema, edema, and other visual parameters may lead to confounding by interobserver and intraobserver variation. Although the eye may be more sensitive than current spectroscopy and chromametric techniques, the reproducibility and increased statistical power of such data may provide greater benefit. A combination of techniques, such as TEWL, capacitance, ultrasound, laser Doppler flowmetry, spectroscopy, and chromametric analysis, in addition to skilled observation may increase the precision of the test. Andersen and Maibach [54] compared various bioengineering techniques, finding that clinically indistinguishable reactions induced significantly different

changes in barrier function and vascular status. An outline of many of these techniques is provided by Patil et al. [13].

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34 Noninvasive Clinical Assessment of Skin Inflammation

Michael K. Robinson and Kevin J. Mills

INTRODUCTION

The study of skin biology has been transformed over the past 20 years, largely owing to advances in measurement technologies that permit evaluation of parameters invisible to the naked eye. Years ago, the late Dr. Albert Kligman coined the term "invisible dermatoses" to emphasize that what appears visually normal can be quite abnormal under the skin surface [1]. He further suggested that the future of dermatology would become so reliant on nonvisual methods of diagnosis that traditional visual assessment techniques would likely be superseded by these noninvasive techniques [2]. Evolving techniques and instrumentation have facilitated the study of many of the skin's physiological and biophysical properties, including water content, barrier properties, tensile strength, and elasticity, and estimates of melanin, hemoglobin, and collagen. However, for those more interested in the immune and inflammatory response of the skin, instrumental methods have been less useful.

Surface assessment and grading of inflammatory skin reactions has long relied on visual assessment methods [3–6]. Certain instrumental methods, such as laser doppler flowmetry and colorimetry, provide some degree of numerical quantification of surface skin reactions, which can be used to supplement simple visual grades [7]. Novel skin imaging techniques are also now providing dynamic profiles of human skin, which are revolutionizing histomorphometric analyses [8,9]. These noninvasive techniques may someday provide molecular profiles of skin inflammation. However, up to now, the detailed study of the cellular and molecular processes underlying inflammatory skin responses has commonly required the use of highly invasive (e.g., biopsy), or moderately invasive (e.g., suction blister), techniques [10–12].

In the mid-1990s, new techniques were introduced to study skin inflammation by attempting to adsorb molecular mediators of inflammation from the skin surface or within superficial layers of the stratum corneum (SC). The first published method used a relatively mild cellophane tape-stripping approach and measured extracted constitutive cytokine levels (and ratios) in sun-exposed, unexposed, and ultraviolet (UV)-irradiated skin [13]. At about the same time, we (MKR) independently developed an even less invasive approach that used minimally adhesive Sebutape™ for mediator adsorption [14–16]. Over the next several years, both groups reported on the additional application of these procedures for the "targeted proteomic" detection of inflammatory mediators (cytokines) in various types of compromised skin conditions and diseases [17–21]. Other investigators have more recently adopted these techniques, using sometimes more aggressive tape-stripping techniques to probe cytokine, lipid, or messenger RNA (mRNA) expression patterns at various levels of the SC [22–27]. As will be described below, these methods have been useful in the detection of diseased or chronically compromised skin and mucosal tissue. Also, changes in the recovery of skin surface cytokines and small molecules have been used as surrogate end points to demonstrate the efficacy of treatments of inflammatory skin conditions [28,29].

METHODS USED TO RECOVER AND MEASURE SKIN SURFACE INFLAMMATORY BIOMARKERS

The original procedure for noninvasive tape adsorption of human skin for assessment of inflammatory mediators was that of Hirao and coworkers [13], who used cellophane tape to extract the constitutive cytokines interleukin-1 alpha (IL-1α) and its competitive inhibitor interleukin-1 receptor antagonist (IL-1ra) from the SC of sun (UV)-exposed and unexposed and UV-irradiated skin. Their basic procedure was to cleanse the sampling site with soap and water, tape-strip once with cellophane tape (which was discarded), and restrip the same site. The second tape was extracted (sonicated in buffer) and assayed for immunoreactive IL-1α and IL-1ra. The cytokines were measured directly by enzyme immunoassay as well as by immunoblotting and functional (induced cell proliferation) assays. In a follow-up study, they simplified the tape sampling approach by applying a single tape-strip procedure [17].

Independently of the work by Hirao and Terui [13,17], we examined a variety of approaches to this problem, including a variety of tapes, extraction methods, and mediators of interest. We settled upon Sebutape™ as the adsorbent tape of choice. An acrylic polymer film manufactured by CuDerm, this tape was much less adhesive and adherent to skin than either cellophane tape or another CuDerm tape product, D-Squame™ (a polyacrylate ester adhesive). Unlike the other two tapes, multiple 1 min applications of Sebutape™ to the same skin site did not strip off the SC. We obtained complete and quantitative recovery of spiked cytokine when the Sebutapes were sonicated and vortexed to extract the

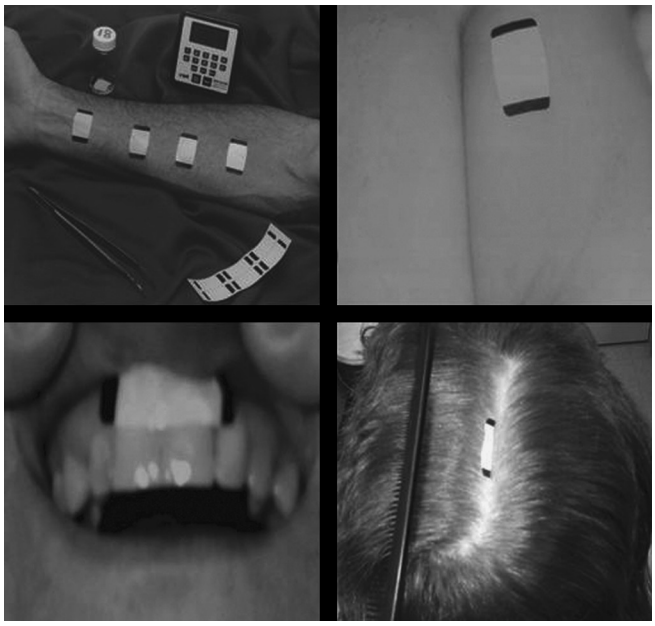

FIGURE 34.1 Photographs of Sebutape™ application to various skin and mucosal surfaces. (From Robinson, M.K., and Perkins, M.A., *Am. J. Contact Dermatol*., 13, 2002. With permission.)

material [14–16]. Sebutape™ turned out to be a very flexible adsorbent tape for our purposes. It could be applied to overtly inflamed skin (including infant skin) without causing pain on removal. It could also be applied to mucosal surfaces and trimmed and applied to tight spaces with limited skin surface area (e.g., scalp skin between parted hairs). Proteins (cytokines) of interest were assayed by individual analyte-specific enzyme immunoassays, and recoveries were normalized to total protein to reduce intersubject variability. Figure 34.1 shows some of the Sebutape™ application methods used in our studies. Reapplication of up to 30 of these tapes to the same area of skin did not produce any erythema—common with cellophane tape stripping—and also tended to recover similar amounts of the cytokine IL-1α (Figure 34.2) with each application, confirming historical findings that the SC acts as a reservoir (sink) for this cytokine [30]. No inducible cytokine (IL-8) was detected even 24 h after the initial tape collections—again indicating a lack of tape-induced irritation.

A source of frustration surrounding our early work in this area was the need to run individual immunoassays on each protein of interest. This limited the number of analyses that could be run on each sample. More recently, multiplex immunoassays (e.g., Luminex beads) or assay services (e.g., Rules-Based Medicine, Austin, Texas) have opened the door to more extensive analysis of adsorbed proteins or other biomarkers from limiting amounts of tape-absorbed samples. One such application, looking at structural skin proteins and serological markers, was recently published by Hendrix et al. [31]. They used D-Squame™ tapes to adsorb and quantify structural proteins (involucrin, fibronectin, and keratins 1, 6, and 10) and plasma biomarkers (cortisol, human serum albumin) from healthy forearm skin. They used a multianalyte-profiling method, SkinMAP™ (Linco Research, St. Charles, Missouri) for the analysis.

Other groups have recently used more aggressive tape-stripping techniques to examine biomarker recovery patterns at different locations within the SC from the skin surface to just above the viable epidermis [23]. While this approach is more "invasive" than techniques using one or a few tape applications, and might not be appropriate for certain types of studies (e.g., infant studies), it is still relatively noninvasive compared to suction blister or biopsy procedures. More gentle techniques can also be used, such as simple scrubbing of the skin surface with a small volume of buffer and collecting

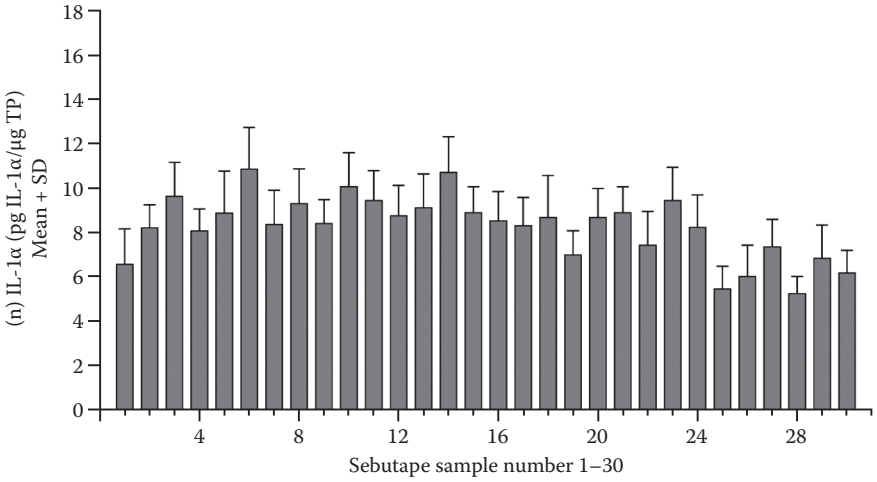

FIGURE 34.2 Recovery of IL-1α from Sebutape™ samples collected from the same forearm skin site. Thirty successive Sebutape™ samples were collected from the same normal-appearing naive skin site on the lower volar forearm of four subjects ($n = 2$ sites/subject). Each bar represents the group mean IL-1α level (± standard deviation [SD], $n = 8$ samples) for each of the 30 Sebutape™ samples collected from each of the two skin sites. The IL-1α/total protein levels from samples 1 to 30 range between 5 and 15 pg IL-1α/mg total protein. There were no detectable levels of the inducible cytokine IL-8 (assay sensitivity 10 pg/mL) in any of the tape extracts, including samples collected 24 h after the initial tape collection. (From Perkins, M.A. et al., *Skin Res. Technol.*, 7, 2001. With permission.)

and assaying the collected fluid (MKR, unpublished), similar to the cup scrub technique used for bacterial collection [32]. Such gentle techniques would not be as useful for collection and quantification of skin structural proteins.

Last, the technique of skin surface tape stripping has not been limited to the study of protein or small molecular biomarkers. Others have adapted the procedure to recover lipid species [27] or RNA fragments from within the SC as a surrogate means to study epidermal gene expression [25,26]. The latter methodology has been patented [33] and has been applied to dermatological diagnostics [34,35]. Details on the utility of these techniques for assessment of diseased or compromised skin, as well as more recently described utility in treatment-associated skin benefits, are described below. Given all of this foundational work, and with recent advances in multianalyte-profiling methods, it should also be noted that noninvasive skin samples can now be processed through various transcriptomics, proteomics, lipidomics, or metabolomics analyses to provide readouts on hundreds or even thousands of biomarkers of interest [36].

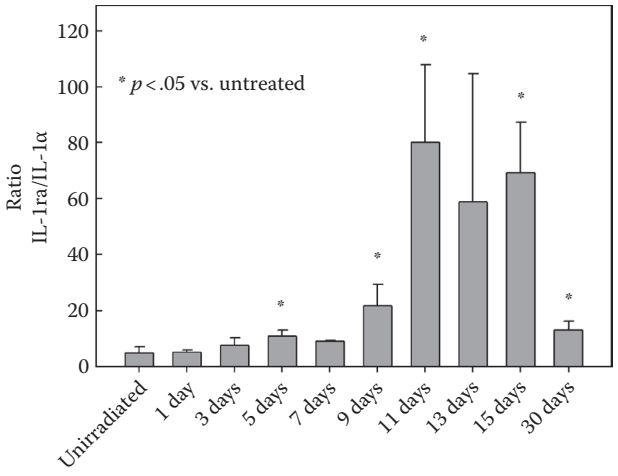

FIGURE 34.3 Time course of IL-1ra and IL-1α recovery, expressed as IL-1ra/IL-1α ratio after 2 MED dose of UVB to back skin. (From Hirao, T. et al., *J. Invest. Dermatol.*, 106, 1996. With permission.)

USE OF NONINVASIVE BIOMARKER SAMPLING OF SC TO INVESTIGATE INFLAMMATION ASSOCIATED WITH DISEASED OR COMPROMISED SKIN

In the original publication on tape-adsorbed skin surface biomarkers, Hirao et al. [13] studied sun-exposed versus sun-protected skin as well as sun-protected skin experimentally exposed to UV irradiation [13]. In comparing sun-exposed (face) and sun-protected (inner arm) skin sites, they saw higher levels of IL-1α on the arm versus the face and higher levels of IL-1ra on the face. Levels of both cytokines were normalized to total recovered protein. The ratio of IL-1ra/IL-1α was approximately 8 on the arm and >100 on the face. IL-1ra activity and the IL-1ra/IL-1α ratio were also increased on UV-unexposed back skin for 1 to 4 weeks after a 2 minimal erythema dose (MED) irradiation of the skin. Here, it took approximately 1 week to see an elevated ratio, which peaked at about 15 days postexposure, and returned to near-baseline levels after a month. The tabular data from their study are displayed in graphic form in Figure 34.3. These results suggested that chronic inflammation due to UV from either natural sun exposure or UV lamp irradiation was associated with elevated IL-1ra production, perhaps a regulatory response to IL-1α–induced inflammation and an effort by the skin to quell this response and restore homeostatic balance.

Two years later, this same laboratory expanded on their initial findings by again demonstrating increased ratios of IL-1ra/IL-1α in inflamed skin [17]. In this second study, they examined involved versus uninvolved skin sites in subjects with psoriasis, atopic dermatitis (AD), and senile xerosis. Their interest in psoriasis stemmed from earlier findings from Cooper's laboratory (using keratomed epidermal skin samples) that involved psoriatic skin had increased IL-1ra/IL-1α ratios compared with uninvolved skin from the same patients [37]. They confirmed increased IL-1ra/IL-1α ratios in sun-exposed versus unexposed skin and also observed increased ratios in all of the inflammatory skin conditions versus uninvolved skin from the same subjects. Because of intersubject variability in the measured cytokine amounts recovered, not all the comparisons were statistically significant; however, there were obvious directional changes even for those comparisons that were not significantly different. Their conclusion from these studies was that an increased IL-1ra/IL-1α ratio in the SC represents a nonspecific phenomenon in any inflammatory skin condition, likely reflecting a regulatory response against unchecked inflammation.

Consistent with the work of Hirao and coworkers, we found a reproducible elevation of IL-1ra and the IL-1ra/IL-1α ratio on sites of the body (face, lower leg, forearm) that were prone to sun exposure and lower ratios on generally sun-protected skin sites (upper leg, back, underarm) [14,16] (Figure 34.4). We also observed elevated IL-1ra/IL-1α ratios and evidence of induced IL-8 (Figure 34.5) on infant skin associated with different types of diaper dermatitis. In contrast, an acute (1 h) exposure to a high concentration of the irritant surfactant sodium dodecyl sulfate (SLS), which is sufficient to produce a weak erythema response 24 h after exposure [5], produced an opposite effect. In this situation, IL-1α (measured 24 h after the SLS exposure) was elevated, and the IL-1ra/IL-1α ratio was decreased. This indicated that IL-1α is induced and mobilized in the acute irritation response and that the IL-1ra levels increase later on, again supporting the role of elevated IL-1ra as a means to regulate more chronic inflammatory responses.

The elevation of the ratio of IL-1ra/IL-1α was a hallmark indicator of every inflammatory condition (skin or mucosal) that we studied over a period of 6 years. In addition to our findings with sun-exposed skin and infant diaper dermatitis noted above, we saw directional or significant elevation in this ratio in dandruff and seborrheic scalp dermatitis [18]

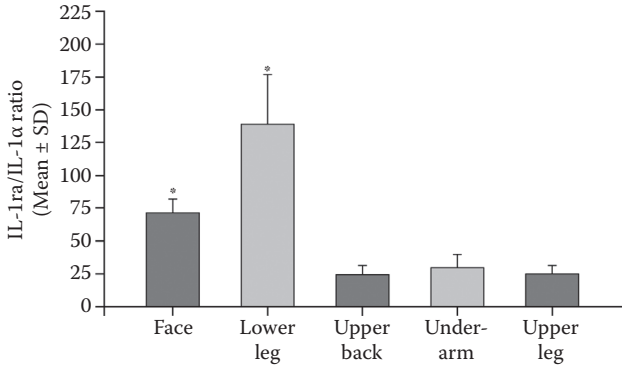

FIGURE 34.4 Cytokine levels in sun-exposed versus unexposed skin. Sebutape™ samples were collected from different body sites of adult (ages 18–65 years) male and female subjects with normal-appearing unblemished skin. The ratio of IL-1ra/IL-1α for sun-exposed facial skin (mean ± SD of all sites) and lower leg were significantly higher (~3–6 times, respectively) than skin that was minimally sun exposed (upper back, underarm, upper leg). (From Perkins, M.A. et al., *Skin Res. Technol.*, 7, 2001. With permission.)

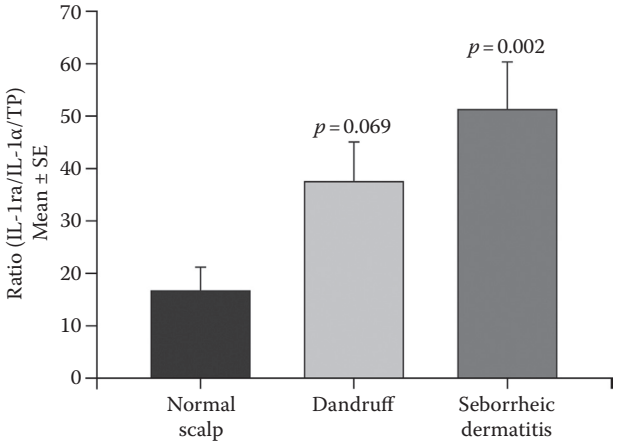

FIGURE 34.6 The ratio of IL-1ra/IL-1α normalized to total protein was significantly increased in the seborrheic dermatitis scalp group compared to normal scalp controls. The dandruff group approached significance when compared to the normal scalp group. (From Perkins, M.A. et al., *Skin Res. Technol.*, 8, 2002. With permission.)

(Figure 34.6). Seborrheic dermatitis and dandruff were also associated with elevated recovery of the inducible immune or inflammatory cytokines IL-2 and tumor necrosis factor-alpha (TNF-α), respectively. Rosacea, an inflammatory skin condition [38], with known cytokine involvement [39,40] was also shown to be associated with elevated IL-1ra/IL-1α ratios [19] (Figure 34.7). Involved skin sites showed elevated ratios compared with uninvolved skin sites from the same subjects. However, even the uninvolved sites showed slightly elevated ratios compared with facial skin sites sampled from normal control subjects.

In addition to our work on skin and overt inflammatory conditions, we expanded this technique to other realms of the inflammatory response. A common technique for the study of oral inflammation (e.g., gingivitis) is to try to recover small amounts of gingival fluid for assay. As shown in Figure 34.1, we were able to apply Sebutape™ over the gum surface and adsorb cytokines as an alternative, somewhat simpler method [20]. The objective of the study was to examine the relationship between changes in cytokine levels and clinical inflammation. Subjects participated in a 14-day experimental gingivitis (EG) model, in which, starting 5 days after a dental prophylaxis treatment, subjects refrained from all oral hygiene measures for 14 days. A gingivitis index (GI) and gingival bleeding were assessed clinically by standard techniques. Sebutape™ samples of each subject's gingival surface were collected from the right posterior buccal quadrant at baseline (pre-EG) and day 14 (post-EG). The tapes were analyzed for both IL-1α and IL-1β. Over a 14-day EG period, statistically significant increases in

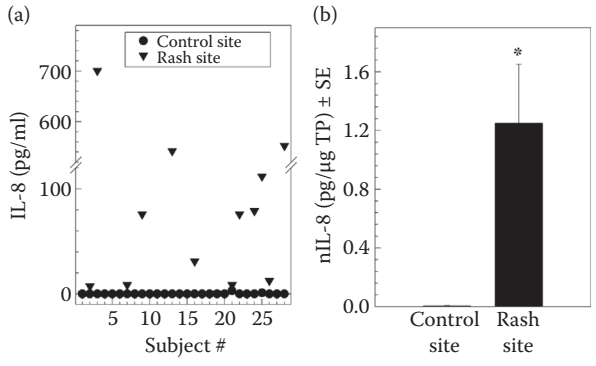

FIGURE 34.5 Comparison between skin reactions in diaper area and IL-8 recovery. Sebutape™ samples were collected from infant skin sites with different rash severity and from control leg sites. Individual levels of IL-8 for each subject (n = 28) for control and diaper rash sites (all rash grades) are shown (a). The normalized IL-8 levels (b) were significantly higher in rash versus control sites ($p \leq .05$, paired Student's t-test). (Adapted from Perkins, M.A. et al., *Skin Res. Technol.*, 7, 2001. With permission.)

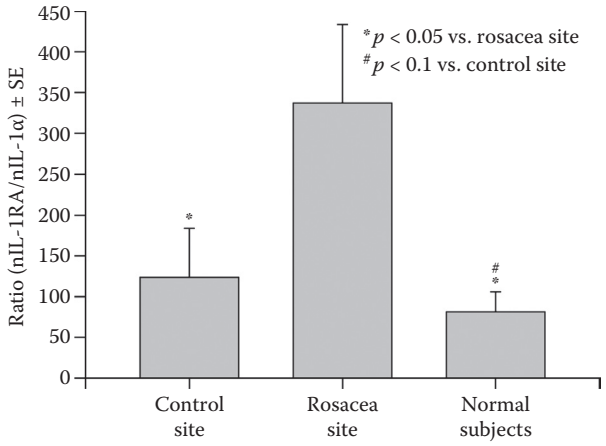

FIGURE 34.7 The ratios of normalized IL-1ra/IL-1α were determined for rosacea subjects (involved and noninvolved control sites) and normal subjects. (From Robinson, M.K. et al., *J. Toxicol. Cutaneous Ocul. Toxicol.*, 22, 2003. With permission.)

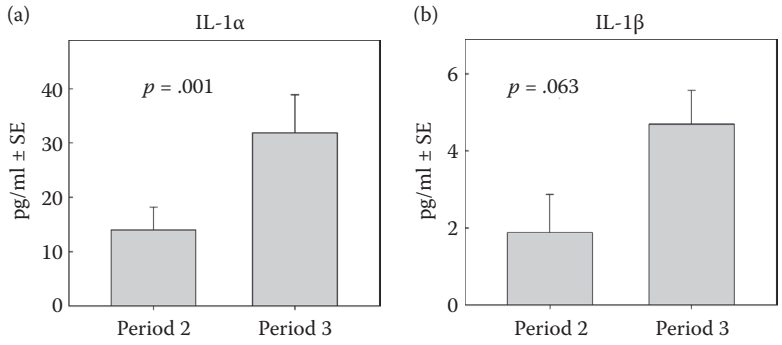

FIGURE 34.8 Sebutape™ samples were collected from 10 adult clinical subjects in an EG model. Baseline samples were collected 5 days after dental prophylaxis (period 2) and then after 14 days of EG (period 3). Samples were extracted in saline and analyzed for IL-1α (a) and IL-1β (b). *p* values denote paired comparisons between the two sampling periods.

GI, gingival bleeding, and IL-1α were observed. A directional increase in IL-1β was also observed (Figure 34.8).

Our final adaptation of this method was to try and identify skin surface biomarkers that could be associated with, and diagnostic of, neurosensory skin irritation. Because sensory irritation is purely symptomatic in nature, we applied this technique to see if a more objective index of the response could be developed through the Sebutape™ biomarker adsorption method. Though we were unable to completely investigate this hypothesis, we did have some early success in demonstrating reduced nitric oxide (NO) recoveries from the skin of test subjects experiencing weak-to-moderate stinging responses to lactic acid or capsaicin [21].

In addition to well-studied cytokines, other molecules have recently been recovered and analyzed from tape-strip samples and shown to correlate with inflammatory skin disease states. Morita and colleagues [41] used tape stripping to recover thymus and activation-regulated chemokine (TARC) from the SC of patients with AD. The TARC levels recovered correlated with several visual clinical indices of AD severity (e.g., erythema and edema) as well as other molecular (immunoglobulin E [IgE]) and cellular (blood eosinophil counts) measures [41]. Yamane and coworkers [42] found that, in comparisons of involved and uninvolved forearm skin between AD patients and healthy subjects, SC levels of fatty acid binding protein-5 (FABP-5), squamous cell carcinoma antigen-2 (SCCA-2), α-enolase, annexin-2, apolipoprotein a-1, and human serum albumin all displayed elevated content in the disease state. To varying degrees, the levels of these markers also showed some correlation to lesion severity, serum IgE levels, and even transepidermal water loss [42]. Amarbayasgalan and colleagues [43,44] recently investigated the question of whether vascular endothelial growth factor (VEGF), an activator of vascular permeability, known to be elevated in AD lesional skin, could be a reliable SC biomarker of lesion severity. By quantifying the levels of SC VEGF, obtained noninvasively by tape stripping, they found an association between SC VEGF levels and localized lesion symptom scores. Specifically, SC VEGF levels were significantly higher in AD patients versus healthy controls, and SC VEGF levels correlated with erythema and edema/papulation scores, as well as with transepidermal water loss and skin moisture content [43]. Via similar techniques, they also identified elevated SC levels of IL-8, IL-18, and transforming growth factor-alpha (TGF-α), which correlated with the severity of the AD skin lesions, with IL-8 showing the strongest correlation [44].

Recent studies in dandruff [28] confirmed some of our earlier cytokine results [45] and expanded upon the profiling of this disease looking at both structural proteins and small molecules [28,29]. These studies also examined treatment effects on both the clinical condition and biomarker profiles. Details on this work are provided below.

The ability to examine skin biomolecular profiles related to inflammatory and immune status, and do so via a painless recovery technique, was most recently illustrated by the work of Narendran and colleagues [46]. They compared structural and immune–inflammatory biomarker profiles in tape-strip samples from adults and both full-term and premature neonates (samples taken a few days after birth). They observed numerous differences in structural protein (keratin 1, 10, and 11 and involucrin), cytokines, and cortisol between preterm and term infants and between infants and adults. They concluded that these differences might be related to stress responses as well as the maturation of the skin barrier [46].

As noted above, studies from the Netherlands used various tape-stripping methods with several different types of tape (including D-Squame™) and examined baseline and SLS-induced cytokine recovery in a variety of ways. They first measured IL-1α, IL-1ra, and IL-8 after single 24 h exposures to 1% SLS and 6 h exposures to 0.1% SLS four times a week (for 3 weeks). The chronic exposures were associated with increased IL-1ra, IL-8, and IL-1ra/IL-1α [22]. They measured cytokines at three different levels from the outer SC to the lower SC just above the viable epidermis [23]. At baseline, normalized IL-1α levels were consistent across the SC. However, in SLS-irritated skin, the IL-1α decreased, and IL-1ra and IL-8 levels increased at increasing depth. They also observed that concentrations of IL-1α and IL-1ra/IL-1α ratios were related to specific polymorphisms in the IL-1 gene cluster [23,47].

RECENT ADAPTATIONS OF SKIN TAPE APPLICATION METHODS FOR GENE EXPRESSION PROFILING

Another application of tape stripping of skin has been to recover mRNA fragments that code for inflammatory or other proteins. Morhenn et al. [25] first reported on this technique in 1999, showing differential recoveries of RNAs coding for the cytokines IL-4 and IL-8 and the enzyme-inducible NO synthase. They recovered the RNA from multiple (up to 23] D-Squame™ tape strips of the skin and used a ribonuclease protection assay for detection and quantification. They showed distinctly elevated recoveries of RNA for all three proteins from allergic contact dermatitis skin sites versus irritant contact dermatitis skin sites [25]. Later, using a more limited four-repeated-tape-stripping procedure, they recovered and amplified mRNA from normal and SLS-irritated skin and examined gene expression profiles via microarray analysis [26]. They demonstrated significantly altered expression in over 1700 genes as the result of SLS-induced skin irritation. This method was later patented and given the nomenclature epidermal genetic information retrieval (EGIR) [33], and it has now found its way into dermatological diagnostics [35]. Benson et al. [35] used four tape strips from psoriatic skin sites or nonlesion sites and measured cytokine and keratin protein recoveries via reverse transcription-polymerase chain reaction (RT-PCR) analysis. Using this approach, they detected changes (overexpression) in biomarkers that were distinct from those seen via biopsy procedures and felt that the more noninvasive approach was a useful adjunct for the study of this disease.

The method has also been used recently to examine differential gene expression profiles from tape-strip samples from in situ and invasive (stages TIS–IV) melanoma, benign or atypical nevi, and normal skin [34]. They identified 312 genes differentially expressed between the three classes of samples. They also discovered a 17-gene signature (or classifier) that they found could efficiently distinguish both the in situ and invasive neoplastic from benign lesions.

USE OF NONINVASIVE BIOMARKER PROFILING TO ASSESS DANDRUFF AND MONITOR THERAPEUTIC EFFICACY

The literature suggests that the key symptoms of dandruff are a consequence of perturbation in permeability barrier function resulting in an autocatalytic cascade of inflammation that disrupts normal cell proliferation and differentiation [28]. On this basis, it seemed possible to define biochemical markers (biomarkers) to indicate the status of these processes in the dandruff condition and use them as surrogates to monitor how these processes are changed during a successful course of therapy.

We (KJM and colleagues) monitored biomarkers of inflammation (IL-1α, IL-1ra, and IL-8), differentiation (K1, 10, 11, involucrin), and barrier integrity (SC lipids, human serum albumin), all processes known to be disrupted in dandruff [28]. We found that changes in the levels of these biomarkers of scalp skin homeostasis tracked well with the reduction in symptoms (flaking and itch) following treatment with a zinc pyrithione (ZPT)-containing shampoo (Figure 34.9). The utility of these biomarkers resides in their ability to be used as molecular descriptors of scalp condition as well as to augment subjective assessments of disease severity [48] and evaluate the therapeutic efficacy of existing and novel antidandruff therapies.

We also sought to determine whether the itch-mediating small molecule, histamine, could be detected in scalp SC by

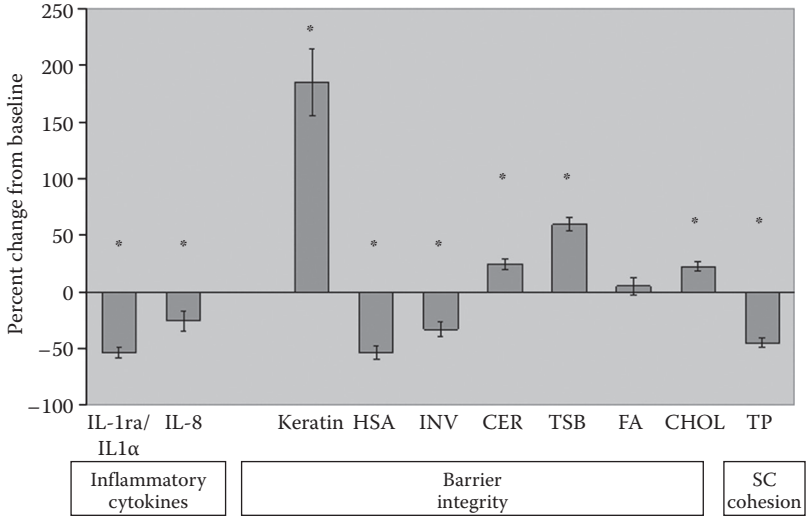

FIGURE 34.9 The levels of all of the stratum corneum biomarkers are expressed as percent change from baseline and are normalized to soluble SC protein. Inflammatory cytokines were measured as pg/mg protein; keratin 1, 10, and 11 and human serum albumin (HSA) were measured as ng/mg protein; involucrin (INV) was measured as pg/mg protein; total ceramide and total sphingoid base were measured as ng/mg protein; total free fatty acids (FA) and cholesterol (CHOL) were measured as mg/mg protein; and total protein was measured in mg. $p \leq .05$. (From Kerr, K. et al., *Int. J. Dermatol.*, 50, 2011. With permission.)

noninvasive techniques and whether there was an association between basal levels of this chemical itch mediator and self-perception of itch in the scalp in dandruff. It was likewise of great interest to determine whether treatment with a therapeutic regimen known to resolve all of the symptoms of dandruff, including itch, could establish a correlation between a treatment-induced change in a well-known biomarker of itch and the perception of itch itself by means of subjective assessments [29].

We found for the first time that histamine could be detected in scalp SC and that dandruff SC displayed a more than two-fold higher specific content of histamine compared with scalp SC from nonsufferers. We also demonstrated that a ZPT-containing shampoo, when used in a treatment regimen known to resolve the symptoms of dandruff, led to an almost complete normalization of basal SC histamine (Figure 34.10), which was accompanied by a highly significant reduction in the subjective perception of itch quantified by means of a visual analog scale. These findings established a strong association between the subjective perception of itch in the scalp and the objectively determined level of a key biomarker known to play a role in itch in the skin.

Finally, we have performed clinical studies that involved transcriptomic profiling of dandruff lesional scalp skin compared with normal scalp skin and also assessed the effect of treatment with ZPT on these profiles [49]. We found that for the inflammatory biomarkers detected in scalp SC by noninvasive tape-stripping methods, there was generally very good agreement between what was found at the level of the proteins (e.g., IL-1α, IL-1ra, IL-8, and S100A7, 8, 9, and 11) and the corresponding gene expression levels. These results provide confidence that noninvasive monitoring of selected biomarkers can provide a very reliable reflection of the biology taking place within the viable compartment of the skin. The results also provide an approach to mining available transcriptomic profiling studies of a variety of skin conditions/diseases to identify potentially high-value biomarkers that can be monitored noninvasively and used in therapeutic efficacy studies to gain mechanistic insight into new treatment modalities without the need of a biopsy.

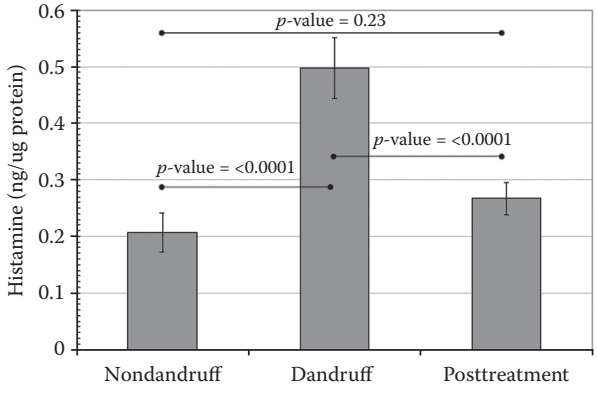

FIGURE 34.10 Subjects used a 1% potentiated ZPT-containing shampoo three times per week for 3 weeks and were assessed for stratum corneum histamine level at baseline and after 3 weeks of product use. Additionally, nondandruff subjects (adherent scalp flake score ≤ 12) were assessed at baseline (as discussed in Ref. 29). The levels of the histamine are standardized to soluble SC protein and reported as ng/mg protein (± standard error [SE]). (From Kerr, K. et al., *Acta Derm. Venereol.*, 91, 2011. With permission.)

CONCLUSIONS

The ability to study the inflammatory response of the skin has traditionally relied on invasive techniques to collect cells and mediators of this complex response. Skin surface adsorption of biomarkers of inflammation certainly does not tell the entire story of the underlying inflammatory processes. By the simple fact that adsorption is from the surface of the nonviable SC, the molecules collected were derived from earlier synthetic processes in response to some stimulus. This may make it difficult to easily discern (from the absorbed molecule profiles) the exact nature or mechanisms of acute inflammatory responses. However, chronic conditions or diseases, for which the molecular responses are ongoing, are much more assessable by this approach. Also, the use of such biomarkers to monitor therapeutic efficacy over a period of time is also a feasible approach, as suggested by the recent work in dandruff/ZPT treatment. The consistent finding (from multiple skin conditions and diseases, and across several laboratories over the years) of elevated IL-1ra/IL-1a ratios, and other biomarkers, is a testament to the validity and utility of the approach and the value of noninvasive tape adsorption of biomarkers as a means to detect, differentially diagnose, and evaluate treatment of inflammatory skin conditions.

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35 Ethnicity as a Possible Endogenous Factor in Irritant Contact Dermatitis

Comparing the Irritant Response among Caucasians, Blacks, and Asians

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INTRODUCTION

Irritant contact dermatitis (ICD) is a common and potentially serious dermatological disorder [1–3]. It is also the second most common occupational illness [4]. Since contact dermatitis can develop into chronic skin disease, understanding the underlying factors of its etiology is clinically important.

This condition is divided into several forms, depending on the nature of exposure and the resulting clinical presentation. Two common entities are acute and cumulative dermatitis. Acute contact dermatitis presents the classic symptoms of irritation such as localized and superficial erythema, edema, and chemosis. It occurs as a result of single exposure to an acute irritant [5]. Cumulative irritant dermatitis presents similar symptoms but occurs when exposure to a less potent irritant is repeated until signs and symptoms develop over weeks, years, or decades.

The ability of the offending irritant to cause dermatitis depends on both the nature of the irritant agent and the initial skin condition. The severity of symptoms depends on exogenous and endogenous factors [6–8]. Exogenous factors include the irritant's chemical and physical properties and the vehicle and frequency of application. Endogenous factors have been speculated to be age, sex, preexisting skin diseases, skin sensitivity, genetic background, and—the subject of this review—race [6], or, in today's parlance, ethnicity.

Ethnic differences in skin physiology and pathophysiology exist [9–11], and so whether ethnicity is, in fact, an endogenous factor affecting ICD is an important question in dermatotoxicology. Ethnic predisposition to ICD has been studied by comparing the irritant responses of blacks and Asians to those of Caucasians as a benchmark. We review these studies to evaluate if ethnic differences in susceptibility to ICD do exist.

The answer to the question of ethnicity as a factor in ICD has clinical and practical research consequences. Premarket testing of topical products (soaps, detergents, perfumes, and cosmetics), risk assessment for occupational hazards, and subject-inclusion requirements for product safety studies require knowledge about ethnic differences in irritation [12].

BLACK VERSUS CAUCASIAN IRRITATION RESPONSE

Using erythema as the parameter to quantify irritation, early studies note that blacks display less redness than Caucasians. In a hallmark paper, Marshall et al. [13] showed that while 59% of Caucasians exhibit acute irritant dermatitis as defined by erythema from 1% dichlorethylsulfide (DCES), only 15% of blacks do. Later, Weigand and Mershon [14] performed a 24 h patch test using orthochlorobenzylidene malononitrile as an irritant, which confirmed that blacks are less susceptible than Caucasians to ICD as defined by erythema (Table 35.1, item A). Further studies, also using erythema as a measure of irritation, showed that blacks are less reactive than Caucasians to irritants (160 and 1280 mM/L methacoline) [19,20].

Weigand and Gaylor [21] showed that if the stratum corneum (SC) of black and Caucasian subjects is removed, there is no significant difference in irritation as measured by erythema between the two groups. They concluded that there might be structural differences in the SC that provide more protection from chemical irritation to black skin than Caucasian skin. Indeed, while the SC thickness is the same in both races [22], the SC of black skin has more cellular layers and stronger cells [12], more casual lipids [23], increased desquamation [24], decreased ceramides [25], and higher electrical resistance [26] than Caucasian skin. Wesley and Maibach [3] also found significant evidence that innate differences exist in skin properties between black and Caucasian skin. They found that blacks had higher transepidermal water loss (TEWL) values, decreased skin surface pH, variable blood vessel reactivity, and large mast cell granules. These variables, they concluded, may play a role in the differences observed in dermatologic skin disorders between blacks and Caucasians.

TABLE 35.1
Black versus Caucasian Irritation Response

Interference	End Point	Comment	Reference
A. Findings that Show a Statistically Significant Difference in the Irritation Response between Blacks and Caucasians:			
1% Dichlorethylsulfide	Erythema	Untreated	Marshall et al. [13]
Orthochlorobenzylidene	Erythema	Untreated	Weigand and Mershon [14]
100 mM methyl nicotinate	PPG	Untreated	Guy et al. [15]
0.05% clobetasol	LDV	Preoccluded	Berardesca and Maibach [16]
0.5%–2.0% SLS	TEWL	Preoccluded	Berardesca and Maibach [17]
B. Findings that Do Not Show a Statistically Significant Difference in the Irritation Response Between Blacks and Caucasians:			
0.5%–2.0% SLS	LDV and WC	Untreated, preoccluded, and predelipidized	Berardesca and Maibach [17]
100 mM methyl nicotinate	LDV	Untreated	Guy et al. [15]
0.1, 0.3, and 1.0 M methyl nicotinate	LDV and erythema	Untreated	Gean et al. [18]

Note: LDV, laser Doppler velocimetry; PPG, photoplethysmography; SLS, sodium lauryl sulfate; TEWL, transepidermal water loss; WC, water content.

It is difficult, however, to conclude that blacks are less susceptible to cutaneous irritation only on the basis of studies using visual scoring. Erythema is notoriously difficult to measure in darker skin. Perhaps the difference in skin irritation between the two test groups is simply a result of the difficulty of assessing erythema in black subjects.

This idea was explored in a paper by Peters et al. [27], who studied an interethnic population of light-skinned Punjabi and darker-skinned Tamil, controlling for confounding variables such as skin evaluator, time, and environment. Sodium lauryl sulfate (SLS) at concentrations of 1.0%, 2.0%, and 3.0% was applied to occlusive patches for 24 h and assessed for a variety of visual parameters including erythema, wrinkling, and glazing. The results showed that if erythema was used as the sole measure of irritation, the darker Tamil subjects were less sensitive to SLS than Punjabi subjects. If, however, glazing and wrinkling were assessed, Tamil subjects were more sensitive than Punjabi subjects. More importantly, a similar dose response was noted in terms of edema and scabbing reaction, showing an equivalent response to SLS between the two populations. The authors conclude that erythema alone is an insufficient measure of irritation and that, especially in darker skin, additional visual parameters must be considered.

To better discuss this issue, it is necessary to analyze studies that use alternative accurate detection methods [28] to assess the level of induced cutaneous irritation. Berardesca and Maibach [17] performed such a study to determine the difference in irritation between young Caucasian and young black skin. They applied 0.5% and 2.0% SLS to untreated, preoccluded, and predelipidized skin. Then they quantified the resulting level of irritation using objective techniques: laser Doppler velocimetry (LDV), TEWL, and water content (WC) of the SC. They found no statistical difference in irritation between the two groups as measured by LDV and WC, but they did find a statistical difference in the 0.5% SLS preoccluded test, which showed blacks as having higher TEWL levels than Caucasians. Further review by Wesley and Maibach observed that six out of eight studies demonstrated higher TEWL values in black skin (Table 35.1, item B) [3].

The significance of higher TEWL values have been shown by Wu et al. [29] to reflect increased skin sensitivity in patients subjected to a lactic acid stinging test. Their results showed a statistically significant decrease in time to stinging as well as an increased clinical score (degree of sting) in patients with higher TEWL and lower capacitance values. A later study by An et al. [30] comparing a number of skin factors in relation to a lactic acid stinging test showed similar results, with higher TEWL levels in "stingers" versus those classified under "nonstingers." Notably, this study also showed no correlation between capacitance or pH and increased skin sensitivities.

In concordance with previous studies, Gean et al. [18] found no statistically significant difference in the maximum LDV response between black and Caucasian subject groups when they challenged skin with topical methyl nicotinate (0.1, 0.3, and 1.0 M). Furthermore, they found no difference in the blood flow and erythema responses between the two groups. Guy et al. [15] support the findings that LDV measurements of induced blood flow after application of 100 mM methyl nicotinate reveal no significant differences between black and Caucasian subject groups; however, a significant difference was found using photoplethysmography (PPG). Caucasians had a greater PPG value than blacks did, suggesting that Caucasians may be more susceptible to irritation. The authors did not explain why blood flow measurements using PPG showed a statistically significant difference between the groups when LDV did not.

Contrary to early studies, Berardesca and Maibach [16] found decreased reactivity in blood vessels in the black test group than in the Caucasian test group. They measured the postocclusive cutaneous reactive hyperemia—temporary increase in blood flow after vascular occlusion—after an

application of a potent corticoid and measured vasoconstriction using LDV; the black subject group had several significantly different parameters of the hyperemic reaction. They found a decreased area under the LDV curve response, a decreased LDV peak response, and a decreased decay slope after peak blood flow, showing that blacks have a decreased level of irritation-induced reactivity of blood vessels. These results are consistent with their previous work.

Older studies have shown, using erythema as an indicator, that blacks have less irritable skin than Caucasians; recent data, however, have shown this method to be a misleading form of measurement. The development of new bioengineering techniques has led to more objective ways of inferring skin irritability, though conflicting evidence shows that it is still too early to conclude on the relative skin sensitivities between blacks and Caucasians.

ASIAN VERSUS CAUCASIAN IRRITATION RESPONSE

An early study comparing Caucasian and Japanese susceptibility to cutaneous irritation was conducted by Rapaport [31]. He performed a standard 21-day patch test protocol on Caucasian and Japanese females in the Los Angeles area, in which 15 irritants (different types or concentrations of cleansers, sunscreen, and SLS) were tested. The results were reported according to the cumulative readings of all subjects in an ethnicity group for each irritant. Japanese women had higher cumulative irritation scores for 13 of the 15 irritants tested; Rapaport interpreted these findings to confirm the common impression that the Japanese are more sensitive to irritants than Caucasians are. Also, this sensitivity was independent of the concentration or exact chemical formulation of the substance tested, suggesting that Japanese are, in general, more sensitive than Caucasians.

While these findings are important, it is difficult to interpret the data. First, as also noted by Robinson [12], Rapaport provides little experimental detail and data. For example, while the study required 21 separate days of irritation readings, only the end cumulative irritation scores are reported. Had he reported daily irritation readings, we would have been able to note the time pattern of response. Further, no statistical tests were conducted to ascertain if the differences between the Japanese and Caucasian subjects were statistically significant. Note, too, that the cumulative irritation test score does not distinguish between the intensity of a subject's response and the number of subjects responding. Thus, it is possible, for example, for a few extremely sensitive Japanese subjects to inflate the overall irritation score. Therefore, at the minimum, it would be helpful to provide standard deviations to rule out such problems.

In what at first seems surprising, Basketter et al. [32] found that Germans are more sensitive than Chinese subjects. Subjects in Germany, China, and the United Kingdom were exposed to varying concentrations (0.1%–20%) of sodium dodecyl sulfate (SDS) for 4 h on the upper outer arm, and the resulting dose-response irritation was measured on the basis of erythema. They concluded that the German subjects tend to be more sensitive than the Chinese, and the Chinese to be slightly more sensitive than the British. This conclusion is the opposite of popular belief and of the Rapaport study, which indicated that Asians are more likely to develop ICD than are Caucasians.

There are, however, inherent flaws in this study, some of which the authors acknowledged. First and foremost, this study does not control the variables of time and location. The German and Chinese studies were performed in 3 to 6 weeks in the winter, while the UK study was spread over 15 months. Also, in particular, German winters are colder and drier than Chinese winters, which in turn tend to be colder than English winters. These variables will distort the results in a predictable way if we assume that an individual becomes more sensitive to ICD in colder and drier climates [2]. We would then expect, on the basis of climatic conditions, that the German subjects would be more reactive than the Chinese, and the Chinese more reactive than the British. As these are the actual results, we cannot necessarily attribute the differences in irritant response to ethnicity, as it is reasonably likely that the differences are possibly due to weather conditions. Also, they mention that 15% of the UK volunteers were black. While they account for this by showing that the black irritant response was similar to the overall UK group response, it is scientifically problematic to mix racial groups in a study testing for racial differences. Furthermore, they supplied no statistical tests for their conclusion that Germans are slightly more sensitive than the other ethnic groups.

To shed more light on the results, we conducted simple binomial tests of the differences in the percentage response of the subject groups. Using the resulting statistics, we found a larger statistically significant difference between the two predominately Caucasian groups than between each of the Caucasian and the Chinese groups (Table 35.2). These results indicate that race may not be the predominant factor affecting susceptibility to ICD in this study; other uncontrolled variables may dominate the results.

Variables such as time and location were eliminated by the Goh and Chia [33] study that tested the susceptibility to acute irritant dermatitis in Chinese, Malay, and Indian subjects. These subjects were exposed to 2% SLS in the right scapular region, and resulting irritation was measured using TEWL. This technique is an objective way to indirectly quantify irritation—as discussed earlier, the higher the TEWL value, the greater the implicit irritation. There was no significant difference in the TEWL level of irritant skin in a three-way statistical test of the three racial groups. There was a significant difference, however, between the TEWL values of Chinese and Malay subjects so that Chinese subjects were more susceptible to contact dermatitis. While this test does not contribute to the discussion of the difference in predisposition of irritation in Caucasian skin versus Asian skin, it does add to the overall question of whether race can be a predisposition to irritant dermatitis.

Foy et al. [34] clearly added to our knowledge of the difference in the acute and cumulative irritation response in Japanese and Caucasian female skin. They reduced some variables that compromised other studies; location, time,

TABLE 35.2
Statistical Analysis of the Basketter et al. Study

	0.1% SDS	0.25% SDS	0.5% SDS	1.0% SDS	2.5% SDS	5.0% SDS	10% SDS	20% SDS
Germany	0.03	0.09	0.23	0.50	0.65	0.72	0.76	ND
China	0	0	0.01	0.21	0.45	0.61	0.79	0.90
UK	0.01	0.01	0.06	0.15	0.33	0.41	0.49	0.76
N	100	100	100	100	100	100	100	100
Z (Germany–China)	1.75	3.07*	4.79*	4.29*	2.84*	1.65	−0.51	NA
Z (UK–China)	1.00	1.00	1.92	−1.10	−1.74	−2.83*	−4.42*	−2.64*
Z (UK–Germany)	−1.01	−2.60*	−3.41*	−5.28*	−4.53*	−4.42*	−3.94*	NA

Source: Basketter, D.A., *Contact Dermat.*, 35, 1996.

Note: SDS, sodium dodecyl sulfate; *Z*, *Z* values; ND, not done; NA, not available. The numbers in the first three rows are the decimal value of the percentage of the group that developed a positive irritant reaction at a specific SDS concentration. The numbers in the last three rows are the *Z* values. We applied the binomial test to ascertain the differences in the percentage response of the subject groups:

$$Z = \frac{r_1 - r_2}{[2r(1-r)/100]^{50}}$$

where r_1 and r_2 are the ratios for the two ethnic groups and r is the weighted average. Since the sample sizes for different groups are equal, r becomes the simple average. Note that all the UK–Germany differences, except one, are statistically significant; however, more than half of the UK–China and almost half of Germany–China differences are not statistically significant. This indicates a larger statistically significant difference between the two Caucasian groups than that between the Caucasian and Asian groups.

* Ratios are significant at the 5% level.

season, and scores were the same for both study populations. Eleven different materials were tested in the acute test; they were applied to the upper arms for 24 h, and irritation was measured on the basis of erythema. The cumulative test consisted of testing five irritants using a four-exposure cumulative patch protocol.

In the acute test, while there is a slight tendency toward greater susceptibility to irritation among Japanese subjects, only four out of the 11 irritants caused a significant difference in reactivity between the two groups—these were the most concentrated irritants used. This shows that perhaps for more concentrated irritants, there is indeed a statistical difference in the acute contact dermatitis response; of course, this study needs to be interpreted in context with others to follow. For the cumulative study, the skin irritation scores between the two test groups are close, but the Japanese tended to have slightly higher numbers. The differences, however, only reached statistical significance in two instances. And as the authors noted, it is difficult to interpret the importance of those two instances since the statistically significant differences are not maintained at later points in the timeline. It is safe to conclude, therefore, that while the acute irritant response to highly concentrated irritants was significantly different between the Japanese and Caucasian subjects, the cumulative irritant response rarely reaches a statistical difference.

Studies that include both acute and cumulative irritant tests, like the one above, are more informative than single tests since they give a more complete view of differences in skin irritation between groups. Robinson [35] conducted a series of studies that tested racial differences in acute and cumulative skin irritation responses between Caucasian and Asian populations. In the first acute tests, Caucasian and Japanese groups were exposed on the upper outer arm to five irritants under occlusion for up to 4 h. The resulting erythema was scored on an arbitrary visual scale. The results are represented as the cumulative percentage incidence of positive test reactions to the different irritants.

It is curious to note that while Japanese subjects again tended to be more susceptible to acute irritation than Caucasians, neither one irritant nor one test time caused a significant response difference between the two groups. Further, note that for three of the five irritants, only Caucasians reacted at early test times, contradicting the hypothesis that Japanese are more reactive to irritants. But even this trend-breaking difference is not considered statistically significant. The acute irritation response data were then reanalyzed in terms of possible differences in temporal response. The analysis showed that Japanese subjects generally react faster than their Caucasian counterparts, as indicated by their shorter TR50 values (the time it takes for the cumulative irritation score to reach 50%). While this result is interesting and adds the new dimension of temporal differences in reactivity between the two groups, hard data were not provided, and statistical analysis was not conducted to see if this temporal pattern difference is indeed statistically significant.

The cumulative irritation test was conducted concurrently and on the same Japanese and Caucasian subjects. Four concentrations of SDS (0.025%, 0.05%, 0.1%, and 0.3%) were applied on the subjects' upper backs for 24 h for 14 days. The resulting skin grades were summed for all subjects for all test days. For the two lower SDS concentrations, the Japanese subjects reacted only slightly more than the Caucasian subjects, but only the difference in skin grades for 0.025% SDS

reached statistical significance. When these data were analyzed in terms of temporal response, for the two lowest concentrations, the Japanese reacted only slightly faster than their Caucasian counterparts. Whether the difference in reaction time is statistically significant is not known.

In the same study, Robinson then applied both the acute and cumulative irritation protocols to compare three new subject groups—Chinese, Japanese, and Caucasian—with each other. The cumulative irritation study found no statistically significant differences between the different groups. In the acute test, he found that, in most cases, the Chinese subjects were more reactive to irritants than Caucasians but that this difference was significant in only one case, and he stated that most likely, this was an anomaly. There was no discernable difference between the Japanese and Chinese groups. And surprisingly, when the Japanese subjects were again compared with the Caucasian subjects, as they were in the beginning of his study, the results showed no significant difference between the two groups.

While Robinson's first two-way irritation response comparison test between Japanese and Caucasian subjects did show some statistical differences, the fact that they could not be confirmed in the second half of the study emphasizes the difficulty in obtaining repeatable results in this type of study. For one, in the statistical sense, Robinson's sample sizes (approximately 20 people) were small; combined with the variability between human skin within an ethnic group, this makes it difficult to make concrete conclusions. His study showed, however, that there were essentially no significant differences between the Asian and Caucasian groups, at least none that could be repeated.

In a later study, Robinson et al. [36] had similar results. Using the 4 h occlusion patch method, they compared the relative acute skin reactivity of Asian and Caucasian subjects using the irritation temporal response to measure the difference in reactivity between the test groups. They tested five chemicals, including 20% SDS and 100% decanol. Unlike the previously described study, they failed to find a statistical difference in the reactivity to multiple irritants between the two groups even at the 4 h mark. Then they did something new: they separated the racial subpopulations into "sensitive" and "normal" groups to test any differences in percentage cumulative scores and temporal responses within these new groups but across race (i.e., he compared sensitive Asians with sensitive Caucasians). There were no statistically significant differences between subjects of the same skin type in different racial groups. This further contraindicates the early-held hypothesis that Asians are more reactive to irritants than Caucasians.

Robinson compiled 5 years of his previous data and compared the acute reactivity differences between Caucasian and Asian (combined Japanese and Chinese) subgroups using the 4 h human patch method [37]. The data were represented in terms of the time it took for subjects to have a positive response to the irritant chemical. Again, as in most experiments, Asians displayed a greater irritation response score than Caucasians. However, this difference only reached statistical difference at the 4 h mark, with SDS and decanol as the irritants. Note that while the results of this study are probably more representative of the population at large because of the relatively large sample size (200 plus), the data from this study were compiled from three different testing centers over 5 years. This could have potentially added uncontrolled and unaccounted-for variables.

To determine whether Rapaport's findings were objective, Aramaki et al. [38] compared the skin response of Japanese and German women to a 0.25% and 0.5% SLS patch. To control for confounding variables, the authors controlled for time, temperature, and humidity. No significant differences in TEWL, LDV, sebum, and hydration measurements were found, suggesting that, objectively, there were no differences in the skin barrier between the two groups.

In the same study, the authors then explored whether a subjective bias could factor into Rapaport's results. Using a lactic acid stinging test, they evaluated for skin tightness, stinging, burning, and itching. Surprisingly, they found that Japanese women had a higher sensitivity to lactic acid than German women; more specifically, while both groups responded to the 10% lactic acid solution, Japanese women scored higher on the pain severity scale, with maximal scoring at 2 min, compared to German women at 4 min. Of note, prior research has shown no differences in epidermal innervations between Caucasian and Japanese subjects, ruling out a structural reason for these results [39]. Another possible explanation suggested by the authors is the increased skin absorption of lactic acid by Japanese women, thus causing the more rapid irritant response. Studies by Lotte et al. [40], however, show no statistically significant differences in the absorption of benzoic acid, caffeine, or acetylsalicylic acid between Asian, Caucasian, and black subjects. Because the authors' objective results showed no significant differences between the two groups of women while in subjective measurements, Japanese women showed increased sensitivity, they concluded that cultural differences likely factor into their observations.

In summary, several studies [34,36,37] have supported the long-held belief that Asians are more susceptible to ICD. Rarely, however, is this trend statistically significant, and even more rarely can the statistical significance be repeated in another study. Additionally, recent studies have suggested the increased irritation response in Asian women to be, in part, due to cultural behavior rather than measured objective differences in skin sensitivity. It would be practical for the time being, in terms of topical product safety, risk assessment for occupational hazards, and global product marketing, to assume that there is a limited fundamental difference between Asian and Caucasian cutaneous irritant reactivity.

CONCLUSION

Table 35.3 summarizes some potent factors that might influence the refinement of interpretation in future investigations. Recent advances in technology using new bioengineering techniques have shown interethnic differences in indirect measures of irritation. Though these studies have

TABLE 35.3
Potent Factors that Might Influence Refinement of Interpretation in Future Investigations

Experimental design
Baseline versus "stress" test differences
Anatomic site
Open versus occluded irritant stresses
Ethnic groups in the same versus varying geography
Comparable climatic conditions
Presentation of hard data and statistical analysis

elucidated new and more convincing studies regarding differences in the irritation response, there is still inconclusive evidence that statistically significant differences in the irritant response between Caucasian and black or Asian groups exist. Intuitively, we suspect that ethnic differences exist in skin function and may have evolved as have those in hair and other differences.

Essentially, the studies suggesting differences in skin [15,17] are "stress" in nature (preoccluded). Additionally, differences in emotional and psychological variations in response should also be considered. Presumably, new insights into physiology, pharmacology, and toxicology may clarify this situation.

Also, it is possible that the well-known, divergent response to irritants is due to intraindividual variations in the skin irritation response [41–43]. This remains a somewhat novel concept, and therefore, further studies need to be conducted in this area before a definitive statement can be made linking intraindividual variation to ethnic differences in the intensity of an irritation response.

The above discussion is clearly limited in scope when considering the wide array of ethnic groups present globally. The Latino population was not discussed herein, but a growing body of literature has begun to focus on this group as well. Considerable work remains to be done before the role of ethnicity in ICD is fully appreciated.

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36 Allergy and Hypoallergenic Products

An E. Goossens

INTRODUCTION

The assessment and detection of the number of contact allergic reactions to cosmetics are not simple. Generally, a consumer who has a problem with cosmetics will consult a doctor only if he or she does not recognize the cause to be a particular cosmetic product or if the dermatitis persists when the suspected product has been replaced by another, determined by trial and error. Consequently, only a small proportion of the population with cosmetic intolerance problems is ever seen by a dermatologist. Moreover, cosmetic reactions may present in unusual clinical forms, which may evoke an erroneous diagnosis.

In general, adverse effects are underreported [1], certainly to the cosmetics industry, which obtains its most reliable information in this regard mainly from the relatively few dermatologists who concentrate on cosmetic intolerance problems and from reports in the literature that are, almost by definition, out of date. Sometimes, beauticians and consumers report adverse reactions, but in most cases, this kind of information is difficult to objectify unless it is verified by a dermatologist.

Application of cosmetic products to the skin may cause irritant, phototoxic, contact allergic, and photocontact allergic reactions, as well as contact urticaria. It is generally agreed that most skin adverse reactions to cosmetic products are irritant in nature and that people with "sensitive skin" as indicated by conditions like atopic dermatitis, rosacea, or seborrheic dermatitis are particularly liable to develop such reactions. However, contact allergic reactions attract much more attention and thus tend to be overestimated, although recently, such reactions are increasingly seen in dermatological practice. The identification of the cosmetic allergen is by no means a simple task. It demands special skills and interest on the part of the dermatologist, although labeling of all cosmetic ingredients is facilitating that task. Moreover, there are many factors involved in the sensitization to a specific cosmetic product, all of which have to be taken into account when one seeks an allergen.

FACTORS CONTRIBUTING TO CONTACT ALLERGIC REACTIONS TO A COSMETIC PRODUCT

Frequency of Use

One may expect frequently used products to cause more skin reactions than more exclusive products simply because more people are exposed to them. This alone does not imply anything about the quality of these products. (The same thing may be said about individual cosmetic ingredients.)

Composition

The complexity of a formula can be either positive or negative as far as its allergenic capacity is concerned. One of the principles of creating "hypoallergenic" cosmetics and perfumes is the simplicity of formula: the fewer the constituents, the easier it is to identify the offending substance should difficulties arise, and the less danger there is of synergism. Some investigators recommend placing upper limits on concentrations rather than advising against the use of any particular ingredient. However, the more ingredients there are, the more chance there is of sensitization by one of them.

Preservatives are needed in water-based or other easily contaminated products and have recently become as important cosmetic allergens as fragrances. It seems that it is very difficult to combine potent antimicrobial and antifungal properties with low allergenic capacity: indeed, it is very difficult to restrict the biological activity of a substance to a single domain. Therefore, some cosmetic manufacturers have developed new packaging devices in order to prevent the introduction of air and microorganisms into the containers during usage, thus avoiding the addition of preservatives into the formulation.

Concentration of Ingredients

Although the use of low concentrations does not assure complete safety, the incidence of sensitization induction is, indeed, a function of the concentration of the allergen, at least to some extent. But once a patient has become sensitized, even low concentrations can trigger a reaction. Cases of allergy to the preservative mixture of methylchloroisothiazolinone and methylisothiazolinone (MCI/MI) illustrate this problem very well. At first, when a 50 ppm concentration of this mixture was allowed for use in cosmetic products in the European Community and when this concentration was actually used in some products, there were "epidemics" of contact allergic reactions to it. Later on, the frequency of positive reactions had diminished considerably, not only because its use was declining, particularly in "leave-on" cosmetics, but also because its usage concentration had been reduced to 15 to 7.5 ppm. However, many problems are actually being seen again with the less sensitizing methylisothiazolinone (MI) alone that, because of lower antimicrobial activity, is allowed up to 100 ppm in cosmetics.

Purity of Ingredients

It is impossible to refine raw materials to absolute purity. More or less strict quality control of raw materials and finished products has long been general practice in modern cosmetic manufacturing. However, one can never rule out the sensitizing potential of impurities in these materials, such as has been the case, for example, with the amphoteric surface active agent cocamidopropyl betaine, in which they were the allergenic culprits.

Common Use of Cosmetic Ingredients in Pharmaceuticals

Patients easily become sensitized to topical pharmaceutical products, which, unlike cosmetics, are most often applied to diseased skin. Once sensitization has occurred, however, they may react to cosmetics containing the same ingredients.

Role of Cross-Sensitivity

Chemically related substances are likely to induce cross-reactions and, contact eczematous lesions may be maintained in this way. This is especially the case with fragrance ingredients, which often cross-react with each other, as well as with natural ingredients, among which are plant extracts. Cross-reactions may, however, occur with many other cosmetic ingredients as well.

Penetration-Enhancing Substances

The chemical environment can substantially affect the sensitizing potential of individual chemicals. For example, emulsifiers and solvents enhance skin penetration and, thereby, contact sensitization. Penetration-enhancing agents can also be the cause of false-negative patch-test reactions: The cosmetic product itself may be clearly allergenic, while the individual ingredients, abstracted from the environment of the product and tested separately, may not cause a reaction.

Application Site

Some areas of the skin, like the eyelids, are particularly prone to contact dermatitis reactions. A skin-care cream applied to the entire face or a hair-care product such as a hair dye may sometimes cause a pronounced allergic reaction on the eyelids, in particular. Moreover, "ectopic dermatitis" (by transfer of the allergen by the hand, as often occurs with tosylamide/formaldehyde resin, the allergen in nail polish), "airborne" contact dermatitis (for example, due to "volatile" fragrance components), as well as "connubial" or "consort" dermatitis (caused by products used by the partner or any other "nearby" person) often only occur on "sensitive" skin areas such as the eyelids, the lips, and the neck.

Moreover, the penetration potential of cosmetics is heightened in certain "occluded" areas, such as the body folds (axillae) and anogenital region, which also increases the risk of contact sensitization. The allergic reactions often tend to persist for weeks following the initial contact with the allergen. This may be partly attributable to residual contamination of clothing, but the increased penetration of the allergen, assisted by occlusion and friction, may lead to the formation of a reservoir from which the allergen is subsequently again released.

Condition of the Skin

Application on damaged skin, where the skin barrier is impaired, enhances the penetration of substances and thus increases the risk of an allergic reaction. This is the case with body-care products used to alleviate dry, atopic skin and with barrier creams for protecting the hands, which often suffer from irritant problems (dryness, cracking). The skin reaction may also be limited to certain areas that have been previously affected, since these react more readily to subsequent application of the same allergen. Sometimes, an already existing dermatitis or another eczematous skin condition such as atopic or seborrheic dermatitis may be aggravated or even mimicked, and also, a noneczematous clinical picture may sometimes point to a cosmetic allergy, such as, for example, with preservative agents that may present as a lymphocytic infiltrate or even have a lupus erythematous-like picture [2].

Contact Time

In the world of cosmetics, a distinction is being made between *leave-on* products, which remain on the skin for several hours (for example, face- and body-care products and makeup), and *rinse-off* products, which are removed almost immediately. The division between leave-on and rinse-off products is not always relevant to the sensitization process because a thin film can remain on the skin and be sufficient to allow ingredients to penetrate. In this regard, particular attention needs to be paid to wipes (moist toilet tissues), with mainly preservatives as the allergenic culprits.

Frequency of Application and Cumulative Effects

Use daily or use several times a day of cosmetics may cause ingredients to accumulate in the skin and thus increase the risk of adverse reactions. In fact, the concentration of an ingredient may be too low to induce sensitivity in a single product but may reach critical levels in the skin if several products containing it are used consecutively. This may be the case for people who are loyal to the same brand of, for example, day and night creams, foundations, and cleansing products, because a manufacturer will often use the same preservative system for all of its products. This should be taken into consideration by companies that use biologically active ingredients such as preservative agents, emulsifiers, antioxidants, and fragrances, because it might well account for many of the adverse reactions to these particular substances. In our experience, intense users of cosmetics are more prone to cosmetic dermatitis than others.

CORRELATIONS WITH THE LOCATION OF THE LESIONS

Like many other contact allergens, cosmetics can reach the skin in several different ways: by direct application; by airborne exposure to vapors, droplets, or particles that are released

TABLE 36.1
Cosmetic and Cosmetic-Related Dermatitis Caused by Direct Application of the Allergen

Area of Dermatitis	Cosmetics That may Contain Allergens
Face in general	Facial skin-care products (creams, lotions, masks); sunscreen products; makeup (foundations, blushes, powders); cleansers (lotions, emulsions); cosmetic appliances (sponges); perfumed products (aftershave lotion)
Forehead	Hair-care products (dyes, shampoos)
Eyebrows	Eyebrow pencil; depilatory tweezers
Upper eyelids	Eye makeup (eye shadow, eye pencils, mascara); eyelash curlers
Lower eyelids	Eye makeup
Nostrils	Perfumed handkerchiefs
Lips, mouth, and perioral area	Lipstick; lip pencils; dental products (toothpaste, mouthwash); depilatories
Neck and retroauricular area	Perfumes; toilet waters; hair-care products
Head	Hair-care products (hair dyes, permanent-wave solutions, bleaches, shampoo ingredients); cosmetic appliances (metal combs, hairpins)
Ears	Hair-care products; perfume
Trunk/upper chest, arms, wrists	Body-care products, sunscreens and self-tanning products; cleansers; depilatories
Axillae	Deodorants; antiperspirants; depilatories
Anogenital areas	Deodorants; moist toilet paper (wipes); perfumed pads; depilatories
Hands	Hand-care products; barrier creams; all cosmetic products that come in contact with the hands
Feet	Foot-care products; antiperspirants

into the atmosphere and then settle on the skin; by contact with people (partners, friends, coworkers) who transmit allergens to cause "connubial" or "consort" dermatitis; by transfer from other sites on the body, often the hands, to more sensitive areas such as the mouth or the eyelids ("ectopic" dermatitis); and by exposure to the sun with photoallergens. In case of a severe allergic reaction, eczematous lesions may spread to body parts other than the application site or even become generalized.

The most common sources of cosmetic allergens applied directly to the body are listed in Table 36.1.

NATURE OF COSMETIC ALLERGENS

Allergic Contact Dermatitis

In cosmetics, fragrances and preservative agents are the most important culprits, but reactions also occur to ingredients of category-specific products such as hair dyes, resins used in nail cosmetics, and sunscreens, as well as to antioxidants, vehicle components, emulsifiers, and so forth.

Fragrance Components

Fragrance components most often produce allergic reactions when present in toilet waters, aftershave lotions, and deodorants, but also, skin-care and other cosmetic products may be involved. The main skin sites affected are the face, neck, axillae, and hands.

The literature confirms that fragrance mix (I), which contains eight components (amyl cinnamal, cinnamal, cinnamyl alcohol, hydroxycitronellal, eugenol, isoeugenol, geraniol, and *Evernia prunastri* [oakmoss] extract, all diluted 1% in petrolatum and emulsified with sorbitan sesquioleate) and which is tested routinely in the baseline series, remains a good screening agent for contact allergy to fragrances because it is said to detect some 70% to 80% of all perfume allergies [3,4]. However, this also depends on how many and which fragrances are tested in order to detect contact allergy, and the literature insists on the need to test with additional compounds. Indeed, additional markers such as, for example, other mixes and individual components, as well as complex natural mixtures [5–8], increase the sensitivity of the testing. This is the reason why a second fragrance mix (II), consisting of hydroxyisohexyl 3-cyclohexene carboxaldehyde (Lyral), farnesol, citral, citronellol, coumarin, and alfa-hexyl cinnamaldehyde, as well as hydroxyisohexyl 3-cyclohexene carboxaldehyde itself, have been introduced into the baseline series as well [9]. Nowadays, the relevance for positive patch tests can be more readily determined, and sensitized consumers are better informed since 26 fragrance components are (since March 2005) labeled as cosmetic ingredients on the packaging (Annex 3 of the Cosmetic Directive 2003/15/EC).

Most fragrance-sensitive subjects present with multiple positive patch-test reactions, which indicate the presence of common or cross-reacting ingredients in natural products, the occurrence of cross-reactions between simple fragrance chemicals, or concomitant sensitization. Moreover, fragrance components may be allergenic by themselves but may also contain strongly sensitizing oxidation products [10–11], as is the case with, for example, the terpenic components limonene and linalool, or they may be contaminated by allergenic impurities. For example, this has been shown for resin acids and their oxidation products, the main allergens in colophonium also detected in *Evernia prunastri* (oakmoss), which was contaminated with or substituted by the cheaper *Evernia furfuracea* (tree moss), which, like colophonium, is also derived from pine trees [12].

Preservatives

Preservatives have recently become more important cosmetic allergens, particularly in water-based cosmetic products. However, within this class, important shifts have occurred over the years [13,14].

Methyldibromo glutaronitrile—which was used in a mixture with phenoxyethanol, better known as Euxyl K400—became such an important cosmetic allergen that, in March 2007, the European Union no longer permitted its further use in cosmetic products.

The mixture of MCI/MI, commonly in use since the 1980s, did cause an epidemic of contact allergies, after which it has been recommended by the manufacturer to

be used only in rinse-off products (up to a maximum of 15 ppm); however, it was still found in several leave-on products on the market such as moisturizers and (baby) wipes, the latter often producing allergic contact dermatitis [15], even in those who took care of babies! In cosmetics, MCI/MI is now largely replaced by MI, which is a weaker sensitizer than the chlorinated derivative but also less efficient as a preservative; hence, larger use concentrations (up to 100 ppm) are required. Not only do MCI/MI-sensitive patients often react to this nonhalogenated derivative [16], but also, MI is revealed to be an important primary sensitizer itself. Most cases are caused by the use of wipes (moist toilet paper) for intimate hygiene; however, also, other cosmetic products may be the sensitization source [17]. The numerous cases and prevalence studies (e.g., ref. 18) recently reported in the literature point to a new epidemic of isothiazolinone (MI) contact allergy all over Europe.

The incidence of positive reactions to formaldehyde and its releasers is slightly increasing again [14]. Besides releasing formaldehyde, imidazolidinyl urea and diazolidinyl urea do contain allergenic degradation products [19,20].

Parabens are rare causes of cosmetic dermatitis, and when allergy does occur, the sensitization source is most often a topical pharmaceutical product. Its withdrawal from cosmetics (but not from pharmaceuticals or food!) is merely a consumer, publicity, and political issue [21].

Exceptional cosmetic allergens are chlorphenesin, which may cross-react with mephenesin, a rubefacient in topical pharmaceutical products [22], and iodopropynyl butylcarbamate, also present in baby and make-up cleansing wipes [23] and reported as a cosmetic allergen by Pazzaglia and Tosti in 1999 [24]. Its presence in cosmetics is being discussed not because of its potentially allergenic properties but because of its iodine content (Ian White, personal communication).

Antioxidants

Antioxidants are only a minor group of cosmetic allergens. Examples are propyl gallate and octyl gallate [25], which may cross-react with other gallates that are also used as food additives. Some antioxidants are used more specifically in sunscreen products and also in moisturizing products to prevent aging but are rare causes of allergic contact dermatitis in such preparations, for example, tocoferol (vitamin E) acetate and retinol palmitate [26], ascorbic acid (vitamin C) [27], and idebenone or hydroxydecyl ubiquinone (a synthetic analog of coenzyme Q10 [CoQ10]) [28]. Also (meta)bisulfites are potential allergens in cosmetic creams and hair dyes [29]. More recently, we have observed six cases of contact allergy to tetrahydroxypropyl ethylenediamine, a chelating compound, due to its presence in skin care products; no cross-reactions to ethylenediamine or edetate were observed [30].

Category-Specific Ingredients

With regard to category-specific ingredients, the number of reactions to oxidative-type *hair dyes* (para-phenylenediamine [PPD] and related compounds) increases in some centers and decreases in others (e.g., ref. 31). Active sensitization to PPD and related compounds from temporary tattoos has been encountered (e.g., ref. 32); however, hair dyes are the main culprits both in clients, in whom they often cause severe reactions, and hairdressers. Moreover, also, immediate-type reactions or contact urticaria syndrome may occur to it, as well as to persulfates in *hair bleaches*.

In *nail* varnish, tosylamide/formaldehyde (toluenesulfonamide formaldehyde) resin and also epoxy resin and copolymers may be the cause of "ectopic" dermatitis, which often gives rise to confusing clinical pictures, even mimicking occupational dermatitis. (Meth)acrylates are important causes of reactions to artificial nail adhesives and gel formulations, being the newest development in this regard, both in clients and, particularly, in manicurists [33].

As for *sunscreens*, because of media attention being given to the carcinogenic and accelerated skin-aging effects of sunlight, they are increasingly used, not only in sunscreen products but also in other cosmetics including moisturizers, and this in rather high concentrations. They are also used to protect cosmetic products from degradation. Sunscreen agents may be responsible for allergic and photoallergic reactions (e.g., ref. 34) and also contact urticaria. Octocrylene, in particular, has become a frequently reported contact allergen, also in children [35]. Moreover, it also often causes photocontact allergy as well.

The contribution of sunscreens to cosmetic allergy has been considered to be relatively small despite the increase in their use, however, the low rate of allergic reactions observed may well be because a contact allergy or a photoallergy to sunscreen products is often not recognized, since a differential diagnosis with a primary sun intolerance is not always obvious. Furthermore, the patch-test concentrations generally used might be too low, in part because of the risk of irritant reaction. Last but not least, photopatch testing is not routinely performed in dermatological practice, which might be the most obvious reason for underreporting.

Excipients, Emulsifiers, and Humectants

Excipients, emulsifiers, and humectants are common ingredients in topical pharmaceutical and cosmetic products, the former being likely to induce sensitization. The classical examples are wool alcohols, fatty alcohols (e.g., cetyl alcohol), and propylene glycol, but these are really rare allergens in cosmetics. Emulsifiers in particular have long been regarded as irritants, but their sensitization capacities should not be overlooked. It is imperative, of course, that patch testing be properly performed to avoid irritant reactions and that the relevance of the positive reactions be determined. A large number of newer emulsifiers, emollients, or excipients have been reported as cosmetic allergens: for example, sodium stearyl lactate [36], polyglyceryl laurate [37], dicaprylyl maleate [38], isononyl isononanoate and trioleyl phosphate [39], as well as ascorbyl tetraisopalmitate [40]. Some humectants are also, because of their low irritant properties and "skin-mildness," often incorporated in skin-care products "recommended by dermatologists," "for use on intolerant," or "for sensitive skin" that have become very popular in recent

years. A low irritant potential, however, does not preclude the occurrence of, albeit rare, allergic contact dermatitis from such cosmetics. Examples are butylene glycol [41] and pentylene glycol [42], that is, aliphatic alcohols with similar uses (solvent, humectant, and antibacterial) to propylene glycol that are considered to be more irritant and allergenic, and ethylhexylglycerin (synonym, octoxyglycerin), a skin conditioning agent [43]. Other occasional contact allergens are alkyl glucosides, that is, coco-glucoside and lauryl glucoside [44], also being used as mild surfactants and cleansing agents in "biological" cosmetics, and decyl glucoside as a hidden allergen in sunscreen products [45].

Copolymers are potential allergens as well, such as, for example, methoxy polyethylene glycol (PEG)-17 and PEG-22/dodecyl glycol copolymers and polivinyl pyrolidone/hexadecene copolymer (see ref. 46 for a review), with the most recent one described being C30–38 olefin/isopropylmaleate/Ma copolymer in a sunscreen [47] and moisturizer [48]. In view of their rather high molecular weight, the exact nature of the allergens involved is not known, and impurities or degradation products might well be the actual culprits, such as has been shown, for example, for hydroxyethyl acrylate in hydroxyethyl acrylate/sodium acryloyldimethyl–taurate copolymer [49].

Natural Ingredients

Plant extracts and herbal remedies have become very popular in recent years and may give rise to (sometimes severe) contact dermatitis problems [50,51] in aromatherapists, beauticians, and clients. Patients found to be allergic to fragrance components should be advised to avoid cosmetics containing plant extracts (separately labeled on the packaging) since these may cross-react.

Proteins and protein-derived ingredients, such as hydrolysates, are often used in skin-care products intended to be used for the treatment of the dry skin in atopic subjects (often children). Allergic contact dermatitis (sometimes located mainly on the eyelids [52] may occasionally develop to oat or *Avena* extract, whose allergenic proteins may be removed, though [53]; hydrolyzed wheat protein [54]; and soy bean extract [55]. However, not only contact dermatitis but also immediate-type reactions [56] to such products may occur [57], particularly in atopic subjects; hence, the use of these products has given rise to controversies since subjects may perhaps get sensitized through topical preparations and develop food allergies afterwards, or vice versa [58,59].

Photoallergic Contact Dermatitis

In the 1960s, most photoallergic reactions resulted from the use of deodorant soaps containing halogenated salicylanilides and related compounds, while in the 1980s, they were most often due to musk ambrette and 6-methylcoumarin, present in, for example, aftershaves, hence affecting men. This sometimes resulted in the development of persistent light reactions. Later on, sunscreens, particularly benzophenones (its presence needs to be labeled on the packaging) and dibenzoylmethane derivatives, became important photoallergens, while methylbenzylidene camphor, cinnamates, phenylbenzimidazole sulfonic acid, and octyl triazone were only occasionally reported in this regard (for example, see ref. 34 for a review). The reactions to benzofenones and, particularly, octocrylene [35] are, in most cases, actually related to photocontact allergy to ketoprofen, a nonsteroidal anti-inflammatory drug widely used to treat muscle pain. The reason why is not clear yet and needs to be further elucidated, since cross-reactivity, as is the case between ketoprofen and benzophenones, is not obvious.

Immunologic Contact Urticaria

IgE-mediated or immediate-type allergic reactions are caused both by low-molecular-weight chemicals and large molecules, such as proteins and derivatives. Cosmetic examples of substances to which severe reactions (even anaphylaxis) also have been reported are permanent hair dyes, for example, PPD [60–62], and also direct hair dyes, such as, for example, basic blue 99 (see, for example, ref. 63), persulfates in hair-bleaching products [64], the sunscreen agents benzophenones [65–67], and protein-derived (i.e., hydrolyzed_ products used in skin- and hair-care products [53,56].

DIAGNOSING COSMETIC ALLERGY

We can only find the allergens we look for; hence, all ingredients in cosmetic products should be regarded as potential contact allergens [68,69]. Beside taking a careful history of the patient and noting the clinical symptoms and localization of the lesions, allergen identification for a patient with a possible contact allergy to cosmetics is performed by means of patch testing with the baseline (standard) series, specific cosmetic-test series, the used products themselves, and all their ingredients. Late readings up to 7 days are advised. If suspected, photopatch tests should be performed as well. However, when a cosmetic dermatitis is suspected and the results of patch testing are doubtful or false negative, usage tests and/or repeated open application tests (ROATs) are additional test procedures to obtain a correct diagnosis. Moreover, if immunologic contact urticaria is suspected, prick tests with the cosmetic product and its ingredients, with immediate test readings, are necessary.

HYPOALLERGENIC PRODUCTS

Most of the cosmetic industry is making a great effort to commercialize products that are the safest possible [70]. Some manufacturers market cosmetics containing raw materials having a "low" sensitization index or a high degree of purity, or from which certain components have been eliminated (generally fragrance ingredients). Sometimes, "active" preservative agents are also omitted, and immunologically inert physical agents are being used more often in sunscreens rather than ultraviolet (UV) absorbers.

Statements such as "recommended by dermatologists," "allergy-tested," or "hypoallergenic" have been put on the

packaging by manufacturers to distinguish their products from those of their competitors. Although there are several ways to reduce the allergenic nature of cosmetics, such as reduction of concentration of recognized allergens, there are no governmentally mandated standards or industry requirements.

The latest trend is target marketing to people with "hypersensitive" or "intolerant" skin, an often-used term for the shadowy zone between normal and pathological skin. These would be people with increased neurosensitivity (e.g., atopics); heightened immune responsiveness (e.g., atopic and contact allergic individuals); or a defective skin barrier (i.e., people with irritable skin such as atopics or those suffering from seborrheic dermatitis) or rosacea. This means that part of the cosmetic industry is moving more into the area of pathological skin and that certain products are in fact becoming drugs, often called "cosmeceuticals." This may cause a great deal of regulatory concern because it suggests some middle category between cosmetics and drugs that does not yet legally exist. In Japan, however, these products fall in the category of "quasi-drugs."

The meaning of most such claims used nowadays is unclear for both the dermatologist and the consumer, the latter being convinced that hypersensitive skin is allergic skin. It is the dermatologist's task to diagnose the skin condition and to provide specific advice about the products that can safely be used. All such problems must be approached individually, not least the contact allergic types, because people sensitive to specific ingredients must avoid products containing them. Therefore, ingredient labeling can be of tremendous help. Moreover, providing the allergic patient with a limited list of cosmetics that can be used is practical and effective [71].

CONCLUSION

The identification of cosmetic allergens is challenging because of the extreme complexity of the problem. This applies not only for the dermatologist who is trying to identify the culprit and advise his patient but also certainly for cosmetic manufacturers, who are extremely concerned about assuring the innocuousness of their products. Precise, current, and rapid information about adverse reactions to cosmetic products is critical in product design. Apparently, premarketing studies are unable to identify all the pitfalls. Therefore, the fruitful communication that is developing between dermatologists and cosmetic manufacturers must be encouraged. Sensitivity to cosmetics can never be totally avoided, but its incidence can be substantially reduced.

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37 Anti-Itch Testing (Antipruritics)

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INTRODUCTION

Itching, or pruritus, is an unpleasant sensation that provokes a desire to scratch. Chemical, mechanical, thermal, and electrical stimuli can elicit itch [1–5]. Mediators of itch, presumably, directly act on nerve fibers or lead to a nerve stimulation cascade whose final common pathway is interpreted in the central nervous system as itching [2–6]. Putative receptors for itching are C-fibers with exceptionally low conduction velocities and insensitivity to mechanical stimuli [4–6]. Histamine, the prototypical chemical mediator of itch, which is released during mast cell degranulation and mediates its effects in the skin via H1 receptor [3,5], is the best-known experimental pruritogen [2,3,5,7]. Other pruritogens such as compound 48/80 [8,9], substance P [10,11], and serotonin [12] have been reported. Recent studies of other pruritogens include cowhage spicules [13] and the bovine adrenal medulla 8-22 (BAM-22) peptide [14].

Antipruritics may alleviate or diminish itching sensation. The traditionally used topical antipruritics such as antihistamines, anesthetics, capsaicin, corticosteroids, and cooling agents are extensively used [8–10]. The topical calcineurin inhibitors tacrolimus and pimecrolimus are added to this armamentarium [15]. Alternatively, the κ-opioid receptor agonists such as butorphanol and the μ-opioid receptor agonists such as naltrexone were added to the systemic antipruritic medications, which are the systemic antihistamines (e.g., hydroxyzine), antidepressants (e.g., mirtazapine), neuroleptics (e.g., gabapentine), ultraviolet (UV) therapy, and the immunosuppressives cyclosporine and azathioprine [15]. To define antipruritic effects, testing methodologies have been developed [11,12,16]. However, the clinical effects of anti-itch vary, and sometimes, it is difficult to compare efficacy between antipruritics. One reason may be inadequate biometrics, as itch is a subjective symptom, and to measure its severity is a challenge; its magnitude (intensity) can be only estimated from reports of patients or volunteers. Methodologies have been adopted to evaluate antipruritics that may aid future development of anti-itch products.

The third edition focused on the evaluation of topical antipruritics and further reviewed recent investigations involving thermal stimuli-modifying itch [17–19]; electronic devices for measurement [8,9,20]; as well as alleviation of itch [21], newly found use of known drugs [11,22], questionnaires for assessment of pruritus in atopic and uremic patients [23,24], and possible models for developing new antipruritics [10,11,20].

The fourth edition focuses on the evaluation of recent advances of pruritogens for models for antipruritics [13,14]; the cutaneous innervation of pain and itch in keloids [25]; a further report on the explanation of the pathway of the antipruritic effect of sertaconazole nitrate (STZ) [26]; the use of a Web-based questionnaire to determine the difference in the characteristics of itch between patients with psoriasis and atopic dermatitis (AD) [27]; and finally, the recent findings on the role of toll-like receptor 7 (TLR7) as a mediator of pruritus and possible target for future antipruritics [28].

METHODOLOGIES

Histamine-Induced Itch Human Model

Rhoades et al. [29] examined the inhibition of histamine-induced pruritus by three antihistaminic drugs using a double-blind crossover study on 28 human subjects. These included diphenhydramine HCl, cyproheptadine, hydroxyzine HCl, and a lactose placebo in identical capsules. All subjects were given intradermal injections of increasing doses of aqueous histamine phosphate in the volar aspect of the forearm to establish their individual threshold levels at which itching occurred. Following the establishment of a baseline, the subjects received two doses of one of the three antihistamines or placebo on four test periods with a 1-week interval between test days. Results revealed a fivefold increase above baseline of the histamine dose required to produce pruritus following both cyproheptadine and placebo. This compared to a tenfold increase following diphenhydramine and a 750-fold increase following hydroxyzine HCl.

Yosipovitch et al. [30–33] performed human studies to evaluate the antipruritics with this histamine injection, as well as histamine iontophoresis-induced itch models in man [14,15]. They also utilized the visual analogue scale (VAS) to measure the itch magnitude (intensity). One study compared the effect of antipruritics of a high-potency corticosteroid, clobetasol propionate (CP) ointment, versus its placebo in a double-blind manner on 16 healthy volunteers. Additionally, they evaluated the effect of CP and its placebo to thermal sensation and pain [30]. They demonstrated that the CP had rapidly decreased histamine-induced itch but did not alter warmth sensation and thermal pain thresholds. Another study determined the effect of menthol and its vehicle (alcohol) on thermal sensations, pain, and histamine-induced experimental itch with 18 human subjects [31]. Menthol showed a subjective cooling effect lasting up to 70 min in 12 of 18 subjects;

however, it did not affect the cold and heat threshold, nor did it affect cold and heat pain threshold. Alcohol produced an immediate cold sensation lasting up to 5 min in 4 of 18 subjects and lowered the sensitivity of cold sensation threshold ($P < .05$). Histamine injection did not change thermal and pain thresholds. Menthol did not alleviate histamine-induced itch magnitude, or its duration. They suggested that menthol fulfills the definition of a counterirritant but does not affect histamine-induced itch, nor does it affect pain sensation.

Later, they examined the effect of topical aspirin and its model vehicle dichloromethane on histamine-induced itch in 16 human subjects [32]. Aspirin significantly reduced itch duration ($P < .001$) and decreased itch magnitude ($P = .04$). Aspirin and vehicle application did not affect thermal and pain thresholds during histamine-induced itch. Further, they tested the antipruritic effect and thermal sensation of a local anesthetic, 1% pramoxine, and its vehicle control in 15 human subjects [26]; pramoxine significantly reduced both the magnitude and duration of histamine-induced itch. The pramoxine also reduced the cold pain threshold but did not affect warm sensation or heat pain threshold.

They further investigated the effect of thermal modulation in histamine-induced itch [17,18]. They first investigated the effect of thermal stimuli and distal scratching on skin blood flow and histamine-induced itch in 21 healthy volunteers [17]. Thermal stimuli included temperatures of 41°C, 15°C, and 49°C while scratching was performed using a 7 in. cytology brush. Assessment of itch was done psychophysically using a computerized VAS (COVAS; Medoc, Ramat Ishai, Israel), and mapping of skin blood flow was done utilizing a PIM II laser Doppler perfusion imager (LDPI) at baseline, in the different thermal stimuli, after histamine iontophoresis treatment, and after scratching. They found that scratching significantly ($P < .01$) reduced skin blood flow and itch; noxious heat significantly increased basal skin blood flow ($P < .001$) but was not significant in reducing blood flow and itch intensity; noxious cold and cooling significantly ($P = .007$) reduced itch intensity but not in histamine-induced skin blood flow; and subnoxious warming had no effect with either itch intensity or skin blood flow. They suggest that heat pain and scratching may inhibit itch through a neurogenic mechanism that also affects blood flow.

The other study involved 21 healthy human volunteers and assessed whether (1) the sensory perception of itch is attenuated by interactions between thermal and mechanical stimuli, as well as afferent information related to itch; and (2) if interindividual differences in itch perception were related to interindividual differences in pain sensitivity [18]. They used a 100 mm COVAS on histamine iontophoresis applied on the flexor forearms. After 30 s, thermal stimuli (noxious cold [2°C], innocuous cool [15°C], innocuous warmth [41°C], and noxious heat [49°C]) were delivered repetitively in a random order by a 16 × 16 mm Peltier device at 3 cm distal to the sight of histamine iontophoresis. A cytology brush was used to simulate scratching at a constant pressure. Results revealed that noxious heat, noxious cold, and scratching significantly ($P < .004$, .001, and .0001, respectively) reduced itch via spinal or supraspinal mechanism. A possible explanation favoring a supraspinal mechanism is that these three stimuli were sufficient to have called attention from the prefrontal cortex, thus diverting attention away from the itch. The study revealed significant interindividual differences in itch sensitivity to histamine. On the other hand, interindividual differences in itch sensitivity were unrelated to interindividual differences in pain sensitivity.

Pfab et al. [19] also used thermal modulation for histamine-induced itch. They evaluated the effect of short-term alternating temperature modulation in nine healthy human volunteers and developed a possible methodology for imaging studies using functional magnetic resonance imaging. Histamine induction was done using the skin prick model [34] to the volar aspect of the dominant right forearm of each subject. Skin temperature was modulated, intensity of itch was determined, and the Eppendorf Itch Questionnaire was done by all subjects at the end of the study. Results revealed that the mean itch intensity was significantly ($P < .001$) higher at 25°C temperature compared with that at 35°C temperature. Alternating changes in mean itch perception between 25°C and 35°C were notably reproducible. And the mean descriptive and emotional ratings were also significantly ($P < .01$) higher at 25°C temperature compared with that at 35°C temperature as well. They concluded that a decrease in short-term moderate temperature enhances histamine-induced itch, providing the possibility of further and more detailed itch investigation by methods usually used for nociception, such as functional magnetic resonance imaging.

Weisshaar et al. [35] evaluated the effect of topical capsaicin on the cutaneous reactions and itching to histamine in atopic eczema (AE) patients and healthy human subjects. Capsaicin 0.05% was applied three times daily over a 5-day period to the same infrascapular region. The effects of pretreatment upon the pruritogenic and wheal-and-flare reactions to subsequent histamine iontophoresis were evaluated on the following day. In control subjects, but not in AE patients, capsaicin pretreatment significantly reduced the flare area. Compared with control subjects, AE patients showed a lack of alloknesis (itchy skin) or significantly smaller areas of alloknesis in pretreated and nonpretreated skin. In control subjects, capsaicin pretreatment significantly reduced itch sensations compared with nonpretreated skin, whereas in AE patients, no differences were seen. Itch sensations in capsaicin-pretreated skin were significantly lower in control subjects than in AE patients. They concluded that capsaicin effectively suppresses histamine-induced itching in healthy skin but has less effect in AE. The diminished itch sensations and the absence of alloknesis in atopic individuals indicate that histamine is not the key factor in itching in AE.

Thomsen et al. [36] conducted a randomized, double-blind, and placebo-controlled human study to determine the antipruritic ability of topical aspirin in inflamed skin. In 24 nonatopic volunteers, an inflammatory skin reaction was induced in forearm skin at five sites by sodium lauryl sulfate (SLS) contained in Finn Chambers. Aspirin 10%, aspirin 1%, mepyramine 5%, and vehicle were applied to the inflamed

and corresponding noninflamed areas 20 min before itch induction with intradermal histamine injection. No difference in itch intensities was found after application of aspirin, mepyramine, and vehicle, but more itch was induced in aspirin- and mepyramine-pretreated sites in inflamed skin compared with normal skin ($P < .05$). In normal skin, flare areas were smaller after pretreatment with aspirin 10% ($P < .05$) and mepyramine ($P < .001$), as were wheal areas after mepyramine ($P < .01$), compared with vehicle pretreatments. In inflamed skin, flare areas were smaller after pretreatment with aspirin 10% ($P < .01$) and mepyramine ($P < .001$), as were wheal areas after aspirin 10% ($P < .01$), aspirin 1% ($P < .05$), and mepyramine ($P < .001$). They concluded that despite a significant skin penetration as measured by the influence on wheal-and-flare reactions, topically applied aspirin did not decrease histamine-induced itch in the model used.

Zhai et al. [37] evaluated the antipruritic effect of hydrocortisone (1% and 2.5%) and its vehicle control on histamine-induced itch and sensory effects in 18 human subjects. In comparison with placebo, 2.5% hydrocortisone significantly ($P = .03$) reduced itch duration from 12.6 ± 11.0 to 8.6 ± 8.2 min (the reducing rate was 32%) as well as itch magnitude (at minutes 3, 6, and 7, and overall). Placebo and 1% and 2.5% hydrocortisone significantly altered ($P < .05$) the cold sensation threshold. No treatment altered cold or heat pain thresholds. They suggested that topical application of 2.5% hydrocortisone might be significantly beneficial for the treatment of histamine-induced itch.

They further ascertained the antipruritic effects of topical strontium salts with the histamine-induced itch model on eight human subjects [38]. Strontium nitrate, in comparison with its vehicle control, significantly shortened itch duration from 28.1 ± 5.4 to 18.5 ± 4.2 min ($P < .01$) and reduced itch magnitude at time points 12 to 20 min and overall ($P < .05$). They concluded that strontium nitrate may act as a topical antipruritic agent in reducing histamine-mediated itch. Furthermore, they utilized this histamine-induced itch human model to screen and to compare the efficacy of a group of topical antipruritics on 10 individuals who were responsive to histamine-induced itch sensation [39]. The pramoxine-containing cream (formulation D) significantly ($P < .05$) decreased itch magnitude (within a 20 min test period), from 2.6 ± 2.1 to 2.2 ± 2.1 cm when compared with its vehicle control; it also significantly ($P < .05$) shortened itch duration (15.0 ± 7.4 min) in comparison with its vehicle control (20.3 ± 7.0 min). Of all the formulations tested, pramoxine-containing cream was the most effective antipruritic in decreasing histamine-induced itch.

Kesting et al. [21] established a reliable model that objectively demonstrates the effectiveness of ear electroacupuncture by reducing alloknesis areas in experimentally induced itch. Thirty-two human healthy volunteers underwent two experiments and had both their volar forearms treated with histamine iontophoresis. In the first experiment, 16 were administered with electrical ear acupuncture on the left ear, and the other half, the right ear. Alloknesis was measured at 5 and 10 min posthistamine iontophoresis treatments. In the second experiment, none of them received acupuncture, serving as control. Results revealed that, after the 5th and 10th minute, the alloknesis areas in the ipsilateral sites, treated with acupuncture, were significantly smaller ($P < .05$) than the contralateral untreated sites. And the contralateral sites of the first experiment, compared with the results of the second experiment, showed increasing areas of alloknesis at both the 5th and 10th minute; results were not statistically significant in terms of the size of the increasing alloknesis areas.

Recently Discovered Pruritogens as Targets of New Antipruritic Drugs for Chronic Pruritic Dermatoses

Most chronic pruritic dermatological, neurological, and other systemic illnesses are unresponsive to antihistamines [13,14]. The cowhage spicules and BAM8-22 were found to induce scratching in humans with AD and scratching behavior in mice, respectively, in a histamine-independent pathway, and are both possible mediators of itch [13,14].

Proteinase-activated receptor-2 (PAR-2) has been implicated with the pathophysiology of pruritus in AD. Cutaneous application of spicules of the plant cowhage (*Mucuna pruriens* var. pruriens) stimulates the nerve fibers differently from those activated by histamine [40]. It is the mucunain ingredient of the cowhage plant that binds to the PAR-2 receptors—the receptors found to be abundant in AD patients [41]. Papoiu et al. [13] tested the validity of cowhage as an itch-inducing agent by contrasting it with histamine-induced itch and the combination of both pruritogens in 15 AD patients and 15 healthy individuals utilizing COVAS [13]. Itch stimuli were alternatively applied to the volar aspect of the forearms in the following sequence: histamine (right forearm), cowhage alone (left forearm), and cowhage + histamine (right forearm, 10 cm away from the cowhage alone). The mean and peak COVAS ratings of both histamine-alone and cowhage-alone groups were significantly influenced by the itch stimulus ($P < .0001$ and $P < .0001$, respectively). The cowhage-alone group showed significantly greater mean and peak levels ($P < .000$) when compared with the histamine group but were not significantly different when compared to the cowhage + histamine group (contrast mean ratings: $P = .89$; contrast peak ratings: $P = .29$). Even if AD patients appeared more sensitive to all stimuli, there was no main disease effect on itch ratings (mean rating: $P = .77$; peak rating: $P = .36$). This study showed that cowhage induced a greater itch sensation when compared to histamine and was the foremost substance in itch induction when both pathways were activated at the same time. Cowhage-induced itch could serve as a suitable model for AD and other chronic itch diseases and can also be used as a model for new antipruritic medications in humans.

The BAM8-22 peptide is a proteolytically cleaved product from proenkephalin A and is a potent activator of the Mas-related G-protein coupled receptors (Mrgprs) [14]. Consequently, activation of two the Mrgprs, that is, MrgprC11 and hMrgprX1, induced scratching behavior in mice in a Mrgpr-dependent pathway [42]. Sikand et al. [14] investigated if BAM8-22 peptide is a possible mediator of

itch in humans, likewise in a Mrgpr-dependent pathway on the volar forearms of 15 healthy human volunteers, by their self-perceptions from particular stimuli (dysesthesia)—alloknesis, hyperknesis, and/or hyperalgesia—introduced using cowhage-soaked and histamine-soaked BAM8-22 and BAM8-18 (a putatively inactive peptide) and histamine. The itch elicited by BAM8-22 spicules was significantly greater than BAM8-18 spicules ($P = .02$ for mean and peak magnitude; $P = .0012$ for total duration in minutes). Similarly, BAM8-22 spicules elicited greater values for pricking/stinging (area under the curve [AUC]: $P = .02$; peak magnitude: $P = .004$; total duration in minutes: $P < .0001$). For the histamine-soaked group, both BAM8-22 and histamine elicited at least one dysesthesia. There was no effect exhibited on the histamine-soaked BAM8-22 in the pretreated areas with antihistamine, while in the in the histamine group, pretreatment with antihistamine produced itch inhibition. Hence, BAM8-22 may be an endogenous source of an itch mediator that activated MrgrpsX1, a novel target for potential antipruritic treatments.

Scratch Behavior Measurement

Tohda et al. [43] studied the effect of Byakko-ka-ninjin-to (BN), which is composed of gypsum, the root of anemarrhena, ginseng, licorice, and rice, on the inhibition of itch using a naive/challenged (NC) mouse model of AD. BN (200 mg/kg, orally) significantly inhibited the scratching frequency in NC mice and decreased the skin temperature by 1.97°C.

Electronic Devices for Accurate Measurement of Itch

Orito et al. [8] developed a model for assessing the duration of scratch behavior in mice by evaluating the time course changes in the distance between the animal's hind limbs and the back of the neck. Ten micrograms (~10 μL) intradermally administered compound 48/80 was used to induce itch to the backs of Imprinting Control Region (ICR) mice, and their scratch behavior was recorded on digital videotape; as well, the distance between the back and the hind limb (hind limbs were color coded) was measured continuously using an image analysis system (SCLABA system, Noveltec, Kobe, Japan). Results for "true" scratching behavior revealed no significant difference among the three thresholds ($P = .1$), while the results for duration of scratching recorded during the observation period increased significantly ($P < .001$) as the threshold lengthened. This study suggests that the SCLABA system is a good tool for studying factors that may cause itch and also for evaluation of efficacy of a new antipruritic drug using experimental animals such as NC/Nga mice, a representative model of AD.

Inagi et al. [9] evaluated and characterized scratching behavior using their new apparatus, MicroAct, in ICR and BALB/c mice. Inductions of scratching behavior were done by (1) intradermally injecting 20 mL of compound 48/80 and 20 mL of physiologic saline in two sets of both ICR and BALB/c mice, the other set serving as control; (2) intradermal administration of 20 mL of appropriately diluted anti-dinitrophenyl (anti-DNP) monoclonal immunoglobulin E (IgE) to induce passive cutaneous anaphylaxis (PCA); and (3) inducing contact sensitivity reaction through nine applications of 0.15% dinitrocholorbenzene (DNCB) diluted in acetone done on the backs of BALB/c mice. Frequency of scratching events (three or more consecutive scratch behaviors or beats), total scratching time, and total number of beats (scratch behavior) detected by MicroAct were the parameters used. Results revealed that MicroAct's tally was comparable with the observer's tally. The frequency of scratching events and total scratching time increased in a dose-dependent manner for both the ICR and BALB/c mice. In the PCA of the ICR mice, the three parameters increased, though not significantly. There was a significant ($P < .001$) increase in the three parameters with induced contact sensitivity in the BALB/c mice.

Benjamin et al. [20] developed a practical method for evaluating scratch behavior by use of portable digital limb-worn accelerometers suitable for children and adults, in seven atopic children (aged 2–9 years) and seven children (aged 5–7 years) without atopy, utilizing a night video recording with infrared light as the gold standard. Parameters of measuring accelerometer readings were epochs (unit of time assayed) equivalent to 2 s and "burst analysis" (successions of 1 epoch), while for the night video recording, the following were observed: sleeping, scratching, restless movements, and movements under covers—which were clearly defined operationally in the experiment. Results from night video recording revealed a statistically significant ($P < .01$) result of 46 min less sleep and a greater "scatter" of readings in atopic patients compared with the control group, while results from the accelerometer readings were significantly ($P < .01$) clear and consistent, and, though not significant, arm movements scores resulted higher than lower limbs scores. Accelerometer scores were highly correlated with the video scores ($P < .01$) for scratching, restlessness, and sleeping time.

Others

These include a contact allergic dermatitis model (poison ivy), contact irritant dermatitis induced by SLS, and so forth [44–46].

Keloid scars are characterized as overproliferation of collagen appearing in a previously "traumatized" site [47]. Trauma may mean actual wounding of skin or just a simple "stretch." A previous study reported that keloids may have the sensations of itch and pain, 86% and 46%, respectively [48], and itch was located in the periphery of the keloid, while the pain was localized in the center of the scar [49]. Tey et al. [25] evaluated 22 subjects—13 with keloids (9 with pruritic keloids and 4 with painful keloids) and nine healthy human volunteers who served as the control—for the following: (1) to determine whether innervations of keloids in the epidermis and dermis are different from healthy skin; (2) to determine if there is a difference of innervations between central and peripheral areas of the keloid scars; (3) if there is difference, to relate it to pain and itch sensations utilizing the VAS and correlate it with immunofluorescent staining using the

pan-neuronal marker protein gene peptide (PGP) 9.5. There was no significant difference between the nerve density of the test subjects and the control ($P = .161$), although a trend of increasing nerve fiber density was observed in the lower epidermis in itchy keloids ($P = .069$). Further, there were no significant differences in nerve fiber density and distribution in the epidermis between the central and peripheral parts of the keloids, and there was less nerve fiber density noted in the central dermis, but it was not significant ($P = .508$). In addition, there was no significant correlation found between itch and pain VAS and the amount of staining of PGP 9.5 in the epidermis and papillary dermis of the central and peripheral sites of keloids. Even if this report did not produce significant results, the authors were able to establish a trend. The authors hypothesized from the studies of chronic pruritic dermatoses such as lichen amyloidosis [50] and prurigo nodularis [51], which were found to have a lower epidermal nerve fiber density, that they may be the result of chronic itch stimulation of itch-transmitting nerve fibers, which then produces a self-regulated hypoplasia of nerve fibers, which is not observed in the dermis.

Recently Discovered Use of an Known Drug

Substance P-induced itch was used by Liebel et al. [11], who found that the antimycotic STZ inhibited contact hypersensitivity and scratching responses in a murine model of pruritus. Fifty microliters (50 μL) of 300 mg of substance P dissolved in sterile physiological saline was intradermally injected in male mice to produce itch response, while 50 mL intradermal administration of sterile physiologic saline served as control. Results revealed statistically significant reduction in scratching in STZ-treated animals ($P < .05$) compared with the reduction in scratching in 1% hydrocortisone-treated animals.

Kaur et al. [26] endeavored to explain STZ's antipruritic activity—through induction of prostaglandin D_2 (PGD_2)—and how PGD_2 works in exerting its antipruritic effect, utilizing rat basophilic leukemia (RBL-2H3) mast cells with compound 40/80 as the pruritogen, in an in vivo scratching model. Mast cell degranulation using granulation marker β-hexoaminidase release, detection of PGD_2 using a PGD_2 competitive enzyme-linked immunosorbent assay (ELISA) kit, and Western blotting for RBL cells treated with STZ lysates were the methods used. Several studies reported that the antipruritic PGD_2 is by histamine suppression from mast cells [52]. STZ diminished the degranulation of RBL-2H3 mast cells induced by compound 40/80, a pruritogen known to promote histamine release, and, enhanced PGD_2 production in mast cells. Additional PGD_2 (from an exogenous source) abated compound 40/80-induced degranulation by acting through prostanoid D receptor 1 (DP1). Compound 40/80-induced scratching behavior was abated by a cyclooxygenase inhibitor, ibuprofen, or a DP1 receptor antagonist (MK0524). STZ also induces the p38 mitogen-activated protein kinase (p38 MAPK) phosphorylation in mast cells, and the p38 MAPK inhibitor SB203580 resulted in the attenuation of PGD_2 levels. All in all, the results suggest that the STZ mediates its antipruritic effects by enhancing PGD_2 production by the activation of the p38 MAPK pathway.

Wikstrom et al. [22] conducted a randomized, double-blind, placebo-controlled study using κ-opioid agonist nalfurafine in 144 uremic patients with end-stage renal disease (ESRD) undergoing hemodialysis. Itch intensity was assessed using a five-point scale, which revealed a significant ($P < .0410$) reduction in itch intensity; as well, a three-point scale showed a significant reduction in the number of excoriations in the body ($P = .0060$). Safety profiles of nalfurafine were evaluated; the most common adverse drug reactions were headache, insomnia, vertigo (mediated by the central nervous system) and nausea, and vomiting (mediated by the gastrointestinal system). These adverse drug reactions were transient and were resolved. The results suggest that nalfurafine seem to be both an effective and safe drug in the treatment of patients with ESRD undergoing hemodialysis.

Questionnaires for Itch Assessment

Yosipovitch et al. [23,24] constructed two questionnaires for itch assessment modifying McGill's pain questionnaire. The first study utilized a predetermined questionnaire that provided a detailed description of pruritus in AD in 100 atopic Chinese patients [23]. The modified questions were aimed at characterizing the clinical pattern and sensory and affective dimensions of itch experience in AD. Itch intensity was also measured using VAS. Results revealed the following: (1) There was prolonged duration of pruritus (descending order) in lower limbs, flexures, upper limbs, and neck. (2) Itch intensity peaked twice as much as mosquito-bite itch. (3) Itching was most frequent at night, and most patients reported difficulty in falling asleep. (4) Daily-life activities that increased severity of the itch were (in descending order) sweat, dryness, stress, physical effort, specific fabrics, activity, and hot water, and males significantly ($P = .004$) differed with females in terms of activity and physical effort ($P = .002$) in increased pruritus. (5) Major factors found to reduce itch included bathing in cold water and cold ambient environment. (6) Associated symptoms were heat sensation, sweating, and pain in the pruritic area. (7) Most antipruritic medications have limited long-term effects. (8) Itch is bothersome and a major distress to the patient. (9) The affective score significantly ($P < .001$) correlated with itch intensity during its peak. Taken together, these results show that the questionnaire was a useful tool in characterizing itch.

The other questionnaire constructed to measure pruritus was based on the short form of the McGill Pain Questionnaire in 145 uremic patients [24]. This modified questionnaire included (1) patient characteristics, (2) the use of antipruritics, (3) effects of pruritus on sleeping and on mood, (4) effects of dialysis and of daily activities on itch, (5) location of pruritic sites, (6) sensory and affective scores, and (7) itch intensity measured using VAS. Revalidation of the questionnaire was repeated in 28 subjects after 2 weeks and revealed no significant ($P > .05$) difference in VAS temporal states (onset,

pattern, course) and no significant ($P > .05$) difference with regard to the sites of the itch between the two questionnaires. Also, the reliability was high ($P < .01$).

Recently, the "Characteristics of Itch" questionnaire was used by O'Neill et al. [27] to determine the differences in itch characteristics between psoriasis and AD patients, as the severity of itch sensations bears a great impact on the quality of life (QOL) of those with chronic pruritic skin conditions. This Web-based previously validated questionnaire was made available through the National Eczema Association for Science Education and the National Psoriasis Foundation Web sites. A total of 524 AD and 194 psoriasis subjects participated. Patients with AD were more likely to experience itch ($P < .001$) than patients with psoriasis, and the mean itch episode severity was also higher in patients with AD than patients with psoriasis ($P < .001$). More than half of the patients in both groups associated pain with itch, while other associated symptoms, (i.e., heat sensation and sweating) were reported more commonly by the patients with AD ($P < .001$). Patients with AD reported more itch in the flexor surfaces ($P < .001$), while the patients with psoriasis reported slightly more itch on the groin and buttocks. A trend toward greater itch in areas of the extensor surfaces, particularly on lower extremities, was reported by patients with psoriasis. Overall, patients with AD reported a greater negative impact of their skin condition in their everyday lives.

Studies of Possible Models for Establishing New Antipruritics

As mentioned earlier, Orito et al. [8] developed a model for itch assessment using the SCLABA system and proposed what may be a potential model for development of new antipruritics, as well as for the recently discovered pruritogens as targets for new antipruritic drugs, the cowhage spicules [13] and BAM8-22 peptide [14].

Thomsen et al. [10,12] also proposed two models that could benefit in developing new antipruritics. The first animal model is to topically apply nonhistaminic antipruritics using serotonin, recognized as a weak local pruritogen in humans [12]. Out of the eight substances screened (histamine, compound 48/80, kallikrein, trypsin, papain, substance P, serotonin, and platelet-activating factor) injected intradermally (50 μL per substance) into the rostral back of rats, only serotonin induced excessive scratching, while the rest of the substances were weak or inactive. A dose–response curve was plotted against $\log_{10}$ using different concentrations of serotonin to evaluate possible systemic effects in (1) 14 rats intradermally and subcutaneously injected with 0.1 and 1 mg/mL (50 μL per dose) to the rostral and caudal back; (2) another four rats given intradermal serotonin of 10 mg/mL to the caudal back; (3) another 10 rats for each group given concentrations of 0.01 to 31.6 mg/mL intradermally; and (4) two rats given a concentration of 100 mg/mL each. Video recording was used to objectively count scratch sequences, viewed separately by two investigators, and showed the following: (1) The number of scratch sequences of injected serotonin related to the rostral back is significantly greater ($P < .001$) than that of the caudal back. (2) The number of scratch sequences in the caudal and "other" sites did not produce a significant result. No systemic adverse effects of serotonin occurred at 1 mg/mL, 50 mL. Scratching was probably not due to histamine, since the screening period revealed that histamine did not produce scratching. They concluded that serotonin is a reproducible pruritogen eliciting scratch in rats.

The second study was a randomized, double-blind and placebo-controlled study involving SLS-induced inflamed skin, as well as in normal skin in 32 healthy volunteers, pretreated with 1% SLS in one of their volar forearms; their opposite forearms served as control [10]. The study evaluated itch intensity, pain, whealing, and redness in 16 subjects given 20 μL of group A battery of substances (substance P, neurokinin A, neurokinin B, histamine [positive control], and physiological saline [negative control]), and the other 16 given were given 20 μL of group B battery of substances (platelet-activating factor, serotonin, trypsin, histamine [positive control], and physiological saline [negative control]), all intradermally injected to both forearms. Results revealed that inflamed skin is significantly more pruritogenic than normal skin with substance P ($P = .024$) and histamine compared with the control. Neurokinin A, trypsin, platelet activating factor (PAF), and serotonin only elicited itch in normal skin, while neurokinin B did not elicit itch in both groups. Wheal area was significantly ($P < .001$) larger in inflamed skin, though it did not show a significant correlation with itch intensity.

Toll-like receptors (TLRs) are a class of proteins that play a critical role in regulating the innate immune system responses to the pathogen-associated molecular patterns (PAMPs) in mammals [28]. Liu et al. [28] have uncovered a function of TLR7 as a novel itch mediator and a potential therapeutic target for anti-itch therapies for pruritic dermatoses in the future. First, investigators established that the *Tlr7* knockout ($Tlr7^{-/-}$) mice and the wild-type mice (WT) mice would be used for the entire investigation as both differ in their responses to external stimuli. $Tlr7^{-/-}$ mice exhibited normal thermal and mechanical pain sensitivity and did not show any developmental defects in the dorsal root ganglia (DRG), the spinal cord, and the expression of neurochemical markers such as the transient receptor potential subtype V1 (TRPV1), as compared with the WT. Although the scratching behavior of the WT and the $Tlr7^{-/-}$ mice were comparable when infused with the histaminergic pruritogens (histamine, HTMT [histamine H1 receptor agonist], and compound 48/80 [known to release histamine from mast cells]), the $Tlr7^{-/-}$ mice showed a marked reduction in scratching behavior compared to the WT when the nonhistaminergic pruritogens (chloroquine, ser-leu-ile-gly-arg-leu-NH_2 [SLIGRL-NH_2], serotonin [5-HT], and endothelin-1 [ET-1]) were infused. Intradermal injection of imiquimod, resquimod (R848), and loxorabine elicited scratching behavior to the WT but was reduced in $Tlr7^{-/-}$ mice, since TLR7 is known to recognize the imidazoquinoline derivatives (imiquimod and R848) and guanine analogues (e.g., loxorabine) [28,53]. This suggests that the itch diminution/inhibition was through the TLR7 pathway [28,53]. However, imiquimod also elicited

TLR7-independent itch, which may ascribe to its off-tangent effect since imiquimod was shown to act on adenosine receptors or inositol triphosphate receptor (IP3R) [53]. In addition, the investigators ascertained that it is the TRPV1-containing C fibers (not the TRPV1 alone) that are needed for imiquimod-elicited itch [53]. Immunohistochemistry revealed the TLR7 ligands are generally expressed in small-sized dissociated DRG neurons, and its application to the DRG induced very rapid inward currents and action potentials [53]. On the other hand, the ligands failed to produce inward currents and action potential in *Tlr7*$^{-/-}$ mice. As a result, activation of TLR7 leads to an immediate increase in neuronal excitability [28,53]. The TLR7's nongenomic action suggests a possible coupling of TLRs with ion channels in the primary sensory neurons that can trigger immediate itch and/or pain sensation.

CONCLUSION

The measurement of scratch behavior is problematic and has been addressed in a variety of ways such as lack of validation and unlikeliness to be reproducible, and so forth [12]. Since human verbalization may be more accurate in describing itch sensation, the VAS may prove superior to other methods [11,12,21,22]. To evaluate antipruritic drugs, clinical methods may rely on either naturally occurring or experimentally induced pruritus. Methods and judgments based on naturally occurring pruritus better reflect the actual clinical setting [11]. However, they have disadvantages, including the following: (1) Pruritus intensity may fluctuate on its own if the study is conducted over several days since the naturally occurring pruritus may not be stable over time. (2) Comparing the pruritic intensity of specific lesions in different patients is often difficult and not always relevant. (3) Adequate controls are difficult to achieve [11].

A histamine-induced itch model was utilized because acute itching is most commonly evoked by chemical stimuli (e.g., histamines) [2]. Some individuals do not itch after histamine injection [13,14,44,45,54]; therefore, to diminish the variation of responses, we suggest that only subjects with histamine-induced itch sensation should be enrolled. This will improve discrimination—an obvious advantage in a screening assay. However, the histamine injection model may induce pain sensation; it may partially interrupt the itch sensation. We note that a 1 mL injection appears high and undoubtedly spreads; this large volume has added reproducibly to previous studies [22,23,44,54–56]. The VAS score was comparatively low; however, this level (3 cm) was adequate for the discrimination noted. Higher concentrations might be considered in the future.

Alternatively, other itch-inducing models may well be justified in the assessment of antipruritic drugs, complemented by new measuring devices [8–14,17–21,28,34,56].

Last, it is essential that studies of topical antipruritics are well designed and double blinded so that resulting data are valid and able to distinguish between effective and noneffective treatments.

Table 37.1 lists a summary of data of models and mediators of itch and the efficacy of antipruritics.

TABLE 37.1
Summary Data of Models and Itch Mediators and Efficacy of Antipruritics

Models	Efficacy	Reference
Intradermal histamine injection-induced itch	Fivefold increase above baseline of the histamine dose required, producing pruritus following both cyproheptadine and placebo. A tenfold increase following diphenhydramine and a 750-fold increase following hydroxyzine HCl.	Rhoades et al. [29]
Intradermal histamine injection-induced itch	Clobetasol propionate ointment rapidly decreased itch but did not alter warmth sensation and thermal pain thresholds.	Yosipovitch et al. [30]
Intradermal histamine injection-induced itch	Menthol failed to show the effect of antipruritics.	Yosipovitch et al. [31]
Intradermal histamine injection-induced itch	Aspirin significantly reduced itch duration and decreased itch magnitude.	Yosipovitch et al. [32]
Intradermal histamine injection-induced itch	Pramoxine significant reduced both the magnitude and duration of itch.	Yosipovitch et al. [33]
Histamine iontophoresis-induced itch	Capsaicin significantly reduced itch sensations.	Weisshaar et al. [35]
Intradermal histamine injection-induced itch	Aspirin did not decrease histamine-induced itch.	Thomsen et al. [36]
Intradermal histamine injection-induced itch	2.5% hydrocortisone significantly reduced histamine-induced itch.	Zhai et al. [37]
Intradermal histamine injection-induced itch	Strontium nitrate showed a good antipruritic effect in reducing histamine-mediated itch.	Zhai et al. [38]
Intradermal histamine injection-induced itch	Pramoxine-containing cream was the most effective antipruritic in decreasing histamine-induced itch.	Zhai et al. [39]
Histamine iontophoresis-induced itch	Noxious heat, noxious cold, and scratching attenuated itch via a spinal or supraspinal mechanism.	Yosipovitch et al. [17]
Histamine iontophoresis-induced itch	Heat pain and scratching reduced itch.	Yosipovitch et al. [18]
Histamine iontophoresis-induced itch	Ear electroacupuncture reduced alloknesis areas on the forearms.	Kesting et al. [21]

(*continued*)

TABLE 37.1 (Continued)
Summary Data of Models and Itch Mediators and Efficacy of Antipruritics

Models	Efficacy	Reference
Skin-pricked histamine-induced itch	A decrease in short-term temperature enhances histamine-induced itch.	Pfab et al. [19]
Intradermal injection of 8 pruritogens	Histamine and substance P were more pruritogenic in SLS-induced inflamed skin.	Thomsen et al. [10]
Scratch behavior measurement	Byakko-ka-ninjin-to significantly inhibited the scratching frequency in NC mice.	Tohda et al. [43]
Intradermal serotonin-induced itch	Serotonin is a reproducible pruritogen eliciting scratch behavior in rats.	Thomsen et al. [12]
Intradermal compound 48/80-induced itch	MicroAct was comparable with the observer's tally in scratching behavior in mice.	Inagi et al. [9]
Intradermal compound 48/80-induced itch	SCLABA image analysis system was as good as the "true" scratching behavior in mice.	Orito et al. [8]
Intradermal substance P-induced itch	Sertaconazole nitrate was comparable with 1% hydrocortisone in reduction of scratching behavior of mice.	Liebel et al. [11]
Lesional skin of atopic patients	Limb-worn accelerometers were comparable with night video recording in assessing scratch behavior.	Benjamin et al. [20]
Uremia-induced itch	κ-opioid agonist nalfurafine was effective and safe treatment in patients with uremia.	Wikström et al. [22]
Predetermined questionnaire (Modified McGill Pain Questionnaire)	Predetermined questionnaire was useful in the assessment of itch in atopic dermatitis patients.	Yosipovitch et al. [23]
Modified McGill Pain Questionnaire	The reliability of Modified McGill Pain Questionnaire was high when compared to the VAS results.	Yosipovitch et al. [24]
"Characteristics of Itch" questionnaire	Patients with atopic dermatitis reported a greater negative impact in their quality of life (QOL).	O'Neil et al. [27]
Cutaneous application of cowhage spicules	Cowhage-induced itch induced a greater itch sensation in AD patients than histamine-induced itch.	Papoiu et al. [13]
BAM8-22 peptide activation of Mrgprs	BAM8-22 peptide produces itch and nociceptive sensations via histamine-independent mechanism.	Sikand et al. [14]

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38 Determination of Skin Color in Relation to Ethnicity, Gender, Age, Site, and Environmental Factors

An Overview

G. Cazorla

INTRODUCTION

The first contact we have with someone else is usually when we look at his/her face. And through this first glance, the impressions we have about that person remain engraved in us for a long time.

The face plays a fundamental role in human interactions. People's faces tell us about their identity and origins. They also help us judge their age, condition of health, and even what mood they might be in. Many studies have focused on the importance of face shape in the evaluation of age, attractiveness, or health [1,2]. But more recently, numerous works have highlighted how skin color contributes to the way in which we perceive these important elements in our social interactions [3–8]. The visible appearance of human skin has also long been used as a major assessment tool in the clinical evaluation of physiological and pathological phenomena [9–13].

The importance of our skin coloring on this medical or social level can be connected to its extreme diversity in the human species. The skin color of different ethnic groups varies dramatically from an almost black/brown color in a few locations on the African continent or among the Australian aborigines to a very fair complexion, almost white, in Scandinavian populations. This diversity in skin coloring is one of the most variable phenotypes in humans. In 2002, Relethford [14] estimated that pigmentation differences among major continental groups can explain about 90% of the total variations in skin pigmentation. In different genetic studies, genetic variation among major groups accounts for only around 10%–15% of the total diversity [15,16]. Even if this value is linked to the number of polymorphisms used to define this diversity [17], we can see here that skin pigmentation diversity shows an atypical distribution. This article presents different aspects of skin color in relation to ethnicity, gender, age, site, and environmental factors.

CHROMOPHORES IN SKIN COLOR

Human skin color is determined by the absorption, reflection, and scattering of light as it strikes the surface of the skin [18–20]. After illuminating the skin, two types of light are returned to the observer, specular light and diffused light. Specular light is determined mainly by the surface properties and represents, according to Anderson and Parrish [21] and Takiwaki [22], less than 10% of the light reflected from the surface, whereas diffused light is a result of the scattering and absorption properties of the skin, known as diffuse reflectance. Different skins yield different reflectance spectrums due to the unique composition of chromophores in each skin. There are three main types of chromophores present in human skin: melanin, a brown/black or red/yellow polymer produced by melanocytes and then packaged into melanosomes that are found dispersed throughout the epidermis; hemoglobin, in red blood vascular networks; and, to a lesser degree, carotenoids, which are lipids of exogenous origin that can accumulate in the stratum corneum [21,23–26].

Melanin plays the dominant role in skin coloring [19,21,26–28]. There are two types of melanin: eumelanin, a black/brown pigment, and pheomelanin, which is yellow/red. Of these two pigments, eumelanin is the dominant chromophore in terms of perceived color, as it provides varying degrees of brown coloration at the skin's surface and plays a fundamental role in photoprotection [21,29]. Hemoglobin, more precisely the oxygenated hemoglobin, which represents the majority of hemoglobin in the arteries (about 90%), is itself responsible for the red color of the skin, the remaining 10% being composed of reduced hemoglobin, which has a bluish red color [30]. Carotenoids are yellow/red organic pigments that are abundant in fruit and vegetables. These phytochemicals are efficient singlet oxygen quenchers [31,32], which facilitate the protection of tissue against oxidative stress. Oxidative stress can accelerate damage to proteins, lipids, and DNA [31] and contribute to many diseases (cardiovascular [33], diabetes [34,35], or cancers [36]). The skin is directly exposed to endogenous or environmental oxidants [37,38]. Thus, as antioxidants, carotenoids are important for skin health.

In addition to the concentration of these chromophores in human skin, their distribution in the dermis and epidermis as well as the thickness of these layers play a significant role in the reflectance spectrum. We see here that the diversity of skin color in humans is linked to a great number of

physiological parameters, themselves depending on a large number of external and internal factors.

NONINVASIVE TECHNIQUES FOR THE DETERMINATION OF SKIN COLOR

In spite of the ability of human eyes to differentiate between colors, it is impossible for us to quantify our perception of color without instrumental means. The quantification of skin pigmentation therefore needs reproducible objective and noninvasive tools. A large number of rating scales and instrumental devices have been developed since the early works of the 1920s and 1930s [39–41]. Readers may refer to different reviews [42–44] to acquire a comprehensive overview of the main techniques used to analyze skin color. We would like to focus here on the two systems that are the more commonly used in skin pigmentation studies, that is, the "tristimulus" system and diffuse reflectance spectroscopy (DRS).

The International Commission on Illumination (Commission Internationale de l'Eclairage [CIE]) established in 1976 a "tristimulus" system based on a psycho-photometric method [12,45] in order to systematize color reproduction and measurement. As the human eye contains three different types of color-sensing cones with broadband sensitivity in the red, green, and blue spectral areas, the perception of a particular color requires three parameters to be accurately defined (a tristimulus system). Thus, the tristimulus analysis converts the spectral information into three numbers that indicate how an object's color appears to a human observer. One particular three-dimensional spacing system, the CIE L*a*b* system, was developed to be closely and linearly correlated with the response of the human eye. This system expresses color using the following parameters: L* indicates light intensity and takes values from 0 (black) to 100 (white), a* indicates the color of the object on a scale that goes from green (negative values) to red (positive values), and b* indicates the color of the object on a scale that goes from blue (negative values) to yellow (positive values). This system has been used widely in the study of skin color, partly because of its ease of use and the commercial availability of instruments that calculate L*a*b* values [27,46–50]. Chardon et al. [51] have proposed the use of a vector representation for the ultraviolet (UV)-induced tanning reaction in the L*a*b* space. Increases in skin pigmentation can be graphed as a shift on the L*–b* plane. In an attempt to evaluate skin pigmentation, the "individual typology angle (ITA)" has been proposed, defined as the vector direction in the L*–b* plane with the following formula:

$$\mathrm{ITA} = \left[\arctan\left(\frac{\mathrm{L}^* - 50}{\mathrm{b}^*} \right) \right] * \frac{180}{\pi}$$

where ITA is given in degrees.

This parameter has been validated as an expression of skin pigmentation [26,52]. Skin classification with this parameter has also been shown to be related to skin sensitivity to UV exposure [52]. Although the CIE L*a*b* system may be a measure of perceived skin pigmentation, it still lacks the information about the molecular origin of skin color.

To have a better understanding of the molecular origin of skin color, we have to take into consideration the entire chromophore absorption spectrum in the visible region and how it contributes to the absorption spectrum of skin. This can be achieved by performing DRS. In DRS, light is delivered onto the skin, and the remitted light is collected and analyzed with a spectrometer. In addition to the L*a*b* values, current spectrophotometers allow the acquisition of a reflectance spectrum in the visible region (400–700 nm) with a typical measurement every 10 nm using an integrating sphere and an 8 mm aperture. Reflectance spectroscopy has found many applications in noninvasive monitoring of biological tissues and particularly of human skin [26,53–60]. With the high information content in this full spectrum, DRS is a versatile and specific method that can describe biological tissue in relevant parameters like melanin content, blood oxygenation, blood stasis, and so forth but can also be used in the study of relatively less abundant chromophores like bilirubin [28,61,62], methemoglobin, carboxyhemoglobin [30], and so forth.

These instrumental systems allow us to measure precisely and reproducibly the diversity of human skin that we see everywhere we look, from Caucasian to Negro skins, and, inside these skin types, all the subtle variations that make the skin a continuous gamut of color.

VARIATION OF RACE IN HUMAN SKIN COLORING AMONG "RACES"

As mentioned before, pigmentation is one of the most variable phenotypes in humans [63]. About 90% of total skin pigmentation variation can be explained by pigmentation differences observed among major continental groups [14]. These differences between main geographical groups are so obvious that skin color has been widely used to define human "races." The density of melanocytes does not differ between various racial/ethnic groups [64–66]. The interracial differences in skin pigmentation mainly depend on the amount and type of melanin [67–70] and on the size and distribution of melanosomes [64,71–75]. There is a gradient in the size and number of melanosomes in dark, intermediate, and light skin: the melanosomes are larger, more numerous, and more pigmented in dark skin than in fair skin [76]. In addition, the melanosomes in dark skin are more widely dispersed and offer better protection to the cell nucleus. Alaluf et al. [67,77] have shown that more pigmented skins (African and Indian) have about twice as many melanosomes in the epidermis than less pigmented ones (European, Chinese, and Mexican). In another study, Shriver et al. [78] obtained similar results comparing African American skins with European American skins. The transfer of melanin to keratinocytes and

their distribution in the epidermis also contribute critically to visible pigmentation [79,80].

Skin pigmentation shows a strong correlation to latitude [81], and this particular geographic distribution of human coloring has been strongly influenced by UV radiation (UVR) [14,81–83]. Melanin is an effective sunblock, protecting against the harmful effects of electromagnetic radiation above 300 nm [29,84,85]. UVR absorption, and hence skin protection by melanin, is maximal at the shorter wavelengths, where damage to nucleic acids and proteins are more serious [86]. UVR exposures have multiple effects on the skin—sunburn, impaired thermoregulation, and even skin cancer [87–89]—but they can also damage essential nutrients, particularly folate. Folate deficiencies can induce complications during pregnancy, are causes of prenatal and postnatal mortality, and have a key role in spermatogenesis [90–92]. These are some of the potential factors that could explain the evolution of highly pigmented skin in equatorial and tropical regions with high exposure to UVR.

Even though UVR on the skin is mostly damaging, there is one important exception: the synthesis of vitamin D. UVB radiation is essential for the synthesis of this component. Vitamin D plays a key role in bone metabolism, and its deficiency results in rickets in children and osteomalacia in adults [93–96]. According to Holick [97], people with dark skin need 10 times more exposure to sunlight than those with light skin to produce the same amount of vitamin D. In some high-latitude regions, there is not enough UVR for highly pigmented skins to synthesize enough vitamin D [82,95,98]. Thus, the distribution of human pigmentation is a result of the balance between natural selection for darker skins around the equator favoring protection against high UVR exposure and selection for lighter skins far from the equator facilitating vitamin D synthesis [82,99,100].

Skin pigmentation according to population is thus a fundamental task in public health: with modern migrations, more and more populations are living under UVR to which they are poorly adapted (for example, the English who settled in Australia in the nineteenth century). The consequences can be disastrous in terms of public health: people with fair skins are at higher risk of several types of cancer, especially in areas with high UVR, and people with dark skins are at higher risk of diseases caused by insufficient vitamin D levels in low-UVR regions.

SEXUAL DIMORPHISM IN HUMAN SKIN COLOR

In most populations, females tend to be lighter than males [82,101–104]. In 1997, Fullerton et al. [138] showed with objective measurements that females have a lower basal a* level than males and a higher L* value on their back. These differences can be explained by the lower amount of melanin and hemoglobin observed in females in comparison to men [82,106]. At relatively unexposed sites, the constitutive sex difference seems to decrease from strongly to weakly pigmented people. Facultative pigmentation, or tanning, also differs between the sexes. Men tan more than women with similar sun exposure time or amount of clothing [107]. And for the same solar radiation, male reflectance declined more than female reflectance [108]. This gender difference seems to emerge at puberty [102,109–111]. The occurrence of this gender differentiation after puberty suggests that the sex hormones are responsible for it. Both androgens and estrogens increase skin pigmentation by promoting the synthesis of melanin and cutaneous blood flow; the effect of androgens, however, is stronger [112]. This stronger effect in itself might explain why the sexes differentiate in skin color during adolescence, except that this differentiation occurs because girls lighten in color and not because boys darken. One explanation of this phenomenon could be the thickening of subcutaneous fat in adolescent girls [113]. In an evolutionary sense, few explanations have been proposed to account for such differences. Sexual selection has been proposed to explain this sexual dimorphism: men seem to prefer light skin color, which can be regarded as a sign of fertility [106,114,115]. Another hypothesis is that vitamin D deficiency has a greater impact for women, due to their need for calcium and vitamin D during pregnancy and lactation [82,116]. In modern societies, we may wonder if this sexual dimorphism is less significant than in the past. Color differences observed in our multiethnic environment could have outweighed the color differences between males and females. Diet and suntanning have also helped reduce the social significance for women. In 2009, Stephen et al. [8] showed that an increase of the CIE L*, a*, and b* parameters enhances a healthy appearance. In this study, the sexual dimorphism in skin color was exaggerated in the participants by lightening more female faces than male faces and by making more male faces more red and yellow than female faces. In conclusion, although it is partly subconscious, sexual dimorphism in skin coloring plays a significant role in gender relations, particularly in the choice of mates [5].

INCIDENCE OF AGE IN SKIN COLORATION

Chronological skin aging and skin photoaging induce pigmentary changes. These two processes are distinct but are difficult to separate in a given individual [117]. Constitutive color designates cutaneous pigmentation in the absence of direct influences by UVR. Constitutive pigmentation plays a critical role in minimizing DNA damage from UVR [66,118] and in reducing the risk of skin cancer [119]. Measurements of constitutive color are generally taken in a sun-protected part of the body, such as the inner arm or the buttocks, for example. Facultative color characterizes the immediate and delayed tanning reactions following direct sun exposure [120].

Concerning constitutive color, several studies have shown that skin pigmentation at the buttocks is at the highest level in the first years and then decreases with aging [121,122]. This pigmentation decrease is explained by the number of active melanocytes, which decreases with age by about 10%–20% per decade above the age of 30 years [123,124]. According to several studies [125,126], the CIE a* parameter increases

with aging in sun protected areas. This can be explained by the thinning of the epidermis with age and thus by increased skin transparency, which makes it easier to observe the subpapillary vascular plexus, and consequently, blood flux and redness increase [127].

Photoaging has an important impact on skin coloring. The effects of photoaging differ according to race: hyperpigmentation and uneven skin tone in the African American population [128], hyperpigmentation spots and skin yellowing in the Asian population [74,129,130], and skin reddening in the Caucasian population [130,131]. A darkening of the skin has been observed in all ethnic groups in unprotected areas [132]. The long-term effects of UVR on the pigmentation of the skin, as published by Coelho et al. in 2009 [133], may persist for several years and explain this overall darkening of the skin in all racial skin types. The increase of hypopigmentation or hyperpigmentation spots during aging is a direct consequence of exposure to solar radiation through the production of local accumulations of melanin or hemoglobin [6]. This heterogeneity of skin in the perception of female attractiveness or in determining age has been studied recently [3,4,6] and has been shown to play an important role in these evaluations, with a preference for homogeneous and smooth faces.

DIFFERENCES BETWEEN BODY SITES

The choice of measurement site is an important element in the development of a study. In one subject, variability in measurements related to the body site may invalidate the results of the experiment. Finding a representative site for what the scientist wishes to demonstrate and that is not too dependent on spontaneous changes related to physiological or hormonal variations is a key factor in the success of a study. This difficulty should not be underestimated; the differences observed in one area can be of the same order or magnitude as those observed in a different anatomical site. In 1996, Ale et al. [134] showed that the CIE L*, a*, and b* parameters were statistically different between different areas of the volar forearms. In this study, conducted on Caucasian subjects, the most important variations were observed in the a* parameter, showing that vascularization plays a significant role in color variation within one anatomical site.

Even though it is quite difficult to separate chronological aging and photoaging, as mentioned earlier, natural color differences do exist between anatomical sites. In the face, for example, which we may consider as quite a homogeneous area in terms of sun exposure, significant differences exist between the cheeks and the forehead. The forehead is darker, less red, and more yellow than the skin in the cheeks. These observations have been made in different ethnic groups: Caucasians, African Americans, Chinese, Mexicans, and Indians [132,135,136]. Comparisons of two different sites both exposed to sunlight on a regular basis showed that the forearm is lighter than the facial sites [130,137]. In another study, higher basal a* and lower basal L* levels were found on the upper part of the back compared to the lower regions of the back [138]. The basal b* level showed no site-related variation in this study. Of course, skin color differences are much more pronounced between protected and unprotected areas of the body, protected areas being lighter than unprotected ones [46,122,139]. The impact of sun exposure is of the utmost importance in skin colorimetric studies. As we highlighted earlier, the consideration of this parameter is essential for an objective interpretation of the results of an experiment.

INCIDENCE OF EXTERNAL FACTORS ON SKIN COLORING

As previously mentioned, the most obvious external factor that regulates skin pigmentation, and thus, skin color, is solar UVR. The increase of skin pigmentation after UVR exposure is called tanning. This defensive response takes place in several phases. An interesting series of clinical studies have recently been conducted on this topic, and for further details, interested readers can see reference [133] and the literature cited therein. Briefly, these studies have revealed four clear and distinct stages in pigmentation responses after solar UVR: the first is immediate pigment darkening (IPD), which develops in minutes and can remain for several hours and is certainly due to the direct effects of UV on existing melanin or melanin precursors, oxidizing them to form darker pigment. The second is persistent pigment darkening (PPD), which occurs within hours and remains for days and which results from newly synthesized melanin. The third, delayed pigmentation (DP), which develops in days and remains for weeks, results from prolonged increases in melanin content. The fourth stage is long-lasting pigmentation (LLP), which remains for more than 9 months after the initial UV exposure and results from prolonged activation of the pigmentary system. The long-term effects of UVR on human skin can last for years, even in the absence of further UV exposure. All these phases are related to constitutive pigmentation and are more pronounced in dark skins [140,141].

Recently, several studies have also focused on the impact of dietary carotenoids on skin color [24,142]. Alaluf [24] have shown that carotenoids contribute significantly to normal skin color, particularly in the appearance of yellowness, as defined with the b* parameter. The impact of carotenoids in skin coloring is certainly more pronounced in Caucasian skin, the quantity of melanin also having an impact on b* values. The accumulation of carotenoids in skin observed after supplements of β-carotene have been ingested over a long period [143] has been shown to be related to lower UV-induced skin damage [24,144,145]. In 2012, Whitehead et al. [142] showed that increasing one's consumption of fruit and vegetables confers a visible effect on the skin's appearance. These results confirmed those of Stephen et al. [25], who showed that higher levels of carotenoid coloration (i.e., higher values of b* parameter) correlate with a higher perception of health. When we look at the impact of inadequate fruit and vegetable consumption in our societies, leading to approximately 2.6 million deaths a year worldwide [146], this

link between better nutritional habits and improved appearance may be an interesting and efficient way to develop better dietary behavior awareness campaigns [147].

CONCLUSIONS

The color of our skin is one of the most perceptible elements that characterize us the best: constitutive color gives information about our ethnicity, or rather, the place where our ancestors lived. The facultative color reveals something about our lifestyles, our sun exposure habits, and even the type of profession we have. Color irregularities due to wrinkles or spots may give indications about our age. Some color shades reflect our current level of stress, our state of health. All these variations in skin color also show how the skin has the amazing capacity to adapt permanently to its environment, due to its particular role: to separate our organism from the external environment and protect us against external aggressions.

Whether it comes from the inside or the outside, the color of our skin is a reflection of what we are, holding onto traces of the past and showing how we are now living. This is somehow a little summary of each one of us.

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39 Skin Care Products
Artificial Tanning

Stanley B. Levy

INTRODUCTION

The desire for a tanned appearance along with increasing awareness of the hazards of ultraviolet (UV) light exposure has generated a renewed interest in artificial tanning products. Better formulations of sunless tanners or self-tanners with improved aesthetics are more widely available. As consumer experience with the newer products has grown, this category has become more popular, and there has been an increasing proportion of overall sun care sales.

Over 20% of young adults in both the United States and Australia reported using these products in the preceding year [1,2]. Individual users were also more likely to have sunburned, consistent with higher use of these products in fairer Caucasians. In other studies, exclusive users of sunless tanners were more likely to practice overall sun protection [3,4] and decrease their use of tanning beds [5].

Dihydroxyacetone (DHA) is the active ingredient in sunless or self-tanners and is responsible for darkening the skin by staining. DHA is classified in the *International Cosmetic Ingredient Dictionary and Handbook* [6] as a colorant or a colorless dye. Other agents with the potential to enhance skin pigmentation, include tan accelerators containing tyrosine and other ingredients. Tanning promoters containing psoralens, which require UV exposure, will not be discussed here [7].

HISTORY

The first mention of DHA as an active ingredient in medicine appeared in the 1920s, when it was proposed as a substitute for glucose in diabetics. In the 1950s, the oral administration of DHA was restudied as a diagnostic procedure for glycogen storage disease when it was given in large doses orally [8]. When children spit up this sweet concentrated material, the skin became pigmented in areas splattered on the skin, without staining clothing. Aqueous solutions were then applied to the skin directly, and the pigmentation was reproduced [9]. In the late 1950s, cosmetic tanning preparations first appeared in the marketplace. Cosmetic acceptance of these initial products was limited because of the uneven orange–brown color they imparted to the skin. With the availability of improved formulations in the 1990s, sales of sunless tanners grew exponentially as a total proportion of sun care product sales. In the last several years, operator-assisted spray tans using DHA have become popular in spas and salons.

CHEMISTRY

DHA (C3H6O3) is a white, crystalline hygroscopic powder. This three-carbon sugar forms a dimer in freshly prepared aqueous solution (Figure 39.1). With heating to effect a solution in alcohol, ether, or acetone, it reverts to the monomer. The monomeric form is less stable but more important in the browning reaction, which leads to the skin color change [10]. DHA is stable between a pH of 4 and 6, but above a pH of 7, efficacy is lost with the formation of brown-colored compounds. A buffered mixture at a pH of 5 is the most stable. Heating above 388°C for long periods of time will also affect stability. DHA needs to be stored in a cool, dry place, ideally 48°C, and at low atmospheric humidity [11]. Glyceraldehyde, the isomer of DHA, is also present in the solution. Glyceraldehyde may degrade into formaldehyde and formic acid. In acidic solution (pH 4), this isomerization and therefore these latter undesirable ingredients are minimized.

The Maillard or browning reaction has been defined as the reaction of an amino group of amino acids, peptides, or proteins with the glycosidic hydroxyl group of sugars. DHA in the context of this reaction may be considered a three-carbon sugar, reacting with free amino groups available as amino acids, peptides, and proteins supplied by the keratin to form products or chromophores referred to as melanoidins [12]. Melanoidins have some physicochemical properties similar to naturally occurring melanin [13]. Electron spin resonance has recently shown that free radicals are produced in vivo by the Maillard reaction [14].

FORMULATION

The concentration of DHA in self-tanning products can range from 2.5% to 10%, with the usual concentration being 5% [10]. Lower-concentration products allow the consumer greater latitude with application since they tend to be more "forgiving" of uneven application or rough surfaces. Labeling products as light, medium, or dark can be particularly helpful with the depth of shade, a function of the DHA concentration.

DHA is predominantly formulated in oil-in-water emulsions. Formulating with silicones allows the formulator to obtain the spreadability of oils, which potentially reduces streakiness with application to the skin. Minimizing particle size of the micelles in the emulsion chosen also improves uniformity of spreading the formulation on the skin surface. On the basis of the chemistry of DHA, formulations should

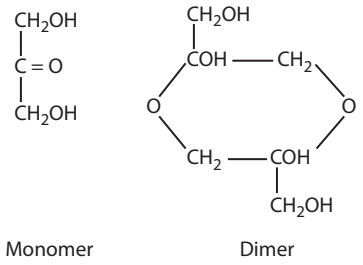

FIGURE 39.1 Chemical structure of dihydroxyacetone.

be buffered to an acidic pH (4 to 5) and not heated in manufacturing to temperatures higher than 408°C.

After incorporation of DHA into a formulation, the pH may drop during storage, suggesting that stability may actually be increased when the pH is kept between 3 and 4 [15]. The use of nonionic emulsifiers as opposed to ionic emulsifiers may also improve stability. Some thickeners such as carbomers, sodium carboxymethylcellulose, and magnesium aluminum silicate can cause rapid degradation of DHA. Hydroxyethylcellulose, methylcellulose, and silica as well as xanthan gum and polyquaternium-10 for thickening DHA-containing emulsions are better choices.

DHA can react with oxygen and nitrogen-containing compounds, collagen, urea derivatives, amino acids, and proteins. They should be avoided in the formulation of the DHA-containing vehicle. Nonnitrogen-containing sunscreen should be used if sun protection is desired. Attempts have been made to take advantage of this effect by adding amino acids to speed up the skin darkening process but with less substantive color results. Methionine sulfoxide, a sulfur-containing amino acid, has been used as an excipient applied before the application of the DHA-containing cream [16]. Two compartment systems have been patented on the basis of this reaction.

As with moisturizing products in general, lotions are more readily accepted by consumers than are creams with ease of spreadability and aesthetics. Creams can produce a more intense tan owing to greater applied film thickness. Products may be formulated for dry-skin types by the addition of emollients and humectants. Products formulated in gel or alcoholic vehicles may be more suitable for oily skin. Newer vehicles include sprays, foams, mousses, and wipes.

MECHANISM OF ACTION

The site of action of DHA is the stratum corneum [17]. Tape stripping of the skin quickly removes the color [18], as does mechanical rubbing. Deeper staining in areas with thicker stratum corneum and no staining of mucous membranes without a stratum corneum are also consistent with this being the site of action. DHA may be used as a substitute for dansyl chloride as a measure of stratum corneum turnover time [19,20]. Microscopic studies of stripped stratum corneum and hair reveal irregular pigment masses in the keratin layers [21] consistent with melanoidins. These melanoidins are formed via the Maillard reaction with DHA as a sugar reacting with the amino groups supplied by the keratin.

APPLICATION

Following application of a typical DHA-containing self-tanning lotion, color change may be observed within an hour [22]. This color change may be seen under Wood's light (black light) within 20 min. Maximal darkening may take 8 to 24 h to develop. Individuals can do several successive applications every few hours to achieve their desired color. Color may last as long as 5 to 7 days with a single application. Depending on anatomical application, the same color can be maintained with repeat applications every 1 to 4 days.

The face requires fewer applications but more frequent reapplication to maintain the color than the extremities. Depth of color varies with the thickness and compactness of the stratum corneum. Palms and soles stain deepest, necessitating washing of hands after application to avoid staining. Hair and nails will color but not mucous membranes lacking a stratum corneum or keratin layer. Rougher hyperkeratotic skin over the knees, elbows, and ankles will color more unevenly as will older skin with keratoses and mottled pigmentation. Color will also be maintained longer in these areas.

As in the formulation, the pH of the skin before application may have an effect on the tonality of the skin color [10]. Alkaline residues from soaps or detergents may interfere with the reaction between DHA and the amino acids on the skin surface, resulting in a less natural-appearing (more yellow) color. Wiping the skin surface with a hydroalcoholic, acidic toner just prior to DHA application may improve results. Ex vitro epidermal studies suggest that skin hydration [23] and relative humidity [24] influence the development of coloration. Careful directions provided with these products are therefore quite important in determining consumer satisfaction. The skin may be prepared with a mild form of exfoliation.

Even application is required, with lighter application around elbows, knees, and ankles to avoid excessive darkening in these areas. Care also needs to be taken around the hairline, where lighter hair may darken. Hands need to be washed immediately after use to avoid darkening of the palms, fingers, and nails. Skill and experience are necessary with using these products, resulting in greater user satisfaction.

ADDITIVES

As commonly occurs, growth in this category has compelled both formulators and marketers to seek points of differentiation between their product and that of their competitors. Besides formulating for specific skin types, active treatment ingredients may be incorporated into DHA-containing formulations. Vitamins, botanical extracts, antioxidants, anti-irritants, and even α-hydroxy acids may be added to broaden the claims made for a given product. Addition of antioxidants can shift tonality to a more natural coloration [25]. The addition of sunscreen ingredients to self-tanners warrants a more detailed discussion in the section that follows.

Some newer formulations have included colorants as used in bronzers, including dyes and caramel, to achieve an

immediate makeup effect. Similarly, tinting with titanium oxide or iron oxides can provide immediate color and allow the user to more easily visualize the evenness of application. Metal oxides may, however, induce degradation of DHA [15]. To compensate for less red absorption by the products of the Maillard reaction, erythrulose may be added as a colorant to add red to the tone.

SUNSCREEN ACTIVITY

In the United States, the Food and Drug Administration (FDA) Tentative Final Over-the-Counter Monograph on Sunscreens (Fed. Reg. 1993) listed DHA as an approved sunscreen ingredient when used sequentially with lawsone (2-hydroxy-1,4-napthoquinone). The final monograph (Fed. Reg. 1999) removed this combination from the approved list. The European Economic Community Directive does not list DHA as a permitted UV filter.

DHA itself has, at most, a modest effect on sun protection factor (SPF) [26], providing perhaps SPF 3 or 4. SPF increases with DHA concentration and number of applications [27]. Low-level SPF persists for several days, decreasing with loss of color [28]. The brown color obtained on the skin does absorb in the low end of the visible spectrum with overlap into long UVA and may provide some UVA I protection [29]. Melanoidins can act as free-radical scavengers as they demonstrate an electron spin resonance signal [14]. Superficial skin coloration induced by frequent topical application of DHA in high concentrations may delay skin cancer development in hairless mice irradiated with moderate UV doses [30].

Individuals using DHA-containing tanning products need to be cautioned that despite visible darkening of their skin, these products provide minimal sun protection. Confusion may be compounded by the addition of UV filters to the formulation providing significant sun protection. The stated SPF for the product is applicable for a few hours after application but not for the days during which the skin color change may remain perceptible.

INDICATIONS

Even with recent improvement in DHA formulations, the color achieved remains dependent on skin type. Individuals of medium complexion with skin phototypes II or III [31], as opposed to those who are lighter or darker, will obtain a more pleasing color. Individuals with underlying golden skin tones will achieve better results than individuals with rosy, sallow, or olive complexion. Older consumers with roughened, hyperkeratotic skin or mottled pigmentation with freckling may be less pleased with their use.

Dermatologists regularly recommend these products for tanning as a safe alternative to UV exposure. They may be used to camouflage some skin irregularities such as leg spider veins. Light to medium complected patients with vitiligo who show increased contrast in the vitiliginous areas with natural or unavoidable tanning in their normal skin may also benefit [32,33]. They may even provide some protection for individuals with certain photosensitivity disorders [34]. Protection of uninvolved skin by DHA during psoralen–UVA treatment (PUVA) allows higher UVA exposures to be tolerated, with fewer treatments resulting in faster clearing, known as turbo-PUVA [35].

SAFETY

The visible color change associated with the use of artificial tanning products might suggest to some users that these products are hazardous. On the basis of the chemistry of DHA and its toxicological profile, it can be considered nontoxic. It reacts quickly in the stratum corneum, minimizing systemic absorption. The acute toxicity of DHA was investigated for diabetics in the 1920s, with oral intake well tolerated [15]. The phosphate of DHA is found naturally as one of the intermediates in the Krebs cycle.

Contact dermatitis to DHA has only rarely been reported [36]. As with other topical products with active ingredients, such as sunscreens, much of the reported sensitivity is secondary to other ingredients in the vehicle [37]. Adverse reactions are more likely to occur on the basis of irritation and not true allergy. Ultimately, all claims related to product safety are based on testing the final formulation.

ALTERNATIVE TANNING AGENTS

Lawsone found that henna plant and juglone (5-hydroxy-1,4,-napthoquinone) derived from walnuts also stain hair, skin, and nails. They have been used for centuries for hair coloring. Both substances lack skin substantivity and readily discolor clothing [38]. The skin color they produce does not resemble a natural tan.

On the basis of the underlying principle of the Maillard reaction, other molecules with a ketone function have been investigated [39]. An α-hydroxy group attached to electron withdrawing groups can also increase reactivity. Substances such as glyceraldehyde and glyoxal [40] have been described but found ineffective. Mucondialdehyde, as described by Eichler [41], is an effective agent, but associated toxicity mitigated against its use [39]. Although several other aldehydes have been shown to have better color properties, stability issues limit their use [39].

CONCLUSION

Increasing consumer awareness to the hazards of UV light should fuel ongoing interest in self-tanning products. The benign toxicologic profile of DHA reinforces the notion that these products represent a safe alternative to a UV-induced tan. The results obtained with these products are dependent on the final formulation, individual application technique, and consumers' complexion type. Greater experience in formulation combined with increasing sophistication on the part of the consumer should lead to continuing growth and satisfaction with the use of these products.

Consumers need to be clearly informed that these products do not offer significant protection against UVB. If formulated with standard sunscreens, consumers should be cautioned that the duration of UV protection is more short-lived than the color change.

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40 Skin Whitening Agents

Michal W.S. Ong and Howard I. Maibach

INTRODUCTION

Pigmentation disorders of the skin are common, and although this condition often does not affect patients' physical health, many patients are greatly affected psychologically [1].

There are different modalities for skin hyperpigmentation. This can be categorized into two major groups: (1) chemical agents and (2) physical therapies [2–4]. Examples of the most commonly used chemical agents are hydroquinone (HQ), arbutin, kojic acid, ascorbic acid, and its derivatives, and examples of physical therapies are cryotherapy with liquid nitrogen, laser surgery, chemical peeling, and superficial dermabrasion [5,6]. Though multiple interventions are available, chemical agents remain as a mainstay of the approach to treat abnormal hyperpigmentation of the skin, such as melasma, freckles, and actinic lentigines in a clinical setting, as well as in the cosmetic field for individuals who wish to lighten their skin color. The efficacy, mechanism, and safety of these modalities have been extensively reviewed [7]. This chapter aims to discuss various types of skin whitening agents that are commonly used and report on current findings of potential skin whitening agents.

SKIN WHITENING AGENTS

Hydroquinone

HQ (1,4-dihydroxybenzene) is the most popular depigmenting agent. It is used in various industries including photographic, rubber, and chemical industries. During the late 1930s, some workers in a rubber factory who came into contact with a monobenzyl ether of HQ (a chemical used in the manufacture of rubber) had depigmented skin [8]. HQ was introduced for clinical use for skin hyperpigmentation. Since then, the efficacy, mechanism, and safety of this chemical have been established in in vitro, animal, and human studies. Due to the hazard of long-term treatment with HQ [9], the use of HQ in cosmetics has been banned by the European Committee (24th Dir. 2000/6/EC), and formulations are available only by prescription. In the United States, concentrations of up to 2% HQ are available over-the-counter (OTC), and higher concentrations are only available by prescription [2,8]. Combination therapy of fluocinolone acetonide 0.01%, HQ 4%, and tretinoin 0.05% is available by prescription (Tri-Luma Cream).

Mechanism of Action

HQ acts as a depigment agent through inhibiting tyrosinase enzyme, which reduces the conversion of DOPA to melanin [2,8,10]. The oxidation products are quinones and reactive oxygen species (ROS), and these products lead to an oxidative damage of membrane lipids and proteins, including tyrosinase, and depletion of glutathione, which contributes to the lightening action [11]. Other proposed mechanisms of action of HQ are

1. Covalent binding to histidine or interaction with coppers at the site of tyrosinase [12]
2. Inhibition of DNA and RNA synthesis
3. Alteration of melanosome formation and melanization extent [13]

In an electron microscopic study, black guinea pig skin treated with HQ showed anatomic consequences of this action [8]:

1. The melanosome structure is disturbed, resulting in decreased production or increased melanocyte degradation of these organelles, or both.
2. Melanocyte degradation.
3. Keratinocytes are spared, showing no apparent injury.

In an animal model, black guinea pigs and black mice were used to predict the depigmenting action of chemicals such as phenols, catechols, and organic antioxidants. There was complete depigmentation on all test sites with monomethyl ether of HQ and *p*-tertiary butyl catechol in black guinea pigs, but there was less pigment loss with these chemicals in black mice.

Skin of hairless dogs has also been used to study HQ. In this study [14], the skin of hairless dogs began to become depigmented after application of 3% HQ for 1 week; a quarter of the body became depigmented after 1 month. The number of DOPA-positive melanocytes in the HQ-treated sites decreased to less than about a fifth of that before treatment; histologically, there was complete absence of melanin pigment.

Efficacy of HQ

Concentration

The ideal depigmenting agent will be one that is effective and does not cause unnecessary side effects. In a nonplacebo-controlled study that tested 2% HQ, 64% of patients showed an overall improvement of hyperpigmentation [15]. In another nonplacebo-controlled study, which tested a higher

concentration of HQ (3%) on patients with melasma, a higher proportion (88%) of patients showed an overall improvement [16]. It is possible that higher concentration has better efficacy; however, patients will be more at risk of side effects. To establish this, a study compared two concentrations of HQ (2% versus 5% HQ). This study showed that HQ was moderately efficacious in 80% of melasma cases and there was no efficacy difference between the two concentrations; however, 2% HQ was less irritating than 5% [17].

Combination Formula

There have been attempts to increase the efficacy of HQ through combining with other chemicals that may enhance the effects of HQ. Chemicals such as tretinoin [18], fluocinolone acetonide [18], retinol [19], retinoic acid [20], ascorbic acid [21,22], mequinol [23], glycolic acid [24], dexamethasone [25], betamethasone [26], and broad-spectrum sunscreen agents [27] have been added to HQ to help with this.

Broad-Spectrum Sunscreen with HQ Furthermore, HQ in combination with a broad-spectrum sunscreen has been shown to be effective in a double-blind and vehicle-controlled study. Of patients who had combination treatment of HQ and sunscreen agent, 96.2% showed improvement, as compared with 80.7% of patients given placebo, who only had HQ [27].

Retinol/Retinoic Acid with HQ 0.15% retinol in combination with 4% HQ proves to reduce pigmentation at weeks 4, 8, and 12 compared with baseline. Patients applied the formulation on the full face twice daily (morning and evening). A broad-spectrum sunscreen was applied once in the morning, 15 min after application of the test product. The lesion area and colorimetry measurements were statistically significant at each visit [19].

Pathak et al. [20] suggested that formulations of 2% HQ and 0.05% to 0.1% retinoic acid provided the most favorable result for treating melasma. Similarly, they suggested that the treatment for melasma should also include avoidance of sun exposure and use of broad-spectrum sunscreens. They also suggested that patients should avoid other agents (such as oral contraceptive pills) that may cause hyperpigmentation.

Ascorbic Acid with HQ A randomized double-blind split-face study compared 5% ascorbic acid cream and a 4% HQ cream in 16 female patients with melasma over 16 weeks. Patients were directed to apply sunscreen daily throughout the observation period. The best subjective improvement was observed on the HQ side, with 93% good and excellent results, compared with 62.5% on the ascorbic acid side of the face. However, objective measures (using colorimetry) showed no statistical differences. Side effects were present in 68.7% (11 out of 16) of patients with HQ creams, and only 6.2% (1 out of 16) of patients experienced side effects with ascorbic acid. Therefore, HQ is a more effective treatment, but the use of ascorbic acid for hyperpigmentation disorders should be considered due to its low side effect profile compared to HQ [21].

A combination of ascorbic acid and HQ called ascorbate–phytohydroquinone complex has been tested in 14 patients with actinic lentigo in a nonplacebo-controlled study. The skin color changes were measured objectively using a chromameter. After 1 month of treatment, a clear depigmentation of those hyperpigmented macules was demonstrated [22].

Triple Combinations: Dexamethasone/Betamethasone/Fluocinolone acetonide, Tretinoin with HQ Topical corticoids have also been used to enhance the efficacy of HQ. Corticosteroids exert an antimetabolic effect, resulting in decreased epidermal turnover, and it was thought to produce a mild depigmenting effect. When it was tested in combination with tretinoin and HQ for melasma, flucinolone acetonide 0.01% was found to suppress biosynthetic and secretory functions of melanocytes and, therefore, reduce melanin production. Fluocinolone acetonide works synergistically with the other two agents (tretinoin and HQ), and there were no significant side effects reported over an 8-week period [28].

A triple combination (TC) regime of 5% HQ, 0.1% tretinoin, and 0.1% of dexamethasone [25] and a combination of 2% HQ, 0.05% tretinoin, and 0.1% betamethasone [26] have been tested in a nonplacebo-controlled study. In the former clinical trial (5% HQ, 0.1% tretinoin, 0.1% of dexamethasone), Kligman and Willis [25] demonstrated that in comparison to monotherapies (such as HQ, tretinoin, or dexamethasone), the combination regime is a more effective treatment for melasma, ephelides, and postinflammatory hyperpigmentationon (PIH) in adult male black subjects. In the latter clinical trial (2% HQ, 0.05% tretinoin, with 0.1% betamethasone), Gano and Garcia [26] reported that this combination of depigmenting agents showed a subjective improvement rate of 95% and objective improvement rate of 65%.

A TC formula (fluocinolone acetonide 0.01%, HQ 4%, tretinoin 0.05%) is more effective than 4% HQ alone. In a randomized, double-blind, placebo-controlled study, improvement of more than 75% was achieved by 73% of TC cream patients, compared to 5% of those who used HQ cream [29]. Patients are more satisfied with the use of TC cream than HQ. However, more patients had related adverse events on TC cream than on HQ cream [18,29]. The most frequently reported side effects were erythema, burning sensation, and desquamation. These side effects were mild and tolerable, and none of those patients discontinued treatment due to adverse effects of TC [18,29]; only one patient who was on HQ discontinued the study prematurely due skin irritation [18].

Though side effects were frequent, they were minimal. Beware of the potential side effects in prolonged use of fluorinated corticosteroids on the face. It may cause epidermal atrophy, telangiectasia, rosacea-like erythema, acne, and perioral dermatitis.

5% Skin Whitening Complex versus 4% HQ In a randomized double-blind split-face prospective study, a 5% skin whitening complex (SWC) was found to be nearly as effective

as 4% HQ, and there was no side effects reported with the use of SWC. Group 1 (half-face HQ and half-face placebo) presented with an improvement of 76.9%, with 25% of patients experiencing side effects (itchy eruptions). Group 2 (half-face SWC and half-face placebo) presented an improvement of 66.7% with 0% side effects [30].

HQ-Fructoside An HQ derivative, HQ-fructoside (HQ-Fru), was first synthesized via the acceptor reaction of levansucrase from L-mesenteroides with HQ and sucrase. HQ-Fru (Ki value, 1.53 mM) showed higher tyrosinase inhibitor activity than β-arbutin (Ki value, 2.8 mM). Besides that, HQ-Fru showed increased 1,1-diphenyl-2-picrylhydrazyl (DPPH) scavenging activity, inhibition of lipid peroxidation, and nitrite-scavenging activity as compared to β-arbutin [31]. HQ-Fru could be a more suitable HQ-derivative whitening agent; the inhibitory effects of HQ-Fru on melanin synthesis in human melanoma cells are currently being studied.

Safety and Side Effect Profiles of HQ

Most common acute reactions are irritant dermatitis, nail discoloration, and PIH [8]. Although generally, HQ is assumed as an allergen causing allergic contact dermatitis, the evidence of this is weak [8]. HQ-induced hypopigmentation of normal skin could occur; however, these changes are temporary and resolve on cessation of HQ treatment [32]. In contrast to monobenzone therapy, this will cause permanent depigmentation and should only be indicated for severe vitiligo [32]. There has been concern regarding HQ-induced ochronosis. In general, this phenomenon only occurs from prolonged used of high concentrations of HQ [32,33]. There has also been concern regarding teratogenic and carcinogenic potentials of HQ and benzoquinone on humans. This area is still inadequately studied, and its cytotoxic effects on human and mouse bone marrow cells are still unclear [34,35].

Topical HQ is considered a relatively safe agent. HQ readily penetrates human skin in an alcoholic vehicle following a single 24 h exposure; the majority of this agent is excreted in the first 24 h [36] and is completely excreted from the body within 5 days [37].

Arbutin and Its Derivatives

Arbutin (β-glucopyranoside conjugate) is a natural form HQ. It is extracted from a bearberry plant in the genus *Arctostaphylos* and contained in leaves of pear trees and certain herbs [38].

Mechanism of Action

In in vitro studies, arbutin inhibits melanin production in B16 cells and decreases melanosomal tyrosinase activity [38–40]. Furthermore, the hyperpigmentation effects of α-melanocyte-stimulating hormone (α-MSH) were found to be abrogated by the addition of arbutin to brownish guinea pig and human skin tissues [40].

To develop a new skin whitening agent, arbutin-b-glycosides were synthesized and evaluated for their melanogenesis-inhibitory activities. Out of the three arbutin derivatives [b-D-glucopyranosyl-(1→6)-arbutin, b-D-glucopyranosyl-(1→4)-arbutin, and b-D-glucopyranosyl-(1→3)-arbutin] investigated, b-D-glucopyranosyl-(1→3)-arbutin was found to have the most profound inhibitory effects on melanin synthesis in B16F10 melanoma cells. The melanin content was reduced to below 70% of that observed in the untreated cells [39].

Kojic Acid and Its Derivatives

Kojic acid is an antibiotic produced by many species of *Aspergillus* and *Penicillum* in an aerobic process from a wide range of carbon sources [41,42]. It was first isolated from *Aspergillus* in 1907 [43]. Skin care products containing kojic acid have been marketed in Japan since 1988. It is readily available OTC at up to 1% concentration. To increase its efficacy, it is usually used at the highest concentration allowed [43].

Mechanism of Action

Similar to other skin whitening agents such as HQ and arbutin, kojic acid inhibits tyrosinase enzyme, mainly attributable to chelation of its copper [43–45]. Kojic acid has been shown to have depigmentation properties, both in vitro [44–47] and in vivo [24,48,49].

Efficacy and Safety of Kojic Acid

Combination Cream with 2% Kojic Acid versus without Kojic Acid

Kojic acid could enhance the whitening effect of whitening combination cream (CC). A randomized double-blind split-face study tested two types of combinations creams (10% glycolic acid and 2% HQ), one with 2% kojic acid and the other without. More than half of the melasma cleared in 60% (n = 24/40) of patients receiving CC with kojic acid, compared to 47.5% (n = 19/40) of patients who had CC without kojic acid. Two patients had complete clearance of melasma, and this was on the side where kojic acid was used. Side effects including redness, stinging, and exfoliation were reported on both sides of the face. These side effects settled by the third week. One patient had an itch with the gel, but again, this settled by the fourth week of use. Three patients (7%) asked to be withdrawn from the study because of the side effects (redness and peeling) and were not keen to continue the gel beyond 2 weeks [24].

Comparing Two Formulas Containing Kojic Acid (Amelan M versus Mela D)

Two different types of formulas of kojic acid creams, Mela D (Mexoryl SX, kojic acid, lipohydroxy acid [LHA]) and Amelan M (kojic acid, phytic acid, butyl methoxydibenzoylmethane), were tested in a double-blind controlled split-face study. There was a decrease in pigmentation with both creams, and it was statistically significant on the Melasma Area and Severity Index (MASI). Only Amelan M showed a statistically significant result with the mexameter. Amelan

M appears to be more effective than Mela D, but a higher proportion of patients (18.2% with Amelan M and 4.5% with Mela D) experienced side effects such as irritation and dryness [48].

Kojic Acid as Chemical Peeling Agent

50% Glycolic Acid and 10% Kojic Acid versus 15%–25% Trichloroacetic Acid Kojic acid in combination with glycolic acid has been tested as chemical peeling agent. It was compared with another commonly used chemical peeling agent, trichloroacetic acid (TCA). Of patients treated with 50% glycolic acid and 10% acid, 30% (n = 6/20) had complete regression of diffuse melasma, 60% (n = 12/20) of patients had partial regression, and 10% (n = 2/20) had no improvement. They had a total of 6 to 12 treatments every 2 weeks. Patients experienced mild erythema immediately posttreatment and mild desquamation a few days after treatment [49].

For patients who were treated with 15% to 25% TCA, 40% (n = 8/20) of patients had complete regression of localized hyperpigmentation; 50% (n = 10/20) of patients had partial regression; and 10% (n = 2/10) had no regression. These patients all had a total of 3 to 8 treatments every 3 to 4 weeks. The immediate side effects of this treatment were white "frost" and evident exfoliation of treated areas for 1 to 2 weeks [49].

Combination of 50% glycolic acid and 10% kojic acid seems more suitable for centrofacial forms of melasma, and TCA is more suitable for patients with localized hyperpigmented lesions.

Ascorbic Acid and Its Derivatives

Mechanism of Action

L-ascorbic acid works by reducing *o*-quinones [50,51]. This prevents melanin production unless all the ascorbic acid is oxidized [50]. Ascorbic acid can reduce oxidized melanin from jet black to a light tan color [50]. However, the problem is that L-ascorbic acid is unstable; it oxidizes rapidly and decomposes in aqueous solution. To overcome this problem, magnesium-L-ascorbyl-2-phosphate (VC-PMG) was synthesized [52]. It was thought that the chemical structure of VC-PMG was magnesium-L-ascorbyl-3-phosphatel later, it was suggested that VC-PMG is magnesium-L-ascorbyl-2-phosphate [53]. VC-PMG is chemically more stable in water, especially in neutral or alkaline solution, which contains boric acid or its salt [54]. VC-PMG is hydrolyzed to ascorbic acid by phophatases (found in liver or skin) to exhibit vitamin C-reducing activity [54] and suppressed melanin formation by tyrosinase and melanoma cells both in vitro and in vivo [55].

Efficacy and Safety of Ascorbic Acid

In a nonplacebo-controlled study, ascorbic acid (VC-PMG) cream was found to have significant lightening effects on 56% (n = 19/34) of patients with chloasma or senile freckles, and only 12% (n = 3/25) of patients with normal skin had significant improvement [55].

Combination Formula

10% L-Ascorbic Acid and 2% Phytic Acid A combination of 10% L-ascorbic acid and 2% phytic acid was found to be effective in subjects with solar lentigines. Thirty healthy subjects were recruited in this randomized double-blind, vehicle-controlled trial, and they were randomly assigned to apply the product (10% L-ascorbic acid and 2% phytic acid) to one side of the body and vehicle to the other side, twice daily for 3 months, with 2 months' follow-up. The pigmentation index and colorimetry results showed statistically significant improvement with the product, and the vehicle-treated lesions remained stable. Fifteen subjects experienced adverse effects; six subjects had halo depigmentation possibly due to the related study drug. Interestingly, six subjects experienced mild to moderate intolerance in the study drug group, and five subjects had same level of intolerance in the vehicle-treated group [56]. Therefore, the reported intolerance may not be due to the active ingredients.

Tetrapeptide (Pro-Lys-Glu-Lys)

Pro-Lys-Glu-Lys (PKEK) has the capacity to reduce ultraviolet B (UVB)-induced skin pigmentation in in vitro and in vivo studies. This agent may serve as a skin whitening agent. In vitro, PKEK significantly reduced UVB-induced messenger RNA (mRNA) expression of interleukin (IL)-6, IL-8, tumor necrosis factors (TNF)-α, proopiomelanocorticotropin (POMC), and tyrosinase. This was also demonstrated in 10 healthy volunteers who were pretreated with PKEK for 4 weeks, once daily [57].

Efficacy of Tetrapeptide (Pro-Lys-Glu-Lys/PKEK)

Combination Formula

PKEK and Sodium Ascorbyl Phosphate Cream versus PKEK Cream or Sodium Ascorbyl Phosphate Cream In a double-blind vehicle-controlled study of 39 Caucasian women, facial pigment spots were significantly faded after 6 weeks' use of PKEK and sodium ascorbyl phosphate (SAP) combined cream compared with SAP or PKEK cream alone. Similarly, when treating hyperpigmented spots on the back of hands, adding PKEK to SAP-containing cream was found to give increased skin whitening potency. According to SCINEXA Validated score to simultaneously assess and differentiate between intrinsic and extrinsic skin aging. score, PKEK and SAP combined formulation reduces skin pigmentation by 26% compared to 18% with a SAP-only formula [57]. Therefore, combination of PKEK and SAP is more efficacious, and PKEK seems to enhance skin whitening effect.

These findings are also supported by another randomized, double-blind vehicle-controlled study that studied subjects with mild acne and melasma. These subjects had skin type V and VI. The PKEK-containing formulation was significantly superior to vehicle at 12 weeks on overall appearance and evenness of skin tone. The results were statistically significant.

N-Acetyl Glucosamine

N-acetyl glucosamine (NAG), an amino hexose, found throughout nature and in all human tissues, is known for its function as a precursor to hyaluronic acid. The role of this polymer is forming the structure of extracellular matrix in joints and skin (dermis and epidermis) and keeps them hydrated [58–60].

Mechanism of Action

Research has shown that NAG [61] and glucosamine [62–64] are able to reduce production of melanin in human cell culture systems. These agents inhibit the glycosylation of tyrosinase to prevent activation of this enzyme and reduce melanin formation.

Efficacy and Safety of NAG/NAG Combination Formula in Humans

Clinical tests demonstrate that NAG is able to reduce skin hyperpigmentation [65,66]. Combination treatment of NAG and niacinamide was found more effective than NAG alone in treating hyperpigmentation [67,68]. A randomized double-blind vehicle-controlled split-face study showed that combination treatment of 2% NAG with 4% niacinamide reduced hyperpigmentation more than NAG alone [67].

Similarly, a 10-week randomized, double-blind, vehicle-controlled, full-face, parallel-group study, comparing a regimen with 2% NAG and 4% niacinamide (test formulation, including SPF 15 sunscreen) with a regimen without NAG or niacinamide showed that the combined test formulation regime was significantly ($P < .05$) more effective than the vehicle control regime in reducing detectable area of facial spots and pigmentation. Overall, both regimens were well tolerated. Seven (3.5%) out of 202 subjects reported adverse effects. Two subjects from each group reported moderate skin irritation and withdrew from the study; further, two subjects from the test formulation regime group and one subject from the vehicle group experienced mild irritation but did not withdraw [68].

Artocarpus

Artocarpus is a genus of about 60 trees and shrubs, belonging to the mulberry family, Moraceae. It is commonly found in Southeast Asia and the Pacific. *Artocarpus incisus* [69] and *Artocarpus lakoocha* [70] were discovered as potential whitening agents, both in in vitro [69,70] and in vivo studies on animals [69] and humans [70].

Mechanism of Action

A. incisus was shown to inhibit tyrosinase activity at a rate equivalent to kojic acid. This extract found was to inhibit melanin biosynthesis of both cultured B16 melanoma cells without any cytotoxicity [69]. Depigmenting effect was also found in the back of brown guinea pigs, which had UVB-induced hyperpigmentation without skin irritation [69].

Through screening a large number of plants for their antityrosinase activity, Sritularak et al. [71] found that *A. lakoocha* exhibited the highest activity. Also, due to the polyphenolic nature of its active constituent, oxyresveratrol, found in this extract, it may also have antioxidative properties [72]. Batch reproducibility has not been reported.

Efficacy and Safety of *A. lakoocha* in Humans

In a parallel clinical trial, comparing *A. lakoocha* and a combined formulation of 3% kojic acid and 0.25% licorice extract, *A. lakoocha* extract was the most effective agent. It gave the shortest onset of significant whitening effect after 4 weeks of application, whereas the kojic acid and licorice extract needed 6 weeks before it showed a significant whitening effect. These results were statistically significant ($P < .05$). The rate of improvement was also dependent on the type of formulation and area of application. The oil-in-water emulsion seemed to give better lightening activity than the propylene glycol-based solution. Since only a low concentration of *A. lakoocha* is needed (0.10% to 0.25% concentration), this extract could have a very promising potential as a safe, effective, and economical skin whitening agent in the cosmetic industry [70].

Mulberry Extract (Morus Alba)

Mechanism of Action

Mulberry extract has been found to have flavonoids and antioxidant properties [73]. Similar to other whitening agents mentioned above, mulberry extract inhibits tyrosinase activity competitively, and there was no suppression of tyrosinase synthesis and gene expression [74–79]. The tyrosinase-inhibiting activity of mulberry extract is comparable to HQ and kojic acid [80].

In an animal study, there was a decrease in melanin production in the brown guinea pig model. Those guinea pigs had UV-induced hyperpigmentation. There was no toxicity observed in animal tests, such as the acute toxicity test, skin irritation test, eye irritation test, skin sensitization test, and acute oral toxicity test [77].

Efficacy and Safety of Mulberry Extract in Humans

Fifty patients with melasma were recruited in a randomized, single-blind, placebo-controlled trial. Twenty-five patients were treated with 75% mulberry extract oil, and the other 25 patients were treated with a placebo. They were followed up on the fourth and eighth week of treatment. The efficacy of mulberry extract was assessed based on MASI score, mexameter reading, and the melasma quality of life score (MelasQOL). The mulberry extract oil gave a significant improvement compared to the placebo. The mean MASI score improved from 4.076 (baseline) to the 2.884 (week 8, posttreatment), whereas the placebo group showed almost no improvement (baseline, 3.383; week 8, 3.392). Mexameter readings also demonstrated a reduction in hyperpigmentation; readings dropped from 355.56 at baseline to 312.52 at

TABLE 40.1
Skin Lightening Agents: In Vitro and Animal Studies

Identification	Enzyme Assay	Animal Assay	Ref.
Bifidobacterium adolescentis	Tyrosinase, B16	–	[83]
Ortho-dihydroxyisoflavone derivatives	Tyrosinase, B16, MelanoDerm	–	[84]
(E)-3-(2,4-dihydroxybenzylidene)pyrrolidine-2,5-dione (3-DBP)	Tyrosinase, B16	HRM-2 melanin-possessing hairless mice	[85]
Flavonoid–polysaccharide conjugate	Tyrosinase	–	[86]
Chinus terebinthifolius Raddi extract and linoleic acid from *Passiflora edulis*	Tyrosinase, B16,	–	[87]
(E)-2-(4-tert-butylbenzylidene) hydrazinecarbothioamide (QNT3-18) or 4-tert- butylphenylthiourea (QNT3-20)	–	Hairless rat UV-induced pigmentation	[88]
Qian-wang-hong-bai-san	Tyrosinase, B16	–	[89]
(2RS,4R)-2-(2,4-dihydroxyphenyl)thiazolidine-4-carboxylic acid (MHY384)	Tyrosinase	–	[90]
Vaccinium bracteatum	Tyrosinase, MelanoDerm	–	[91]
Eupatorium triplinerve Vahl leaves	Tyrosinase, B16	–	[92]
Tetrapeptide PKEK (Pro-Lys-Glu-Lys)	Primary human epidermal keratinocytes (NHEK)	–	[57]
Homochlorcyclizine (HC)	Tyrosinase, B16	–	[93]
Anisomeles indica methanol extract (ovatodiolide)	Tyrosinase, B16	–	[94]
Magnolia grandiflora L. flower (Magnoliaceae)	Tyrosinase, B16	–	[95]
Gnetin C, a resveratrol dimer (found in melinjo (*Gnetum gnemon*) seeds	Tyrosinase, B16	–	[96]
Sargassum polycystum ethanolic extract	Tyrosinase, B16	–	[97]
Paeonia suffruticosa (Ps) extracts (Ps-1 to Ps-8)-phenolic and flavonoid	Tyrosinase, B16	–	[98]
Allium cepa (red onion)—quercetin	Tyrosinase	–	[99]
Pemphis acidula	Tyrosinase, B16	–	[100]
Allium cepa (red onion)—quercetin	B16	–	[101]
Biochain A from *Trifolium pratense* extract	Tyrosinase, B16	–	[102]
[6]-Gingerol	Tyrosinase, B16, reactive oxygen species (ROS)	–	[103]
Rosa canina L. (rose hip)	Tyrosinase, B16	Brownish guinea pig with UVB-induced pigmentation	[104]
Astragalus membranaceus (Astragalus extract)	Tyrosinase, B16	–	[105]
Magnolia officinalis (MOE)	Tyrosinase, B16	Zebrafish	[106]
Nelumbo nucifera	Tyrosinase	–	[107]
Lonicera japonica Thumb	Tyrosinase	–	[108]
Panduratin A from *Kaempferia pandurata* Roxb.	Tyrosinase, B16	–	[109]
HQ galactoside 4-hydroxyphenyl-β-D-galactopyranoside	Tyrosinase, B16	–	[110]
Polygonum cuspidatum, 5,4′-dihydroxystilbene-3-O-β-D-glucopyranoside (piceid)	Tyrosinase	–	[111]
Saponified evening primrose oil (sap-EPO)	Tyrosinase, B16	–	[112]
Bawang tiwai (*Eleutherine americana* L. Merr.) naphthoquinone compound	B16	–	[113]
Hesperetin	–	Guinea pigs with UVB-induced pigmentation	[114]
Zeolite 4A, synthetic salicate	Tyrosinase, B16	–	[115]
Oxalis triangularis (purple shamrock or purple clover)	Tyrosinase, B16	–	[116]
Sulforaphane (found in broccoli)	Tyrosinase, B16	–	[117]
Cardamonin, a calchone from *Aplinia katsumadai* Hayata	Tyrosinase, B16	–	[118]
1,3-thiazine derivative [4-hydroxy-2,6-dimethyl-5,6-dihydro-4H-1,3- thiazine (TZ-6)]	Tyrosinase, B16	Zebrafish	[119]
Tiliroside (from raspberry)	Tyrosinase, B16	–	[120]
Imidazole, AVS-1357	Tyrosinase, B16	–	[121]
Sophora japonica L. (Fabaceae)	Tyrosinase	–	[122]
Hydroquinone fructoside (HQ-fru)	Tyrosinase	–	[31]
Erigeron breviscapus extract, (2Z,8Z)—matricaria acid methyl ester	Tyrosinase, B16	–	[123]
Salicornia herbacea extract	Tyrosinase, B16	–	[124]
Cimicifuga heracleifolia extract	Tyrosinase, B16	–	[125]
Arbutin	Tyrosinase, B16	Brownish guinea pig	[40]

(*continued*)

TABLE 40.1 (Continued)
Skin Lightening Agents: In Vitro and Animal Studies

Identification	Enzyme Assay	Animal Assay	Ref.
Synthetic hexapeptide, Angio-S (SFKLRY-NH2)	Tyrosinase, B16	–	[126]
Cucumis sativus	Tyrosinase, B16	–	[127]
Mulberry plant obtained from *Morus alba* L. and *Morus rotundiloba*	Tyrosinase	–	[74]
Cinnamic acid (phenylpropanoid component found in *Cinnamomum cassia* BLUME and Panax ginseng)	Tyrosinase, B16	Brownish guinea pig with UVB-induced pigmentation	[128]
Myristica fragrans HOUTT (dried rhizomes)	Tyrosinase, B16	–	[129]
Arbutin-b-glycosides	Tyrosinase, B16	–	[39]
Disodium isostearyl 2-O-L-ascorbyl phosphate (VCP-IS-2Na) (synthesized from a hydrophilic ascorbic derivative)	Tyrosinase, B16	–	[130]
Resorcinol derivatives were synthesized and screened for their activity on melanogenesis. KI-063	Tyrosinase, B16	–	[131]
Phenyl-imidazole sulfonamide derivatives, [4-t-butylphenyl]-*N*-(4-imidazol-1-yl phenyl)sulfonamide (ISCK03)	–	Brownish guinea pig with UV-induced pigmentation	[132]
Alpinia officinarum Hance (galangin and flavonoid)	Tyrosinase, B16	–	[133]
Extract of *Gastrodia elata* Blume (Orchidaceae)-*p*-hydroxybenzyl alcohol (4HBA)	Tyrosinase, B16	–	[134]
Longan seed (*Euphoria longana* Lam.), (corilagin, gallic acid, and ellagic acid)	Tyrosinase	–	[135]
KHG22394, a 2-imino-1,3-thiazoline derivative	Tyrosinase, B16	–	[136]
Gallic acid	Tyrosinase, B16	–	[137]
Kojic acid–tripeptide amide conjugates	Tyrosinase	–	[46]
M. alba L. (mulberry plant)	Tyrosinase	–	[75]
Powdered barley shochu	Tyrosinase, B16	–	[138]
Cycloalpioside D (3-O-b-D-xylopyranoside of cycloalpigenin D)-*Astragalus alopecurus* Pall extract.	Tyrosinase	–	[139]
Artocarpus lakoocha Roxb.	Tyrosinase	–	[70]
Pharbitis nil, Sophora japonica, Spatholobus suberectus, and *M. alba*	Tyrosinase, B16	–	[76]
Deoxyarbutin(dA), 4-([tetrahydro-2H-pyran-2-yl]oxy)phenol	Tyrosinase, B16	–	[140]
Angelica dahurica	Tyrosinase, B16	–	[141]
Idesia polycarpa fruits	Tyrosinase, B16	–	[142]
2,6-Dimethoxy-*N*-(4-methoxyphenyl)benzamide (DMPB), synthesized using a combination of benzoic acid and aniline	Tyrosinase	Brownish guinea pig with UVB-induced pigmentation	[143]
Punica granatum L. pomegranate extract (contain 90% ellagic acid)	Tyrosinase	Brownish guinea pig with UV-induced pigmentation	[144]
Octadecenedioic acid	Tyrosinase, B16	–	[145]
Safflower (*Carthamus tinctorius* L.) seeds (*N*-feruloylserotonin, *N*-(*p*-coumaroyl)serotonin, and acacetin)	Tyrosinase, B16	–	[146]
Linoleic acid	–	Brownish guinea pig with UVB-induced pigmentation	[147]
Kinetin	–	Hairless hybrids	[148]
Radix Ginseng (RG) and Radix Trichosanthis (RT)	Tyrosinase, B16	–	[149]
Ramulus mori (young twigs of *M. alba* L.)	Tyrosinase, B16	Brownish guinea pig with UVB-induced pigmentation	[77]
Derivative of kojic acid, 5-([3-aminopropyl]phosphinooxy)-2-(hydroxymethyl)-4H-pyran-4-one (Kojyl-APPA)	Tyrosinase, B16	–	[47]
Mulberroside F (leaves of *M. alba*)	Tyrosinase, B16	–	[78]
M. alba Linne, oxyresveratrol (2′,3,4′,5-tetrahydroxystilbene)	Tyrosinase, B16	–	[79]
Oxyresveratrol (from Mori Cortex)	Tyrosinase, B16	–	[150]
Vitamin E derivative, (6″-hydroxy-2″,5″,7″,8″-tetramethylchroman-2″-yl) methyl 3-(2′,4′-dihydroxyphenyl)propionate (TM4R)	Tyrosinase	Brownish guinea pig with UVB-induced pigmentation	[151]
Sanguisorba officinalis L.	Endothelin-converting enzyme	Brownish guinea pig with UVB-induced pigmentation	[152]
a-Tocopheryl ferulate (a-TF), vitamin E	Tyrosinase, B16	–	[153]
Areca catechu L.	Tyrosinase, B16	–	[154]

(*continued*)

TABLE 40.1 (Continued)
Skin Lightening Agents: In Vitro and Animal Studies

Identification	Enzyme Assay	Animal Assay	Ref.
Artocarpus incisus	Tyrosinase, B16	–	[69]
Linoleic acid	Tyrosinase, B16	Brownish guinea pig with UVB-induced pigmentation	[155]
A. incisus	Tyrosinase, B16	Brownish guinea pig with UVB-induced pigmentation	[69]
Areca catechu L.	Tyrosinase, B16	–	[156]
3% hydroquinone	–	Hairless dogs	[14]
Neoagarobiose	B16	–	[157]
Arbutin	Tyrosinase, B16	–	[38]
Magnesium L-ascorbyl-2-phosphate (VC-PMG)	Tyrosinase, B16	–	[55]
Dried leaves and the bark of *Myrica rubra*	Tyrosinase	–	[158]
Kojic acid	Tyrosinase	–	[45]
Kojic acid	Tyrosinase	–	[44]

TABLE 40.2
Skin Whitening Agents: Human Studies

Identification	Design	Results	Ref.
Glutathione	Randomized, double-blind, placebo-controlled study	60 participants enrolled and completed the study. At 4 weeks, the melanin indices decreased consistently at all six sites in subjects who received glutathione. The reductions were statistically significantly greater than those receiving placebo at two sites, namely, the right side of the face and the sun-exposed left forearm (P values = .021 and .036, respectively).	[159]
Tetrapeptide, Pro-Lys-Glu-Lys (PKEK)	Four double-blind vehicle-controlled studies	In a study enrolling 39 Caucasian women, facial pigment spots significantly faded after 6 weeks when PKEK was combined with the skin whitener sodium ascorbyl phosphate (SAP), whereas PKEK or SAP alone led to less pronounced fading of the pigment spots. Addition of PKEK enhanced the skin whitening potency of a SAP-containing preparation if applied for 8 weeks to the back of hands of 19 Caucasians. 27 Japanese women were treated on their faces twice daily with a SAP-only or a PKEK+SAP-containing formulation for 8 weeks. Application of PKEK+SAP significantly reduced skin pigmentation by 26% and by 18% according to SCINEXA score.	[57]
Lignin peroxidase (LIP) cream vs. 2% hydroquinone (HQ) cream	Randomized, double-blind, placebo-controlled, split-face study	A statistically significant change from baseline in the melanin index was observed in LIP-treated skin, with a mean reduction of 7.6% ($P < .001$) on day 31. Conversely, HQ and placebo did not provide a statistically significant lightening effect when instrumentally measured.	[160]
L-ascorbic acid 10% + phytic acid 2%	Randomized, double-blind, vehicle-controlled study	The pigmentation index for product-treated solar lentigines was reduced [maximum reduction 1.3 at 3 months (M3)] while that for vehicle-treated lesions remained stable. Statistically significant. Colorimetry results indicated a statistically significant improvement in brightness (L*) between study drug and vehicle at M5.	[56]

(*continued*)

TABLE 40.2 (Continued)
Skin Whitening Agents: Human Studies

Identification	Design	Results	Ref.
75% mulberry (*Morus alba*) extract oil vs. placebo	Randomized, single-blind, placebo-controlled, full-face, open two-arm study	The mean melasma area and severity index (MASI) score significantly improved from 4.076 (±0.24) at baseline to 2.884 (±0.25) at week 8 for the 75% mulberry extract oil group, while the placebo group showed an improvement of a lesser magnitude. Mexameter readings for the mulberry group showed a significant drop from 355.56 (±59.51) at baseline to 312.52 (±57.03) at week 8 compared to the placebo group, whose Mexameter readings deteriorated from 368.24 (±46.62) at baseline to 372.12 (±44.47) at week 8. The MelasQOL score also improved tremendously for the 75% mulberry extract oil group, falling from 58.84 (standard deviation [SD]: ±3.18) at baseline to 44.16 (SD: ±4.29) at week 8, unlike the placebo group, which showed a less dramatic improvement from 57.44 (SD: ±4.66) at baseline to 54.28 (SD: ±4.79) at week 8.	[81]
Tetrapeptide, Pro-Lys-Glu-Lys (PKEK)	Randomized, double-blind full-face, placebo-controlled study; skin type V and VI	This study demonstrated the efficacy of PKEK on subjects with skin types V–VI. On comparing the two treatments, the skin-lightening peptide-containing formulation was significantly superior to the vehicle at 12 weeks on overall appearance ($P < .05$) and evenness of skin tone ($P < .01$).	[161]
10% GA, 12% sunscreen filters, 3% milk proteins, 1% *Rumex occidentalis*, 1% squalene, 0.30% salicylic acid, and 0.25% vitamin E (DP cream)	Clinical trial	There was a significant difference between 0–12, 0–24 (L*, C* and h* values), and 12–24 weeks (L* value) ($P < .001$). For C* and h* values, the difference was not significant between 12 and 24 weeks ($P < .464$ and .151, respectively). Statistical significance was detected only between 3 and 6 months for C* value ($P < .05$) for the lesional and the perilesional areas. Clinical response rate was significant ($P < .05$).	[162]
Saponified evening primrose oil (sap-EPO)	3 healthy men; controlled clinical trials; UVB-induced hyperpigmentation on both arms	Significant differences in the melanogenic index were observed from 4 weeks after the initial UVB exposure through the end of the experiment. No erythema was observed during the sap-EPO treatment.	[112]
Morning sun protection factor (SPF) 15 sunscreen moisturizing lotion and evening moisturizing cream each containing 4% niacinamide + 2% NAG (test formulation, $n = 101$) vs. SPF 15 lotion and cream vehicles (vehicle control, $n = 101$)	Double-blind, vehicle-controlled, full-face, parallel-group clinical study conducted in women aged 40–60 years	Niacinamide + NAG formulation regimen was significantly ($P < .05$) more effective than the vehicle control formulation regimen in reducing the detectable area of facial spots and the appearance of pigmentation.	[68]
5% orchid extracts including 3% of a new patented extract of *Brassocattleya marcella* koss leaf/stem vs. standard whitening quasi-drug 3% vitamin C derivative (magnesium ascorbyl phosphate) plus similar moisturizing agents, mainly glycols and hyaluronate	Randomized, placebo-controlled, split-face study	Orchid-rich plant extracts possess efficacy similar to vitamin C derivative in whitening the skin as well as melasma and lentigo senilis on the face of Japanese women.	[82]
Triple combination (TC: fluocinolone acetonide 0.01%, HQ 4%, tretinoin 0.05%) vs. HQ 4%	Randomized, controlled, investigator-blinded, parallel comparison study	TC had superior efficacy to HQ for the primary variable 77/120 patients (64.2%) on TC had melasma global severity score (GSS) "none" or "mild" at week 8 vs. 48/122 patients (39.4%) on HQ ($P < .001$). The secondary efficacy variables confirmed these results. Patient satisfaction was in favor of TC (90/127, 70.8%, vs. 64/129, 49.6%; $P = .005$). More patients had related adverse events on TC (63/129, 48.8%) than on HQ (18/131, 13.7%), but most were mild, and none were severe.	[18]

(*continued*)

TABLE 40.2 (Continued)
Skin Whitening Agents: Human Studies

Identification	Design	Results	Ref.
TC (fluocinolone acetonide 0.01%, HQ 4%, tretinoin 0.05%) vs. HQ 4%	Randomized, double-blind, placebo-controlled study	TC cream was significantly more effective than HQ cream from week 4 onwards: Lesions were approximately equivalent to the surrounding skin in 35% of all TC-treated patients, compared to 5% of those who used HQ cream ($P = .0001$). Improvement of more than 75% was achieved by 73% of TC cream patients and 49% of HQ cream patients ($P = .007$).	[29]
(A) *N*-acetyl glucosamine (NAG); (B) 2% NAG with 4% niacinamide	Double-blind, vehicle-controlled, left–right randomized, split-face study	2% NAG reduced hyerpigmentation; NAG + niacinamide reduced hyperpigmentation more than NAG alone.	[67]
Artocarpus lakoocha Roxb. vs. licorice and kojic acid	Parallel clinical trial with self-control	The *A. lakoocha* extract was the most effective agent, giving the shortest onset of significant whitening effect after only 4 weeks of application ($P < .05$), followed by 3% kojic acid (6 weeks) and 0.25% licorice extract (10 weeks). The effect also increased with time with maximum whitening observed at week 12 for *A. lakoocha* extract.	[70]
Amelan M (kojic acid, phytic acid and butyl methoxydibenzoylmethane, Mesoestetic, Barcelona, E) vs. Mela D (kojic acid/Mexoryl XL and LHA, La Roche Posay, Paris, France)	Double-blind controlled, split-face study	The mean decrease of pigmentation was statistically significant on the MASI with both creams. Amelan M was statistically significant with the mexameter. Some adverse side effects were observed.	[48]
HQ 4% with retinol 0.15%	Full-face clinical trial	Improvement in disease severity and pigmentation intensity was statistically significant at weeks 4, 8, and 12 compared with baseline ($P < .001$). Lesion area and colorimetry measurements also were significantly improved at each visit ($P < .001$).	[19]
5% ascorbic acid cream vs. 4% HQ cream	Randomized, double-blind, placebo-controlled, split-face study	The best subjective improvement was observed on the HQ side with 93% good and excellent results, compared with 62.5% on the ascorbic acid side ($P < .05$); however, colorimetric measures showed no statistical differences. Side effects were present in 68.7% (11/16) with HQ vs. 6.2% (1/16) with ascorbic acid.	[21]
Skin whitening complex with HQ vs. placebo	Randomized, double-blind, split-face study	25 patients completed the study, with an overall improvement of 72% in comparison with placebo. Group 1 (HQ and placebo) presented an improvement of 76.9%,with 25% experiencing side effects, and group 2 (skin whitening complex and placebo) presented an improvement of 66.7%, with 0% side effects.	[30]
2% 4-hydroxyanisole [4HA (mequinol)]/0.01% tretinoin solution (trade name Solage)	Randomized, double-blind, placebo-controlled study	The 4HA/tretinoin combination was clinically superior to each of its active components and to the vehicle in the treatment of solar lentigines.	[23]
50% glycolic acid and 10% kojic acid vs. 15%–25% trichloroacetic acid	Clinical trial	Complete regression of diffuse melasma was observed in 6/20 patients (30%), a partial regression in 12/20 patients (60%), and no regression in 2/20 patients (10%) treated with 50% glycolic acid and 10% kojic acid. Complete regression of localized hyperpigmentations was observed in 8/20 patients (40%), a partial regression in 10/20 patients (50%), and no regression in 2/20 patients (10%) treated with 15%–25% trichloroacetic acid.	[49]
2% kojic acid in a gel containing 10% glycolic acid and 2% HQ (combination cream with kojic acid vs. without kojic acid)	Randomized double-blind split-face (right/left comparison) study	More than half of the melasma cleared in 24/40 (60%) patients receiving kojic acid compared to 19/40 (47.5%) patients receiving the gel without kojic acid. In 2 patients, there was complete clearance of melasma, and this was on the side where kojic acid was used.	[24]

(*continued*)

TABLE 40.2 (Continued)
Skin Whitening Agents: Human Studies

Identification	Design	Results	Ref.
Magnesium L-ascorbyl-2-phosphate (VC-PMG)	Nonplacebo-controlled study	The VC-PMG cream was effective or fairly effective in 19/34 of subjects. The lightening effect was significant in 19/34 patients with chloasma or senile freckles and in 3/25 patients with normal skin.	[55]
2% vs. 5% HQ	Nonplacebo-controlled study	HQ was a moderately effective depigmenting agent in 80% of cases. There was no efficacy difference between the two concentrations; however, 2% HQ was less irritating than 5%.	[17]
2% HQ	Nonplacebo-controlled study	64% of patients showed decrease in hypermelanosis	[15]
5% HQ, in combination with 0.1% tretinoin and 0.1% dexamethasone	Nonplacebo-controlled study	Enhanced efficacy with combination treatment for melasma, ephelides, and postinflammatory hyperpigmentation on adult male blacks. These patients experienced poor results with each of the monotherapies. Actinic lentigines were resistant.	[25]
2% HQ, in combination with 0.05% tretinoin and 0.1% betamethasone valerate	Nonplacebo-controlled study	Objective improvement rate of 65% and a subjective improvement of rate of 95%. Side effects were frequent but minimal.	[26]
2% HQ, in combination with 0.05%–0.1% retinoic acid	Nonplacebo-controlled study	Cream and lotion formulations of 2% HQ and 0.05% to 0.1% retinoic acid provided the most favorable results.	[20]
3% HQ	Nonplacebo-controlled study	Overall improvement was noted in 88% of the patients, moderate to marked improvement in 35% of patients.	[16]
HQ, in combination of a broad-spectrum sunscreen agent	Double-blind and vehicle-controlled study	96.2% of patients who used sunscreen agent showed improvement as compared with 80.7% of placebo group.	[27]
Ascorbate–phytohydroquinone	Nonplacebo-controlled study	After 1 month of treatment, a clear depigmentation of the macules was measured.	[22]

week 8; in the placebo group, there was an increase in mexameter readings (from 368.24 to 372.12 at week 8). Patients also had better quality of life postmulberry extract treatment based on MelasQOL score. MelasQOL score was improved from 58.84 (at baseline) to 44.16 at week 8; the placebo group showed significantly less improvement (from 57.44 at baseline to 54.28 at week 8). Regarding the 75% mulberry extract oil safety profile, only mild itching was reported in four patients, and interestingly, 12 patients reported either itching or erythema from the placebo group [81]. Mulberry extract has been considered as a safe whitening skin agent. No toxicity was found in the human skin irritation test [77]. Result reproducibility from different batches has not been reported.

Orchid Extract

Efficacy of Orchid Extract

Orchid-Rich Plant Extract versus 3% Vitamin C

Orchid extract has also been thought to have a skin whitening effect. Forty-eight Japanese female volunteers, aged between 30 and 60 years, were recruited in a randomized controlled split-face study. They applied orchid extract on one side of their face and 3% vitamin C derivative formula cream to the other side of the face. The efficacy of each formula was assessed objectively using colorimetry and subjectively using a questionnaire and clinical evaluation by dermatologists. There was good agreement between the results of clinical evaluation and dermatologists. Results showed that orchid extract has similar efficacy to melasma and lentigo senilis [82]. Batch-to-batch reproducibility has not been reported.

CONCLUSION

Most skin whitening agents are considered effective. However, HQ remains a most effective agent for the treatment of skin hyperpigmentation. There is an ongoing search for a novel skin whitening agent, both effective and with fewer side effects. In recent years, new agents are being discovered, especially from plants. These agents were extracted from their source, and their depigmenting effects were tested in vitro and in vivo. Tables 40.1 and 40.2 show a summary of potential skin whitening agents.

Taken together, many populations desire more effective preparations for lightening and/or depigmenting and await enhanced efficacy (and safety). Today's methodologies, skillfully utilized by efficient research and development teams, should permit significant and probably cost-effective means of meeting those needs.

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41 Decorative Products

Rodolphe Korichi and Jean-François Tranchant

INTRODUCTION

History showed that makeup has been used since as early as the Ancient Egyptians, and the Ancient Egyptians had many reason for using makeup. Indeed, makeup was used to increase the attractiveness of their appearances, but also for sun protection and medical applications. For a long time considered as an art, makeup entered, with the development of the cinema and the television, a phase of science and technique geared toward helping the aesthetic preoccupations of the women. Over this century, the role of the makeup has been changing with the changing culture of society. However, central themes are easily recognizable through each decade. The natural look of perfection continues today, as does the goal of restoring youth and looking younger. To be always performing to satisfy consumers, who are more and more knowledgeable and demanding, the cosmetics industry has experienced some significant changes to create more sophisticated and innovative cosmetic products that perform more than their basic role, for example, foundations with high sun protection factor (SPF) and ultraviolet (UV) A/B protection, or lipsticks with moisturizing, no-transfer, and long-lasting effects. All these evolutions could not have appeared without the progress of chemistry, which made pigments easier to formulate and brought about polymers that improve the sensory and physicochemical properties of makeup products. Scientific research has also generated pigments with new optical properties in order to create spectacular effects on the skin surface and has made possible the incorporation of certain raw materials currently used in skin care products. Thus, in the last few years, new hybrid products (all-in-one creams) are breaking the line between makeup and care. For example, blemish balm (BB) creams and, more recently, color control (CC) creams are used to instantly correct the complexion of and balance the skin and to protect its youthful appearance. They can also combine the antiaging and moisturizing power of a skin care product with the immediate complexion-enhancing and even-toning properties of a tinted moisturizer and defend the skin against environmental stress with broad-spectrum UVA and UVB protection. Makeup products contribute also in a significant way to the general health and well-being of women. They have a major role, which is to provide psychological stimulation to satisfy personal desires for self-improvement, self-adornment, and good grooming for one's own sense of well-being and for the general attention or attraction of others [1,2]. This heightened technology of makeup products, the sensory interactions based on the skin–brain connection, and ever-stronger claims are today accompanied by the development of specific evaluation methods used to quantify these improvements and to prove the claimed efficacy.

MAKEUP FORMULATIONS

In makeup products, pigments play an essential role because they provide the chromatic modifications necessary for these qualities. Pigments used in current makeup applications can be classified into organic pigments, mineral pigments, and nacreous pigments (Table 41.1). Twenty years ago, the cosmetic industry introduced the treated pigments. The surface properties of pigments differ with the size and the shape of the pigment particles as produced by the various manufacturing methods. It is known that the behavior of pigments is closely related to surface properties and can be classified into hydrophilic and lipophilic. It is very important to know these properties of powders when makeup products are formulated. Generally, pigments and substrates used (mica, talc, sericite, borum nitride, etc.) for makeup cosmetics are hydrophilic. Changing the surface properties of these powders from hydrophilic to hydrophobic can be made with several of the aforementioned coatings depending upon the final product's function. There are many processes by which pigments may be surface-treated. The most common methods used today are chemical interactions, electron–charge reactions, mechanochemical processes, and mechanical processes. Other processes are essentially variants of these four basic methods, as witnessed by an introduction in surface-treatment manufacturing called ultramicronization. Surface treatments impart easier dispersibility, better stability, and flocculation resistance to pigments. The second basic important advantage for the cosmetic chemist in using treated pigments is in the development of hydrophobic systems (silicon media, for example). Hydrophobic pigments of both types are available, including treated inorganic colors, D&C colors (dyes and pigments considered safe in Drugs and Cosmetics when in contact with mucous membranes or when ingested), nylon, titanium dioxide, talc, kaolin, mica, and other minerals. These pigments and the products made with them show water-resistant properties, improved skin adhesion, improved color consistency, and better smooth-skin feel than when uncoated materials were used in the same formulations.

LIPSTICK

Lipsticks are mixtures of waxes, oils, and pigments in varying concentrations to yield the characteristics of the final product [3]. Waxes will give rigidity and solidity to the stick. They

TABLE 41.1
Pigments Used in Current Makeup

Pigments	Description
Organic pigments	Selection of pigments for use in make-up is limited to those allowed by regulations of the Food and Drug Administration in the United States. The pigments are formed by precipitating the colorant onto a substrate, often aluminum or calcium hydroxide, thus forming an insoluble salt.
Mineral pigments	Titanium dioxide and iron oxides are the most commonly used materials.
Nacreous pigments	Nacreous pigments or pearlescents are used to create a frosted appearance, often with other special effects. There are three classes of nacreous pigments used in make-up: Natural pearl essence (for nail enamel), known as guanine (2-amino-6-hydroxy-purine), is derived from the scales of Atlantic herring. It provides a soft luster. The pigment's density is the lowest of the three types noted above, and it is therefore the easiest to suspend. Natural pearl essence exists in the form of platelets or needles. Bismuth oxychloride A commonly used synthetic nacreous pigment is bismuth oxychloride. This material's luster is more metallic than that of the other two types. The particles also have a higher density (7.7), making then much more difficult to suspend. TiO_2–mica or mica coated by several different layers (silica, iron oxide, etc.) Another type of pearlescent material is mica coated with a thin layer of TiO_2. The interference effects change according to the thickness of the layer of TiO_2.

can be of vegetable origin like candelilla wax, which brings brightness, or carnauba wax, which gives hardness. They can also be of mineral origin, like ozokerite wax for adhesion, or of synthetic origin, like polyethylene, which is compatible with silicones and prevents the exudation of oils. Usually, lipsticks contain a combination of these waxes (approximately 20%), which are selected and blended carefully to achieve the desired melting point. Oils (40%–50%) are used to give the lipstick its slippery and soft aspect when applied. To form a film suitable for application of the lips, the formula contains castor oil used to disperse pigments, white mineral oils or oleyl alcohol. Coloring agents are present at between 2% and 10% and can be of several types. One mainly finds synthetic pigments, but minerals like iron oxides (red, yellow, or black) are also used to give the color, and titanium dioxides bring coverage, opacity, and intensity. Pearl pigments can also be used to give color highlights. It should be noted that pigments of vegetable origin are rarely used in cosmetics because most of them are unstable to heat or light. Those of mineral origin also create a problem because they contain too many heavy metals according to the cosmetic legislation. In relation to the claims, additives can be added, such as sun filters, which protect the formula or add a sun protection index, antioxidizers, vitamins (E, C, B5), moisturizing agents, or ceramides. A light fragrance (<1%) will be added to give the lipstick a pleasant taste on the lips and mask possible smells of raw materials. Overall, a standard lipstick manufacturing process can be simplified in four stages: *mixing waxes, oils, and extenders at high temperature; producing a concentrated dispersion of pigment (generally, the pigments are ground in an oily base such as castor oil); adding the colored paste at high temperature with the rest of the formula from the first stage;* and *molding the colored paste*. The lipstick chemist must develop a formula that has a good cosmetic feel for the range of formulated shades. Therefore, it is in his/her interest to estimate the influence of the pigments on the texture of the stick. From rheological studies, Tranchant and Poulin [4] showed that one can monitor the manufacturing stages. Knowing the physicochemical properties of pigments and their rheological behaviors after grinding, they demonstrated and explained the influences on the texture and the mechanical properties of the stick. One of the disadvantages of conventional lipsticks, which almost all consumers point out, is the deterioration of its fresh appearance in a short period of time. In order to solve this problem, one of the most efficient techniques to improve the long-lasting properties has been using a film-forming polymer in combination with volatile oils (cyclomethicone) [5], which evaporate on contact with lips (no-transfer effect). However, incorporation of the volatile oil into the lipstick's composition is accompanied by a loss in the application gloss over time and comfort on lips. For very glossy lipstick, the formulation is different from that of classical and no-transfer lipsticks, because the level of the waxy phase goes up to about 80%. The ratio of oil/wax is higher, whereas the ratio of pigments is lower (0%–5%). For matte lipsticks, we use talcs and some nylon powder, silica, or polymethylmethacrylate, which will give a powdered, slippery, and very soft touch.

Nail Enamel

Nail enamel constituents can be grouped into six families: lacquers, agents for adhesion and gloss, solvents, plasticizers, pigments, and thixotropic agents, which, of course, must be compatible. The heart of the nail enamel formula is the lacquer, which may be defined as a coating that hardens and dries by evaporation of the solvent. The lacquers determine application properties, gloss, wear properties such as adhesion, flexibility and abrasion resistance, water resistance, viscosity, and suspension ability. The polymers selected must be soluble in cosmetic solvents that dry rapidly, leaving a smooth, glossy film with excellent adhesion properties and a good pigment wetting ability. Nitrocellulose (10%–20%), a polymer obtained by nitration (12% nitrogen) of the cellulose, is the filmogenic agent currently used in nail enamel.

However, used alone, it produces films that tend to shrink and become brittle, with only moderately good adhesion to the nail surface. Different modifying resins (5%–10%), such as toluene sulfonamide resins, are added to improve the properties of the lacquers, for example, to increase wear resistance and gloss. These effects are usually adjustable by selection of an appropriate plasticizer (5%–10%), a film-forming agent that acts on the hardness of the film, giving it flexibility. Indeed, the nail is deformable and elastic. A film adhering to the nail surface must therefore be able to withstand any motions. Typical plasticizers used include phthalates, citrates, and camphor. These polymers, resins, plasticizers, and pigments are mainly dispersed in organic acetates and aromatic solvents, with an amount of solvents in the nail enamel of roughly 70%. For formulation reasons, the ratios of solvents can be modified because the choice of solvent can affect the drying time, flow characteristics of the film, and the flexibility of the film. Note that regulations such as Proposition 65, California's Safe Drinking Water and Toxic Enforcement Act, limits the use of certain solvents, such as toluene, which is considered a reproductive toxin. Then, more and more, the new formulations were used without aromatic solvents. The physicochemical factors that determine the suspension stability of nail enamel are complex, such as the particle size and distribution, density differences between particles and continuous medium, viscosity of continuous medium, particle concentration, and particle–particle interactions. However, several authors [6–8] showed that one can predict dispersion stability through dynamic studies of rheology, by relating directly the viscoelastic parameters (viscosity, elastic modulus, and viscous modulus) to the dispersed system structure. Thus, different formulation systems need different viscosity profiles to achieve equivalent aesthetic results. Indeed, the viscosity of nail enamel has to be sufficiently high to avoid the sedimentation phenomena of pigments in the bottle, remain on the brush before the application, and facilitate precise application, but the viscosity has to be low enough for the nail enamel to be taken out of the bottle. This can be done if the viscosity is not linear. This nonlinearity is obtained by using modified clays (thixotropic agents). To prevent settling, pigments and pearlescent pigments are suspended by a rheological agent, and the organic clays are the most commonly used material. Note that smectites are the mineral group of the clays that swell, have high cation exchange properties, and can be observed as thin plates. The organic treatment of these clays consists of replacing the cations that are in the natural product (Na, Ca) by some quaternary ammonium salts. The rheological properties of the medium originating from the hydrogen bridges between the hydroxyl groups of the dispersed plates can be summarized as follows: in undisturbed nail enamel, the plates of clay are packed and linked together by hydrogen bonds [high viscosity]. When shearing, all these bonds are broken, plates are all oriented in the same direction [low viscosity]; after application, there are associations between the plates; and after application, there are associations between the plates, and thus, the viscosity increases. In the early 1990s, environmental laws and regulations as well as the green movement in Europe gave rise to new formulations and an interest in a water vehicle, because it is known that organic solvents can damage nails (for example, moisturization and lipids).

Face Makeup

These products are used to make the skin look natural and beautiful for as long as possible, as they unify the color of the skin, improve a dull and tired complexion, give a matte finish, and mask possible imperfections like dark spots, small wrinkles, dark rings under the eyes, and the pores of the skin surface. Their application must be easy and give coverage for a natural complexion. They must have a pleasant texture and a good adhesive property, be comfortable, and have a consistent color and smooth finish. There are more and more no-transfer and oil-free products. New pigments are also used, such as pigments with an action on light, such as soft focus, photochromic [9], or auto-adaptive effects, as are pigments coated with silicone or fluoride oil, giving a very specific sensory touch. Foundations can contain ceramides, used to strengthen the skin barrier; moisturizing agents like glycerin and hyaluronic acid; vegetable oils, which improve the hydration and flexibility of the skin; and free radical scavengers (vitamin E) as well as UV filters (UVA–UVB), giving makeup products a sun protection index (SPF5 to SPF30). Firming complexes to tone up and restructure the skin and seboregulating ingredients can also be added.

Face Foundations

The formulations vary according to the qualities required for the product. Foundations are available in various forms: liquids, gels, creams, solid creams, sticks (pen stick), cakes (pancakes), or mousse. There are four basic facial foundation formulations that are the most popular products for complexion: oil-in-water, water-in-oil, oil-free, and water-free or anhydrous forms. Oil (silicone)-in-water emulsions are better for normal and oily skin types, whereas water-in-oil (silicone) emulsions are better for normal and dry skin types. Oil-free formulations are used for women with oily complexions, and anhydrous forms are used by women with facial scarring who require camouflaging. Oil-based foundations are water-in-oil emulsions containing pigments suspended in oil, such as mineral oil. Vegetable oils (e.g., coconut, sesame) and synthetic esters (octyl palmitate, isopropyl myristate) may also be incorporated. Oil-based formulations also contain water (30%–45%), siliconed tensioactives (5%), and some specific actives (vitamins, UV filters, moisturizing agents, etc.). The water evaporation from the foundation just after application leaves the pigment in oil on the face. Water-based foundations are oil-in-water emulsions containing a small amount of oil in which pigments (10%–15%) are emulsified with a relatively large quantity of water, which is the dominant substance (50%–60%). Oil-free foundations contain vegetable or mineral oils but also other oily substances, such as the silicones dimethicone or cyclomethicone, which leave the skin with a dry feeling. They come in three forms: alcohol-based,

glycerine-based, and creams or lotions (ideal for oily or acne-prone skin). These foundations go on smoothly but dry fast, so they must be blended quickly for even coverage. Water-free or anhydrous foundations are waterproof, and high concentration of pigments can be incorporated. These several formulations, and the effects of pigments can control the darkening phenomenon from the sebum, hide wrinkles, protect from UV radiation through diffused reflection, give a smooth finish, and give a long-lasting and no-transfer effect of makeup. No-transfer formulations are an ingenious combination of volatile oils, which fix the pigments on the skin after evaporation, giving permanent effects. The most-used ingredients in face makeup were mica mat and micro TiO_2 and SiO_2. Mica is transparent and easy to use. TiO_2 has good covering effects and is very efficient against UV radiation, but its touch is hard. The silica is a multiporous ingredient, which absorbs the oil and sebum. Nowadays, the surface treatments are a wonderful technique to give some special functionalities to the raw material pigments and sensory properties [10]. The smooth feeling of a foundation mainly depends on the physical properties of the raw material pigments, such as particle size, shape, and so on. Furthermore, adding the moisturizing and water-absorbing effect to the raw material pigments with surface treatment, the much more elegant and smooth feeling in use can be completed, and these days, polysiloxane treatment is very popular for makeup products. The long-lasting effect is also a very important functionality for makeup products and especially for face makeup. A lot of women use makeup products in order to maintain their soft and smooth skin for a long time. For women with oily complexions, lipids secreted from skin tissue collapse the makeup finishing, and the surface of the finishing skin gets glossy, since lipid from skin tissue is miscible with pigments and binder in makeup cosmetics. This disadvantage will not be solved with silicon treatment, since polysiloxane is miscible with oil. In order to overcome these problems of raw material pigments for cosmetics, such as poor dispersibility, high activity against skin, and collapse of makeup finishing by lipid from skin tissue, there exists a surface treatment using perfluoroalkyl phosphate [11]. Fluoro compounds are utilized for several kinds of fields in order to avoid water and oil. This treatment showed excellent water and oil repellency and also good dispersibility to organic and inorganic pigments, and it was shown to depress the activity of ultrafine pigments. Other surface treatments can be carried out to give, for example, much more UV cut effect to pigments. Several types of organic low-molecular-weight UV absorbers are commonly used, such as benzophenone, *p*-amino benzoic acid, and so on. It is also known that ultrafine titanium dioxides and zinc oxides absorb UV light, especially zinc oxides, which absorb UVA and UVB both. However, it is recognized that titanium dioxide is not so stable against UV rays. To overcome the disadvantage in photostability, new technology to coat titanium dioxide with high uniformity was explored, and a novel silica-coated titanium dioxide with broad-spectrum protection against UV rays was developed [12]. Other surface treatments with polymer materials were carried out in order to give much more UV cut effect to pigments [13]. This allows the formulator to avoid the main disadvantage of physical sunscreens, namely, the visible whitening that occurs when TiO_2 and/or ZnO are used in high concentrations to obtain a high SPF. These novel polymer surface treatments, such as organic polymers (Teflon, silicones) or active ingredients (collagen, elastin, vitamin E), give better properties of cosmetic makeup, and polymer or polymer matrix systems are very safe compared with using ultrafine titanium dioxides or zinc oxides.

Face Powders

Face powders provide coverage of complexion imperfections, oil control, a matte finish, and tactile smoothness to the skin. Powders give a good lasting effect to foundation makeup and possess oil-absorbing properties very useful for oily skin types. Free powders are used to fix the foundation, and compact powders, to retouch one's face during day. Face powder is more complicated and made by a mixture of products: talc and serecite (to help to spread), chalk or kaolin (to give moisture absorbing qualities), magnesium stearate (gives adherence), zinc oxide and titanium oxide (to help cover the skin thoroughly), and pigments (for color). The use of mica in powder formulations improves skin feel, product application, and skin adhesion. The favorable effects are obtained with wet ground micas that have a particle size of <15 microns. Mica can also be modified by coating with inorganic or organic materials to produce another large group of fillers (spherical, special, and surface modified). Spherical fillers are widely used to improve skin feel. There are a variety of materials available, the organic types consisting mainly of polyamides and nylon spheres and inorganic types consisting mainly of silica, both as solid and as hollow spheres. The improvement of skin feel is attributed to the ball-bearing-like action of the spheres between other powder ingredients in the formulation and the spherical filler. When spherical materials are used, there is also an increase in the viscosity of the emulsion, allowing for a reduction of viscosity modifiers in the final formulation. Bismuth oxychloride (BiOCl), a fine white powder with a high bulk density, is well known as a pearlescent pigment, but it can also be used as a filler with no luster when in a particular crystal form. It has relatively low oil absorption characteristics and also gives rise to excellent compressibility when used in pressed powder formulations. Its hydrophobic character and good affinity with the skin also improve the skin adhesion and wear properties of powder and makeup products. However, one disadvantage of BiOCl has been is a low light stability. Nowadays, BiOCl is much more stable to UV light. Special fillers are a group of fillers that are made up of several components, which combine their individual advantages when they are processed together into a composite material. Coated mica pigments, for example, are often found as light-diffusing pigment agents in color cosmetics, where fine-particle-size micas enhance the light diffusion properties of the material coated on the surface of the mica. Mica can also be coated with very small particles of metal oxides, allowing ease of incorporation into liquid

TABLE 41.2
Possible Raw Materials for Foundations and Powders

Pigments and Mineral Charges					
White	**Colored**	**Unifying Charges**	**Mineral Charges Light**	**Silicones**	**Active**
– Titanium dioxide – Talcs – Pearl pigments (*mica, titanium dioxide coated mica, bismuth oxychloride*)	– Iron oxides (*yellow, red, black*) – Ultramarine blue – Colored pigments and pearls (*mica + iron oxides, mica-titanium dioxides + iron oxides*)	– Nylon (*orgasol*) – Polymethylmetacrylates – Silica – Boron nitride	– Soft focus (*light-diffusing*) – Photochromic – Light correcting (*auto-adaptive*)	– Dimethicone – Volatile cyclomethicone – Silicone gum blends Emulsifying – Cyclomethicone and dimethicone copolyol	– Vitamin C, A, and E – UV filters – Enzymes – Phospholipids – Moisturizing agents (*glycerin*)

formulations. Examples of coating materials for micas are titanium dioxide (e.g., low-luster pigment), barium sulfate, and BiOCl, as well as organic compounds. For surface modified fillers, most coating materials are organic polymers (collagen, elastin, vitamin E). Powders also contain between 10% and 20% organic texture agents (polymers) or mineral agents (boron nitride, silica); preservatives, antioxidants, and perfumes (neutral or more sophisticated) can also be added there. Table 41.2 lists some possible raw materials for foundations and powders.

Eye Makeup

Eye makeup consists of three major categories: mascaras, eye shadows, and eyeliners. Mascaras thicken, sheathe, separate, and lengthen eyelashes to obtain an intense look. Eyeliners help draw a precise line at the base of eyelashes, and eye shadows bring light to the look and highlight the color of the iris.

Mascaras

Among all makeup products, mascara's formula requires a particular development. The choice of mascara depends on the type of eyelashes (short or long, stiff or curved, poor or bushy, fair or brown) and on the required effect (lengthened, curved, and/or thicker eyelashes). Liquid mascaras are the most popular modern formulation, and they can be divided into water-based, solvent-based and water/solvent hybrid varieties. Water-based mascaras are formulated from waxes (e.g., beeswax, carnauba wax, synthetic wax), water, and pigments, which are often iron oxides and resins dissolved in water. The water evaporates readily, creating a fast-drying product that thickens and darkens the eyelashes. Some water-based mascaras very rich in wax (30%) are labeled waterproof or water-resistant. To color eyelashes, inorganic pigments are the most commonly used because the vast majority of mascaras are black. Note that certain pigments, like cochineal carmine, may generate some problems, such as the coloration of contact lenses. The formulae also contain antioxidizers to avoid the rancid smell of fatty substances and preservatives, which protect the eye from any risk of infection. Vitamins and hydrocarbon volatile or silicon solvents can also be used to improve the performance of the makeup. Concerning the solvent-based mascaras, they are formulated with petroleum distillates to which pigments (e.g., iron dioxides, ultramarine blue, etc.) and waxes (candelilla wax, ozokerite, hydrogenated castor oil) are added, making them waterproof. If it is clear that the makeup effect depends on the formula, it is also important to consider the type of brush and the diameter of the aperture of the mascara tube. Indeed, it must automatically adjust the quantity of product on the brush to avoid loads on the eyelashes during application. The packaging also must be totally airtight to avoid the degradation and oxidation of the formulation. Thus, to obtain a good application on the eyelashes, it is necessary to develop a compromise between the mascara formula's viscosity and the brush type. A rheological approach can be made through the rheological characterization in situ of mascara pastes with the brushes [14]. This procedure used to quantify the take-up of the mascara brush in the container allows us to visualize the influences of the shaft, the bristle length, and the hardness and the pattern on the take-up and, therefore, to characterize the product transfer.

Eye Shadows and Eyeliners

Eye shadows are a cosmetic designed to impart color primarily to the upper eyelid. The formulations of eye shadows are identical to those of compact powders for the face, but the color range is wider. Application is helped by tiny particle size, flattering the eyelid by giving a smooth rather than crepey appearance. We find that some creamy textures often claim to be long-lasting sticks and pencils. Eyeliners are mainly liquid formulations using ultrafine pigments.

INTERACTIONS BETWEEN SKIN AND MAKEUP PRODUCTS

Physical appearance, and more particularly, physical attractiveness, is one of the most important determinants of interpersonal attraction in the early stages of many relationships [15,16], and it is obvious that the face is the part of the human body that attracts the most attention [17,18]. Thanks to makeup products, it is quite possible to bring out certain characteristics of the face or, on the contrary, to lighten them

in order to achieve a degree of attraction for all types of faces [19–21]. In all cases, the perceived effects will depend on the nature of the makeup formula, raw materials that compose it, and the interactions between the made-up skin and light. Overall, two main types of interaction can be observed: visual interactions, where the optical way of light is modified by the optical properties of cosmetic ingredients and the relief of the skin surface, and biophysical interactions, where the finished makeup is perturbed by the biochemical and mechanical functions of the skin surface. Concerning the visual interactions, they are mainly generated by pigments with specific effects [22]: pearlescents, iridescent (color changes according to the angle of observation), thermochromic (color changes according to the temperature), photoadaptive (preserve a radiant complexion following the lighting conditions), or soft focus effects [23–25]. These soft focus pigments, mainly composed of polymers, micas, and talcs covered with rough or spherical particles of small diameters, such as silica or titanium dioxide, are used to optically reduce the appearance of wrinkles. These effects are obtained by optimizing outlines of wrinkles and reducing the difference of brightness due to diffuse reflection. Concerning the biophysical interactions, it is known that behavior of a makeup product can be modified by the biochemical and biophysical properties of the skin. For example, a woman's skin type and her facial movements throughout the day actually remove the foundation from the face. Foundation tends to shift and wear off during the day and migrate into the fine lines of the face. The outside environment, such as air pollution, smoke, sun, and stress that we feel in the current world, are also factors that can disrupt the normal balance of the skin (for example, skin discoloration or extreme dehydration) and therefore modify the behavior of makeup products. Other phenomena can be associated with the makeup effects, such as, those based on the relation existing between the pleasure felt during and after application of a makeup product and psychological and social behaviors [26–28]. Indeed, it is clear that individuals psychologically unhappy with themselves or their appearance seek body alterations as a means of improving their self-image. And one of the most important ways today's women increase their psychological well-being and feel confidence about their physical appearance is through the use of commercial cosmetics. For example, women who were particularly concerned about their appearance (those high in public self-consciousness) wore more makeup and were more apt to believe that makeup enhances their social interactions [29]. Wearing makeup is also important across all of the stages of life, associated with greater or lesser sensitivities to social interactions. With changes in age, the psychological effects change [30]. A young woman will use makeup to capture the eye of others and is thus perceived as having a more favorable personality, whereas a mature woman will use makeup to enhance her feelings of self-satisfaction. Finally, beyond the simple application of colorful products on the face, makeup appears as a holistic technique that modifies not only one's appearance but also help to cope with self-image, emotions, and mood.

EFFICACY EVALUATION OF MAKEUP PRODUCTS

Psychophysiological Approaches

The skin is one of the most important components of an individual's physical appearance and facial attractiveness [31,32]. And physical appearance (skin color distribution and texture are known to have a major influence on the perception of female facial age and judgments of attractiveness and health) plays a crucial role in the development of personality and social relations [15,33]. Indeed, beyond simple aesthetic functions, makeup can act on psychosocial functions such as self-assertion, social adjustment, or nonverbal communication. For example, a study showed that beauty modulated by makeup has a significant positive effect on judgment of competence, a universal dimension of social cognition, but has a more nuanced effect on the other universal dimension of social warmth [33]. Makeup can also have a beneficial impact on one's mood and well-being and modulate emotional states during social stress. To explore and describe the links existing between makeup, the external appearance, self-image, emotions, and mood, self-assessment questionnaires can be used in order to clarify reasons that motivate women to use makeup. Well-validated psychometric tests [34–37] can also be used in order to establish if the need for makeup is related to psychological features. In a recent study, we have investigated in women the underlying correspondence between personality and self-experience with makeup and showed a high implication of psychological traits in the makeup functions [2]. From interviews of women on quality of life and makeup, and well-validated psychometric self-questionnaires, two clearly distinctive classes of subjects according to their functional use of makeup and their emotional and psychological profiles have been established (Table 41.3). The first class, which we labeled as "Camouflage," is characterized by women using makeup as a camouflage tool, rather concerned with anxiety and neuroticism, with low self-esteem and low assertiveness. The second class that we labeled as "Seduction" was characterized by women using makeup as a seduction tool, more oriented to the desire to please and characterized by lower anxiety and higher self-esteem, extroversion, and assertiveness. Overall, women of the first class can be classified as having a negative self-perception, worrying more often, and dwelling on frustrations and disappointments. On the other hand, women of the second class tend to perceive themselves as better than average, both on communal traits, with more experience of positive emotions and appearing as more sociable, optimistic, and active. In the psychophysiological domain, other approaches can be considered in the demonstration of makeup effects, for example, the study of the facial expressions known to be linked to emotional responses [38] or, again, the facial skin temperature, salivary hormonal dosages, and vocal acoustic parameters, allowing us to describe physiological responses [39–41]. Cognitive mechanisms involved in the domain of physical appearance and attractiveness are also interesting to explore. Indeed, facial appearance is something that is intuitively perceived (by oneself or by others), and this aesthetic

TABLE 41.3
Statistical Analysis of the Psychometric Variables: Two Distinct Psychological Classes Revealed

	Camouflage (n = 21)		Seduction (n = 49)		
Variables	**Mean**	**SD**	**Mean**	**SD**	**Significance**
STAI (T-anxiety state)	46.6[B]	8.1	35.6 [A]	5.5	S ($P < .01$)
SEI (general self score)	12.4 [A]	3.9	20.6 [B]	2.4	S ($P < .01$)
RAS (assertiveness)	–5.2 [A]	16.2	26.1 [B]	17.8	S ($P < .01$)
EPI Form B (extraversion–introversion)	11.9 [A]	2.8	15.3 [B]	3.6	S ($P < .01$)
EPI Form B (neuroticism–stability)	13.5 [B]	3.4	7.6 [A]	3.6	S ($P < .01$)

Note: The first class (A), "Seduction," is characterized by high self-esteem (SEI), extraversion (EPI), and assertiveness (RAS), while the second class (B), "Camouflage," is rather concerned with anxiety (STAI) and neuroticism (EPI).

appreciation, modulated or not by cosmetics, affects the attentional mechanism sign of sophisticated cognitive processes [42]. Now, if it is often said that beauty is subjective, that beauty is "in the eye of the beholder," David Bohm [43] explains that beauty is the result of dynamic, evolving processes conceived in objective terms.

Measurement of Facial Expressions

Paul Ekman [44], known around the world for his studies on emotional expression and nonverbal communication, found that certain facial expressions were universally associated with seven basic emotions (anger, sadness, fear, surprise, disgust, contempt, and happiness). From these standard emotions, behavioral scientists developed objective coding standards, the Facial Action Coding System (FACS). This system is a comprehensive and widely used method to objectively describe facial activity, as it is based on the identification of specific actions of facial muscles, called action units (AUs). Ekman and Friesen defined 46 of these to describe each independent movement of the face and 12 to describe changes in head orientation and gaze [45]. From these AUs, the emotional state of the human subject can be inferred. In a recent study, we showed that the negative emotional experience of looking at oneself in the mirror, for some women, is reduced by makeup. Indeed, during subjects' facial self-observation before and after the makeup process, we observed that the number of women having expressed AUs 15 and 17 before makeup (AUs corresponding to mouth depression and chin wrinkling and known to be components of some negative emotional expressions) was significantly reduced by the makeup process (Figure 41.1). Finally, the coding of facial expressions to access emotional

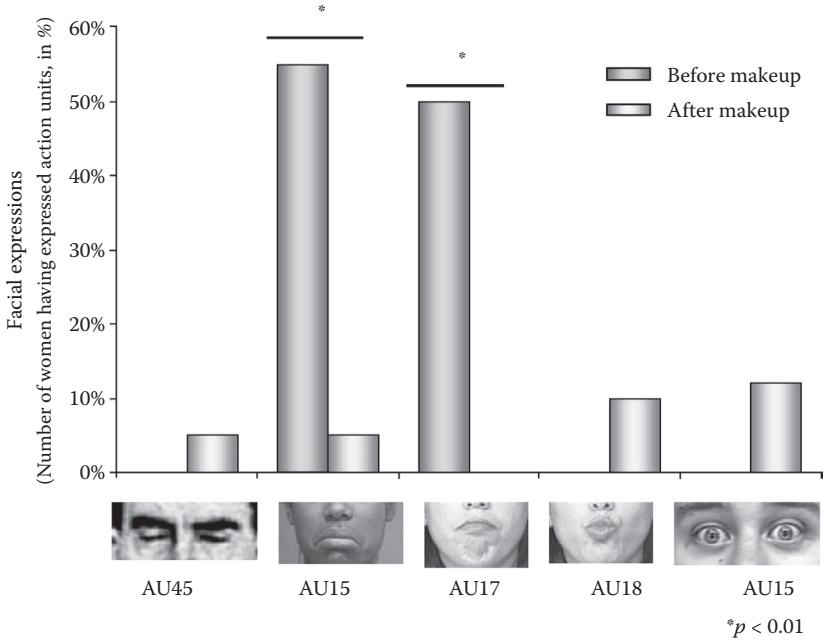

FIGURE 41.1 Emotional expressions during subjects' facial self-observation before and after the makeup process. Ratings were transformed into binomial data, with the presence of an AU scored with "1" and absence with "0." Before makeup, 53% of subjects expressed facial movements coded as AU15 (i.e., lip corner depression, muscle *triangularis*) and AU17 (i.e., chin raised, muscle *mentalis*). After makeup, female subjects observed a significant decrease in AUs 15 and 17 (55% to 5% and 50% to 0%, respectively).

responding before and after applying makeup to the face allows us to illustrate the way in which makeup transforms negative facial expressions. It is clear that our next step will be to further study the emotional impact of different types of facial makeup in order to select those that will be able to give access to positive emotional experiences.

Measurement of Facial Skin Temperature

Certain events can cause distortion of psychosocial attitudes, such as stressful situations. Indeed, through social stressors, people living with physical differences and/or disabilities frequently internalize negative judgments about their bodies and lives and view their bodies as inadequate, unacceptable, and a source of negative emotions such as shame, stress, and/or anxiety [46]. In cosmetology, some studies are able to relate some cosmetic effects to autonomous endocrinological and immune reactions [47,48], but also to various physiological responses, by producing, for example, an increasing of the heart rate and modifications of the skin conductance and facial skin temperature [49,50]. In a recent study [51], we showed by infrared thermal imaging (FLIR system, ThermaCAM SC500) that subjective feelings of social anxiety and emotional reactivity were reduced by improvement of the facial appearance by makeup (Figure 41.2). This result tends to prove that women with negative self-perception use makeup in order to reduce their psychosocial stress and to improve their self-confidence to obtain a higher and more stable social adjustment during stress events.

Measurement of Ocular Behavior

The direction of the eyes corresponds to the allocation of visual attention; thus, measuring gaze behavior can provide insights about mental processing [52]. Eye tracking is a technique able to accurately monitor and record the rapid physiological movements of the human eye to a given scene. Generally used for marketing purposes, few studies were carried out with cosmetics [53,54]. Among number of methods for measuring eye movements, the most widely known uses video images from which the eye movements are extracted. We use head-mounted eye trackers (SensoMotoric Instruments, USA; Pertech Company, France), and some remotely measure the gaze movements but require the head to be stable (Tobii Technology, Sweden). From these systems, we can investigate fixations (looking at the same place for a while), saccades (fast eye movements), and pupil dilation responses (linked to cognitive and emotional events). In a recent study, we showed that two parameters (fixations represented by dots, larger dots indicating a longer fixation time, and saccades indicated by lines between fixations) were most relevant to discriminate the perceived beauty and to inform us about the level of facial attractiveness. Thirty female subjects belonging to two different age groups (27 ± 3.2 years and 55 ± 5.3 years) were recruited, and two digital color frontal-view face images were used (i.e., with and without makeup). All these images were presented to two different age groups of female and male observers in order to evaluate the level of facial attractiveness (according to a 10-point scale ranging from 0 to 9) and monitor the observers' eye movements. Overall, the judgment of facial attractiveness is improved thanks to makeup (whatever the age group of volunteers), and facial attractiveness involved eye-movements in a triangular scanning pattern through the eyes, nose, and mouth, which we called "triangle of beauty." From fixation and saccade data, graphically mapped to a heat map image or a gaze plot to visually demonstrate fixations and gaze paths, we noted that the more the face was judged as attractive, the more the visual attention of the observers was well defined in the center of the face to define a "triangle of beauty." In comparison, the more the face was judged as less attractive, the more the visual attention of the observers was dispersed and outside of the "triangle of beauty" (Figure 41.3).

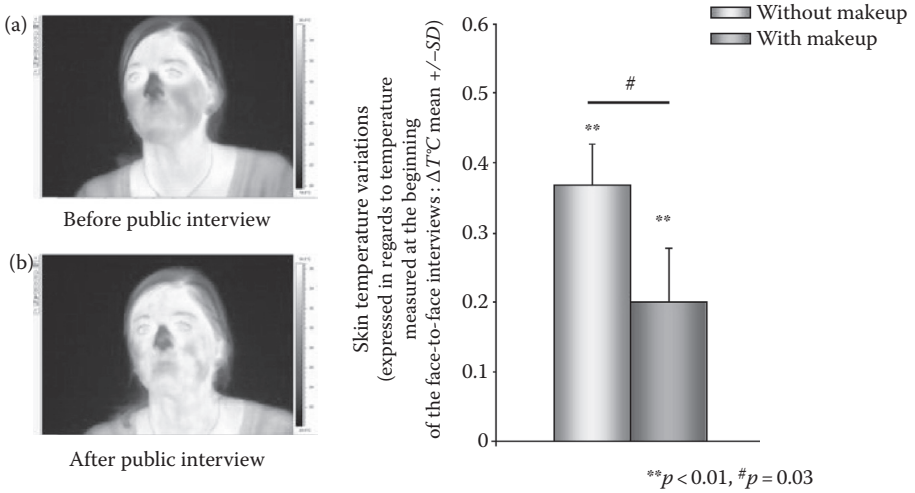

FIGURE 41.2 Facial thermograms showing the skin temperature distribution of the whole face at (a) the beginning of the stressful challenge and (b) the end of *face-to-face* interview. The dark areas correspond to the lower temperature whereas the clear ones correspond to the highest temperature. The public interview generated in women an increase in skin facial temperature (Δ*T*°C mean), and skin facial temperature variations were significantly reduced in women allowed to use makeup in comparison to those not allowed.

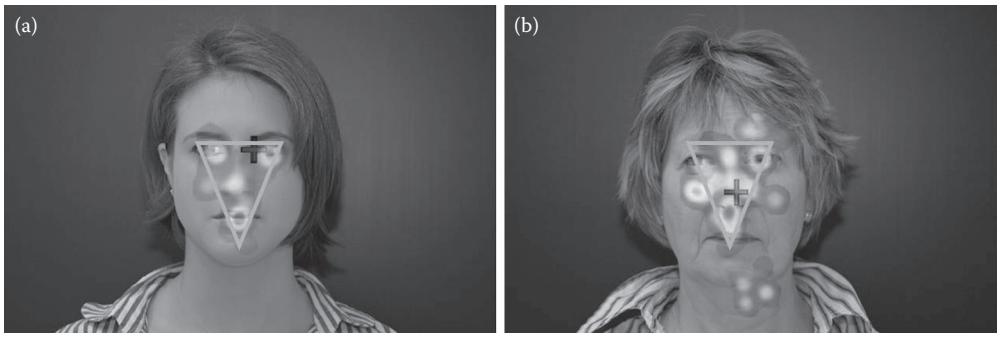

FIGURE 41.3 Heat map representation of the viewer's experience and visual activity in the "triangle of beauty": (a) women judged as attractive, (b) women judged as less attractive. Warmer colors reveal areas that most users looked at, while colder colors show areas that few users noticed.

These variations in the visual processing and judgment of beauty find explanations in the perceptual fluency theory. Indeed, authors proposed that the aesthetic experience is, in part, a function of the perceiver's processing dynamics and suggested that "the more fluently perceivers can process an object, the more positive their aesthetic response" [55–57]. In this perspective, and knowing that certain variables such as symmetry, contour shape, skin color distribution, clarity, proportion, and contrast are known to affect the aesthetic appreciation, it is easy to understand that certain facial features (skin surface quality and morphological aspects) can generate perturbation of visual processing, contributing to the negative reactions to the face as observed in our study.

Instrumental Approaches

Although qualitative evaluation (mainly obtained through consumers' perception and experts' evaluation) is commonly used and remains indispensable to prove the effects of makeup products, and although facial attractiveness is something that is intuitively perceived rather than measurable with instruments, we often associate it with some quantitative data. Some may be obtained with devices already used for the evaluation of skin care products [58], but the most representative are those obtained from methods able to describe visual effects, the main property of makeup products. Video imaging appears as a pertinent approach because, thanks to high-resolution video cameras and sophisticated image analysis software, we can visualize and quantify some properties perceived by consumers (concerning either the whole face or smaller areas). However, video imaging has its limits, and in certain cases, biophysical methods could be very useful.

Lip and Eye Makeup

Applying lip makeup is one of the most important parts of any woman's makeup routine. First of all, lips have a significant effect on the aesthetic judgment of the face [59]. And second, they are associated with estrogen levels, sexual arousal, and health, which in turn lead to an increase in the positive perception of women's faces [60,61]. But beyond the psychosocial aspect, what are the ideal physical lipstick properties like? Often, it has to have high capacity for color preservation (long-lasting effects), moisturize, and protect the tender lip skin. To measure lip color preservation, several devices can be used, such as spectrophotometers (CM-508i, CM-2002, Minolta) and chromameters (CR-200®, CR-300®, Minolta). However, these approaches have some limits because they require direct contact with the made-up skin surface, have a small surface of measurement, and generate shrinking at every measurement, modifying the aspect and the long-term behavior of the product. To avoid these phenomena, lip color preservation can be quantitatively evaluated by image analysis. Thanks to this technique, it is possible to study skin areas of various sizes, to take measurements without any contact with the skin, and to analyze the image pixel by pixel ("picture element," which is the basic unit of programmable color), providing quantitative information according to localization. For example, the global color of the made-up area and the shades most represented in the global color can be quantified. It is also possible to evaluate the streakiness, which represents the amount of lipstick in the small lines surrounding the lips, the brightness [62], and the degree of chapped lips due to moisture loss (Figure 41.4a). Concerning eye makeup products, their performance can also be evaluated by video imaging. In the case of mascara giving an extra lash-curling effect, a video camera positioned close to the eye at a fixed distance, enabling a clear view of the median part of the eye, allows us to calculate the radius of the curve of the lash (in pixels) corresponding to the radius of the circle describing the lash (Figure 41.4b). The more the radius of the circle decreases, the more the lash is curved.

Face Makeup

To evaluate the impact of facial makeup on the visible skin condition and judge the performance of makeup products (for example, foundations and powders), certain characteristics can be studied, for example, the long-lasting effects that represent the behavior of the facial skin texture and color after makeup. To follow the behavior of makeup in time, methods based on the determination of changes in the intensity of dermis autofluorescence excited by UV irradiation [63,64] can be used. The principle consists of measuring the fluorophores

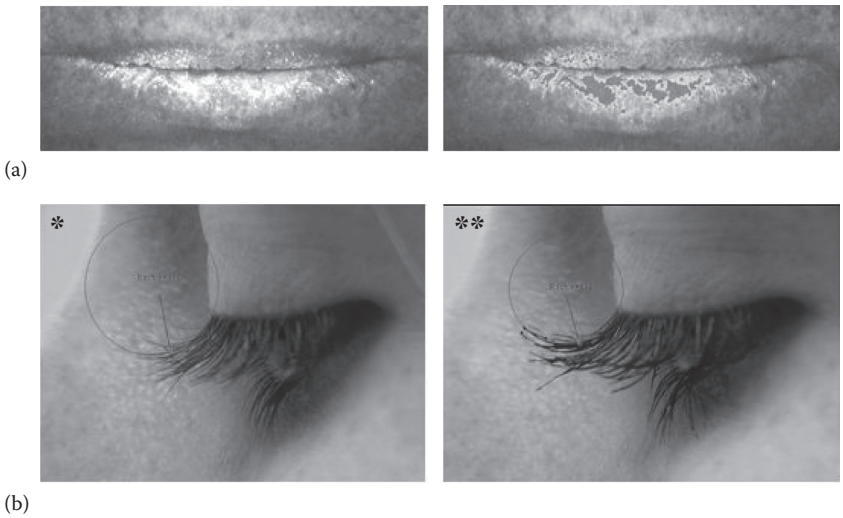

FIGURE 41.4 Video imaging approaches. (a) Quantification of the degree of chapped lips (VISIA's UV photography). The hyperkeratotic surface of the lips is detected, and pixels contained are quantified by image analysis. (b) Curving effect of Mascara expressed by the radius of curve of the lash (in pixels): (*) before makeup and (**) just after makeup.

of the skin, such as collagen and elastin [65], and following over time the skin's autofluorescence response in order to estimate the quality of the physical and chemical barrier created by makeup. The more stable skin autofluorescence is over time, the more made-up skin will keep all these properties. The long-lasting effects can also be evaluated by colorimetric approaches well known in cosmetic [66] and image analysis. In this domain, multiple lighting systems (VISIA-CR, Canfield Imaging System, USA; VISIOFACE, Kourage & Khazaka, Germany) appear very interesting because besides conventional photos, special UV flashes for fluorescence images or different polarizations offer a wide range of optical proofs of cosmetic efficacy. As an example, thanks to facial UV reflectance images and quantification of UV spots (melanin coagulates below the skin surface), the behavior of facial makeup products can be estimated. As shown in Figure 41.5, an unstable made-up surface over time will be characterized by an increase in the number of UV spots. The radiance of the complexion is also a major expectation in makeup effects because that is the most visible component of young and healthy skin. But the skin radiance is complex to explore in a scientific way because there is no universal definition, and each individual perceives it in a different way because it often includes physiological and psychological attributes.

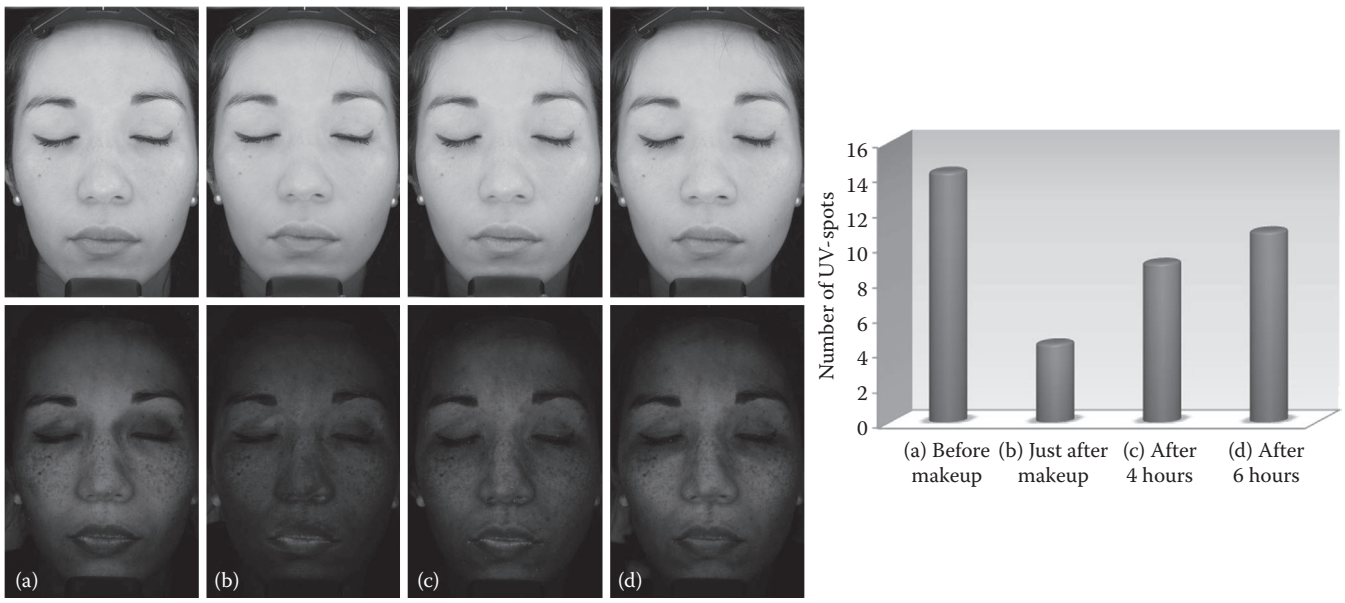

FIGURE 41.5 Study of the long-lasting makeup effect by using facial UV reflectance images (VISIA-CR UV flash mode). Quantification of the number of UV spots (a) before makeup, (b) just after the makeup process with foundation, (c) after 4 h, and (d) after 6 h.

Then, what can we consider as parameters characterizing the skin radiance? How can we rigorously quantify these parameters? If the literature argued that the main factors affecting the skin complexion are the skin color, surface texture, luminosity, and mainly, microcirculation, which gives the skin a rosy appearance [67–69], it is essential to consider their interactions in the demonstration of the skin radiance perceived. Indeed, skin radiance is a psychophysical parameter that involves quite complicated surface and internal qualities of the skin, and it involves more than simply the quantity of light that is reflected from the skin. By taking into account all these elements, we have developed in our laboratories a method based on a multidisciplinary approach in order to obtain skin radiance information closer to the consumers' perception [70]. More recently, after several validation steps with a dermatologist, this method has been upgraded, and a new mathematical equation combining images from the VISIA-CR imaging system and several new algorithms has been proposed (publication in progress).

CONCLUSION

The human face communicates an impressive number of visual signals, and all these signals, modulated by cosmetics, have to be evaluated: first of all, to judge their performances once applied on the skin surface, and second, to prove the effects claimed on cosmetic products. If in these two domains, qualitative and quantitative assessments (consumer's perception, expert evaluation, and instrumental data) are often used, the complexities of human beauty and the science of attractiveness suggest that we associate psychophysiological and cognitive approaches. Indeed, the role of facial cosmetics is not only restricted to simple aesthetic functions, generally seen as superfluous or trivial ones. Cosmetics are part of behavioral and psychosocial dynamics acting on dimensions such as self-esteem, social adjustment, social interactions, or nonverbal communication.

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42 Removal Methods and Evaluation of Removal of Makeup Products

Nattaya Lourith and Mayuree Kanlayavattanakul

INTRODUCTION

Skin appearance is a significant factor that impacts self-esteem and also contributes to physical well-being and positive social relationships [1]. The application of facial cosmetics results in young and healthy-looking skin that is of particular importance in social interactions [2] and when predicting overall attractiveness [3]. Therefore, the application of cosmetics, in particular, makeup cosmetics, aims to increase positive social attractiveness [4].

Makeup cosmetics have been formulated and developed for facial decorative effects. The history of this cosmetic type can be tracked back to ancient Egypt, where it played an important role in religious functions. However, a complete understanding of ancient makeup is rarer than perfume. The corresponding data described the probable composition of oils and/or resins and powder pigments in makeup cosmetics [5]. Today, makeup application is a statement associated with youth [6] that regulates the pleasure of attraction [4] by manipulating one's appearance using a variety of facial makeup products. Higher standards and positive attraction are achieved by makeup application, as evidenced by research into tipping behavior that showed that waitresses who used facial cosmetics [7], particularly those who wore red lipstick, gained higher tips [8]. A full facial makeover contributed the most to female attractiveness in rating scores by males and females. The second most popular makeup cosmetics application that enhanced the perception of attractiveness by men was females who wore foundation, whereas women preferred the use of eye makeup products [4]. Thus, facial makeup undoubtedly encompasses the perception by the individuals and others.

MAKEUP COSMETICS CATEGORY

Makeup cosmetics are primarily categorized into base and point makeup [6,9].

Base makeup is mainly applied on the face and includes face powder and foundation.

1. Face powder is used to adjust and brighten the skin tone, resulting in a transparent feeling with a reduction of sebum secretion. Additional ultraviolet (UV) protectors are usually added.
2. Foundation is applied to change the facial color as desired and make the skin more vibrant and transparent. Foundation is composed of oils/fats, waxes, fatty acids and esters, alcohols, surfactants, hydrocarbons, plasticizers, thickeners, water, and inorganic powders as base materials. The coloring agents may be organic and/or inorganic materials with pearly pigments. Foundation is the primary makeup product routinely applied to hide blemishes or defects on the face.

Among base makeup cosmetics, foundation is of importance as it is a base product with versatile functions that is applied before the point makeup cosmetics. Foundation is proposed to hide fine lines, provide a smooth and flawless skin surface, and even out skin tone. Furthermore, antisolar activities and moisturizers are normally added to enhance the light protection and hydration of the skin underneath the foundation. These functions contribute to the wearability or longevity of the subsequent point makeup application. Foundation is available in a variety of formats, for example, liquid, cream, cake, stick, and powder. Among the commercial liquid foundations commonly sold over-the-counter are water-, emollient- and mineral-based formulations, in addition to silicone-based formulations that improve the efficacy of the foundation.

Foundations with different shades of color have been developed to meet the requirement to match skin tones that are differentiated by both ethnic origin and living style. The preference of foundation in the makeup strategies of women was studied by L'Oréal among different ethnic groups using their own choice of cosmetics that were monitored by a chromameter. African American women used liquid foundation mainly to cover up blemishes. A foundation shade that was darker than the skin tone was used for the covering effect. Therefore, these women preferred foundation shades that were at least as dark as their darkest skin area. Furthermore, mixing several products was their usual practice, to more satisfactorily fulfill their makeup preference for redder tones. In contrast, Hispanic American women selected their preferred foundation based on the lightness, darkness, or redness of their skin. The divergent preference of this group reflected their different ethnic origin and further affected their makeover practice. Meanwhile, American and European Caucasian women consistently applied foundation to slightly darken their skin tones. The preferred foundation tone of Asian women was comparatively determined by studying

representative Japanese women. These women used foundation to improve the evenness of their skin by choosing the cosmetics that make skin lighter and more yellow [10].

Point makeup is differentiated based on its application to lips, cheeks, and eyes.

Lipstick

Cream lipsticks have high pigment contents, providing the coloring effect of the lips with additional moisturizers, UV protectors, and antiaging agents. Sometimes, lipstick is mixed with frost or glitter to provide a glamorous effect. This smooth and shining lip care product differs from matte lipstick, which is flat and without shine. Matte lipstick is formulated with a dense pigment and claims a long-lasting wearing effect. The application of lipsticks should provide the longest-lasting effect while maintaining dermatological safety, which includes nontoxic fragrances to pleasantly cover the base odor of the lipstick. Furthermore, the shine and persistence of lipstick is a currently popular consideration. The applied colored lipstick may be coated with lip gloss, followed by the application of lip liner to define or enhance the lip shape or reshape the lip as desired.

Rouge

Rouge or blush can be formulated in creams, powders, and gels. This highly pigmented makeup is used to color the cheeks and define the cheekbones. However, rouges are most commonly in powder form.

Eye Makeup

Mascara

A mascara cake is the most widely used mascara that is applied with a brush with no clumps. Liquid mascaras, particularly nonwaterproof products, are prone to microbial contamination [11], although preservatives are added. This contamination can occur at a slower rate for a single user compared to a greater rate when there are multiple users. Because of this drawback to the consumers' safety, multiple-user mascaras are rarely formulated and used currently.

Eye Shadow

This point makeup is formulated and sold in a variety of formats, including creams, powders, gels, liquids, and pencils or pens. Eye shadow in any form is developed to be a water-resistant product to improve the product's wearability. However, liquid eye shadows, which require a wand or brush for application, are prone to deterioration, which results in allergies and lower consumer preferences.

Eyeliner and Eyebrow Makeup

Although eyeliners and eyebrows can be formulated in several formats, similarly to eye shadow, the pencil formats are important because of the current application practice.

More details and information on the makeup products and their ingredients are extensively addressed in other chapters of this book.

Advanced research is leading to the formulation of water-resistant makeup products that have excellent moisture-holding capacities with good resistances against sweat and sebum even during workouts or swimming. The preference is for a makeup product that prolongs the wearing period without the need for reapplication. In addition, those products that claim to be waterproof typically have to be removed using makeup remover [12].

These preferred characteristics of facial coloring products in turn make them difficult to be cleaned using a typical cleansing product that employs detergent surfactants. Therefore, specific makeup cleansers, commonly called makeup remover, have been developed and are available in cream or liquid formats or are absorbed onto pads, wipes, or cotton balls for conventional cleansing practices. Bilayer makeup removers are composed of water and oil phases that are vegetable, mineral, or silicone based.

MAKEUP REMOVER

The advanced development of makeup cosmetics enhances their duration of wearing, including addressing the water resistance and cleaning strategies for greasy skin obstructions when the products have finished their daily propose. The difficulty of makeup removal, which leaves behind cosmetic residues, in turn causes drawback effects. As discussed in this chapter, the discussion on makeup removal is focused particularly on foundation and lipstick because of their prevalent role in social attraction [4,8].

The general practice in makeup removal may begin with using a tissue to wipe off the makeup, which may damage the skin because of the tissue texture and scrubbing motions. Furthermore, this method incompletely removes the applied makeup, and cleansing products are critically required to rinse the oily materials and pigments remaining from the facial decorative products. A lipophilic cleanser is typically used. However, other hydrophilic ingredients remain on the skin surface. Therefore, a combination of oil- and water-soluble materials should be considered and mixed into a remover product to enhance the cleansing efficacy.

EVALUATION OF THE REMOVAL EFFICACY

Acceptability or Sensory Test

Sensory evaluation by means of a questionnaire is extensively presented in claims for cleansing efficacy. The acceptability test is a score rating on the basis of a hedonic system of 1 to 4, 1 to 5, or 1 to 8, depending on the protocol of the cosmetic firms [13–16]. The score is determined based on a visual evaluation and skin feeling in a comparison with commercialized benchmark products, with image scoring either before or after cleansing [13]. In addition, the resulting fresh skin feeling following the application of the cleansing

product with barely any oil left on the skin contributes to the remover's preference and, accordingly, to the acceptability score [14].

However, an efficacy evaluation using human volunteers may face several obstacles, particularly in the reliability of the panelists and the availability and variation in the budget of different cosmetic companies. Therefore, an in vitro evaluation during the course of the development of the makeup remover is more feasible, and results from different laboratories can be better compared than a preference test before the product launch. The reliability of instrumental evaluation is consequently challenged and worthy of development.

Instrumental Evaluation

Instrumental evaluations are therefore adopted, among which the chromameter is largely employed. The evaluation of the removal efficacy mainly relies on a color measurement with respect to the central function of removing the pigmented product in either foundation or point makeup.

Therefore, the cleansing efficacy of foundation, lipstick, and mascara are discussed subjectively in this article as representatives of base and point makeup cosmetics.

REMOVAL METHOD AND EVALUATION

Foundation Remover

Several formulations are currently used in foundation remover. A liquid crystal foundation remover, which works by dissolving and dispersing the oily materials, was formulated, and an efficiency was claimed for the practice of wiping and rinsing off as tracked by a skin image analyzer. The product was applied on the forearms (1.25 mg/cm^2) and rubbed with the remover (400 mg) for 25 s prior to rinsing with water. For the skin image before washing, the amount of residues covering the skin surface was taken into account. The cleansing ability was more obviously exhibited by visual scoring [13].

The experiment can be conducted on human skin either on the forearm or on the face. A clinical study would be unpractical in the course of product development, although a sensory evaluation can be conducted alongside product development. Despite the preferred characteristic of using a lipid on the skin surface, which is an important key in the makeup remover formulation, skin hydration can be observed at the same time as the cleansing ability. The cleansing ability was assessed by means of a chromameter. The foundation was applied to an area of leather (120 cm) that was stored for 6 h before being cut into a small square (1 cm^2) and macerated in a remover (50 mL) for 10 min while shaking. The brightness (L) of the artificial surface before and after applying the makeup and the cleansing processes was used to calculate the cleansing ability according to the following equation [14].

$$S = \frac{(L - L_{FD})}{(L_0 - L_{FD})}$$

where

S = cleansing efficacy
L = brightness after cleaned
L_0 = brightness of bare leather
L_{FD} = brightness after foundation application

However, the colorimetric method mainly tracks the colorant ingredients in the foundation. The color residue after the foundation was cleansed from the surface was compared with the bare surface. However, the residues of the chromophore ingredients that are colorless were not determined, casting doubt on the accuracy of the cleansing efficiency. Furthermore, the accuracy and precision of the protocol were not reported. Therefore, a validated cleansing ability of foundation remover is needed and should be proposed as an accurate protocol to be used in the development of makeup remover.

UV-Vis spectrophotometry is generally used in the quality control of cosmetics, is widely implemented in the cosmetic industry, and is an appointed technique for quantifying cleansing ability. This method was validated and offers a simple, rapid, accurate, precise, and inexpensive technique for the routine evaluation of the makeup remover efficacy in vitro. A liquid foundation (35 mg) was applied onto a glass plate (9 cm^2) with the addition of a remover product (two drops) prior to wiping the plate with a stack of four cotton sheets (6.25 in^2). The protocol found that ethanol was a specific solvent that could extract the cleansed foundation and remove oil with a linearity of 0.9977 over a range of foundation density of 0.540 to 1.412 mg/mL and recovery of 78.59%–91.57%. The method was accurate, and its precision was confirmed with relative standard deviation (%RSD) of 0.59%–1.45% [16]. This spectrophotometric method is therefore reliable and able to evaluate the efficacy during the course of foundation remover development.

Mascara Remover

Mineral oil and/or wax (15%–25%) mixed with isoparaffin (5%–40%) was formulated and patented as a mascara remover with a cleansing efficacy claimed by means of a color measurement on the basis of the Commission Internationale de L'éclairage (CIELAB) system [15]. A heavy, uniform coverage of mascara (0.5 g) was applied on the volunteers' inner forearm (3.5 × 2.5 cm), and the preliminary color (C) was measured by the chromameter and recorded. The mascara application used a spatula or a brush and was left to dry for 12 min, after which a second color measurement was conducted (M). A known remover was applied onto the target skin site with a circular rubbing motion for 20 s before being wiped off with a tissue, and the color (R) was recorded and the efficacy was calculated as follows.

$$\text{Removal efficacy (\%)} = \frac{\sqrt{(L_M - L_R)^2 + (a_M - a_R)^2 + (b_M - b_R)^2}}{\sqrt{(L_M - L_C)^2 + (a_M - a_C)^2 + (b_M - b_C)^2}} \times 100$$

Although this chromatometry method predominantly monitors the color residue after the cleansing, some chromophore ingredients are neglected. In addition, the accuracy of this colorimetric method was not mentioned.

Lipstick Remover

The removal efficacy of lipstick cleanser was tracked by using a chromameter [17]; therefore, the removal ability was focused on monitoring the colorant residues. The color of a white rectangle (3 × 2 cm) plastic plate was measured and recorded as a baseline (*A*). A known weight of lipstick was then applied onto the plate, and the color measurement was recorded (*B*). Then, 0.2 mL of 10% of the makeup remover was applied on the plate by gently rubbing for 20 s followed by a rinse of tap water with a fixed flow rate and temperature for 20 s. The plate was left to air dry before the final color measurement (*C*), and the cleansing efficacy was calculated as follows.

$$\text{Removal efficacy } (\%) = \frac{B - C}{B - A} \times 100$$

Similarly to the other study that was based on a chromameter, the accuracy was not addressed. Furthermore, the remaining colorless compounds remained undetermined.

PROSPECTIVE MAKEUP REMOVER

In addition to the cleansing efficacy of the remover, other factors must be verified. To formulate makeup remover with good quality, while meeting the preference and expectations of consumers, some requirements must be met.

BASIC REQUIREMENTS OF MAKEUP INGREDIENTS

The physicochemical properties of makeup ingredients in either form were comprehensively summarized, and the reader should consult the work of Schlossman [18]. Of these properties, the safety of the colorants is the prime property of concern, and the requirements of the food, drug, and cosmetics (FD&C) and drug and cosmetics (D&C) must be appropriately met. However, additional properties resulting from novel ingredients in current makeup products are encouraged.

Makeup cosmetics may be formulated using organic and inorganic colorants such as pigments, lakes, or dyes of natural and/or synthetic origins. However, new colorant candidates for makeup application are in high demand, especially naturally derived colors and ecological colorants. Therefore, the color stability of natural colors must first be evaluated, and their other functions that are adoptable for cosmetics, that is, biological activity and chemical activity, should also be evaluated. The CIELAB system is generally used to monitor the color stability under different concentrations, temperatures, and pHs, and UV spectrophotometer tracking is used to monitor the chemical stability based on the λ_{max} and absorption pattern [19,20].

Several makeup products are formulated in powder form. as addressed previously. Therefore, the flowability in terms of the bulk and tapped densities, Hausner ratio, and Carr index are required [21], in addition to the sun protection efficacy in terms of the sun protection factor (SPF), UVA/UVB, critical wavelength, and Boots star rating [22]. Moreover, some herbal powders have also exhibited colorant properties [21].

MULTIFUNCTIONAL MAKEUP REMOVER

Removal efficacy can be tracked by spectroscopy techniques such as the use of a chromameter or UV spectrophotometer. However, a skin assessment following the use of makeup remover should be done to ensure the product's safety and evaluate the suppression of skin friction caused by the product following cleansing [23]. The potential parameters for an adequate makeup removal product are summarized as follows.

Moisturizing Effect

Skin hydration can be used to evaluate the safety of the product in that higher water loss from the skin will be noticed if the skin is irritated. Furthermore, skin moistening is additionally required for more beneficial condition of skin to compensate for the moisture removed by the cleansing product. Therefore, several moisturizers are included in the composition of makeup remover. Thus, it is worthwhile to monitor their moisturizing effect. The skin water flux or transepidermal water loss (TEWL) is measured by an evaporimeter, models of which include the Tewameter (Courage & Khazaka) and AquaFlux (Biox). The skin water content is determined on the basis of either conductance, that is, Skicon (IBS), or capacitance, that is, Corneometer (Courage & Khazaka).

Skin Surface Lipids, pH, and Temperature

The cleansing of the skin surface by any product solubilizes the skin surface and the epidermal lipids that work as a skin barrier and regulate the skin permeability. An oily skin feeling after the application of makeup remover decreases the preference for the product. Cleansing with surfactants obviously reduces the greasiness of skin. However, overremoval of the skin surface lipids leads to the friction of the skin, suppressing the skin barrier. On the other hand, cleansing with an oil-based cleanser leaves an oily skin feeling. Therefore, the balancing of the skin lipids with the removal of makeup adds to the product's preference. The greasiness of the skin can be determined by the transparency of a lipid-coated medium. Plastic strips are a commonly used opalescent medium, which include Sebutape (CuDerm), DualTape (Cortex), and Sebumeter (Courage & Khazaka). In addition to the skin surface lipids, the pH and temperature of the skin are other parameters indicating the homeostasis of the skin that consequently alter skin hydration and erythema. These parameters are tracked by the Skin-pH-Meter and Skin-Thermometer (Courage & Khazaka), respectively.

Scaliness of Skin

Skin scaliness occurs once the skin pH and temperature are altered, reducing the skin's lipids and hydration. The dryness of the skin consequently leads to skin wrinkles. This can be traced initially by the skin scaliness, and further, severe dry skin can be monitored in terms of skin flakes. A skin image analyzer is used to monitor the skin scaliness and flakes, that is, the SEsc parameter in Visioscan (Courage & Khazaka). Severe dry skin that causes skin flakes can be easily determined by a noninvasive technique using D-squame (CuDerm).

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43 Skin Radiance Measurement

A. Jeudy, S. Mac-Mary, J.M. Sainthillier, T. Lihoreau, F. Fanian, and P. Humbert

INTRODUCTION

The term "complexion" has always attracted attention; it had to be white from the antiquity to contemporary times, and then tanned from the 1920s. Nevertheless, we are at the beginning of a new era: a tanned complexion is no longer fashionable; a young homogeneous complexion without imperfection is now the preferred option. Today, the essential preoccupation is not so much the color of the complexion but its texture, which should evoke youth, health, and happiness [1].

The term "radiance" refers to shine and light. A vivid red is a red that shines. Radiance also defines what arouses admiration, attention, glitter, and magnificence.

In fact, the radiance/complexion is the mirror of general health, both physical and psychological. A gray skin, a "blotchy complexion," may reflect bad looks because of great fatigue, weariness, hormonal status, emotional states (sadness, stress, etc.), illness (dysfunction of vital organs such as the liver), nutrition deficiency or excess, tobacco, alcohol, pollution, seasons [2–6], etc. Inversely "peachy-colored skin" reflects a smooth, pink, and velvety skin and good health. The complexion plays essential psychological and social roles [1].

Skin radiance is referred to without any precise definition and quantifiable appreciation data. All the parameters that constitute it and their relative proportions are difficult to list. Authors showed that the perception of aging is highly influenced by the skin tone uniformity [7], and that the perception of a skin visual appearance for its age involves the large-scale inhomogeneities in Chroma, the light-diffusing property of skin, and the large-scale inhomogeneities in lightness [8].

The skin radiance seems to be a balanced mixture of color, itself mostly influenced by the skin microcirculation, of light reflection, and more globally of texture of the skin surface [1]. Its study therefore implies the quantification of each of these components.

This is in agreement with Fink and Matts [9], who showed in 2008 that the surface topography cues had a significant influence on facial age perception and that the absence of facial discoloration is a key indicator of health, two attributes that are probably most influential in the evaluation of human facial attractiveness.

METHODS

When the light meets the skin surface, a small quantity (about 5% [10]) is reflected directly while the major part penetrates the different layers of the skin. The specular light gives its luminous aspect, whereas the back-scattered light (Figure 43.1) gives the complexion a peachy-color. In other words, specular reflection (depending on the nature and state of the surface of the skin) explains the glow, absorption phenomena (depending on the skin's chromophore content) explain its hue, whereas diffusion phenomena (depending mostly on the collagen content) define its saturation.

The skin color depends mainly on the melanin as well as the hemoglobin concentrations and distributions. The melanin (brown) absorbs all wavelengths, but this absorption decreases considerably from purple to red, which makes the melanin look like a mixture of gray (global absorption) and yellow (significant absorption of blue). Hemoglobin looks red because it absorbs selectively the green and therefore retrodiffuses a light where this complementary color is missing.

The importance of the distribution of color was observed by Matts et al. [11], who found that melanin evenness had a stronger effect on perceived age, health, and attractiveness than hemoglobin in female skin. Similarly, Fink et al. [12] found that hemoglobin distribution in males was associated more strongly with health and attractiveness perception, whereas the evenness of melanized features was a stronger cue for age perception.

The hemoglobin plays an important part in the skin radiance, which is usually perceived as "pink" skin. An indirect way to assess the radiance is consequently to study the skin microcirculation.

Assessment of Skin Microcirculation

The hemoglobin transports the oxygen inside the red globules: if there is not enough oxygen, the face will be gray and dull. If the microcirculation is effectively stimulated, the light will more easily reflect off the red blood cells at the source of the skin color. The skin microcirculation is therefore an important factor of the dull complexion:

- Under stress, catecholamines are delivered in the skin, inducing vasoconstriction (and thus skin pallor) [2,14,15].
- With tiredness (lack of sleep and/or intensive activity), the body reacts by sending more blood to vital organs and the cutaneous microcirculation is "sluggish" [6].
- With pollution and smoking, the microvessels lose their colors (grayish aspect of the skin) [4–6,16,17].
- With aging, papillar capillary loops disappear, inducing a loss in dermal nutritional vessel density and surface area for exchange [18].

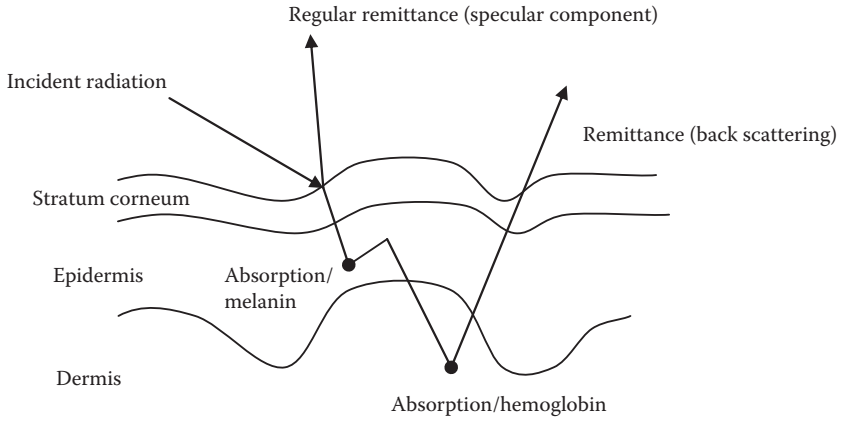

FIGURE 43.1 Optical pathways in the skin (the layer is so thin in the stratum corneum and the epidermis that its contribution to remittance other than specular is minimal over the entire visible and near-infrared regions [13]).

Among the existing available techniques, capillaroscopy and videocapillaroscopy allow direct visualization of the capillary network in vivo. After depositing a drop of oil to enhance skin transparency, an optical magnifying system is used to visualize its vascular network. Different levels of magnification can be used to assess the surface of the microvasculature structure as well as its color (Figure 43.2) [19,20].

Colorimeters

The most logical way to assess skin complexion is to study its color directly. Taylor et al. [21] described in 2006 the different techniques available. Among them, reflectance spectroscopy, chromameters, and narrowband reflectometers should be the most frequently used. The latter are dedicated to the assessment of erythema or pigmentation (by measuring more specifically the skin chromophores) [22,23].

The color variations of the skin surface can be measured by reflectance spectroscopy using a spectrocolorimeter (CM2600D, Konica Minolta Sensing) or a chromameter (CR400, Konica Minolta Sensing). These devices have sensitivities corresponding to those of the human eyes, but the measurements are performed in standardized conditions using the same light source. The Commission Internationale de l'Eclairage (CIE) has defined the spectral characteristics of several types of typical illuminants (the D65 corresponds to average daylight and is usually used as the reference) [24].

The results are expressed in the standard L*a*b* system (CIE LAB 1976 [25]). The L* in this space corresponds to the luminance axis (0 for the black and 100 for the white) characterizing the separation between bright and dark; a* and b* form the other two color orthogonal axes, with a* evolving from green to red (usually selected for the study of redness) and b* from blue to yellow (study of pigmentation).

The skin radiance can therefore be described by a*, which corresponds to the redness, and L*, which defines the luminosity. However, additional information can be extracted in order to qualify the skin color heterogeneity (color difference metric DeltaE = $[(L_1-L_2)^2 + (a_1-a_2)^2 + (b_1-b_2)^2]^{1/2}$ [26]) and/or vividness (chroma = $((a^*)^2+(b^*)^2)^{1/2}$) [24]), which are key factors in the problems of a dull complexion.

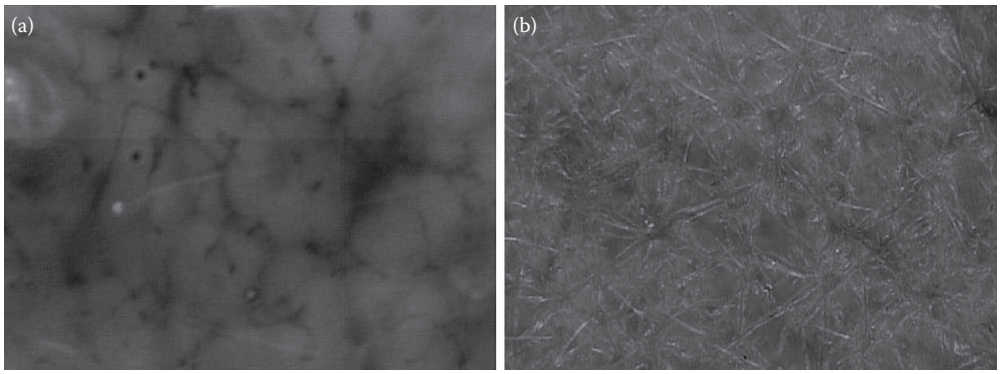

FIGURE 43.2 (a) (7 mm^2) magnification of 100× allows one to quantify the vascularization network (arrows show the capillary loops and the vessels) of the skin, whereas (b) (28 mm^2) a magnification of 50× allows one to quantify the skin redness.

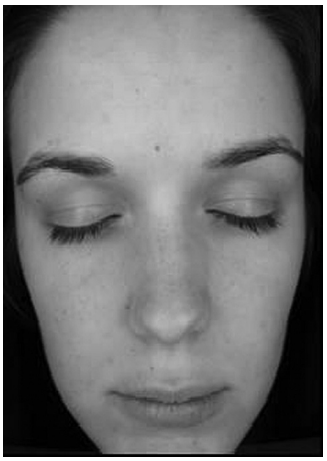

FIGURE 43.3 Photography taken in an integration sphere, with a homogenous and symmetrical light.

To conclude, videocapillaroscopy and colorimeters assess color hue, saturation, and heterogeneity directly or indirectly. They can be usefully combined with standardized photography, which allows one to illustrate and even quantify it.

Photography

Once calibrated and standardized, photography offers many applications to study skin complexion. It appears more appropriate to study texture or color heterogeneity than the glowing aspect because of the influence of the light. The image can be easily disturbed by brightness induced by the flashlight or by the environment. The nature as well as the positioning of the light source and the posture of the subject are of major importance and must be taken into consideration to avoid visual artifacts (Figure 43.3).

Polarized light photography provides more information than usual in terms of heterogeneity of structure. Its principle consists of differentiating the regular reflectance (glare) from the light "back-scattered" (complexion) from within the skin. The regular reflectance contains information related to the surface relief/shininess and the other one to the color. A filter is placed in front of the camera and another one in front of the flashlight. When the orientation of the filters is perpendicular (cross polarization), the regular reflectance is blocked and only the back-scattered light from the tissues reaches the lens (revealing the complexion [skin redness or paleness, color heterogeneity, etc.]) (Figure 43.4), whereas when they are parallel (parallel polarization),

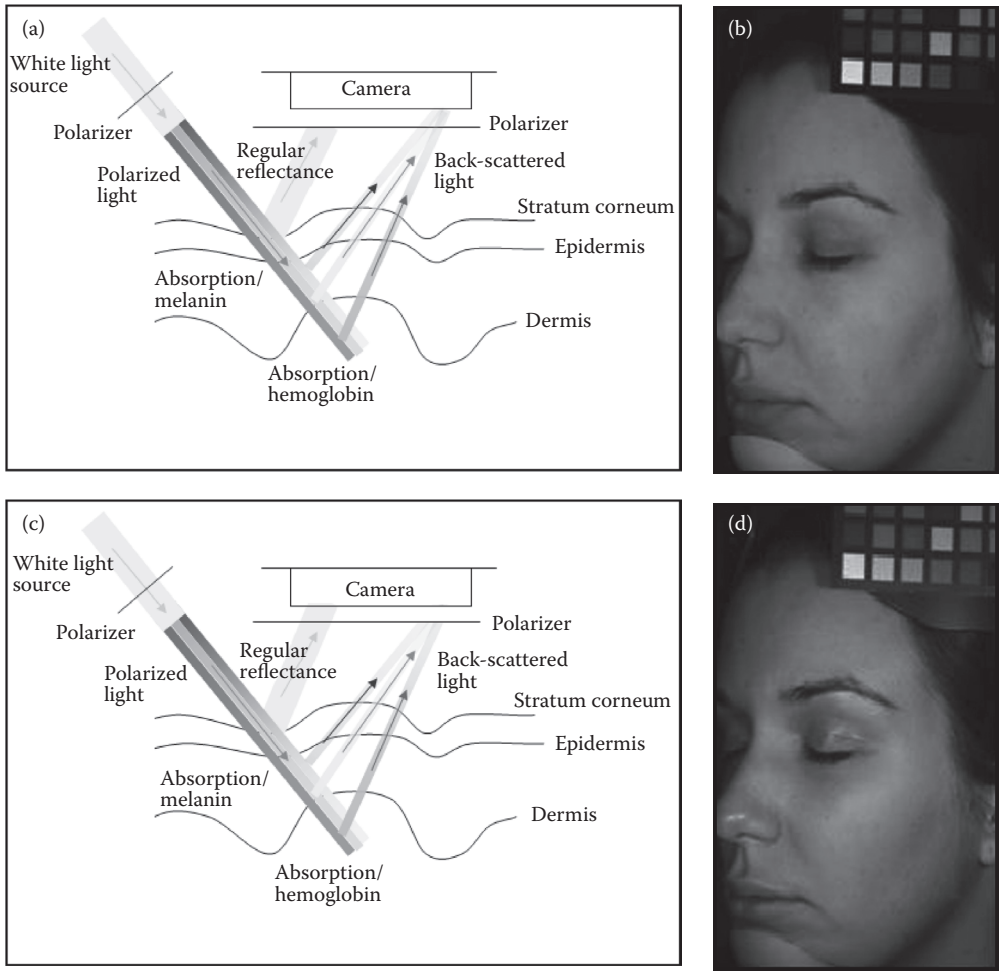

FIGURE 43.4 Simplified schema of the principle of polarized light photography. (a) With cross-polarization, only the back-scattered light reached the lens (revealing the complexion (b)). With parallel polarization (c), only the reflected polarized lights pass (revealing the shininess and relief of the skin (d)).

only the reflected polarized light passes through the lens filter, and details of the surface are enhanced (wrinkles, shininess, scaling) [17,26–28].

Imperfections, which can be revealed by parallel polarization in photography, can be directly assessed thanks to profilometry.

Assessment of Skin Relief

Radiant skin is usually considered as smooth, homogeneous, and without imperfections. Although innate, the microtopography of the skin is affected by the environment as well as aging and undergoes changes in quantity, depth, and direction [29,30].

Profilometry (mechanical, transparency, or optical) initially developed for microtechnics has been applied for many years to study the skin relief [31–34]. From 2000, devices using fringe projections specifically dedicated to the skin have been created (DermaTop, Eotech, France, and Primos, GFM, Germany). Interestingly, this in vivo technique allows one to study the skin imperfections via the assessment of the volume and depth of the wrinkles and in particular the roughness and heterogeneity of the microrelief (dilated pores, comedones, fine wrinkles, etc.) (Figure 43.5).

Assessment of Skin Ability to Reflect Light

The relief of the skin produces different image contrasts depending on the brightness level of the surface (the same relief appears more easily in the case of a bright surface). When the skin surface is irregular, it absorbs light with difficulty and reflects it in many directions. This is particularly typical of dry and scaly skin. The assessment of the specular component provides direct information on the glare of the skin. Sandoz et al. have underlined the influence of the surface bidirectional reflection distribution function measurements (BRDFs) for the visibility of the skin surface [35,36]. The light intensity directed toward the observer's eye is proportional to the coefficient of reflection of the surface. The behavior of bright and matt surfaces is different since the BRDFs are different for the direction of observation (Figure 43.6).

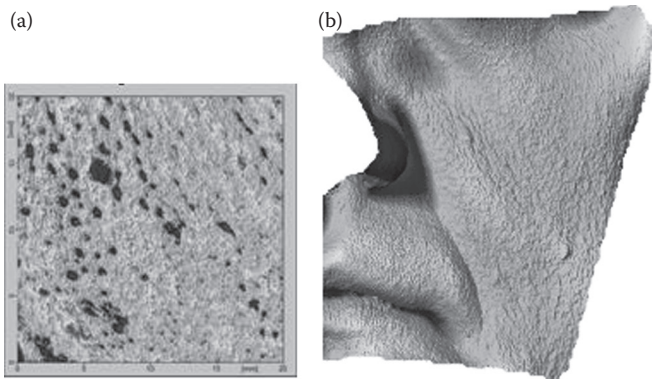

FIGURE 43.5 3-D reconstructions obtained from the cheek (20 × 20 mm area) allowing one to detect dilated pores (a) or from hemiface (60 × 80 mm area) (b) allowing one to quantify the roughness/heterogeneity of its relief.

From this principle, a specific device has been developed in Besançon [36,37]. Its aim is to assess the quantity of light reflected, which depends on the brightness (Figure 43.7). It consists of a system that illuminates an area with white light through an objective at one end of an optic fiber. The light reflected is measured by imputing a profile of intensity of light for various angle values. It is generally admitted that radiant skin tends to act as a mirror, that is, to reflect rays in a specular manner, and dull skin tends to diffuse light more. This instrument has shown its good sensitivity and specificity to characterize the dull to glowing radiance of the skin [37].

Based on this principle, Courage and Khazaka have developed the Glossymeter. In the probe head, parallel white light is created by the LEDs and is sent via a mirror in a 60° angle onto the surface. The glossymeter measures the portion of directly reflected light via a mirror, which is related to the gloss and the scattered portion from the surface by a measure of the diffuse reflected light vertically above the surface.

The SkinGlossMeter developed by Delfin Technology measures the specular light from skin using a built-in 635-nm red semiconductor diode laser.

Gillon et al. [38] have also developed a specific contactless device (brillanometry). Its principle consists of directing a polarized light beam perpendicularly to the skin surface and assessing the reflected beam in the same direction.

The application of systems of polarization of the light currently used in photographs gave the opportunity for Bossa Nova Technologies to develop a system called SAMBA a few years ago. It evaluates the gloss by assessing the scattering properties of the skin or the hair [39].

Recently, the Orion concept developed GonioLux, a device that measures light intensities reemitted in all directions of space. Parameters are expressed as a volume or quantity of reemitted light in different directions of space (total quantity, specular, diffuse, left and right reflection).

Finally, no instrument can quantify today the skin radiance globally, but there are devices that allow one to assess its different components.

With contrast to the color, the glow remains difficult to quantify. The development of simulation systems would perhaps be one solution [40]. Many terms are usually employed to characterize the skin radiance: shininess, brightness, glow, glare, gloss, etc. Shininess, brightness, and gloss refer to the reflection of light to something and are more frequently used to characterize seborrheic skin or to assess the effect of makeup on lips. Glare seems to evoke a shine with a dazzling light, whereas the glow associates an intense color with a slight shine. The glowing aspect of the skin has thus to be differentiated from its brightness, which is the characteristic of greasy skin and is inaesthetic. If gloss is what women expect for their hair [39], glow is a better representation of radiant skin. All these notions show a real need to define the skin radiance more accurately.

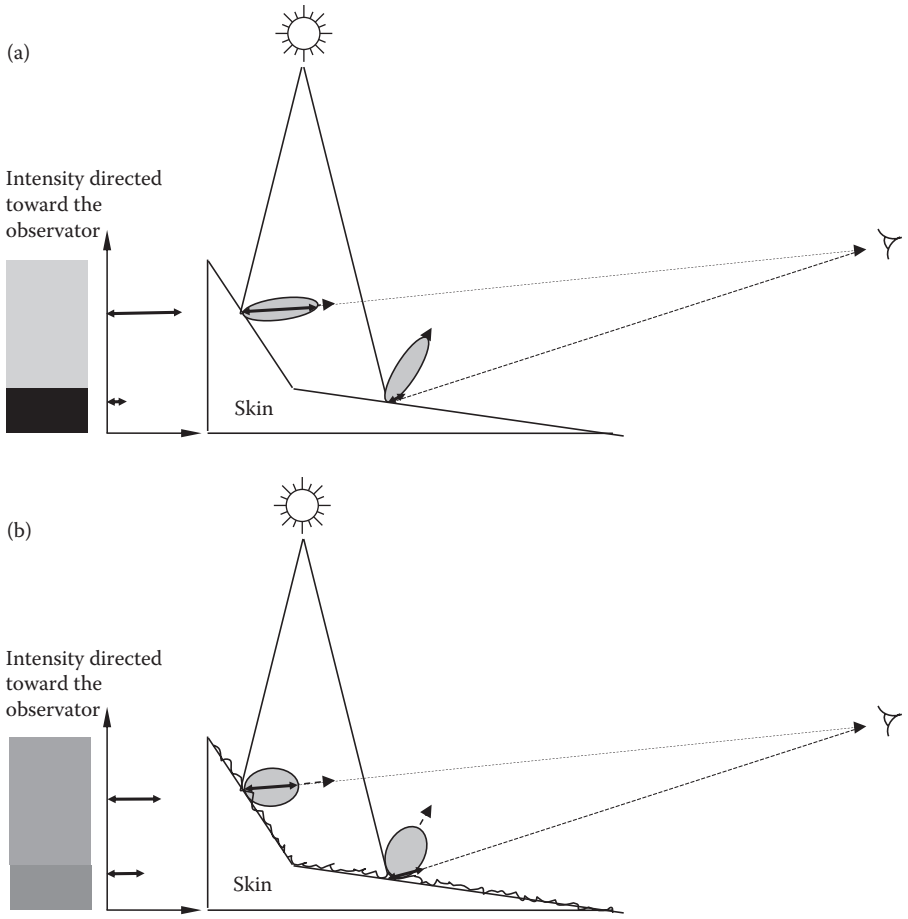

FIGURE 43.6 Simplified schema of the visibility of the skin relief: the contrast is given by the amount of light reflected in the direction of the observer. The perception of a wrinkle will be modified by the quality of the skin surface. If the skin is bright (a), the wrinkle will be more contrasted and more visible. If the surface is matt (b), it will be more blurred. ⬭ wider reflection cone ↑ specular component of the light ▰ intensity of the light perceived = simulation of the contrast of the skin perceived by the observer.

Clinical Scoring

In 2006, Baret et al. [28] organized a round table meeting with women. As a result of these exchanges, many items (including the skin grain, brightness, and color) were selected and studied in volunteers of different age groups from image analysis of video data of their face. Experts performed clinical scoring and then divided the volunteers into two groups: the young one, whose radiance was considered more dependent on skin luminosity and color ("rosy color"), and the old one, who was mainly characterized by irregular skin surface aspects and color variations.

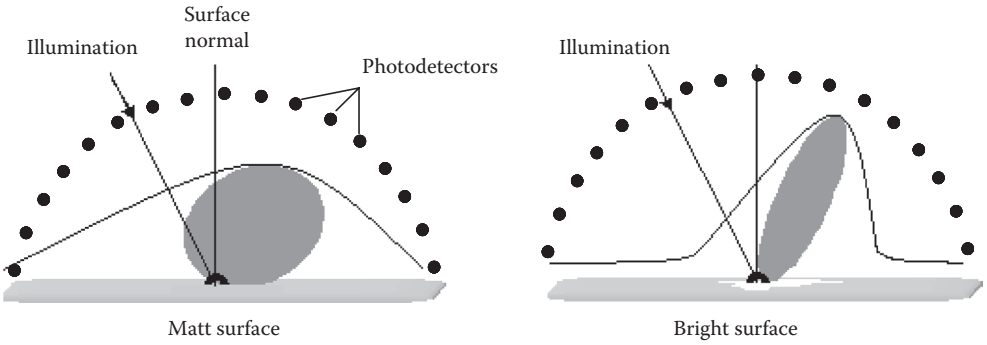

FIGURE 43.7 Principle of the instrument developed for BRDF measurement on the skin. Photodetectors distributed in the place of incidence sample the angular distribution of the reflected light. BRDF of a matt surface distributes the reflected light in a wider cone of diffusion (dull complexion). A bright surface concentrates the reflected light in a narrow cone of reflection (glowing complexion).

These definitions corroborate the idea that radiance is multifactorial and naturally include an important subjective part.

As previously mentioned for photography, it is of great importance to perform the scoring in controlled conditions. The subjects have to be seated between "daylight" lamps and to wear white clothes as well as a white paper cap to avoid any influence from extrinsic colors, and to stay at rest in a temperature-controlled room [41].

In 2004, Musnier et al. [41] organized a brainstorming session with several beauticians to develop a model of sensory evaluation of the skin radiance. One hundred volunteers were also interviewed to self-appraise their complexion. The synthesis of the data allowed one to determine four skin coloring descriptors and to construct a model "C.L.B.T.", based on the visual perception of skin color (C), luminosity (L), brightness (B), and transparency (T). Recently, this score has been adapted to Asian skin [42].

Clinical scoring offers the possibility of evaluating the different parameters implicated in skin radiance.

CONCLUSION

Facing the increasing importance of appearance, consumers wish to reflect the image of their inner well-being represented mainly by a beautiful skin and healthy looks [39]. The cosmetic industry has perfectly understood this concept and developed specific products designed to enhance the complexion, illuminate the skin, or fight off the detrimental effects of pollution.

Several options are available to improve dull complexion. Most of the products currently on the market claim an action on the skin texture. They act either by optical effects with the help of pigments, or by a vitamin contribution to the antioxidant properties, or with exfoliating active principles that attenuate the imperfections and smooth the skin, making the complexion more glowing. Others claim an action on the microcirculation. The products often associate several of these effects.

All these data confirm that the assessment of skin radiance implies the study of the geometry of the light reflection as well as the interaction between the light (interaction with surface/glow) and the color (interaction within the skin/complexion). However, skin relief seems to be, therefore, one of the key factors of skin radiance [1].

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44 Anticellulite Products and Treatments

André O. Barel and Peter Clarys

INTRODUCTION

Cellulite is a localized condition of subcutaneous fat and connective tissues with the typical visual appearance of the orange-peel look of the skin. Cellulite, or more correctly, gynoid lipodystrophy (GLD), affects mostly women and, rarely, men and is considered as a common aesthetic problem for many women. Cellulite appears generally after puberty and worsens with age. There are preferential places of cellulite: buttocks, thighs, upper part of the arms, knees, and more rarely, the lower parts of the legs and the back of the neck. It is interesting to note that these preferential cellulite sites are areas in which the typical pattern of adipose deposition is observed. Although cellulite may be found in areas with excess adipose tissue, obesity is not necessary correlated with the presence of cellulite. It is known that that men and women have a different connective tissue organization at the first layer of subcutaneous fat. With females, the adipose tissue is contained in chamber-like structures (septae) that favor the expansion of adipose tissue onto the dermis. On the contrary, men have a network of criss-crossing connective tissue architecture, forming smaller spatial units, which allow for subcutaneous fat deposits to expand more laterally and internally but with little herniation into the dermis. Furthermore, men have thicker epidermis and dermis tissue layers in the thighs and buttocks than females.

Important review articles concerning the etiology of cellulite have been published in the past by Nünberger and Müller [1], Curry and coworkers [2,3], Di Salvo [4], Draelos and Marenus [5], Barel [6], Rosenbaum et al. [7], Piérard et al. [8], Rossi and Vergnanini [9], and Goldman [10].

Hormones, specifically estrogens, are thought to influence the formation of cellulite. Estrogen is known to stimulate lipogenesis and inhibit lipolysis, resulting in adipocyte hypertrophy [8]. This may explain the onset of cellulite at puberty, the condition more prevalent in females, and the augmentation of cellulite with pregnancy, nursing, and estrogen therapy (oral contraceptive use and postmenopausal hormone replacement). For recent review articles, see the following references: Avram [11], Quatresooz et al. [12], Rawlings [13], Terranova et al. [14], Goldman et al. [15], Warner [16], Barel [17], Goldman and Hexsel [18], Warner and Avram [19], Khan et al. [20], Draelos [21], Al-Bader et al. [22], Kravitz and Achenbach, [23] and De la Casa Almeida et al. [24].

The aims of this chapter are to describe

1. Etiology: the histological, physiological, and biochemical characteristics of subcutaneous lipodystrophy
2. The different objective evaluation methods of lipodystrophy
3. The different anticellulite treatments available and their efficacy

Etiology of Cellulite

Cellulite is probably a multicausal condition, and many hypotheses have been proposed regarding the origin of fat lipodystrophy [1–10]. The term *cellulite* refers to a localized complex skin disorder caused by the herniation of subcutaneous fat into the dermis resulting in structural alterations of the surface of the skin that are visually characterized by a dimpling appearance of the skin (*orange-peel surface* of the skin). The exact causes of cellulite remain poorly understood; however, studies have shown that a number of mechanisms are involved in the pathophysiology of this skin condition, including changes in the connective tissue structure, the structure of subcutaneous fat, the extracellular matrix, the microcirculatory system, and perhaps also inflammatory alterations, hormonal and genetic influences, as well as other cellulite aggravating factors including stress, weight changes, and aging [11–13,15,16,18,20–25].

Histological Structure of Subcutaneous Fat

There is sexual differentiation in the histological distribution of subcutaneous fat lobules in women and in men. The differences between the sexes can be found in the structure of the septal connective fat tissue. The anatomy of cellulite can be clearly seen from the studies of Piérard et al. [8]. The superficial fat lobules (papillae adiposae) that protrude into the dermis can be clearly seen in autopsy sections of the skin. In women, one observes a higher percentage of septae perpendicular to the skin surface and a smaller percentage parallel to the surface as shown in men. Furthermore, in women with cellulite, deep indentations of adipose tissue into the skin were recorded. Using in vivo high-frequency ultrasound imaging, Querleux et al. [25], Lucassen et al. [26], and Nuijs and Van Herk [27] confirmed an irregular dermo-hypodermal interface in women with cellulite. Mirrashed et al. [28] and Querleux [29,30] confirmed by magnetic resonance imaging (MRI) the existence of indentations of adipose tissue into the dermis. Also, observed in women with cellulite, was an increase in the thickness of the inner fat layer, a higher percentage of septae in the direction perpendicular to the skin surface. Since cellulite is widely present in women, some

authors consider cellulite as a secondary sexual characteristic. Although cellulite is not always synonymous with being overweight, there is clearly a relation between cellulite and hypertrophy of fat tissues.

Vascular Modifications

These consist in alterations in the microvascular network (mostly venous blood circulation) in the fat tissue leading to a venous stasis [2,3,10,11,15,20,21]. The superficial microcirculation appears to be less efficient, and this results in subcutaneous edema because of the altered permeability of blood vessels and the presence of plasmatic exudate in the subcutaneous connective tissue. This edema is probably a noninflammatory symptom. Increased interstitial fluid protein concentrations and interstitial pressure have been reported, provoking reduced blood flux into the tissue culminating in decreased skin temperatures on affected sites [13]. Furthermore, some sources have suggested that cellulite is a lymphatic disease, with limitations of fluid movement and lymph drainage, which may contribute to the appearance of cellulite [2,3], but there are no scientific publications to support this hypothesis [23,29,30]. In addition, other authors have mentioned an inflammatory basis for its pathophysiology [6,11]. Furthermore, alterations in the reticular fibrillar network surrounding the blood vessels and adipocytes are observed. This fibrosclerosis provokes stiffening and decrease in mobility of fibers. Also, alterations in the interstitial fundamental substance (proteoglycans) are reported [2,3]. GLD is probably associated with chronic venous problems. Venous insufficiency shows typical symptoms such as possible presence of telangiectasias, heaviness in the legs, cramps in the lower limbs, pain on deep palpation of the skin, and irregularities in skin surface temperature as detected by thermographic examination.

Alterations in the Matricial–Interstitial Unit Surrounding the Fat Cells

The matricial–interstitial unit is formed by fibroblasts (synthesis of macromolecules of the cellular matrix), collagen, elastin, and reticular fibers, and by the ground substance (proteoglycans, glycoproteins). Alterations in the structure of the glycosylaminoglycan (GAG) in the perivascular tissues provoke hyperpolymerization and an increase in their hydrophilicity and the interstitial osmotic pressure: edema and hypoxia [2,3,31]. Also, suggested in the case of cellulite was the hypothesis of an increase in the concentration of GAGs, presumably leading to a rise in the amount of water retained in the skin. Querleux et al. [25,29,30] did not confirm the hypothesis of increased water content in the adipose tissue of women with cellulite, except if such water would be located in the connective septae. Modifications in the structure of the proteins of the cellular matrix are observed: alterations of the fibers are followed by sclerosis [2,3]. Finally, the hypothesis that cellulite is an inflammatory process resulting in breakdown of the collagen in the dermis, providing for the subcutaneous fat herniations as seen on ultrasound, has not been confirmed experimentally [5,11,21].

Predisposing Factors

A genetic predisposition factor plays an important role in the development of cellulite [8,32]. Recently, Emanuele et al. [33], identified in a multilocus genetic approach two susceptible genes for cellulite, one coding for angiotensin-converting enzyme (ACE) and another coding for hypoxia-inducible factor (HIF).

Aging seems to be also an aggravating factor [34]. Ortonne et al. [35] have demonstrated that a female population with cellulite presents earlier skin aging characteristics than the control group without cellulite. More controversial is the observation that Caucasians tend to have more cellulite than Asians. Also, it appears that Latin women develop more GLD on the hips and thighs than Nordic women. The sale of anticellulite creams and treatments is far more popular in Mediterranean countries. A nonbalanced diet with excessive intake of fats and carbohydrates provokes the hypertrophy of fat tissues. A sedentary lifestyle contributes to the aggravation of cellulite, and wearing tight clothes makes venous return more difficult [6]. Furthermore, a synergism of smoking and the ACE/HIF polymorphism on the risk for cellulite was recently reported by Stavroulaki and Pramantiosis [36]. Other coexisting disorders (hormonal, circulatory, metabolic, gynecologic, nephrotic, and gastrointestinal) may be important and contribute to the development of cellulite.

Modifications and Hypertrophy of Adipose Tissues

Although cellulite is not always synonymous with being overweight (some lean persons could present symptoms of cellulite), there is a relation between cellulite and hypertrophy of fat tissues [11,13,21]. In many studies, there is confusion between obesity and cellulite (although adipose volume is clearly an aggravating factor for cellulite). Patients confuse weight gain with the appearance of cellulite, and many commercial anticellulite treatments are in fact slimming treatments. As a consequence of this confusion, the use of anthropometric measurements is widely applied to measure the efficacy of the various antiweight treatments: circumference measurements of the hip and both thighs.

As a conclusion, it must be mentioned that some authors (e.g., Terranova et al. [14]) have clearly emphasized the limitations of the above-described theories. It appears that adipose tissue does not play a significant role in the onset of cellulite: it participates only as a pure inert physical function, producing mechanical tension through its hypertrophy. Today, we know that the adipose organ performs complex functions by acting as a system controlling the systemic energy balance, by modulating the food intake and the metabolism of other tissues, and as a glandular system for multiple hormonal secretions. It is known that adipose tissue is able to modulate the blood flowing through it and can secrete numerous substances with the power to regulate the

activity of the endothelial cells. In conclusion, these recently identified properties of the adipose tissue are also involved in the pathogenesis of cellulite.

DIFFERENT OBJECTIVE EVALUATION METHODS OF CELLULITE

Description of the Different Stages of Lipodystrophy of Fat Tissues

The typical symptoms of cellulite, namely, orange-peel skin surface can be observed visually by the subjects themselves and in a more objective way by trained investigators. There is the presence of the typical orange-peel skin upon normal visual examination and/or after pinching of the skin. Table 44.1 shows the different grades of cellulite from 0 to IV.

Based on visual or digital photographic pictures, a grade scale of 0 to 4 at rest or after gluteal contraction has been proposed by Rossi and Vergnanini [9] and Curri and coworkers [2,3], whereas using digital photography, Perin et al. [37] used a scale of 0 to 7 after a standardized compression of the thigh area using a slide mechanical system. It is difficult to detect cellulite by visual examination and by palpation at the first stages: at grade I, orange-peel skin is not permanently present, only visible after pinching the skin. The clinical symptoms are clearly more visible at later stages of cellulite: permanent orange-peel, colder skin areas, diminution in mobility of fat tissue upon palpation, and increased skin sensibility [6].

Skin surface contact thermographic pictures using thermographic foils give an indication of the degree of cellulite, as the skin surface temperature patterns correlates to some extent with the clinical symptoms of cellulite. On the basis of these thermographic patterns and clinical symptoms, Curri and coworkers [2,3] confirmed the visual classification of cellulite in four stages. Stages 1, 2, and 3 of lipodystrophy are not considered clinically as pathological symptoms but considered more as aesthetic–cosmetic problems of the skin. Only in stage 4 are some clinical symptoms, such as increased skin sensitivity, extensive fibrosclerosis of connective tissue, and very advanced edema, considered as light pathology symptoms. Furthermore, it is believed that the first stages are more or less reversible, whereas the latter stages are almost irreversible and consequently very difficult to treat [6].

TABLE 44.1
Different Grades of Cellulite

Grade 0	No cellulite
Grade I	Vertical standing position, minimal cellulite based on visual observation of the thigh and buttock without or with gluteal muscle contraction, without or with pinching of the skin
Grade II	Irregular skin topography, cellulite present, enhanced by gluteal muscle contraction and by pinching
Grade III	Typical orange-peel dimpling, cellulite clearly present even without pinching or gluteal muscle contraction, small nodularities present on palpation
Grade IV	Same symptoms as grade III but more severe, more palpable nodules, and irregular skin surface temperature pattern

Sources: Curri, S.B., and Ryan, T.J., Panniculopathy and Fibrosclerosis of the Femal Breast and Thigh, in Ryan, T.J., and Curry, S.B., eds., *Cutaneous Adipose Tissue*, Lippincott, Philadelphia, 1989. Rossi, A.B., and Vergnanini, A.L., *J. Eur. Acad. Dermatol.*, 14, 2000.

Objective Evaluation of the Symptoms Of GLD

There are a variety of physical and pharmacological anticellulite treatments ranging from topical products to oral food supplements or regimens, from manual to mechanical massage, laser, infrared light, continuous or pulsed radio frequencies, and so forth. As a consequence of this, there is a need, in addition to visual evaluation of cellulite, for accurate, sensitive, noninvasive bioengineering methods for the quantitative evaluation of the degree of cellulite, particularly at early stages. Furthermore, noninvasive bioengineering measurements are necessary for the objective evaluation of the efficacy of various cosmetic treatments [11,38–45]. However, the clinical grading of cellulite based either on direct visual examination or macrophotography of the orange-peel skin remains very important since the visual evaluation is more closely related to the consumer's considerations and expectations.

The different noninvasive bioengineering measurements are as follows:

- Macroscopic digitalized photographic pictures of the skin surface
- Contact skin surface thermographic measurements using liquid crystals
- Ultrasonic skin analysis of skin density; measurement of thickness of the hypodermis at 10 to 14 MHz and measurement of the surface of the interface between dermis and hypodermis at 20 MHz
- MRI
- Skin surface topographical imaging and fringe projection analysis

Digital Photographic Pictures

The relief of the skin can be evaluated by taking digital macrophotographic pictures under standardized experimental conditions [43,46]. The pictures must be taken according the following guidelines: constant and reproducible vertical position of the subjects on a stand system, constant illumination of the skin surface, neutral background, and fixed position of the digital camera. These photographic pictures are then graded visually using numerical scales in a double-blind manner by expert independent reviewers for the intensity of cellulite and the efficacy of various anticellulite treatments [43–45]. These digitalized macrophotography pictures of the external part of the thighs can be taken normally or after application of a pinching system around the thigh to increase the orange-peel look of the skin surface [37].

Skin Surface Contact Thermography

The principle of the encapsulated cholesteric liquid crystal contact thermography consists of color plates presenting a pattern of different colors corresponding to skin surface temperature. Application of the color sheet with uniform pressure on the skin surface and photographic recording of the thermographic pattern using a camera can be made. A qualitative and quantitative global analysis of the thermographic pictures in relation with the different stages of cellulite can be carried out [38,39,46,47]. A cellulite-free skin surface thermography shows a uniform color pattern without hypothermic and hyperthermic areas. A cellulite skin surface thermography shows a nonuniform color pattern with the presence of hypothermic (cold spots) and hyperthermic (warm spots) areas. Quantitative analysis of the thermographic pictures can also be carried out by image analysis. Computerized color image analysis gives the mean temperature of the thermogram, and, respectively, the number and the percentage area of the hypothermic and hyperthermic areas present on a well-defined skin area. One observes large interindividual variations in skin surface temperature and a long acclimatization time for temperature equilibrium of the skin (influence of external temperature). This method remains a qualitative or semiquantitative testing of cellulite at different stages.

Ultrasonic Imaging of the Skin

High-frequency ultrasound C-mode imaging (10–20 MHz) appears to be the most interesting method. This noninvasive method has been frequently used in research for studying the epidermis, dermis, and hypodermis [45,48–52]. Different authors have used the technique of the measurement of the thickness of the subcutaneous fatty layer using ultrasound imaging at 10, 14, or 20 MHz [45,48–52]; however, the determination of the echographic borderline between subcutaneous fat and connective tissues/muscles is very delicate. As a consequence, the determination of the mean thickness of the hypodermis is not very accurate. The interface between the dermis and the subcutaneous fat can be measured using ultrasound imaging at 20 MHz [26]. The interface between the echogenic epidermis–dermis and the hypoechoic subcutaneous fat is clearly visible, allowing measurements of skin thickness and the surface of this border. In normal cellulite-free skin, the interface between the dermis and the fat tissue is rather smooth. In skin with cellulite, this surface is not smooth and very irregular due to herniation of the fat layer. The surface of this interface is quantified and can be used as a measure of the degree of cellulite.

Measurement of Skin Surface Topography

Cellulite skin surface presents irregularities (orange-peel skin). The classical skin surface roughness measurements, which are used in cosmetic research, could be applied for studying cellulite [41,53–56]. These involve stylus profilometry, image analysis by shadow method, optical focus laser profilometry, and fringe projections analysis. The roughness parameters can be directly measured on the skin or through the use of silicone replicas. Quantification of the skin surface macrorelief involves a computerized correction for the curvature of the thigh skin surface with cellulite. Actually, the skin surface topography of skin with cellulite can be easily evaluated using 3-D map topography from fringe projections (GFM Bodyscan Primos System) [56].

MRI

Recently, high-resolution MRI data were published [25,28,29], allowing investigation of subcutaneous adipose tissue in men and in women with and without cellulite. As previously mentioned in this chapter, MRI is very efficient for measuring the thickness, surface, and volume of the adipose tissue. In women with cellulite, an increase in skin thickness and presence of deep indentations of adipose tissue into the skin were noticed. Unfortunately, because of the high cost and limited accessibility of this instrument, this promising technique will not be available for cellulite research for most laboratories and cosmetic companies.

TREATMENTS OF CELLULITE

Different reviews concerning the possible treatments of cellulite have been published: Smith [57], Draelos and Marenus [5], Barel [39], Avram [11], Goldman [10], Brewster [40,44], Rawlings [13], Biefeldt et al. [43], Hexsel et al. [58], Bazela et al. [45], and Kravitz and Achenbach [23]. The importance and the use of noninvasive bioengineering techniques in order to quantify the efficacy of various anticellulite products has been pointed out in these review articles.

Diet and Physical Exercise

Dietary influences. The theory that diet contributes to the pathophysiology of cellulite has been popularized by the women's press. Articles stating that a low-carbohydrate, low-fat, low-salt, high-fiber diet can minimize cellulite are abundant in these public journals [21]. A controlled medical study to verify the effect of diet on minimization of the appearance of cellulite has never been conducted and published. But probably, a low-calorie diet corresponding to low carbohydrates and lipids may decrease adipose tissue and extracellular fluid volume and consequently minimize vascular effects [11].

Some investigators have observed that cellulite is more common in overweight obese women [3]. These authors suggest the presence of copious fat lobules within the subcutaneous tissue encased in fibrous septae with dermal attachments, leading to the rumpled appearance of the skin typical of cellulite [21]. Thus, weight loss, which reduces the size of the fat lobules and decreases the influence of excess adipocity, improves the appearance of cellulite [8]. A low-calorie diet and exercise are excellent ways to start improving cellulite

appearance [11]. It has been shown that visible cellulite is reduced in females who lose weight [59]. A calorie-restricted diet plan combined with a comprehensive exercise program (cardiovascular exercise and resistance training) should be implemented to reduce some of the underlying body fat. Histological research (examining the microscopic structure of tissue) shows that the fat cells do retract slightly from the dermis with weight loss [13].

Physiotherapeutic Treatments

The physiotherapeutic treatments (massage), laser and pulsed light treatment, topical application, topical application under occlusion, topical application combined with massage treatment, and oral food supplement are disponible to treat cellulite. In this chapter, only a few of these numerous therapies that have been advertised in order to treat cellulite will be described.

Physiotherapeutic treatments such as deep manual or mechanical massage stimulate the blood and lymph microcirculation and increase the removal of the extra fluid in the adipose tissues. In addition, these massage techniques will retard the further development of fibrosclerosis and the aggregation of fat cells in nodules [5,13,23,60]. These physiotherapeutic treatments can be combined with the topical use of anticellulite dermato-cosmetic products (during massage or premassage or postmassage).

Endermology is a suction massage technique developed in the 1980s in France. This type of electrically powered device has a two-roller system with a suction device created by a vacuum system. The surface of the skin is pulled into the machine and kneaded by the two revolving rollers. Claims of the Endermologie apparatus are that it modifies fat tissue structure, makes changes in dermis connective tissues, and improves lymphatic drainage and capillary circulation [61–63]. When this deep mechanical massage is applied two to three times weekly over a few months, it will induce changes in the connective tissues and subcutaneous fat layer and perhaps diminish the degree of cellulite. However, upon reviewing the very little published research on Endermologie, the suction device with roller seems not to fulfill its claims [64]. Despite the multitude of therapeutic approaches, there is little scientific evidence assuring the efficiency of such a low vacuum. A majority of the evidence is anecdotal, subjective, or based upon patient self-evaluation. Only a few anticellulite treatments are validated using noninvasive bioengineering measurements to quantify the degree of cellulite. Using a similar commercial massage and suction device (Cellesse Philips), Lucassen et al. [26] were able to show a temporary decrease of cellulite after 3 months' treatment (twice weekly massage sessions). The degree of cellulite was quantitatively evaluated by macrophotography of the skin surface followed by rating the grade of cellulite as carried out by three independent experts, ultrasonic determination of the dermis–subcutaneous fat layer interface, and 3-D skin surface roughness by fringe projection.

Photothermal therapy has also been considered in the past as anticellulite treatment [65]; also, the effect of volumetric cellulite tissue heating with radiofrequency was investigated by del Pino et al. [32]. A combination of radiofrequency, infrared light, and mechanical tissue manipulation was used by Alster and Tanzi [66] to treat cellulite.

Recently, two new physiotherapeutic techniques have been developed in the treatment of cellulite [59,67,68]. One technique combines the use of near-infrared laser light of a continuous radiofrequency wave and mechanical suction (two rollers with suction), and another instrument combines near-infrared light with a mechanical massage apparatus. These combined physiotherapeutic instruments seem to improve the symptoms of cellulite.

Topical Dermato-Cosmetic Products

The topical anticellulite products can be divided in different major groups according to their proposed mechanism of action [10,58].

1. Agents that increase microvascular blood flow. Some authors think that alterations in blood flow and lymph circulation play a role in the pathogenesis of cellulite [2,3]. Most of the active ingredients in cellulite treatment are supposed to increase microvascular flow and perhaps also lymphatic drainage. Plant extracts and chemicals that are supposed to act on the microcirculation of the skin include horse chestnut, sweet clover, red grapes, pineapple, *Ginkgo biloba*, Butcher's Broom, *Centella asiatica*, artichoke, and silicium.
2. Agents that reduce lipogenesis and promote lipolysis. The purpose of these products is to reduce the size and volume of adipocytes, to decrease tension on the surrounding connective tissues. As a consequence, a diminution of fat invaginations in the profound dermis is observed, and the clinical appearance of cellulite is reduced. Chemicals that that have a lipolytic effect on adipose tissue include the methylxanthines (theobromine, caffeine, aminophylline, and theophylline).
3. Agents that restore the structure of the dermis and connective tissues surrounding the adipocytes, preventing fat herniation in the deep dermis. Retinol (vitamin A) has been evaluated for its effectiveness in this category as an anticellulite agent.
4. Agents that prevent alterations in the fibrillar connective tissues (stiffening of collagen fibers, loss of elasticity, and alterations in the viscoelastic properties [proteoglycans]) due to aging and free-radical formation. Vitamins (ascorbic acid and vitamin E) may work as antioxidants, as well as *Ginkgo biloba* and red grapes. Antioxidants may protect the dermal and subcutaneous cell membranes and inhibit cellulite formation

In conclusion, the use of various topical products, generally applied with massage, in the treatment of cellulite

and/or for slimming has been known for many years. The most used pharmacological topic agents are xanthines, retinoids, and plant extracts.

Xanthines

Xanthines are common ingredients used in anticellulite products (caffeine, aminophylline, theophylline, or plant extracts rich in xanthines) [4,48,61,69,70]. Xanthines are used because of their proposed effect on adipocyte lipolysis via the inhibition of phosphoesterase, provoking an increase in adenosine mono phosphate (AMP). In vitro metabolism studies on fat cells have shown that caffeine (the most used and safest methylxanthine, normally used at a concentration of 2%) could slow down lipogenesis (uptake of glucose and free fatty acids to synthesize triglycerides) and stimulate lipolysis (degradation of triglycerides and release of the free fatty acids) in different ways. Furthermore, it appears that caffeine increases the level of a class of uncoupling proteins (UCPs) in subcutaneous white adipose tissue adipocytes and may help to reduce the adipose tissue mass.

Lupi et al. [69] have published a 30-day clinical study using a 7% caffeine solution on women with cellulite showing a modest improvement in thigh diameter but no significant effect in intercellular edema and microcirculation. Greenway and Bray [71] demonstrated a significant reduction in anthropometric measurements of the medial part of the thigh after twice-daily topical application for 30 days of a mixture of a beta-adrenergic antagonist, aminophylline, and yohimbine (an alpha-adrenergic antagonist). The effects of methylxanthines can be enhanced by coenzyme A and the amino acid L-carnitine [4]. These chemicals act by stimulating the mobilization of free fatty acids and by inducing their transport through the membrane of mitochondria [58]. On the other hand, a review published by Warner and Avram [19] presents some doubts about the anticellulite efficacy of methylxanthines. A modest decrease in thigh circumferences and subcutaneous fat thickness is reported, but there was practically no significant reduction of cellulite.

Retinoids

The use of topical retinol to improve cellulite was proposed by Kligman et al. [72], and they demonstrated an improvement in cellulite. However, Piérard-Franchimont et al. [73] could not find any change in the orange-peel aspect of the skin surface but did observe an increase in skin elasticity and a decrease in the viscoelastic properties of the skin. Finally, Bertin et al. [74] tested the effects of retinol combined with caffeine and ruscogenin: a decreased orange-peel appearance and improved cutaneous microcirculation were observed. Retinol will be metabolized to retinoic acid. These effects may be due to the known effects of retinoids in the dermis modifying the collagen fibers and the network of elastic fibers. It can be also noted that retinol has an "antiadipocyte" activity.

Plant Extracts

Many botanical extracts are used as topical agents in the treatment of cellulite and in slimming products [75,76]. The following plants were used: artichoke, barley, Butcher's Broom, *Centella asiatica*, fennel, *Fucus*, *Ginkgo biloba*, green tea, horse chestnut, ivy, kola nut, lemon, marjoram, papaya, pineapple, red grapes, strawberry sweet clover, verbena, and so forth.

Using a commercial cream containing various herbal extracts silver terminalia, *Visnaga vera*, Indian coleus, and *Cola* together with cyclic AMP, Perin et al. [37] showed, after 2 months of treatment, a significant decrease in the cellulite score, a decline in the thickness of the thigh subcutaneous adipose tissue as measured by ultrasound, and positive self-perception data concerning smoothing and firming of the skin and silhouette appearance.

Buscaglia and Conte [48] examined the combined effect of caffeine, horse chestnut, ivy, algae, bladderwrack, plankton, Butcher's Broom, and soy protein. After 30 days' treatment, a significant decrease in subcutaneous fat thickness was observed. Plant extracts are also used as topical application under occlusion. A novel approach to topical anticellulite treatments consists of combining the topical application of the plant extracts under occlusion as enhancement (bioceramic-coated neoprene short). Rao et al. [77,78] evaluated anticellulite cream containing black pepper, sweet orange-peel, ginger root extract, cinnamon bark extract, capsaicin, green tea, and caffeine, which was applied under occlusion for 4 weeks. The subjects noticed an improvement in the degree of their cellulite, and the dermatologists who treated the thighs observed a greater improvement compared to the placebo thigh and a decrease in thigh circumference. The use of plant extracts such as *Centella asiatica*, Butcher's Broom, horse chestnut, ivy, *Ginkgo biloba*, witch hazel, white oak, green tea, lemon, orange, cranberry, kola, fennel, algae, barley, strawberry, marjoram, sweet clover, aloe vera, and so forth should be noted. The "active" molecules of these plant extracts are probably flavonoids (rutins, rutinosides) or terpenes (ginkgolides).

These slimming/anticellulite plant extracts present properties of stimulation of the peripheral blood circulation and lymph circulation and inhibit further the fibrosclerosis of the fat-surrounding collagen matrix. Various algae species such as *Fucus vesiculosus*, *Laminaria flexicaulis*, and *Ascophyllum nodosum* are incorporated in anticellulite cosmetic preparations for their hypothetical beneficial effect on the skin surface. There are very few in vivo scientifically reported studies examining the effects of these plant extracts improving the condition of cellulite [58]. The use of anticellulite creams containing various plant extracts seems to be acceptably safe [79]; however, the risk for adverse allergic reaction must be taken into account.

Alpha-Hydroxy Acid

Alpha-hydroxy acids (AHAs), more particularly lactic acid, have been proposed in the treatment of cellulite [57]. At the present time, there are no scientific reports showing any anticellulite effect of the AHAs [13]. However, the AHAs have an antiaging effect (stimulation of collagen synthesis) and improve the signs of photodamaged skin [80], as well

as promoting epidermal differentiation and barrier function [81]. So it is likely that AHAs in conjugation with other ingredients will improve the skin surface orange-peel appearance in cellulite [13].

Oral Treatments

Many of the above-mentioned active ingredients are also used in oral anticellulite or slimming treatments of cellulite, and similar to the topical treatments, there are very few scientifically proven clinical studies reported [13]. The following examples of scientific studies with oral treatments (double blind, placebo controlled) were noticed.

Cellasene is an herbal supplement presenting claims for improving the appearance of cellulite (Cellasene, Medestea, Italy). It contains *Ginkgo biloba*, sweet clover, seaweed, grape seed oil, primrose oil, and lecithins [13]. In one study based on clinical appearance and not on bioengineering methods, the investigators failed to observe any improvement in the cellulite condition after 2 months' treatment [82]. Furthermore, Leibaschoff et al. [83], testing a different Cellasene formulation (replacement of primrose oil by fish oil and borage oil) found improvements in lipoedema and skin muscular fascia diameter, and about 71% of subjects showed improvements in the symptoms of cellulite. Birnbaum [76] compared the effect of an undisclosed herbal anticellulite pill containing also increasing concentrations of conjugated linoleic acid (CLA). The beneficial effects observed in 75% of the women who took the herbal anticellulite pill plus CLA were improvement of cellulite appearance, decrease of thigh circumference, and improvements of the microcirculatory pattern.

Hachem and Borgoin [84] reported the effect of oral supplementation of 60 mg daily during 90 days of taking *Centella asiatica*. At the gluteofemoral region, a significant reduction of the diameter of adipocytes was observed as well as a decrease in interadipocyte fibrosis. These preparations contain mostly various plant extracts and xanthines (caffeine or plant extracts rich in caffeine). The anticellulite food supplements can be used alone or in combination with massage and/or topical creams. The use of plant extracts such as green tea, grape, ginkgo biloba, and *Centella asiatica* are particularly noticed. It is possible that both oral and topical routes may have a synergic effect and may be the best way to ameliorate the symptoms of cellulite.

CONCLUSIONS

Although there are numerous possible topical treatments available, one observes very few double-blind, placebo-controlled published studies demonstrating the efficiency of any of these treatments [11,13]. Furthermore, it is important to note that many of the cellulite treatment studies have been carried out on a relatively small sample of subjects and generally do not follow up the treatment during extended period of time [23]. The methods used to analyze the subjects give results based often on subjective human visual observations rather than objective noninvasive measurements such as digital macroscopic pictures, roughness parameters, and ultrasonic observation of the subcutaneous fat layer. Finally, during anticellulite studies, it is difficult to rule out external factors such as changes in diet and lifestyle when interpreting the results of some studies.

In a critical overview of some published clinical studies, one can make the following remark. In the case of trials where one thigh is treated with the *active cream* and the other thigh is treated with the *inactive cream* as control, improvements are often observed on both sides. One question remains: are the improvements the result of the combined action of massage and the active ingredients or solely the result of the massage?

It must be noted also that many anticellulite or slimming claims are based on in vitro studies and partially in vivo [22,85]. In vitro metabolism studies on adipocytes and fibroblasts have shown that different molecules (caffeine, plant extracts, *Fucus*, etc.) can be considered as active ingredients in order to slow down the synthesis of triglycerides and to stimulate the degradation of triglycerides in the adipocyte. However, when using these active molecules in vivo as anticellulite ingredients, one must take into account the limitations in percutaneous penetration of the active molecules through the skin to reach the hypodermis. For example, caffeine penetrates readily into the skin, but scant information is published about the penetration of these plant extracts.

Another problem is related to the concentration of the active products in commercial anticellulite products. It must be assumed that the concentrations of plant extracts are rather low considering the high cost of these extracts and the potential danger of these plant derivatives as allergens. The possibility of problems of photoallergy and photoirritation must be considered.

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45 Skin Healing
Integrating Scientific Advances into Cosmetic Practice

Ethel Tur and Laura L. Bolton

BACKGROUND

Cosmetic procedures frequently result in acute cutaneous wounds. Acute wounds arise from a brief event recognized as the wound cause, including surgery, physical trauma, and chemical trauma such as a chemical peel or burn. Chronic wounds are generated by unresolved continuing insults. For example, pressure ulcers may occur as a result of damage to tissue by prolonged pressure, friction, and/or shear forces. Venous ulcers occur as cells "drown" in local edema resulting from venous insufficiency. Diabetic neuropathic foot ulcers arise from trauma associated with loss of protective sensation with up to 50% complicated by peripheral arterial disease.[1] Arterial or ischemic ulcers are caused by tissue damage when deprived of the blood supply by peripheral arterial disease.

Wounds caused by cosmetic procedures share the same causative and healing pathways as wounds caused by other factors. Therefore, a lot can be learned about, interpreted about, and applied to these wounds from studies involving all kinds of cutaneous wounds. To heal any wound, acute or chronic, three important steps need to be taken: (1) identify and resolve all causes of tissue damage, (2) remove necrotic tissue or foreign matter that may obstruct healing, and (3) provide a physiological environment for repair.[2] Evidence described below supporting ways to accomplish these three steps is accessible by searching for each wound procedure among international guidelines found at www.guideline.gov, the Web site of the Agency For Healthcare Quality and Research *National Guideline Clearinghouse*. Applying principles, procedures, and modalities with evidence that they function to achieve these goals efficiently is a functional approach to wound *management*[3] that helps wound care professionals heal wounds, not just "care for" them.

PRINCIPLES OF WOUND MANAGEMENT

Healing of acute or chronic wounds can be delayed by a variety of morbid factors. Avoiding or treating these factors is the first step toward healing. These include infection (bacterial, viral, fungal, parasitic); nutritional deficits (e.g., protein, vitamin C, iron, or zinc deficiency); immunologic conditions like sensitization; pharmaceutical agents (steroids, drugs, "recreational drugs"); external conditions like irritation; reactions to physical (heat, cold, light) or chemical (antimicrobial agents, cosmetic formulations) conditions; endocrinologic disorders (e.g., diabetes, thyroid disease); impaired circulation (arterial disease, venous insufficiency); lifestyle (stress, smoking); or genetic abnormalities. The same factors that cause chronic wounds may also cause an acute wound to become chronic, so it is important to be aware of and resolve all sources of tissue damage in patients with acute or chronic wounds.

Removing debris or necrotic tissue is a much simpler step but as important as the first.[4] Foreign matter, such as strands of gauze, suture materials, or debris deposited during trauma can act as a prolonged inflammatory focus and potentiate infection.[5] Nonionic surfactants applied with a force of 4 to 15 lb/in.^{2}, for example, through an angiocath or 19-gauge needle delivered from a 30 cc syringe, can facilitate better removal of foreign matter from wounds compared to saline or fluids delivered with less or more force.[6] Debridement options to remove necrotic tissue include autolytic debridement, for example, with hydrogels and moisture-retentive dressings; enzymatic debridement, using pharmaceutical enzymes; and surgical debridement.[4] Mechanical debridement applying wet gauze, then removing the dried gauze and local wound tissue, is now recognized as substandard practice,[7] no longer recommended because it is painful and indiscriminately removes healthy as well as dead tissue. Further research is needed to evaluate the efficacy of high-powered irrigation and allay concerns that it may force debris or necrotic tissue deeper into the wound. The only form of debridement currently with evidence of superior efficacy compared to another modality, wet-to-dry gauze, is autolytic debridement with hydrogels.[4]

Optimizing the environment for healing of acute and chronic wounds is as old as history. Though ancients like Hippocrates recognized the value of physiologically moist wounds, opinion shifted through the centuries.[8,9] The concept reemerged in 1948, when Oscar Gilje, a Danish dermatologist, first systematically reported the benefits of moist wound healing on portions of venous ulcers covered with tape beneath Unna's Boots.[10] George Winter, a British plastic surgeon,[11] elegantly replicated his findings in controlled studies on partial-thickness swine wounds, laying the foundation for

a multidisciplinary evidence base of hundreds of controlled studies supporting faster,[12,13] less painful,[13,14] more economical[14,15] wound healing with lower likelihood of infections,[16] using moist wound healing as compared to air exposure or saline- or medication-impregnated gauze. Supporting evidence spans all continents addressing acute wounds such as skin graft donor sites[17,18] or burns,[19,20] chronic wounds,[14,16,21] diabetic foot ulcers,[22,23] pressure ulcers,[24–27] radiated skin conditions[28,29] and venous ulcers.[30]

The basic principles of local moist wound management to achieve the major goals of wound care including exudate, odor, and pain management and autolytic debridement are outlined in Table 45.1, which illustrates how to integrate them into a functional, efficient evidence-based local wound care protocol designed to achieve patient, wound, and professional goals for most wounds. Within this functional approach to wound care, the main goals flow from documented wound assessment and patient evaluation, leading to principles and modalities with evidence of safety and efficacy in achieving wound and patient goals identified. In summary, a functional approach to moist wound healing addressing patient and wound conditions and needs can optimize healing and minimize complications such as infection, scarring, and associated pain or odor. Healing outcomes can be improved by addressing patient conditions, such as vascular or endocrinologic function and nutritional status, and by addressing local wound needs. Local wound needs would be attended to by maintaining a physiologically moist wound environment that (1) speeds healing,[13–16,31] (2) minimizes wound pain,[14,15] and (3) reduces the likelihood of infection,[32] and by removing foreign matter and necrotic tissue.[4,33] This functionally integrated approach heals most wounds in a timely and economical manner while optimizing patient quality of life and reducing side effects and frequency of dressing changes.[34,35] In this chapter, we describe the wound healing process and how it is affected by applying these functional principles of wound management to meet patient and wound goals.

PATHOPHYSIOLOGY OF WOUND HEALING

If causative factors are resolved, wound healing progresses in an orderly process, as described in detail by Chin et al.[36] and by Hunt et al.,[37] progressing through hemostasis, inflammation, granulation (if dermis is injured), epithelization, and maturation. Healing begins with *hemostasis* if blood vessels are broken. During the first few minutes after injury, exposure of plasma proteases to extracellular matrix molecules initiates the clotting cascade, which ends by converting prothrombin to thrombin, the proteolytic enzyme that converts fibrinogen to fibrin. The resulting fibrin net traps platelets and red blood cells and plugs the injured vessel. The injured vessel then constricts in response to cytokines released by mast cells, which stimulate local endothelial cells and smooth muscle cells to contract.

Attracted by growth factors (platelet-derived growth factor [PDGF] and transforming growth factor-beta [TGF-β]) released by the trapped platelets, polymorphonuclear leukocytes in circulating blood arrive at the injured site within minutes after injury as the *first inflammatory response*, mainly intravascular neutrophils (polymorphonuclear neutrophils [PMN]).

TABLE 45.1
Functional Approach to Local Wound Management

Evaluation/Assessment to Establish Goals of Care and Measure Progress Toward Those Goals	Principal Goal or Expected Outcome	Local Wound Modality to Achieve Goal
Wound length, width, duration, location, etiology.	Heal fast with minimal scarring and infection.	Moisture-retentive (occlusive) film or hydrocolloid dressing.[32]
Wound-related pain reported on visual analog scale or Likert rating scale.	Identify and resolve source of pain. Minimize intraoperative and postoperative wound pain.	Preoperative topical or systemic anesthesia or analgesia. Postoperative moisture-retentive dressings.[19]
Wound odor reported on visual analog scale or Likert rating scale.	Identify and quell odor source. Prevent, minimize, or contain wound odor.	If microbial source, apply local strain-specific antimicrobial. Use odor-managing dressing.[74]
Foreign matter in wound, e.g., presence and location of debris, gauze strands, or closure materials.	Remove all foreign matter with minimal injury to wound.	Irrigate with nonionic cleansing solution applied at 4–15 lb/ in.^{2}.[5]
Necrotic tissue in wound documented as percent of wound covered with necrotic tissue.	Remove necrotic tissue.	Autolytic debridement using an occluded hydrogel is optimal if feasible. Evidence is needed for enzymatic or surgical debridement options.[4]
Excessive exudate rated as moderate or heavy.	Identify and resolve exudate source.	Alginate or absorbent fiber primary dressing or exudate management modality, with occlusive secondary dressing.[75]
Dry wound, with exudate rated as none or scant or requiring autolytic debridement of necrosis.	Hydrate to provide moist environment	Hydrating gel primary dressing with occlusive secondary dressing.[76]
Clinical infection signs: odor, pain, erythema, edema, or unexplained systemic or local elevated temperature.	Confirm inflammation not due to unresolved chronic wound etiology. Identify and kill source organism.	If microbial source, apply local and/or systemic strain-specific antimicrobial.[74]

Endothelial cell adhesion molecules (CAMs) expressed near the injury initiate and facilitate PMN extravasation. Once outside the blood vessel at the injured site, PMNs phagocytose or ingest local microorganisms, dead tissue, and debris, killing ingested organisms by generating reactive oxygen molecules. They also release packets of lysozymes and metalloproteases (MMPs)[38] to digest dead tissue and debris, serving simultaneously as "clean-up detail" and the body's first line of defense against microbial invasion. PMN numbers peak within 1 to 2 days after injury, so dense populations in any wound over 2 days old may be interpreted as a sign of fresh injury.

The second line of inflammatory defense, monocytes, recruited to the wound site and activated to become macrophages by growth factors released by the trapped platelets, arrive in large numbers after 24 to 48 h. In addition to serving similar functions to PMNs, macrophages release all the growth factors in the right sequence to orchestrate healing, guided and stimulated by cytokines, chemokines, and growth factors released by local platelets. The collective inflammatory response may also include T and B lymphocytes and reportedly may last for up to 2 weeks in normal wounds.

For up to 2 weeks in most normal wounds, the *proliferative phase* of healing ensues. Growth factors released by the macrophages stimulate keratinocytes, fibroblasts, endothelial cells, and smooth muscle cells to repair the damage. Fibroblasts proliferate and migrate through the provisional fibrin matrix, synthesizing and extruding collagen to reconstruct a new though imperfect extracellular matrix of collagen, elastin, glycosaminoglycans, and other molecules. Using actin and myosin to contract like tiny muscles, specialized "myofibroblasts" pull the wound closed and then differentiate into quiescence when these activities are no longer called for. Endothelial cells and their companion smooth muscle cells proliferate to form buds from local vasculature migrating through the extracellular matrix to form and differentiate into new blood vessels that perfuse the extracellular matrix, forming richly vascularized granulation tissue. Proliferating and migrating from local appendages, epithelial cells (keratinocytes) cover the granulation tissue with a new epithelial barrier to protect from invasion and seal in physiological fluids. Communicating with each other and with the extracellular matrix in which they dance, these healing cells repair the lost tissue, releasing and responding to chemokines, cytokines, growth factors, and enzymes that aid and guide their work of healing.

Repairing dermal tissue continues *remodeling* beneath the regenerated epithelium for months to years in the process known as maturation. Cell density and metabolic activity decrease, except if excessive scarring ensues in the form of either a keloid or a hypertrophic scar. Tensile strength increases from 25% that of intact skin as a newly epithelized wound to a maximum of 80% that of intact skin as the new Type III collagen is replaced by more organized, more heavily cross-linked Type I collagen. The maturing scar tissue never becomes as strong as intact skin. Local cells release enzymes, mainly metalloproteases and serine proteases (e.g., elastase released by local PMNs), which facilitate and guide extracellular matrix remodeling as the host *reorganizes* the scar tissue. Locally released enzyme inhibitors usually exert tight control over this process to prevent excessive scar plasticity.

Healing follows a different path if causes of tissue injury, such as repeated trauma, drying, infection, malnutrition, or impaired circulation, persist. Unusually prolonged or increasing granulation, erythema, pain, edema, or exudate may signal infection[39–41] or contact dermatitis[42] and merit immediate attention to allay long-term side effects such as scarring or discoloration.

Such conditions may prolong or reinitiate the inflammatory response so that the wound becomes "chronic," not progressing toward healing in the expected time sequence.[42,43] Each time a wound is repeated, as with repeated chemical or laser treatments required to achieve the desired cosmetic effect,[44,45] the healing process begins anew with inflammation.

Systemic factors like disease, drugs, infection,[46] and even sex hormones[47] may also influence skin healing. Stress was also proven to have an impact on wound healing.[48] Thus, all aspects of the subject's physical, metabolic, endocrinologic, and emotional status should be taken into consideration.

HISTOLOGY PATTERNS OF WOUNDS AND WOUND HEALING

Acute wounds show histologic patterns that reflect their depth typical of their stage of healing. Histology of different levels of chemical peels and dermabrasion on normal and photodamaged skin were characterized by Stegman.[49] Mild inflammatory infiltrates extend several microns below the exfoliated skin of a very light superficial peel or up to 500 μm below surface necrotic tissue in deeper peels. These consist of an immediate influx of neutrophils, followed by a more gradual increase in monocytes activated to become macrophages by 48 h postpeel.

Laser peels increase in width and depth of tissue damage with increasing energy dose,[50,51] which is correlated with clinical improvement of photodamage. Basophilic coagulation of subepidermal collagen accompanies erythema, edema, and discomfort. These symptoms are proportional to increasing fractional CO_2 laser energy density, which is also associated with an increased effect of photorejuvenation.

A burst of epidermal proliferation occurs within 24 h after any level of peel. In superficial peels, basal keratinocytes proliferate, and their offspring differentiate to form a briefly hyperkeratotic epidermis before restoring a normal stratum corneum. After deeper peels, regenerating epidermis proliferates from local appendages and adjacent intact skin. It then migrates across the remodeling dermis, achieving full differentiation with a normal stratum corneum barrier. The time to achieve this is weeks for medium-depth peels and up to months for deep peels. Melanocytes are subsequently restored, usually more uniformly than in previously photodamaged skin.

Fibroblasts proliferate, migrate, synthesize, and extrude a new dermal collagen/elastin extracellular molecular matrix, with fibroplasia lasting several months—depending

on penetration depth of the resurfacing technique.[52] During this time, local endothelial and smooth muscle cells migrate through the new extracellular matrix and populate it with new blood vessels. Degree of organization of collagen and elastin is occasionally used as a measured outcome for skin resurfacing. The medium-depth to deep chemical peel or laser surgery wound will heal in weeks to months, while the dermis may continue to remodel for several months. Increased dermal elastin has been reported 8 weeks after 585 nm pulsed dye laser treatment of striae.[53] During the months following treatment, there is a gradual smoothing of the skin infrastructure.

Acute partial-thickness incisions experience vigorous mitotic responses during the first 7 days after wounding that are briefer and less intense in wounds occluded with a moisture-retentive film.[54] One day after wounding, the epithelium has completely bridged the moist wound but not the air-exposed one (Figure 45.1). Cracks in the eschar covering the air-exposed wound leave an avenue of entry for microorganisms to invade its dermis as well as a dried zone of dead tissue extending 200–250 μm on either side of the original incision. These findings may help to explain the increased likelihood of clinical infection reported in gauze-dressed or air-exposed wounds.[17,19]

Histology of chronic wounds may help clarify another unresolved mystery. While it is often suggested that chronic wounds experience delayed healing due to deficiencies of growth factors or other healing molecules or unresponsive, senescent cells, histology and clinical findings suggest a different interpretation for some common chronic wounds. Healing appears to proceed in some areas, creating a "great amount of granulation tissue" in pressure, venous, and arterial ulcers.[55] This finding suggests that many of these wounds possess intact healing capacity, as confirmed clinically by the rapid healing when pressure or diabetic ulcers are consistently protected from pressure or trauma or as venous ulcers heal with compression adequate to reduce edema.[56,57] While some chronic wound patients may also have inherent nutritional, metabolic, or genetic deficiencies, these would be identified during patient evaluation and addressed as part of the patient's care plan along with resolving recognized causes of the specific chronic wound diagnosed. The clinical implication of these findings may be that adequate growth factors, cytokines, and chemokines are present in many chronic ulcers, and sealing them near the physiologically moist wound surface may help trigger repair as long as the tissue is protected from further insult. Relative healing benefits of active healing agents may become clearer if randomized prospective clinical trials test them against moisture-retentive dressings. Another hypothesis generated by these histologic findings is that chronic wounds may be more accurately described as having "prolonged wounding" rather than "delayed healing," with the clinical implication being that more effective strategies to heal many chronic wounds may be focused on identifying and alleviating causes of tissue injury and using active agents with evidence of healing efficacy superior to moisture-retentive dressings.

CHEMICAL AND LASER PEEL STUDIES

Chemical peels are typically classified as superficial, medium depth, or deep,[42] while lasers generally affect medium-depth or deep dermal tissue and are classified as ablative, fractional, or nonablative.[58] Clinical judgment is required to optimize peel depth for each patient and skin condition, because the extent of epidermal or dermal wounding depends on the following variables: interaction of patient (skin type, lifestyle, medical history); procedural details (skin preparation, application method, duration, repetition frequency); chemical peel formulation (agent, concentration, vehicle); and laser characteristics (wavelength, energy density or fluence, pulse frequency, and duration). Healing time ranges from days for light superficial peels to a few weeks for deeper procedures. Tissue depth and days to heal vary with procedure, site, and postoperative care. Deeper chemical or laser peels usually offer more dramatic cosmetic results but require longer healing time and have a higher risk of complications. Clinical outcomes rated on valid, reliable scales, such as the Glogau photodamage scale,[59] can provide important feedback regarding comparative efficacy of skin resurfacing modalities and enhance professional communication of results.

Side effects include erythema and edema of varying intensity in all resurfaced wounds. The incidence of facial postresurfacing bacterial infections is between 4.3% and 12%,

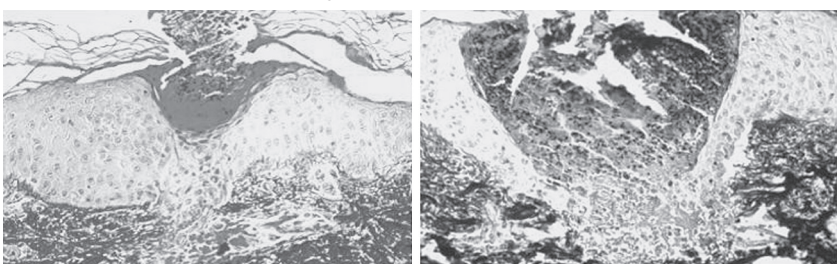


FIGURE 45.1 Histological photograph of human ventral forearm 500-μm-deep incisions, 24 h postoperative, Masson trichrome stain. Left: dressed with a moisture-retentive dressing. Right: air exposed.

and fungal infection rates are seen in 1.8%–2.2% of patients despite the use of prophylactic antibiotics. These are higher than reported for cancer or rhytides scalpel excisions.

Postfacial resurfacing infection incidence with herpes simplex (HSV) was reduced from 3% to less than 1% with acyclovir prophylaxis. It is recommended that an appropriate antiviral dose be administered to patients at risk of HSV infection for 1–3 days before and 7–10 days after facial resurfacing.[60]

Different modalities are used for different conditions.[61] Healing, cosmetic results, and side effects are reportedly similar for 0.350-mm-deep excisions made using CO_2 laser electrocoagulation compared to a steel scalpel[62] and short-pulse CO_2 laser compared to erbium-doped yttrium aluminum garnet (ER:YAG) laser. One treatment with CO_2 laser therapy removed all decorative tattoo pigment, while similar sites treated with infrared coagulation were easier to care for and healed faster but left residual pigment. Similar cosmetic results were achieved when comparing dermabrasion and superpulsed CO_2 laser resurfacing of contralateral perioral rhytides.[63] While the CO_2 laser treatment initiated faster hemostasis, dermabrasion resulted in slightly faster healing, less crusting and pain, and shorter postoperative erythema.

Scanning CO_2 laser surgery elicited more erythema, epidermal loss, dermal fibrosis, and underlying thermal damage in skin on the temple than two passes with a radiofrequency electrosurgery device did,[64] though dermal fibrosis was seen 3 months after both procedures. Higher laser fluence focused on a smaller spot reduced healing time and resulted in more distinct histological edges in cutaneous genital excisions.[65]

MODALITIES THAT IMPROVE WOUND HEALING

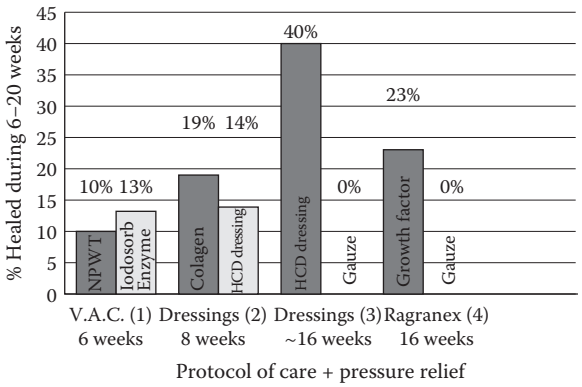
Aids to healing can be divided into procedures or modalities applied before, during, or after the wound is made. Preoperative procedures reported to improve wound healing and scarring have shown promising results.[66] In a blind-evaluated randomized controlled trial, pretreatment injection of botulinum toxin into facial laser exfoliation sites improved cosmetic results similarly for crow's feet areas treated with either CO_2 or erbium lasers.[67] Preoperative topical or systemic vitamin A can reverse healing inhibition typical in patients receiving corticosteroids such as prednisone doses of 40 mg or more given within 3 days preoperatively.[68] Though high-dose corticosteroid pretreatment of skin sites exposed to ultraviolet B prevents sunburn damage,[69] no research has explored preoperative corticosteroid effects on laser or radio frequency dermatologic surgery.

Optimizing the intraoperative procedure can help limit damage to the epidermis, which regenerates without scarring for ideal cosmetic results. In 2008, Alexiades-Armenakas et al.[58] reviewed techniques to optimize laser procedures, limiting their depth to the minimum level needed to accomplish the desired cosmetic or ablative result.

Wound healing progresses more rapidly in a physiologically moist environment that is maintained by moisture-retentive or "occlusive" dressings than when a wound is either air exposed or dressed with gauze (Table 45.2). With all causes of tissue damage resolved, superficial epidermal wounds regenerate without a noticeable scar within 4 to

TABLE 45.2
Healing Time Effects of Wound Depth, Environment, and Continuation of Tissue Damage Comparing Acute to Chronic Wounds

Wound Etiology (Reference)	Moisture-Retentive Dressing	Impregnated Gauze or Air Exposure
Acute Wounds		
Epidermal or nonablative resurfacing[77]	1–3 days	
Epidermal tape-stripped wounds[54]	5–10 days	7–14 days
Partial-thickness incision—epidermis[54]	4 days	7 days
Skin graft donor site[18]	6–10 days	10–15 days
Mid-dermal resurfacing[79]	7 days	>7 days
Shave biopsy[78]	~14 days	~21 days
Burn, second degree[14]	10 days	15 days
Deep dermal resurfacing[79]	30 days	>30 days
Mohs surgical excisions[80]	20 days	26 days
Chronic Wounds		
Arterial ulcer[81]	91 days	
Partial-thickness pressure ulcer[70]	31 days	
Stage II–III pressure ulcers[15]	70 days	84 days
Full-thickness pressure ulcer[70]	62 days	
Venous ulcers[82]	50 days	58 days
Partial-thickness venous ulcer[70]	29 days	
Full-thickness venous ulcer[70]	57 days	



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4. Rees R *et al. Wound Rep Reg,* 1999, 7:141–147. (RCT, p = 0.005)

FIGURE 45.2 Full-thickness (Stage III or IV) pressure ulcers. A perspective of healing results reported in controlled studies.

10 days if kept moist, while if air exposed, it takes 7 to 14 days.[54] The epidermis of partial-thickness dermal incisions repairs in 7 days in moist environments as opposed to 14 days when air exposed. Wiechula[18] concluded that hydrocolloid-dressed skin graft donor sites experienced less pain, faster healing, and fewer infections than those dressed with gauze. Among the most challenging chronic wounds to heal are full-thickness (Stage III or IV) pressure ulcers. A perspective of best results reported in controlled studies (Figure 45.2) suggests that moist wound healing matches or exceeds results reported for negative pressure or growth factor modalities.

Other variables affecting healing time include wound depth and etiology (Table 45.2). Superficial epidermal wounds heal fastest. Partial-thickness wounds, extending to but not through the fascia beneath the dermis, heal in about half the healing time for similar-etiology full-thickness wounds extending below the fascia into the subcutaneous fat, muscle, or other structures.[70] To optimize wound outcomes, one should use the minimum wound depth to achieve the desired outcome. For surgical, chemical, or laser procedures, the minimally invasive effective procedure should be applied. For burns, the burn should be cooled to ~10°C for 20 min, as soon as possible after its occurrence.[71] This reduces or stops the spreading zone of stasis[72] that continues to expand for up to 24 h after the tissue is burned.

MANAGEMENT OF THE WOUND THAT RESULTS FROM A COSMETIC PROCEDURE

Most superficial peels involve minimal pain and are performed as an office procedure without anesthesia or topical anesthetic. They can improve the uniformity of melanin distribution in the epidermis and "plump" the dermis, improving the appearance of wrinkles or scars. When applied with care and consideration for patient, product, and procedural variables, wound resurfacing techniques can achieve satisfactory cosmetic results while minimizing procedure invasiveness.

Side effects should be weighed against those of alternative treatments. Earlier improvement in scar pliability resulted from corticosteroid injection compared with treatment with a 585 nm pulsed dye laser, but patient assessments were similar, and the pulsed dye laser avoided the 50% incidence of hypopigmentation, telangectasia, and skin atrophy associated with the intradermal corticosteroids.[73] The most common side effects of dermatologic chemical or laser surgery are burning pain, pruritus, infection, delayed healing, and persistent erythema. Pain or pruritus is usually milder and less likely for patients undergoing superficial peels. Pain can be controlled with cool compresses or managed with a topical or local anesthetic, in which case vital signs should be monitored throughout the procedure.

Frequent postoperative visits aid early recognition of infection, which can also be prevented preoperatively by recognizing and addressing patient risk factors and, throughout the course of care, by professional adherence to infection prevention and management protocols. Preserving a moist wound environment will also help to minimize wound infection and optimize healing, wound-related pain, and scarring.[31] Persistent erythema, lasting more than 3–5 days for a superficial peel, 15–30 days for a medium-depth peel, or 60–90 days for a deep peel, is considered abnormal and should be treated appropriately with steroids and skin protection to minimize exposure to allergens. One should always consider quality of life issues, like avoiding odor, which is very important to patients' quality of life, especially in the cosmetic patient setting.

We found no guidelines for laser skin resurfacing or chemical peel, when we searched the National Guideline Clearinghouse Web site (www.guideline.gov), a global compendium of health care guidelines. Based on evidence reviewed here, we compiled an example algorithm for wound care following laser resurfacing (Figure 45.3). This algorithm may serve as a basis for further consideration and improvement, as more data are gathered in future studies.

CONCLUSIONS

In summary, wound healing varies with depth, etiology, and management of both patient and wound. Superficial wounds heal faster with less likelihood of scarring, especially if limited to the epidermis, the only layer that regenerates without scarring. If chronic wound causes are not resolved, the healing cascade of hemostasis, inflammation, proliferation, and remodeling begins anew. Even the best local wound care, autolytic debridement and a moist wound environment, cannot foster healing unless

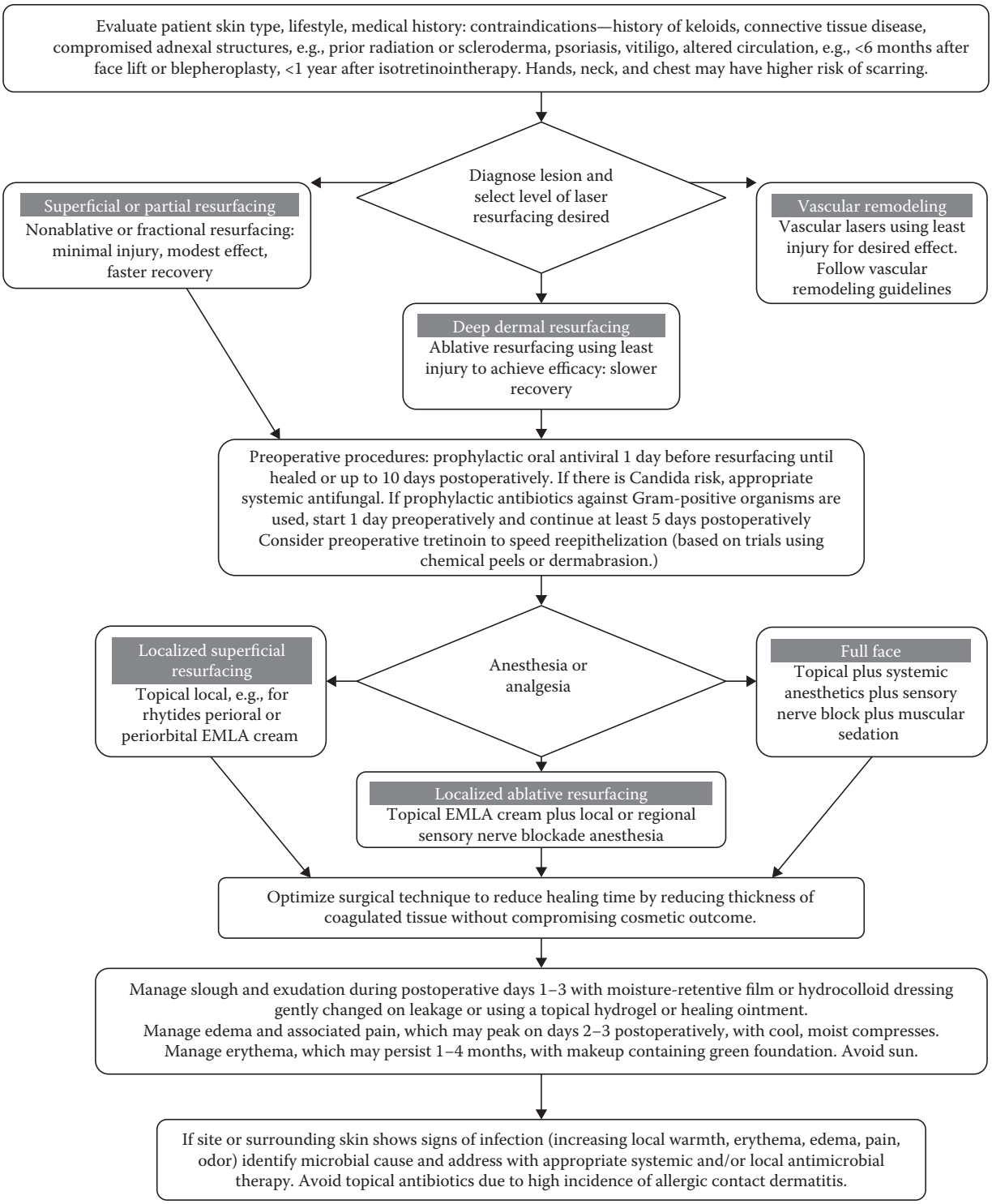

FIGURE 45.3 Wound care algorithm for laser resurfacing.

all causes of tissue damage are resolved.[83,84] Managing the healing wound includes addressing patient concerns such as pain, odor, and excess exudate as well as removal of foreign matter and optimizing the environment for healing and preventing complications such as infection and scarring. Currently available evidence suggests that moist wound healing helps achieve these goals at least as well as growth factors or negative pressure modalities. Randomized controlled trials help in clinical practice decisions by providing information regarding the use of techniques and practices to optimize wound healing outcomes.

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46 Baby Care Products

Bart Desmedt, Susanna Brink, Ralf Adam, Vera Rogiers, and Kristien De Paepe

INTRODUCTION

A great variety of baby skin and hair care products are brought to the market. These products should be specifically suited for the target group. In order to understand whether babies need different skin and hair care cosmetics than adults, it seems necessary to analyze potential anatomical and physiological differences of the skin and annexes between both groups.

A further distinction could be made between the skin of full-term and premature babies, but in this chapter, only the former group is considered. Cleansing and protective cosmetics are available for babies, and their safety is, in the European Union (EU), based on exposure-based risk assessment for the individual ingredients as well as for the finished product. Finally, some common baby skin problems and the applicability of cosmetic products under impaired skin conditions are discussed.

ANATOMICAL DIFFERENCES BETWEEN BABY AND ADULT SKIN AND SKIN ANNEXES

A complete overview of the morphological characteristics of baby skin is present in several comprehensive reviews [1–4]. Here some key differences in comparison to adult skin and annexes are given. Human skin barrier developments begin in utero, and it is believed that skin maturation is nearly complete at gestational age of 34 weeks in order to prepare for the abrupt transition from an aqueous to a dry terrestrial environment. In general, a full-term baby possesses all skin structures of adult skin, and anatomically, these structures do not undergo dramatic changes after birth. The skin of term neonates provides a competent barrier after birth. Transepidermal water loss (TEWL) and percutaneous absorption data favor mature adult-like barrier properties [5]. The skin of the newborn adapts quickly to environmental conditions after birth. Certain functions, however, are believed to progressively adapt during the first weeks and months of life.

This adaptation hypothesis is supported by published research, in which skin parameters investigated are skin thickness, pH, and hydration [6,7]. Importantly, though, these adaptations do not appear to prevent a fully competent barrier function to be expressed in the term neonate. These adaptations possibly are responsible for the morphological or physiological differences observed between baby and adult skin, as summarized in Table 46.1.

- On the *skin surface*, babies have smaller corneocytes and a denser microrelief and the cell turnover is faster [8,9]. At birth, skin is dry and rough but smoothens during the first month. Skin roughness was correlated to skin hydration [10].
- *Corneocyte size* was found to be dependent on body site and age. Anatomic sites with larger corneocytes are associated with a lower desquamation zone. Corneocytes are smaller when skin is exposed to the environment and not protected by clothing. Studies analyzing corneocytes from infants, children, and young and mature adults revealed that corneocyte size increases with age [8].
- *Overall skin thickness* of infants is thinner than that of adults, and skin thickness increases with age [11–13]. This difference is mainly attributed to the thinner dermis layer.
- Results on comparing *stratum corneum thickness* between adults and infants differ strongly depending on the method used. Stratum corneum thickness is comparable when investigating autopsy samples [2,14] and using ultrasound echography [15]. It was shown to be 30% thinner in infants using confocal laser scanning microscopy [9]. Optical coherence tomography, on the other hand, showed a negative correlation between age and epidermal thickness [16]. Differences are believed to be due to sample preparation methods and the fact that each method compared skin from different body sites.
- The *epidermis* of term infants is well developed, and the anatomy and ultrastructure do not differ much from those of adults [17]. In the stratum germinativum—besides a majority of cylindric keratinocytes—dendritic cells, including melanocytes, Langerhans cells, and Merkel cells, are present in a normal number. However, their functionality—namely, photoprotection, immunological barrier, and receptor function, respectively—still has to develop progressively. The melanocytes are less pigmented, which explains the pale color of the newborn's skin and which makes sunburn an important risk factor [3]. The cohesion and adhesion of epidermal cells in newborn skin are not fully developed, and thus, the connection at the epidermal–dermal junction is weaker than in adult skin. At the basal membrane of newborn skin, cohesion structures are present in a normal number in comparison with adult skin [1,17].

TABLE 46.1
Summary of Skin Parameters

Parameters	Newborn/Baby Skin	Adult Skin
Total skin thickness	1.2 mm	2.1 mm
Surface of epidermis	Dry at birth, covered with vernix caseosa, more hydrated after 1 week, denser microrelief	Dry
Skin pH	pH 6.34 (amniotic fluid) After 4 days similar to adult pH	pH 4.5–6, average 4.8
Thickness of epidermis	40–50 μm	ca. 50 μm (20 μm face, 1 mm sole)
Stratum corneum	7.5–10 μm, 15+ cell layers	9–15 μm, 15+ cell layers
Barrier function	Effective	Effective
Eccrine sweat glands	Not fully active, still in upper dermis	Active, in lower dermis
Dermis	Thickness 3.5 × lower versus adult	
Hypodermis	Thin subcutaneous fatty layer	Depending on nutrition

- In the *dermis*, numerous fibroblasts produce elastic and collagen fibers but fewer in number and less dense than in adult life [9,18]. Most of the development and maturation of the elastic fibers occurs after birth, and it is only at the age of 3 years that the elastic fibers become completely mature. It is known that the dermal matrix differs in composition depending on age. Indeed, during development, the water, glycogen, and hyaluronic acid contents of the matrix decrease, but its dermatan sulfate content increases. This difference in composition probably interferes with the particular turgescence of the newborn's skin [19]. Infant dermal papillae are more homogenous, and there is a 1:1 relationship of the papillae with the surface stratum corneum glyphics, which is not seen in adults. For infants, no clear transition between papillary and reticular dermis is observed [9]. In the embryonic dermal skin, primitive dermal vessels can be seen that differentiate into arterioles, venules, and capillaries. With respect to the vascular system, the subpapillar plexus is not yet completely developed, and the upper layer of the dermis contains a rich but disorganized capillary network, causing the erythematous aspect of the newborn [3,19]. Capillary loops for the cutaneous blood flow are not detectable at birth and are first observed by the second week after birth, but not in all skin sites. The cutaneous vascular system is only fully developed in all skin sites as of 14–17 weeks after birth [20]. Laser Doppler (periodic oscillations of skin blood flux) showed that oscillation frequencies in neonates reached the lower range of adult levels at the end of week 1 after birth [21]. The cutaneous nerve system is also not yet finalized [3,19].
- The immature *hypodermis* of baby skin consists of small lobules of roundly shaped adipoblasts that are richly vascularized. The fatty acid composition of the triglycerides is more saturated, which results in a higher fusion point of the lipids than measured for adult skin [1,17].
- The *hairs* of a newborn are well developed. Hair follicles develop in the 14th gestational week. Babies are sometimes born with lanugo hair, which develops by the end of the second trimester [22–24]. After birth, the hairs pass from the anagenic into the telogenic phase. As a consequence, a baby's hairs fall out about 8 weeks after birth. Afterwards, the hair cycle becomes similar to the one observed for adults, and hairs will be present in different phases. The hairs, however, are very thin and only faintly pigmented, but these phenomena normalize as a function of time [25]. In total, 5 million hair follicles cover the body of the newborn infant, which—in relation to the body surface—is 10 times more hair structures per skin surface compared with adults [26,27]. It has also be pointed out that erythematous lesions can often be found all over the body except for regions without hair follicles such as the palms of the hands, soles of the feet, and the penis. This could indicate that microbes gain access into the skin through hair follicles [26].
- *Sebaceous glands* are visible as of 18 weeks of gestational age and are able to produce lipids soon after, when stimulated by the androgens originating from the mother [28,29]. Their secretions constitute the largest part of the vernix caseosa. That is why at birth, the skin is covered with a white fatty substance. The vernix caseosa is a naturally occurring fetal barrier film produced in late pregnancy [30]. Besides the secretion of sebaceous and epidermal lipids, desquamation of maturing fetal corneocytes also takes part in the development of the fetal barrier. The vernix is thought to have multiple overlapping biological functions like moisturization, anti-infective, antioxidant, wound healing, and waterproofing [31,32]. Because it lacks desmosomal interconnections between corneocytes,

it is also referred to as the "mobile phase" stratum corneum. Removal of vernix lipids can modify the water sorption–desorption profile [33,34]. The vernix caseosa is taken away during the first washing of the baby. After loss of this protective layer and the onset of a desquamative stratum corneum, the skin is exposed to a much dryer environment than the one present during fetal development [35]. Erythema occurs that changes in appearance and gets a more marbled aspect that progressively disappears. This is an adaptation of the microvascular system [19].

Because of the different biological effects of the vernix caseosa, the question is often raised whether it would not be better to leave this natural film on the baby instead of washing it away. Several publications investigating the effect of immediate bathing of newborns, however, are contradictory [36–39]. Vernix distribution is dependent on gestational age, delivery mode, gender, race, and meconium exposure and positively affects skin hydration, skin pH, and erythema. These multiple effects would support its retention on the skin surface after birth [30]. Vernix films also retain endogenous chymotrypsin, thus preventing loss of this epidermal enzyme and protecting the epidermal barrier from noxious substances [40]. In this respect, the World Health Organization (WHO) developed general guidelines recommending that neonatal bathing should not be undertaken within the first 6 h of birth [41].

Generally, sebaceous glands occur at a normal frequency but are not yet fully functional in infants. An increase in sebum secretion was reported after birth, with values reaching those in adults at the end of the first week after birth [42,43]. In certain cases, large sebaceous glands are observed together with the occurrence of the typical symptoms of so-called *acne neonatorum*. This particularly happens in male newborns and can persist for a few months. It is seen as a temporary effect of the androgens that are present in the mother's blood. At 6 months of age, the values are lower than in adults, and reactivation of the sebaceous glands only occurs later on, around puberty [39,44].

- The *hydrolipidic layer*, mainly composed of sebum from the sebaceous glands and water originating from eccrine glands and TEWL, is not fully developed in babies. This protective water-in-oil (w/o) mixture is sometimes even nearly absent, which also has an effect on the skin pH of the newborn [45]. Consequently, the observed skin pH imbalance might be responsible for a lower capability to neutralize the alkalinization, especially seen in the diaper area due to urine and defecation. Studies have shown that infants in general have less surface lipids, and the lipid composition differs with age [46,47]. Sebaceous free fatty acids have antimicrobial properties, and sebum delivers vitamin E to skin, which assists in the antioxidant network [48,49].
- The *eccrine sweat glands*' structure is mature, but their intervention is still immature, affecting thermoregulation. Eccrine sweat glands in soles and feet develop in the first trimester and have an adult structure by 24–29 weeks of gestational age [28,29]. In baby skin, eccrine sweat glands are in the upper dermis and only later move down to the lower dermis [43,50]. The number of glands per area unit of skin is higher in the newborn than in adults, and the glands primarily serve a thermoregulatory role. Both thermal and emotional sweating have been observed for full-term neonates. Term infants can react to a warm environment as of the first day [50,51]. Transdermal potential (TDP) is generated largely in sweat glands. The magnitude depends on activity, density, distribution of glands, hydration of epidermis, and integrity and permeability of the epidermis. It results from the balance of active ion transport across the epithelium and the permeability of the epithelium to passive ion diffusion along electrochemical gradients by the active transport process. Certain skin diseases cause a decreased reabsorption of chlorine from sweat and increased negative TDP and high chloride concentration in sweat [52]. Eccrine gland secretion contains the antimicrobial peptide "dermicidin," and this could suggest that sweat plays an important role in the innate immunity of the skin barrier [53].
- *Apocrine glands* only become functional around puberty [19,54].

PHYSIOLOGICAL DIFFERENCES BETWEEN BABY AND ADULT SKIN

Soon after birth, the stratum corneum of the full-term infant is remarkably capable in fulfilling its key functions, especially that of providing an effective semipermeable barrier between the inside and outside of the body [5,6].

On the basis of TEWL and dermal absorption studies, term infants seem to possess a fully developed stratum corneum with adult barrier properties [5]. The barrier is believed to be mature at 30–37 weeks of gestational age [5,55,56]. Other parameters, such as skin thickness, skin pH, and stratum corneum hydration, show that neonatal skin adjusts very well to the extra uterine environment. This is rather in contrast to a more steady-state situation of adult skin [7,19,25].

Dermal Absorption

Dermal absorption in newborn skin is similar to that observed in adult skin. For babies, however, a number of potential risk factors exists [57–59].

1. It is believed that the *hair follicle* density of babies is highest at birth and density of hair follicles decreases with growth [60,61]. Skin appendices may potentially behave as imperfections in the stratum corneum [62–64].
2. The *surface area/body weight ratio* is 2.3-fold higher in newborns than in adults, decreasing to 1.8-fold and 1.6-fold at 6 and 12 months, respectively [65]. Application of the same amount of product on a similar body surface of a baby versus an adult could result in higher blood and tissue concentrations in the newborn. This fact is, however, currently addressed in guidelines for product safety assessments, which account for individual variations adequately and usually normalize for daily exposure per body weight [66,67]. The inter-individual variation is always taken into account by the generally accepted threshold value of 100 for intact skin [68].
3. *Pharmacokinetic parameters* differ widely between babies and adults and result in reduced clearance and/or a longer half-life of bioavailable substances, thus increasing the potential risk for adverse reactions in babies (Table 46.2). However, this may depend on the characteristics of the xenobiotic [69,70].

   Concerning the total body water content, it is known that infants have a higher water content (80%–90%) compared to that of adults, which steadily decreases to 55%–60% [69]. Another pharmacokinetic difference is a decreased protein binding capacity, which can be linked to lower concentrations of glycoproteins in the plasma of infants.

   Premature and full-term neonates tend to show a three- to nine-times-longer pharmacokinetic half-life than adults. However, once the neonatal period is over, often, a greater elimination and higher clearance are observed compared with adults bringing back the normal equilibrium [65,71]. As mentioned by Renwick et al. [66], this neonatal period would coincide with the period of lactation [65,66,71–73].
4. The *stratum corneum thickness* is reported to be the rate-limiting part for percutaneous penetration and, thus, dermal absorption. Although there are contradictive publications as to the stratum corneum thickness of baby versus adult skin, the implication of a potentially thinner stratum corneum in infants, as measured by confocal laser scanning microscopy [9], needs to be considered. Referring to the brick-and-mortar model of the stratum corneum, the walls would be thinner, which potentially creates a shorter path that molecules have to follow to penetrate this layer [74,75]. However, as already mentioned in the sections above, the literature published up to date reported that the stratum corneum barrier and dermal absorption for neonates are similar to what is observed for adults; therefore, even a potentially thinner stratum corneum does not seem to impact the barrier efficiency.
5. *In-use conditions of topical products* also play a role. Cosmetic skin care products often are applied onto large body surfaces, for example, cleansing lotions, sunscreens, and so forth, increasing not only the potential risk for local effects but also dermal absorption and potential systemic toxicity. This factor is considered in exposure-based risk assessment.
6. The *diaper area* and nondiapered regions are indistinguishable at birth but show differential behavior over the first 14 days, with the diapered region having a higher pH and increased hydration [6,74,76]. Special circumstances arise because of the close confining clothes and diapers and the uncontrolled urination and defecation. The close-fitting diaper provides a warm nutritive environment for the proliferation of bacteria [77]. Because of the interaction between urine and the feces, urease becomes activated and converts urea into ammonia, giving rise to alkaline skin pH levels. As a consequence, fecal enzymes such as lipases and proteases can become activated and may damage the skin in the diaper area. Despite modern improvements in diaper technology [78–80], irritant diaper dermatitis cannot completely be avoided, and the resulting damage to the epidermal barrier may potentially favor

TABLE 46.2
Potential Differences in Pharmacokinetic Parameters between Newborns and Adults

Parameters	Newborn	Adult
Plasma binding	+	++
Plasma protein concentration	+	++
Body water	++	+
Fat distribution	+	++
Brain development	+	++
Brain–blood barrier	+	++
Brain volume	++	+
Cyt P_{450} biotransformation	+	++
Conjugation reactions	+	++
Relative liver mass	++	+
Glomerular filtration	+	++
Tubular secretion	+	++

Sources: Renwick, A.G., *Food Addit. Contam.*, 15, 1998. Renwick, A.G. et al., *Regul. Toxicol. Pharmacol.*, 31, 2000. Fernandez, E. et al., *Pharmaceutics,* 3, 2011. Kearns, G.L., *J. Allergy Clin. Immun.*, 106, 2008. Ginsberg, G. et al. *Toxicol Sci*., 66, 2002. Dorne, J.L., *Fundam. Clin. Pharmacol.*, 18, 2004. Dorne, J.L. et al., *Food. Chem. Toxicol.*, 43, 2005.

dermal absorption of xenobiotics. A number of molecules, which historically have been used in the diaper area, are known to induce systemic toxicity and should only be used very carefully and only when indicated, for example, hexachlorophene, dichlorophene, corticosteroids, boric acid, and ethanol [58,81]. In risk assessment of cosmetics, the margin of safety (MoS) approach is used when defining acceptable human exposure levels (see the section "Baby Care Products for Skin and Hair—General Safety Considerations for Baby Care Products"). When extrapolating from experimental studies to human, the magnitude of the uncertainty factor must take into account a variety of considerations, such as species differences, sensitive subpopulations, duration and route of exposure, and vehicle or matrix effects. In addition, when the diaper area is highly irritated, special consideration needs to be made on dermal penetration depending on the frequency, area, and severity of compromised skin concerned [41,82]. Innovative hygiene absorbent and baby care products, however, have been shown to significantly reduce the frequency and severity of diaper dermatitis [83–85].

7. Other important factors for dermal absorption are *stratum corneum hydration* and *skin pH*. For instance, differences in skin pH change the ionization grade of molecules and will influence dermal absorption [70,75]. Hydration favors penetration of hydrophilic substances.

Transepidermal Water Loss

The barrier function of the skin not only prevents absorption of toxic substances but also controls TEWL. In particular, when skin is damaged, excessive TEWL occurs [86–88]. In a healthy, fully developed newborn, TEWL values of 6 to 8 g/m^{2}h water are measured, depending on the measuring technology [89]. A slightly increased value is observed during the first hours after birth, with values decreasing to those in adults within hours. This sudden TEWL decrease is explained by the drying of the skin immediately after birth. TEWL measurements during the first 2 weeks show high variability compared to adults [44,90].

TEWL increases proportionally with immaturity, which means that premature children have an increased evaporative heat loss and, subsequently, poor temperature control [56,88]. Although skin maturation occurs rapidly, fluid and electrolyte shift as well as body temperature have to be controlled frequently [91]. Also, increased risk of local and systemic toxicity from topically applied substances rises with increasing TEWL or barrier damage [92,93]. In the diaper area TEWL is often defined as skin surface water loss (SSWL) and is used to measure the capability of a diaper to keep the skin dry [94,95].

Defense Against Infection: Stratum Corneum Hydration, Skin Lipids, Skin pH, and Microbial Colonization

1. The *stratum corneum hydration* level influences the barrier function, dermal absorption, reactivity to irritants, and the skin's mechanical properties [96]. Although healthy infants and adults tend to have similar TEWL values, newborn skin is dryer at birth, and water content increases during the first 2–4 weeks and then stabilizes. Infants (until 6–24 months) therefore present somewhat higher water contents in the horny layer and a greater variation than adults up to 1 year. The highest hydration levels were observed for age groups 5–6 weeks and 6 months [6,88,89,97]. Newborns also have reduced water holding capacity versus adults. Skin of older infants absorbs larger amounts of water than adult skin and also desorbs the water more easily [44,76,97,98].

   To retain water in the upper thin layers, the stratum corneum contains natural moisturizing factors (NMFs), which are a group of hygroscopic molecules composed of organic acids, sugars, and ions [74]. According to Raman measurements, newborns have higher NMF concentrations in the stratum corneum than any other age group. The NMF concentration drops over the next months to reach the lowest values at 6 ± 1 months. After that time point, the values increase but never get as high as the newborn levels. From these results, it can be concluded that the lower hydration immediately after birth cannot be attributed to a low content of NMF constituents. Similarly, at 6 months, skin has a high water concentration in the stratum corneum despite low NMFs [6,97].
2. Infants have less *skin surface lipids* than adults, and the lipid composition differs with age [46,47]. Sebum forms a waterproof barrier and is important for barrier function [99–101]. Skin of atopic patients, for example, has less sebum compared to normal skin [21]. Sebaceous free fatty acids have antimicrobial properties, and sebum has been shown to deliver vitamin E to skin and assists the antioxidant network [48,49].
3. Infants have higher *pH* (6.6–7.5) at birth versus adults (4.5–6.7), but it decreases rapidly after birth (2–4 days) and continues to decrease for 1 month before it stabilizes at an acidic average (pH = 4.5–6) similar to that of adults [76,87,102,103]. Amongst other factors, this higher pH during the first days of life might reflect the influence of the vernix caseosa and the amniotic fluid (both pH values above 7) [88].

   Stratum corneum acidification is described as a necessary prerequirement to ensure normal stratum

corneum barrier homeostasis, since it not only protects the skin from certain microorganisms but also ensures pH-dependent lipid processing and formation of functional lipid lamellae [104,105]. The pH values in atopic infants and infants with ichthyosis are reported to be significantly higher, even in the postnatal period, which could indicate a role of pH for an intact lipid turnover and barrier function [106,107]. The newborn skin needs time to acidify, and pH-regulating enzyme systems are triggered by the exposure to a dry environment.

The postnatal development of skin pH is determined by exogenous components such as lactic acid, eccrine sweat, and free fatty acids derived from sebaceous gland lipids [43] and via metabolic pathways such as the generation of free fatty acids from phospholipids via enzymatic activity of phospholipases A2 [108], urocanic acid via enzymatic degradation of histidine, pyrrolidone carboxylic acid, and Na+/H+ isoform 1 (NHE1) [104,109,110]. Products associated with the desquamation process of the stratum corneum, such as breakdown products of filaggrin and keratohyalin, have been reported to contribute to skin pH. The outer skin surface pH, however, is not representative of the pH value across the stratum corneum. The stratum corneum pH changes dramatically by 2 to 3 pH units in less than 20 μm of tissue. This pH gradient [104,111] is essential for stratum corneum differentiation and barrier repair, which depend on pH-dependent enzymatic activity; synthesis of ceramides, which requires pH-dependent enzyme activation [112]; and equilibrium of desquamation and cell cohesion, which depend on regulated functions [37].

Whereas the pH value of baby skin is, after a few days, comparable to the pH value of adult skin, the buffering capacity of baby skin is much lower. Therefore, baby skin is more susceptible to pH changes.

4. During the first days of life, rapid *skin microbial colonization* coincides with significant skin barrier changes like reduction in pH and increase in water content [7]. Immediately after birth, bacteria colonies are undifferentiated across body sites. The composition of the skin microflora seems to be site-specific like in adults only from 3 months onwards. Originally, baby skin is dominated by staphylococci, and numbers decrease over the first year. The composition of skin microbial communities evolves over the first year of life, and it becomes more diverse [113,114].

   Acidic skin pH favors colonization of commensal bacteria, discourages growth of pathogenic microorganisms to which the baby is exposed after birth, and serves in the defense against infections [115]. Indeed, microbial colonization of the skin starts immediately after birth by so-called saprophytes that are not pathogenic and are credited with protective properties against some harmful microorganisms [17]. They require acidic surroundings for optimal living conditions [89]. This microflora is also believed to play a role in skin immune function [114]. Bacterial communities contribute to cutaneous homeostasis by directly affecting inflammatory responses [116]. Proteases secreted by certain species can affect the barrier function [117].

   In adults, there are differences in bacteria composition, population, and evenness between sites that are sebaceous, moist, and dry [118]. Infants with a more hydrated stratum corneum are expected to have a bacterial profile similar to a moist skin profile of adults. Indeed, there is a higher abundance of *Staphylococcus*, which agrees with moist adult sites. Despite low sebum levels, propionibacteria increase on the infant forehead at 4–6 months. The microbe colonies are different in the diaper area, with *Finegoldia* and stool bacteria dominating [119,120]. This difference could also be due to the higher pH and water content of skin due to diaper wearing [76].

   Alterations of the skin surface conditions can change the skin microflora and can promote the growth of *Candida albicans*, the microorganism that is mainly associated with severe instances of diaper rash. This mold is present in as many as 77% patients with diaper dermatitis [121,122]. It has been shown that modern diaper technologies can contribute to keeping *Candida* in the diaper area under control [123].

BABY CARE PRODUCTS FOR SKIN AND HAIR

General Safety Considerations for Baby Care Products

Similar to adult skin frequent skin contact with xenobiotics could damage or disrupt the barrier function of the stratum corneum and change the skin pH. Barrier function disruption could lead to an increased TEWL, and the onset of infections [17,89,92]. Therefore, exposure-based risk assessment for baby products is key to bringing safe baby cosmetics to the market. This has also been recognized in Europe by the Scientific Committee on Consumer Safety (SCCS) in its Notes of Guidance, 8th Revision (SCCS 1501/12) [124]. It was concluded that in exposure-based risk assessment of baby cosmetic products, there is no need for the addition of an extra uncertainty factor when intact skin is involved. Thus, in this case, the MoS—used to judge potential systemic toxicity—remains at least 100 as proposed by the WHO for cosmetic products in general (factor 10 for interspecies and factor 10 for interindividual differences). There might be the need for an additional safety factor if

substance-specific data clearly demonstrate that interindividual variability would result in a value, higher than 10. For products used in the napkin area special circumstances can be present, resulting from the close confining clothes and nappies, uncontrolled urination and defecation, and resulting problems with potential damage of the skin in the napkin area. Modern nappy technology has been shown to provide increasingly good skin compatibility, leading to a decline in the frequency and severity of diaper dermatitis. However, irritant diaper dermatitis cannot be completely avoided and might have an impact on dermal absorption of substances.

The SCCS concluded as follows [124]:

- As cosmetic products are meant to be used on intact skin, medical consultation is necessary in the case of real skin damage, and pharmaceutical products (and not cosmetics) should be used.
- For the development of baby cosmetics and the risk assessment of products intended to be used in the napkin area, the potential impact of irritation on dermal absorption of the chemical needs to be considered by the safety assessor in the final quantitative risk assessment of their products.
- The skin structure of full-term neonates/newborns and early infants is similar to that of adult skin, and the dermal absorption is comparable. However, a distinction should be made between the skin of the nappy zone and the rest of the baby skin, since for this particular area, risk factors exist, which are not present for the rest of the body. Therefore, the napkin zone should be further considered, independent of the substance(s) under question.
- The SCCS is of the opinion that, in general, no additional safety factor needs to be included for substances used in children's cosmetics applied to intact skin, as an intraspecies assessment factor of 10—covering the toxicokinetic (3.2) and toxicodynamic (3.2) differences between children and adults—is already included in the MoS calculated for individual substances.

During the development of baby products, a number of criteria are taken into consideration:

- High quality of raw materials in terms of purity, stability, and microbiology via appropriate certificates of analysis.
- Skin irritation, which is dose dependent, can be controlled by avoiding well-known irritative ingredients and/or reducing concentration or frequency of application.
- Skin sensitization, triggered by an immunological response, is not restricted to the area of application. Therefore, induction of sensitization—in particular, for perfume ingredients, even when International Fragrance Association (IFRA)-tested or excluding the 26 allergens identified in the 7th Amendment of the EU cosmetic legislation—should be avoided [125].
- As is the rule for adult cosmetics, safety data of baby cosmetics are taken up in a technical information file (TIF), and the risk assessment—approved by a safety assessor—is the driving force behind the safety of baby cosmetics. Usually, special attention is given to the concentration of (1) reactive colorants; (2) promotional additives, "natural" and "exotic" ingredients, in particular, poorly identified mixtures, plant extracts and ingredients of animal origin, or a questionable, impure source; (3) potential allergens, penetration enhancers, organic solvents (ethanol, isopropanol, highly reactive substances, highly detersive or foaming agents, and antiseptics in particular in daily-use products); and (4) concentrations of preservatives.
- It is considered to be good practice (1) to add antioxidants to protect unsaturated lipids from oxidative reactions; (2) to adjust the pH of the final product, resulting in a skin-friendly pH value between 4.5 and 6 after product application; (3) to add chelating or sequestering agents, when appropriate, to prevent heavy metal precipitation and protect the preservative system; and (4) to use skin barrier protective ingredients.

Types of Baby Care Products

Baby cosmetics can be mainly subdivided in two groups, namely, cleansing and protecting cosmetics.

Cleansing Cosmetics

Bath Products

Bathing a baby for 5 to 7 min in lukewarm water (35°C–36°C) usually is sufficient [89,126]. Recent studies also showed that bathing is milder than washing and use of mild detergent is milder than using water alone [127]. Daily bathing is general practice but not optimal because of the risk of drying out and irritating baby's skin, in particular when aggressive anionics with high degreasing properties are involved [128]. It is better to use so-called secondary tensides, including nonionics and amphoterics, or mild anionics such as sulfosuccinates, isothionates, and protein fatty acid condensates. The use of bath oil is preferred over bath foam and bath cream additives, particularly when dry skin, sensitive skin, or atopic eczema is present. For optimal effect, the baby is bathed for 5 min in plain water, and then the bath oil is added, and bathing continues for another 5 to 10 min [126]. Also, adding starch to the bath water or using starch-containing bath additives may help to restore an impaired skin barrier [129]. In general, the use of bath foam is not suitable for babies because of its high content of primary tensides producing the excessive foam.

A series of studies has shown that the pH of cleansing products can change the microbiological spectrum of the skin. High soap pH values encourage propionibacterial growth on skin, whereas syndets (i.e., synthetic detergents) with a pH of 5.5 did not cause changes in the microflora [130]. Also, different cleansing regimens (water alone or mild wash gel/soap) did not impact the bacterial colonization [127].

Shampoo

Baby shampoo usually has a pH of 6 to 7 and ideally should contain only mild tensides, for example, mixtures of nonionics and amphoteric substances [25]. The shampoo should not be irritating to the eyes. To avoid eye contact, the viscosity of the shampoo could be increased. Parents often think that foam is important for its cleansing properties, but foam has no cleansing function, and the ingredients required to produce a sufficient amount of stable foam are often quite irritating and not suitable to be used alone in baby shampoos, for example, alkyl sulfates and alkyl ether sulfates [89,126]. Furthermore, it is not necessary to wash a baby's hairs every day since they are neither dirty nor greasy. As the hydrolipidic layer is not yet formed on a baby's skin, and sebum production is low, the amount of lipids distributed on the hairs is limited and is easily washed away.

Soap Bars and Syndets

Soaps (salts of fatty acids) liberate, in contact with water, alkali and increase the pH up to a value of 10. Also precipitation occurs with calcium and magnesium ions from hard water. On the contrary, syndets do not precipitate with hard water and have an adjustable pH to neutral or slightly acidic. As syndets cover the whole range of synthetic tensides—with exception of the legally protected soap formulations—they can be aggressive (e.g., alkyl sulfates) or mild (isothionates), depending on the choices and mixtures made. Like soaps, they can dry out the skin when not containing lubricant additives. In addition, when soap and washcloth are used in the diaper area, the buffering capacity of the skin is further compromised [84,126]. Extensive washing with aggressive tensides disturbs the flora of the newborn skin and can lead to infections [37]. In addition, perfumed cleansing products may cause contact allergic reactions due to enhanced skin penetration of the perfume by the presence of anionic soap ingredients [128].

Cleansing Milk

For cleansing of the baby and, in particular, the diaper zone, liquid cleansers based on oil-in-water (o/w) emulsions are often used, especially when water and washcloth are not well tolerated by the baby skin. Also, soft tissues or towelettes impregnated with these emulsions are present on the market. They are easy to use and contain anionic and/or nonionic tensides [126].

When a baby is prone to contact dermatitis, it is advised to screen the ingredient list because those tissues often contain high concentrations of preservatives, necessary to prevent microbiological contamination of the tissues [131]. Mineral-oil-impregnated tissues can increase the presence of *Candida* in the diaper area and change the composition of the skin surface lipids.

Baby Wipes

Over the last decade, disposable baby wipes have been developed as an alternative to traditional cleansing methods. They usually consist of a nonwoven carrier material soaked with an emulsion-type, watery, or oily lotion. Mineral oil wipes do not efficiently clean hydrophilic components and potentially slip over fecal contaminations. Most emulsion-type lotions are o/w and enriched with emollients and surfactants. Because of their high water content, the preservative system is very important to ensure that the product will not be contaminated during its normal lifetime. Products for sensitive skin have also been developed, which offer a choice regarding the nonuse of fragrances or additives with which problems have previously occurred. Clinical studies confirmed that high-quality baby wipes are suitable for daily cleansing of the diaper area, of healthy babies as well as babies with compromised, irritated skin; newborn infants; and atopic and premature populations [84,132,133]. A cleansing baby wipe with sufficient pH buffering capacity offers an opportunity to stabilize skin pH at physiologic levels and thus helps to overcome the potentially detrimental effects of an elevated skin pH in the diaper area. Several papers report the importance of buffering capacity in this respect to obtain sustainable and stable skin effects. There is a clear correlation between the mean change in skin pH after washing and the necessary recovery time back to normal levels, which stresses the need for cleaning practices capable of sustainably maintaining the physiologic skin pH value. Furthermore, the stratum corneum pH gradient is important for barrier homeostasis. The barrier function of the stratum corneum could be improved via topical application of an acidic buffer [85,134–137].

Protecting Cosmetics

Face/Body Creams and Body Lotions

Protective creams for the napkin zone are preventive or protect the skin against aggressions from urine, feces, and their interactions. O/w creams do exist, but in case of starting skin damage, those mostly w/o creams or water-free ointments with talc, kaolin, and zinc oxide are advised. Allantoin, bisabolol, aloe vera extract, and silicones are often added to improve water resistance. In the winter, barrier creams protect a baby's face against the freezing cold and wind. The lipid phase often contains petrolatum. These products are particularly effective around the nose and mouth. They usually also contain moisturizers, soothing active ingredients, and nonionic emulsifiers [89].

Powder

Talc powders are not often applied anymore in the napkin area. They absorb moisture, decrease maceration, and

prevent irritation of the baby's skin [126]. Powders, however, pose a potential inhalation risk and can form on the skin little granules that induce friction. Furthermore, talc is susceptible to contamination with microorganisms and needs sterilization [77,138].

Sunscreens

During the past years, a steady increase of all types of skin cancer has been observed. It is, therefore, very important to inform parents and children about good sun protection. Educational programs can play an important role to prevent overexposure to sunlight [139]. Indeed, sun exposure in childhood is seen as a risk factor for skin cancer later in life, as it is known that there is a relationship between skin burning in the prepubertal period and the occurrence of malignant melanoma 10 to 20 years later [140]. Therefore, babies and infants should, in the first instance, be kept out of the sun and protected by appropriate clothing and hats. Almost 90% of clothes provide an equivalent protection to sunscreens of SPF 30 or higher, although the protection offered is dependent on weave, color, weight, stretch, and wetness [141,142]. Even special protective clothes for children exist today. They have undergone special treatment to filter out ultraviolet (UV) light [143,144].

It has been reported that sunscreens often are ineffective in preventing sunburns completely, because parents tend to forget to reapply or limit their use to just the upper part of the body. The importance of using multiple sun protection methods to maximize effective sun protection clearly has to be promoted [145]. Extreme care should be taken especially during the first weeks and months of life since pigmentation and thermoregulation are not yet fully developed [3]. The Australasian College of Dermatologists recommended that children up to 6 months of age should not be exposed to direct sunlight. However, the use of sunscreens in small children is advised when sun exposure cannot be avoided by other means, including shade, adequate clothing, and wide-brimmed hats, which are the best measures to protect small children. Sunscreens must then be applied in skin areas that are not protected by clothing [146]. The American Academy of Pediatrics recommend the use of sunscreens also in children of less than 6 months in small areas of skin, if adequate clothing and shade are not available [146]. These are conclusions provided in a recent review of the most relevant articles indexed between 1999 and 2012 in MEDLINE/PubMed on photoprotection in childhood [147]. When sunscreens are used, preferably, products containing UVA and UVB screens and scattering powders or a sunscreen mix with a high sun protection factor (SPF) should be applied [148].

Studies have shown that the application thickness of sunscreen products in adults usually lies between 0.5 and 1.3 mg/cm^2 [124,149], although the SPF of a product is assessed in vivo at an internationally agreed application thickness of 2 mg/cm^2 [150]. Application thickness could have an effect on the expected protection of the sunscreen, although opposite data have been shown [149]. It is important to apply a uniform layer with special attention to areas like the ears, neck, and feet, as experience shows that these are commonly skipped [145,151].

The type of UV filter(s) used is important. In the EU, only UV filters taken up in Annex VI of the Cosmetic Regulation n° 1223/2009 are allowed [152]. For babies and children, often, micronized and nano forms of ZnO and TiO_2 are used as an alternative to chemical sunscreens [153,154]. The popularity of these products results from the fact that they are effective and thought to be safe. As nanotechnology in general is questioned with respect to human health, safety assessment has recently been carried out by the SCCS for the topical application of ZnO and TiO_2 as nanoparticles in sunscreen products. These products were considered to be safe. Their use in spray formulations, however, is not recommended [155–157].

Earlier, the European Commission also expressed its concern with regard to the efficacy of sunscreen products and the claims made thereto. Aiming at ensuring sufficient protection against UV light and providing proper information to the general public, Commission Recommendation 2006/647/EC unambiguously states minimum efficacy criteria for sun products: the UVB SPF must at least amount to 6, and the UVA protection factor may not be inferior to one-third of the SPF. In addition, the Commission restricts the UVB SPF values to 8 possibilities, namely 6, 10, 15, 20, 25, 30, 50, and 50+. Higher SPFs are not allowed, and mentions such as "100% protection," "sunblock," or "all-day prevention" are considered misleading and therefore forbidden [158]. For nanomaterials in general, article 16 of Regulation 1223/2009 foresees specific provisions [152].

The viscosity of sunscreens is important since the products must remain on the skin, even with bathing and sweating. Today, most commercially available sunscreens have some water resistance or carry the label of being water resistant, very water resistant, or waterproof, determined in Europe according to Colipa (Cosmetics Europe) guidelines [159].

Some of the organic UVB filters, like 4-methyl-benzylidene camphor, benzophenone-3, homosalate, octyl methoxycinnamate, and octyl dimethyl para aminobenzoic acid (PABA), have been accused of being endocrine disrupters. The safety profiles of these UV screens were revised by the European Scientific Committee on Consumer Products [160] and considered to be safe for human use. Today, a selected number of the most contested UV screens are under SCCS consideration. Indeed, in the meantime, several articles appeared, pointing to potential endocrine-disrupting properties of sunscreens [161–167]. The issue, however, is not limited to sunscreens alone, and whenever possible, appropriate in vivo tests are now being included in the risk assessment process of consumer products in general. As in vivo testing of cosmetics is not possible in Europe as of March 11, 2013 (testing and marketing bans), much effort is going on to develop in vitro alternatives that can be applied to cosmetic products and their ingredients [168].

APPROPRIATE CARE OF FREQUENT SKIN PROBLEMS

Diaper Rash

Diaper rash is a common condition that refers to irritation on the groin, thighs, buttocks, genitals, and perianal area of the infant. It is caused by the combination of incontinence and the fact that diapers are used for hygienic reasons [76]. Excessive wetness caused by urination and the potential occlusive effect of some diapers can cause overhydration of the skin and therefore make it more fragile. Consequently, hydrated skin is more prone to mechanical damage and chafing of the skin since an increased coefficient of friction is observed, and it may allow irritants to penetrate the stratum corneum more easily. As explained before, not only occlusion but also a higher pH can be an underlying factor, which induces several enzyme-mediated irritations. Alkalinization of the skin increases skin penetration of microorganisms and activates fecal enzymes [83,169].

The most appropriate strategy for diaper rash is prophylaxis, and this includes keeping the skin dry. The selection of suitable diapers and frequent diaper changes are important because friction between skin and diaper is often an additional factor. Keeping the baby in an appropriately warmed room with naked buttocks for some limited time period is also quite effective.

In good skin care of the diaper zone, the application of emollients plays an important role, and the application of a cream layer creates an effective protective barrier. Also diapers containing a petrolatum based lotion has been shown to help prevent diaper rash [78,79]. ZnO is an often used component in diaper rash protection products. It adheres well to the injured skin, has astringent and some mild anti-inflammatory properties, and prevents skin injury or further damage. If the diaper rash shows evidence of *Candida* infection—often seen as satellite lesions extending the rash—antifungal therapy can be indicated [17,89,169,170].

Acne Neonatorum

Mild acne may occur in newborn infants. It consists of closed comedones on the nose, forehead, and cheeks. Pustules, open comedones, and inflammatory pustules may also occur but are less frequent. The cause of neonatal acne is not clearly defined, but it is believed to be secondary to the stimulation of the neonatal sebaceous glands by maternal androgens. In boys, it is often more pronounced as they have some additional production of testosterone. Neonatal acne requires no treatment as the lesions spontaneously resolve within 1 to 3 months [171].

Miliaria

Miliaria is a dermatose frequently observed in neonates. It is a generic term denoting retention of eccrine sweat. Miliaria can be subdivided into three groups: *miliaria crystalline*, *miliaria rubra*, and *miliaria profunda*. The difference between these three types is the level of the skin where the obstruction of the sweat gland occurs. *Miliaria crystalline* refers to an obstruction in the stratum corneum, *rubra* an obstruction within the stratum Malpighi, and *profunda* below the dermoepidermal junction. There is no specific treatment of miliaria. Measures that can be taken consist of regulating the heat and humidity of the environment to reduce sweating. Eventually, the poral obstructions are relieved, but this can take up to 2 or 3 weeks [172].

CONCLUSION

Full-term newborns have a skin that progressively develops towards adult skin. Anatomically speaking, the differences are limited, but some changes occurring at birth are responsible for the physiological differences observed between baby and adult skin. The skin of newborns exhibits the same barrier function and dermal absorption characteristics as adult skin, but external circumstances for infants may be different than for adults, and these differences, in turn, may result in differential dermal absorption profiles for infants compared to adults. Also, the thermoregulation (TEWL and sweating) of the baby is not yet fully developed, and the skin is easily invaded by infections, the latter often due to subtle pH changes and the immaturity of the defense systems of the skin. This implies that only safe cosmetics with safe ingredients guaranteed by exposure-based risk assessment should be used for newborns. During the development of new baby care products, ingredients known to be potentially eye or skin irritative or sensitizing are usually limited to a minimum, and profound exposure-based risk assessment has become common practice. One usually aims at simple, pure, mild, and pathogen-free formulations. Systemic side effects are not to be expected with mild rinse-off products (shampoos, bath additives, toilet bars) but should be carefully looked for when leave-on products for babies are being developed (body milks, hydrating creams, ointments, powders, sunscreens). In particular, attention should be given to cosmetic products to be used on the napkin area, where diaper rash might occur. If necessary, extra safety measures should be taken for this type of product.

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47 Cosmetics for the Elderly

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INTRODUCTION

Skin is the only organ where signs of aging are evidently visible at the soonest in phenomena like wrinkle formation, loss of elasticity, uneven pigmentation, loss of moisture, increased roughening, and cutaneous itching. Aging itself is understood as the result of a complex interaction of biological processes, which are caused by both genetic (chronological or intrinsic aging) and environmental or behavioral processes (premature or extrinsic aging).

Current expansion of knowledge in modern biogerontology widely extends the theories and explanations on mechanisms of aging. They are the basis for scientific approaches in research, aiming to identify new concepts for antiaging treatment of skin. In terms of scientific research activities, skin's accessibility to noninvasive and slightly invasive biophysical measurements and procedures is definitely advantageous for studying underlying mechanisms of cutaneous aging. In addition, experiments can also successfully be performed on cultured skin cells or on three-dimensional cultured skin models.

Today, consumers of cosmetic products are increasingly expecting a deceleration, cessation, or even a reversal of the underlying physiological processes contributing to the signs of cutaneous aging. These advancing demands require state-of-the-art technological endeavors in cosmetic research and formula development activities, with novel active ingredients, which can perfectly exert their antiaging efficacy in optimized new formula technologies.

PHYSIOLOGICAL CHANGES IN CUTANEOUS AGING

Unlike internal organs, the skin, being the outermost protective barrier, is particularly exposed to external influences. As the result of environmental challenges, the aging process in the skin is not only influenced by genetic, intrinsic factors, but also accelerated to a far greater extent (80%) by extrinsic factors, especially by sun exposure [1]. Intrinsic mechanisms of skin aging seem to be only basically involved in formation of fine lines and shallow wrinkles in advanced age. Therefore, the research in the cosmetic industry is focused on the identification and qualification of new active principles mainly to fight against extrinsic factors found to be harmful for the skin. One of the most important fields of research is thus the prevention and the repair of sun-induced skin damages, which can occur through several intracellular as well as extracellular mechanisms.

Reactive Oxygen Species

It is widely accepted that ultraviolet B (UVB) irradiation causes DNA damage more or less directly, whereas UVA light induces damages via generation of reactive oxygen species (ROS) in a more indirect manner [2,3]. During sun exposure, endogenous absorbers of UV light and photosensitizers in human skin (riboflavin, porphyrin, tryptophan, urocanic acid, etc.) can be involved in generation of ROS. These UV-induced ROS are believed to be the main factors for age-related damages in skin apparent as deep wrinkles and furrows, which are mechanistically summarized by the term "photoaging."

Dermal Changes

With increasing age and due to UV irradiation, aging skin shows an increasing imbalance between assembly and breakdown of collagen—one of the primary compounds of the dermis—towards breakdown. This results in an overall collagen decline of approximately 1%/year per unit area of skin surface [4]. The reasons for this are lower levels of new synthesized collagen, a shift in the ratio of collagen types [5], and an increased activity of collagen-degrading enzymes such as the collagenase matrixmetalloproteinase-1 (MMP-1) [6]. Aside from this, UV light also influences other important dermal components like elastin and glycosaminoglycans. It causes the accumulation of elastotic material, a nonfunctional mass of elastic fibers [7], and loss of glycosaminoglycans in the dermis. This lack of regeneration of the dermal connective tissue, i.e., structural reorganization of collagen and elastin, decreased glycosaminoglycans, together with a reduction in the tissue fluid content and water-binding capacity, seems to play an important role in the formation of wrinkles. Even in young skin, the regeneration of collagen (over a period of months) is a relatively slow process. Thus, the tensile characteristics of the skin are altered, so that it becomes generally thinner, less elastic, and less resistant against stress. Phenotypically, this extrinsically caused and accelerated premature skin aging is manifested in an advanced state as the formation of coarse, deep furrows and folds, as well as aggravated elastoses. Even though the mechanisms of both extrinsic and intrinsic skin aging cause a fundamental change in

the appearance of the skin, the contribution of the extrinsic portion, however, seems to be predominant.

Epidermal Changes

A characteristic feature of aging skin is the declining ability to regenerate, being particularly evident in the longer time span needed for renewal of the epidermal layer. This so called "epidermal turnover" takes about 28 days in young adult skin and may increase to 40–60 days with age [8]. Furthermore, as skin gets older the UV-induced tanning intensity becomes more irregular, but the scientific knowledge about the physiology on development of age spots or melasma is still insufficient. The appearance of age spots can be a result of the decreasing ability of melanocytes to distribute the produced melanin packets (melanosomes) to the surrounding keratinocytes equally or a localized overproduction of melanin. One can only speculate whether this is primarily caused by an uneven distribution of melanocytes in skin or, rather, a dysregulation of physiological processes in melanocytes.

Other Aspects

Furthermore, the process of premature skin aging leads to an impairment of the denticulation of the epidermal/dermal junction zone and to a reduction of the number of so-called papillae, each of which harbors a blood capillary growing out of the dermis. These structural changes are considered as histological hallmarks of aging skin morphology, which is accompanied by the reduction of the capillary diameter, as well as capillary density in aged skin [9]. A well-functioning blood capillary system contributes to an adequate nutrient supply to the upper skin layers, and thus to the structural integrity and complexion of skin.

The immunological defense system is also significantly reduced with increasing age. Thus, intensive sun exposure can promote neoplastic cell transformation (e.g., melanoma), and the incidence of skin tumors increases with age. In consequence, protection of skin of all ages against the negative effects of sun irradiation is the most important task to keep skin healthy and young. Often described and subjectively felt, dry aged skin cannot be attributed only to the distinctive defect of the epidermal water barrier of the horny layer. Rather, it can be attributed to regenerative processes, as well as a worsening in the water storage capacity, caused by a diminished production of cutaneous moisturization factors (e.g., amino acids, hyaluronic acid, pyrrolidon carbon acid, and glycerine) able to bind water in the horny layer. Besides the reduced water retention capacity, the age-dependent reduction in sebum secretion of the sebaceous glands also plays a role in the formation of dry aged skin. As the sebaceous glands seem to be predominantly hormonally regulated, age-dependent decline and changes in the hormonal system worsen the condition and function of aged skin.

An overt example of the endocrine influence on skin aging is the exacerbation of dry skin and increased wrinkle formation that occur with menopausal hormonal changes, and specialized hormone treatments can lead to an improvement of old skin. These new scientific insights have facilitated a scientific merging in the fields of dermatology and endocrinology.

ACTIVE COSMETIC INGREDIENTS AND THEIR POTENTIAL

Aging consumers experiencing dry skin tend to favor rich skin-care formulations that include *moisturizers* with high water-binding properties, for example, glycerine. Increasingly, modern cosmetics attempt to satisfy these consumer demands for products with preventative or even regenerative performance. Besides preventing early skin aging, products must also smooth or improve the appearance of wrinkles as well as retard the weakened regenerative potential of the skin (epidermal turnover). Modern skin research in the cosmetic industry has already revealed several ways to specifically target the biological needs of aged skin.

In addition, to avoid intensive sun exposure, protection of the skin against UV-dependent oxidative stress can be provided by the use of products with highly efficient *UV filter* technologies. It has to be considered that older skin is even more sensitive to UV exposure. Due to the age-dependent atrophy of the skin [10], UV radiation penetrates more deeply, and damages increase and can accumulate. This effect leads to a need for high photoprotection, so product formulations should employ an efficient UVA/B filter combination. Besides UVB protection, which delivers erythemal protection, UVA filter performance plays a decisive role in the prevention of photoaging, because UVA radiation is a key factor in the production of ROS and the subsequent activation of collagen-degrading enzymes.

As physiological events in skin are based on physical and chemical processes including redox cascades, skin has developed an *antioxidative defense* system as a direct protective barrier against endogenous and exogenous environmental oxidative stress factors (e.g., UV light). These endogenous antioxidative protectants of enzymatic and nonenzymatic antioxidant systems [11] are concentrated to a higher value in the epidermis compared to the dermis. Substances such as flavonoids, vitamins A, C, and E, coenzyme Q10, as well as carotinoids are components of a healthy diet and can replenish and support the cutaneous system in its protective function. Topical application of substances such as vitamin E and vitamin C as well as, in particular, the plant-derived flavonoid derivative alpha-glucosylrutin (AGR) show a large protective potential against premature UV light-induced skin aging [12]. This positive activity, however, preempts that topically applied antioxidants will adequately interact with the natural endogenous redox system of the skin. Therefore, not all of the known oxidative substances achieve the desired protective effect when applied to the skin. The water-soluble and thus bioavailable flavonoid, AGR can build up a skin-protective depot in the living layer of the skin, in which the inherent glutathione redox system protects against oxidative

damage, and UV-induced inflammation is reduced [12–14]. Similarly, the water-soluble antioxidant vitamin C can, amongst other activities, function as a cofactor in collagen synthesis, thereby supporting skin regeneration in deeper layers. Knowing the causative involvement of UV-induced oxidative stress reactions in the cutaneous aging process [15], the best prevention and radical modulation can be reached, and to some extent improved, by providing focused, customized topical treatment strategies.

For treatment of age-damaged skin, particularly for antiscaliness and antiwrinkle efficacy, countless principles are available on the market, which are based on the removal of the outer horny layers of skin (*exfoliation* or peelings). Commonly used agents are so-called alpha hydroxy acids (AHAs), most often endogenous metabolites (lactate) or other naturally occurring substances such as fruit acids. Depending on the substance used, the respective depth of treatment in skin can be determined by adjusting the topical concentration and treatment time applied. The activity of these agents is generally based on induction of skin regeneration by exfoliation and subclinical inflammation, which appears to be comparative to a superficial wound healing process.

Several *antiaging actives* are proven to have beneficial effects on skin aging. Vitamin A and its derivatives have been used as active ingredients in the cosmetic industry for many years. Their activity is essentially based on the interaction of specific nuclear receptors, whose activation regulates, for example, collagen synthesis, improving the structure of the skin. Regrettably, besides concentration-dependent skin irritant properties, these vitamin A ingredients are also highly sensitive to light-dependent and oxidative processes, greatly reducing their activities. New cyclodextrin-based formulation technologies enable the efficient stabilization of these active ingredients without limiting their activity [16].

All cells, and thus also skin cells, need energy. It is needed to grow, for protection and repair, and most importantly, for regeneration and cell division. To maintain this capacity for cellular life, the mitochondria, small intracellular organelles operating as small power plants in the cells, are imperative. Beside the mitochondrial energy supply, cells also have a system named the creatine/phosphocreatine pathway. According to latest insights findings, this occurs in the human skin and is responsible for an extremely fast energy supply [17].

Creatine as well as another energy metabolite, coenzyme Q10, can both be synthesized in human cells, but from the age of about 30, a reduction in the cellular concentration of these compounds in the skin can be determined [18]. As a fat-soluble oxidative substance, coenzyme Q10 protects the cell membrane and organelles [19]. It especially plays a role in the electron transport system during the energy production (ATP) in cellular respiration of the mitochondria, preventing a chronic energy deficiency in aging cells [20]. The topical application of coenzyme Q10 and creatine, respectively, can activate countless synthetic processes, ultimately resulting in a reduction of wrinkle depth by balancing energy deficits [21]. Besides this, the regeneration activity of aged skin can be stimulated by the external application of these active ingredients.

There are different causes for skin irritation. Independent of age, skin is more sensitive to irritation in cold, dry wintertime than in summertime. Especially, people with so-called sensitive skin have to protect their skin against dryness, intensive sun exposure, mechanical stress, and environmental noxae. But also, age-related old skin is reported to be more sensitive to irritation due to restricted defense and repair mechanisms. In consequence, a prophylactic anti-inflammatory treatment appears to be recommendable for the skin of the elderly.

Special skin-care regimens adapted to the specific needs of sensitive skin are developed and provided by the cosmetic industry. Actives isolated from herbal extracts (e.g., licochalcone A) proved to be effective against mechanical stress, like razor burn, but can be also effective in skin care for dry atopic skin or rosacea.

The accumulation of advanced glycation end products is a general phenomenon of aging skin cells investigated in recent years. This nonenzymatic reaction in skin cells, also known as Maillard reaction, was first identified in the skin of young patients with type I diabetes mellitus [22], leading to an increased collagen-linked fluorescence [23]. This autofluorescence is currently used to quantify the accumulation of advanced glycation end products in the skin. Further investigation revealed that as skin ages, there is a continuing process of glycation, leading to a significant increase in skin autofluorescence [24]. On the molecular level, the uncontrolled binding of excessive glucose to proteins (e.g., to arginine and lysine residues of the proteins) leads to the formation of carboxymethyllysine and carboxyethyllysine residues. The important filament protein vimentin is especially prone to targeting by the Maillard reaction in human skin [25].

To date, the accumulation of advanced glycation products in the skin is not reversible; therefore, an optimized cellular energy metabolism by supplementing with creatine and Q10 may have valuable effects [17,21].

Promising in vitro results, however, demonstrate the protection of proteins against the formation of new reaction products by certain chemicals. The cosmetic industry strives to develop special skin-care regimens introducing active ingredients into cosmetic formulations to protect against the accumulation of glycation end products.

PERSPECTIVES

The physiology of skin aging is a complex, multifaceted, and dynamic phenomenon. Even though many molecular causes of the aging process are not understood in complete detail, there is consensus that chronological age alone is not crucial to this process.

In the future, the application of modern molecular and biological methods in skin research such as the DNA chip technology (microarrays) and the proteomic technology will allow new insight into the aging process—genes and gene products involved with their genetic control mechanisms. These new technologies, the accessibility of the skin, and

the improvement in culturing in vivo resembling skin models will increasingly contribute to a better understanding of the regulation of the intrinsic and extrinsic aging process and the positive effects of topically applied age-specific agents. In the long term, these technologies can provide a new fundamental knowledge about the control mechanisms of the human aging process as a whole.

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48 Antiperspirants

Jörg Schreiber

INTRODUCTION

This chapter presents an overview concerning the current knowledge of antiperspirant actives and their interactions with the human axilla. It is the author's intention to give the interested reader a short introduction about formulation work, drug delivery systems, and application forms developed for antiperspirant actives. The final section lists references that should be useful for anyone who wants to learn more about a specific topic of antiperspirant technology.

BIOLOGY OF SWEAT GLANDS IN THE HUMAN AXILLA

The axilla region of humans contains apocrine, eccrine, and sebaceous glands. Approximately 25,000 sweat glands per axilla can produce up to 12 g sweat per hour (1). The current understanding concerning the structure and function of sweat glands is that thermoregulation is the only aspect of the body participating in immunological, metabolic, and hormonal aspects of human life (2).

Eccrine Glands

This is the gland responsible for the majority of sweat production. It has a sensory and an excretory function and can be stimulated by emotional and thermal stimuli (3). It produces clear, colorless, and odorless liquid containing 98% to 99% water and 1% to 2% inorganic and organic compounds (4). Inorganic components include NaCl and traces of $K^{þ}$, $Ca^{2þ}$, $Mg^{2þ}$, $Fe^{3þ}$, and $Cu^{2þ}$ ions. Organic components include lactic acid, citric acid, formic acid, propionic acid, butyric acid, urea, and ammonia. Underarm wetness comes mostly from the secretion of eccrine glands. Antiperspirants reduce the amount of sweat only from eccrine glands.

Apocrine Glands

Apocrine glands are apparently a relic from the phylogenetic development of man. These glands start to produce a milky, viscous fluid during puberty on special locations of the body, especially the underarm pit (5). In contrast to eccrine glands, the openings of the glands are not at the skin surface but appear at the hair follicle. Decomposition of apocrine sweat by skin bacteria is responsible for the characteristic malodor of human sweat. Apocrine sweat consists of, besides water, proteins, carbohydrates, and ammonium salts (6). Other investigators have reported that these glands secrete lipids, cholesterol, and steroids (7). Furthermore, it has been shown that androgen-converting enzymes in the apocrine glands are responsible for circulating androgens to dihydrotestosterone (5).

ANTIPERSPIRANTS

Antiperspirants are topically applied products designed to reduce underarm wetness by limiting eccrine sweat production. In the United States, these products are regulated by the Food and Drug administration (FDA) as over-the-counter (OTC) drugs because they are intended to affect a ''function of the body'' (i.e., in this context, perspiration). Products containing antiperspirant actives have to reduce perspiration to a minimum of 20% in 50% of the test population under validated test conditions. Test protocols (in vivo clinical trials), to develop a safe and an effective product, have been designed to substantiate the desired claims (8–14).

Comparative quantitative determination of the activity of sweat glands on the forearm after application of aluminum chlorohydrate (ACH) solutions is now possible by combining the classic starch iodine visualization technique with digital image analysis (15). A noninvasive optical technique that allows the analysis of the function of a number of glands, simultaneously, in vivo was recently reported (16). A new method for parallel testing of up to eight formulations on the backs of volunteers allows a very fast evaluation of product prototypes (1).

Sweat Reduction by Antiperspirants: Current Model/Theory

The reader should be aware that theories concerning the action of sweat-reducing agents depend strongly on the type of actives (aluminum salts, nonionic agents, or ionic agents). The efficacy of antiperspirants based on aluminum and/or aluminum zirconium salts can be understood by the formation of an occlusive plug of metal hydroxide in the eccrine duct (17). Tape-stripping experiments followed by analysis of transmission electron micrographs of an ACH-treated eccrine sweat gland duct show an obstructive amorphous material supporting the theory of a mechanical blockage of sweat glands from diffusion of the soluble ACH solution into the sweat gland and subsequent neutralization to a polymeric aluminum hydroxide gel (18,19). There seems to be no correlation concerning the efficacy of aluminum salts and the location of the plug in the duct, because it is known that, compared with ACH, the

more effective Al–Zr compounds do not penetrate as deep as the also highly effective $AlCl_3$ solutions (17). The reader is referred to the literature concerning other theories of sweat reduction by aluminum salts (20).

Active Ingredients for Controlling Underarm Wetness—State-of-the-Art

Buffered Aluminum Salts (ACH)

The first antiperspirant, Ever Dry, based on $AlCl_3$, was introduced to the market in 1903 (21). The first cream-containing aluminum sulfate was introduced during the 1930s. The acidic pH value (2.5–3.0) was a drawback of these products, leading to skin irritation in the underarm pit. History tells us that the development of antiperspirant actives with a higher pH value, so-called buffered aluminum chlorides (ACH, pH 1/4 4.0–4.2), was an appropriate step with the additional benefit of reduced destruction of fabric clothes. The formula of this buffering salt is $[Al_2(OH)_5]^{þ}$ þ (Cl^{-}), or more conveniently, $Al_2(OH)_5Cl$.

The historical development from $AlCl_3$ to $Al_2(OH)_5Cl$ can be easily understood by the following consideration:

$$AlCl_j\ 1/4\ ^{x}h\ Al_2\ Cl_6\ \text{ðsubstitute 5 } Cl^{-}\text{ ions against OH ions}^{þ}\ 1/4>$$

$$Al_2\ ðOHþ_5\ Cl$$

$Al_2(OH)_5Cl$ is a 5/6 basic aluminum trichloride. The accepted definition of ACH is the ratio of Al to Cl 1/4 2.1 to 1.0. Lower levels lead to ACH $[Al_2(OH)_4Cl_2]$ or to aluminum sesquichlorohydrate $[Al_2(OH)_{4.5}Cl_{1.5}]$—both actives are also generally regarded as safe (GRAS). ACH is supplied as a powder or a 50% solution in water. It can be formulated up to 25%, calculated on an anhydrous basis. The 20% aqueous solution reduces perspiration by 35% to 40% on average (22). Some dyes used in clothing may be acid sensitive and will change color when in contact with an antiperspirant.

The structure of the Lewis acid ACH is very complex because ACH in water forms the so-called isopolyoxo cations with chloride ions as counterions (23–25). There exist several polymer equilibria of the polycationic aluminum species in water-based systems. Short-chain polycationic species are more effective in reducing sweat.

Aluminum Zirconium Chlorohydrate–Glycine Complexes

Aluminium zirconium chlorohydrate-glycine (AZG) complex is obtained by reaction of ACH with zirconyl chloride. Reaction of the former ingredient in the presence of glycine leads to zirconium aluminium glycine (ZAG) complexes. Glycine is used as a buffering agent. These antiperspirant actives form very complex polymeric structures in water. The actives are defined by the ratio of Al þ Zr metal-to-chloride ratio and the Al–Zr atomic ratio. The interested reader is referred to the literature concerning available antiperspirant actives (26,27) and nomenclature of the Al–Zr complexes (21,22). These antiperspirant actives were developed especially for anhydrous formulations because they show, compared with ACH, enhanced sweat reduction (28–30). The maximal concentration of ZAG calculated on an anhydrous basis is 20%. They are not allowed to be formulated for use in aerosols.

New Concepts for Controlling Underarm Wetness

Titanium Metal Chelates

The understanding of the complex solution chemistry of aluminum-based antiperspirants gave input to the search for alternative antiperspirant salts. Titanium derivatives, like partially neutralized ammonium titanium lactate (ATL) salts, were shown to be effective in in vitro efficacy tests (31). The titanium metal chelates can be synthesized from the corresponding titanium alkoxides and organic acids allowed by neutralization with ammonia. Under acidic to neutral pH conditions, the ATL active seems to be relatively stable to hydrolysis and, therefore, probably is a suitable antiperspirant active in water-based or anhydrous drug delivery systems.

Film-Forming Antiperspirant Polymers

The so-called polybarrier technology is another approach to reduce perspiration by using a polymer that forms an insoluble occlusive film barrier on the underarm skin (32). It was mentioned that the occlusive film is a barrier to the passage of moisture. The main advantages of this technology are reduced skin irritation, applicability after underarm shaving, and higher sweat reduction compared with today's classic antiperspirant salts. The preferred polymer is an olefinic acid amide/olefinic acid or ester copolymer-like octylacrylamide/acrylate copolymer (Versacryl-40). This copolymer can be used alone or in combination with poly vinyl pyrrolidine (PVP)/eicosene copolymer in sticks, roll-ons, or alcohol-based products (33). The reduction of sweat depends on the choice of vehicle and extends in some formulations to 40%.

Lyotropic Liquid Crystals

Certain surfactant/cosurfactant combinations form in water depending on the variables of concentration/temperature instead of micelles' lamellar, hexagonal, inverted hexagonal, inverted micellar, or even cubic phases. The cubic phases can be of micellar or bicontinuous type (34). The water domains in lamellar or cubic phases can swell to a certain degree, while taking up water. The use of this swelling behavior is the basis of a patent where a surfactant/cosurfactant combination is applied to the underarm pit (35). Sweat (water) transfers the applied composition to a lyotropic liquid crystal of cubic structure, thus creating a sweat-absorbing system in the axilla. Oleic acid/glycerol monolaurate is one of the surfactant combinations in the patent. Both components are also well known as deodorizers.

DRUG DELIVERY SYSTEMS AND APPLICATION FORMS FOR ANTIPERSPIRANT ACTIVES

Antiperspirant actives can be formulated in a variety of delivery systems like anhydrous suspensions, water- or hydroalcoholic-based solutions, and emulsions. Typical application forms for antiperspirants are sticks, roll-ons, creams, pump sprays, aerosols, gels, and powders. On a global basis, the three most important product forms are sticks, roll-ons, and aerosols.

Formulation Work

After the decision for the desired application form has been made, the formulator has to decide on the vehicle system for the antiperspirant active. It is the intent of this section to summarize some of the current knowledge concerning the influence of actives with the formula, efficacy of different delivery systems, and the function of the ingredients used in antiperspirants.

Antiperspirant actives, like ACH or ZAG complexes, are soluble in water. Application of a concentrated aqueous solution of an antiperspirant active gives a rather tacky feeling (36). Reduction of tackiness can be best achieved by silicone oils (cyclomethicones) or ester oils like di-(2-ethylhexyl) adipate (27). The acidic pH value (4.0–4.2) has to be taken into account by selecting additional components for the desired drug delivery system. Loss of viscosity and problems of a final formula with color stability are often hints to change the gellant and/or perfume. Aluminum powders in anhydrous systems (aerosols and suspension sticks) often leave visible white residues on skin or clothing. Liquid emollients, like alkoxylated glucose ether (PPG)-14 butylether or the aforementioned adipate ester, minimize these residues. Another approach is to use the solid emollient isosorbide monolaurate (Arlamol ISML, ICI) (37). In anhydrous aerosol formulations, the ACH powder settles down and forms a hard-to-redisperse cake at the bottom of the aerosol can. Suspending aids, like quaternium-18 hectorite or quaternium-18 bentonite, prevent settling of the antiperspirant active and additionally thicken the cyclomethicone oil phase. Usage of fine powders of ACH is another approach to overcome nature's law of gravity.

The reader should be aware that hydrophobic ingredients, like emollients, have an influence on the effectiveness of an antiperspirant active, because a cosmetic oil phase or wax can cover the pores of the eccrine duct. The efficacy of an antiperspirant active, like ACH, is higher in water-containing systems compared with anhydrous formulations. The following rules concerning efficacy might be helpful:

1. Efficacy: aqueous solution > anhydrous suspension.
2. As diffusion of an antiperspirant active in the vehicle and from the vehicle to the skin after application has to be considered, one can further differentiate the expected efficacy trends. Efficacy: aqueous solution > sprayable oil in water (O/W) emulsion > O/W emulsion roll-on > O/W emulsion cream.
3. It is accepted that antiperspirant actives in the outer phase of an emulsion have a higher efficacy than in the dispersed phase. Efficacy: O/W emulsion > water in oil (W/O) emulsion.
4. In water-free systems, the viscosity of the drug delivery system might be of relevance. Suspended ACH in anhydrous vehicles needs to be solubilized after application to the axilla by sweat (water). The effectiveness of suspension sticks depends on the rapidity of active solubilization. The usage of ultrafine powders of ACH is expected to boost efficacy compared with fine powders. Efficacy: low viscous suspension > suspension stick.

The reader is referred to the literature concerning vehicle effects on antiperspirant activity (7,38,39).

Lipophilic ingredients might have an influence on the efficacy of a product, because it is known that the water-soluble propylene glycol can form complexes or hydrogen bonds with aluminum polycationic species, thereby altering the efficacy of the salt (40). Also, propylene glycol in high concentrations may result in skin irritations (41). Successful formulation work aims at finding the right viscosity for the product in the desired application form, a lower viscosity during the flow into the underarm pit, and a higher viscosity after application so that the product stays where it was applied. Conventional shear shinning flow curves are characteristic for antiperspirant products. The reader is referred to the literature concerning rheology aspects of cosmetic products (42).

Deodorant/Antiperspirant Sticks

It is, at present, not easy to give the reader an overview about sticks, because nowadays, there exist many technologies to develop this solid delivery system. In Figure 48.1, an attempt was made to summarize this area. In the following section, only systems of major importance are discussed.

Sticks can be divided into different classes like suspension sticks, gel sticks, and emulsion sticks. Soft sticks have some properties of all three categories (Figure 48.1).

Suspension Sticks

Dry deodorants, or antiperspirant solids, are synonyms for an application form where the active in the form of a powder is suspended in a silicone oil phase. Stearyl alcohol is usually used as the hardening agent. The molten mass crystallizes into a matrix of stearyl alcohol saturated with the silicone oil and suspended particles (43,44). Quaternium-18 hectorite can reduce the settling of the actives. Cyclomethicones give the stick a dry, silky feel; nonvolatile oils, like PPG-14 butylether, minimize white residues on the skin (43). Low-residue sticks can be obtained by using a combination of high- and low-melting waxes and a volatile and nonvolatile silicone oil combination (45) (Table 48.1).

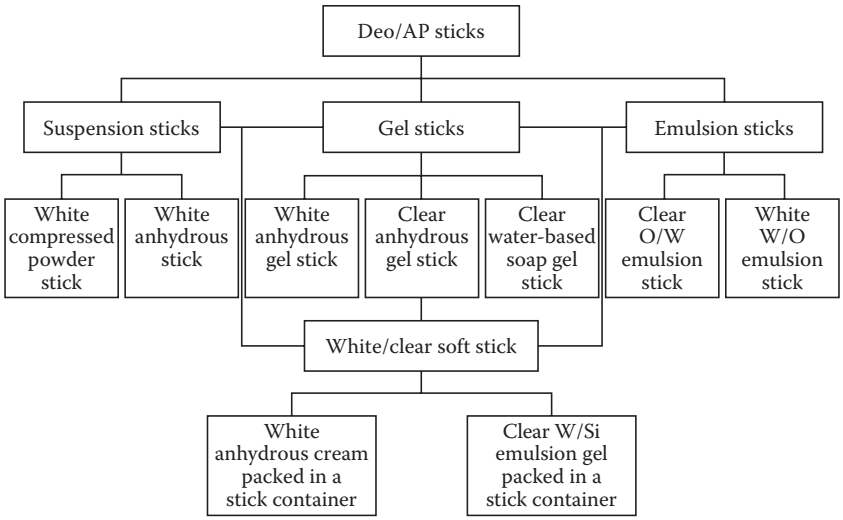

FIGURE 48.1 Overview of cosmetic deodorant/antiperspirant sticks.

TABLE 48.1
Suspension Stick

	Wt%
Stearyl alcohol	20.0
Cyclomethicone	54.0
PPG-14 butylether	2.0
Hydrogenated castor oil	1.0
Talc	2.0
Antiperspirant	20.0
Fragrance	1.0

Note: PPG, photoplethysmography.

TABLE 48.2
Gel Sticks

White Anhydrous Gel Sticks	Wt%	Clear Anhydrous Gel Sticks	Wt%
N-lauroyl-l-glutamic acid dibutyl amide	5.0	Dibenzylidene sorbitol	2.0
12-Hydroxystearic acid	5.0	Dimethicone copolyol	2.0
Cyclomethicone	40.0	Di-isopropyl sebacate	2.0
Hydrogenated polyisobutene	15.0	Glycine	1.0
Di-isopropyl myristate	15.0	Dipropyleneglycol	10.0
Antiperspirant powder	20.0	Propyleneglycol	33.0
		Antiperspirant powder	50.0

Source: Motley, C.B., Gel stick compositions comprising optically enriched gellants. US patent 5 552 136.

Gel Sticks

This class can be subdivided into the following groups: white anhydrous gel sticks, clear anhydrous gel sticks, and clear water-based soap gel sticks. The last class mentioned is discussed in Chapter 49.

White Anhydrous Gel Sticks

Shear solids, or ultraclear solids, are synonyms for sticks with improved washout performance compared with the classic suspension sticks. They contain *N*-acyl amino acid amides (*N*-lauroyl-l-glutamic acid dibutylamide) and 12-hydroxyacid as gelling agents for an oil-phase mixture (e.g., silicone oil/mineral oil). The washout agent is an ethoxylated solubilizer, like Ceteareth-20. These white sticks turn clear after application to the skin (no-residue stick) (46).

Clear Anhydrous Gel Sticks

They are quite popular in the United States because clarity is associated by the consumer with a lack of white residue on skin, no dangerous ingredients, and high efficacy. A typical gelling agent is dibenzylidene sorbitol (dibenzylaldehyde monosorbitol acetal [DBMS A]). This acetal is not stable in an acidic aqueous environment (47). The sticks usually contain a high level of alcohol and/or polyols. At high polyol concentration, the active is regarded to be solubilized instead of suspended in the gel matrix (48). An alternative gelling agent is a polyamide (49) (Table 48.2).

Emulsion Sticks

They can be grouped into clear O/W emulsions, white W/O emulsions, and clear water in silicon oils (W/Si) emulsion gels. The last mentioned is discussed below.

Clear O/W Emulsions

They contain a high surfactant combination with the active solubilized in the external water phase. The high concentration of surfactants is a disadvantage; no products based on this technology are known to the author (47).

TABLE 48.3
W/O Emulsion Stick

	Wt%
Stearyl alcohol	19.0
Volatile silicone	26.0
Mineral oil	1.0
2-Methyl-2,4-pentandiol	2.0
Polyglycerol-4 isostearate	2.0
ACH solution (50%)	50.0

Source: Hourihan, J.C., and Krevald, H., Water-in-oil emulsion antiperspirant sticks, US patent 4 704 271.
Note: ACH, aluminum chlorohydrate.

W/O Emulsion Sticks

The water phase containing the active is solubilized by a surfactant, like polyglycerol-4 isostearate. A typical example for an oil/wax phase combination is a mixture of silicone oil/stearyl alcohol (50) (Table 48.3).

Soft Sticks (Soft Solids, Smooth-Ons)

These sticks can be differentiated into two subgroups, namely, white, anhydrous creams (suspensions) and clear water-in-silicone emulsion gels. Both delivery systems are packed in a container that gives the impression of a stick. The suspension or gel is extruded onto the skin from holes in the top of the stick container to a wide smooth area around the holes.

White, Anhydrous Creams

These creams contain an antiperspirant active, a volatile and nonvolatile silicone oil, and a thickener (*N*-acyl glutamic acid amide).

Clear Water-in-Silicone Emulsion Gels

These formulations can be achieved by adjusting the refractive index of the water and silicone oil phase. Silicone formulation aids (Dow Corning 3225C) are mixtures of cyclomethicone and dimethicone copolyol helping to solubilize the active (7,46,48,51). Low surface tension of cyclomethicones facilitates good spreading of the product on the skin and reduces the tackiness of antiperspirant actives.

Antiperspirant Roll-Ons

Roll-on products can be differentiated into several categories (Figure 48.2). O/W emulsion-based delivery systems are quite popular in Europe, whereas anhydrous suspension roll-ons or transparent water-in-silicone emulsions are preferred in the United States. A new trend concerning the size of the roll-on applicator has been identified. Consumers prefer the big-ball format (3.0–3.5 cm) because of the ease of applying the product to the underarm pit (52). The popularity of roll-ons, in general, is because of the nongreasy and nonoily feel in the axilla and the good spreadability of the content on the underarm skin.

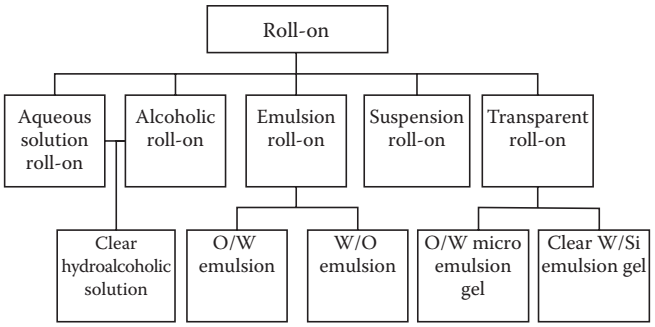

FIGURE 48.2 Overview of cosmetic deodorant/antiperspirant roll-on types.

Clear Hydroalcoholic Roll-On

This delivery system contains a water/alcohol solution of the antiperspirant active thickened with a water-soluble polymer like hydroxyethylcellulose. The alcohol in the formula gives, compared with the clear aqueous solution-based roll-ons, a fresh sensation in the axilla and facilitates drying of the product. Excellent antiperspirant efficacy is another benefit of hydroalcoholic roll-ons.

O/W Emulsion Roll-On

This delivery system uses ethoxylated surfactants, like poly ethylene glycol (PEG)-40 stearate, to solubilize an oil phase like mineral oil. The active is dissolved in the outer phase, allowing the formulation of a highly effective product. In alcohol-free formulated systems, microbiological stability has to be checked (Table 48.4).

W/O Emulsion Roll-On

They are weaker in efficacy because the actives are encapsulated and the external oil phase often gives a sticky feeling.

TABLE 48.4
Roll-On

O/W Emulsion Roll-On	Wt%	Hydroalcoholic Roll-On	Wt%
PEG-40 stearate	5.0	Antiperspirant active	20.0
Cetyl alcohol	3.0	PPG-5 Ceteareth-20	2.0
Mineral oil	2.0	Water	35.4
Polysorbate-80	1.0	Ethanol	42.1
Glycerin	1.5	Hydroxyethylcellulose	0.5
Magnesium aluminum silicate	0.8		
Antiperspirant active	20.0		
Water	66.7		

Note: Mg, magnesium; PPG, photoplethysmography.

W/Si Emulsion Roll-On

Silicone oils allow products to formulate on the basis of a "W/O technology" because the skin feel is not comparable with traditional oily components, like ester oils or triglycerides. The concentration of the thickener is reduced compared with sticks based on this type. The technology is discussed under soft sticks (see p. 509).

O/W Microemulsion Gel

An alternative approach to transparent products uses the phase inversion temperature (PIT) technology. A suitable mixture of surfactants, oils, and water is heated from 60°C to 90°C to give a W/O emulsion above the PIT. During cooling, the mixture shows phase inversion to give white or transparent O/W emulsions; O/W microemulsion gels are obtained in the presence of hydrophobically modified water-soluble polymers (53). The technology is explained in more detail in Chapter 49.

Suspension Roll-On

The antiperspirant active in powder form is suspended in cylomethicone. The roll-on can be formulated with or without ethanol. Quaternium-18 hectorite is used as a thickener to prevent settling of the active. Consumers in the United States prefer this delivery system as it does not give a wet feeling after application and because of the easy drying (39). Actives like ZAG complexes give high efficacy to underarm products (Table 48.5).

TABLE 48.5
Suspension Roll-On

	Wt%
Volatile silicone	65.0
Quaternium-18 hectorite	13.5
Silica	0.5
Antiperspirant powder	20.0
Fragrance	1.0

Antiperspirant Aerosols

Aerosols, in Europe and Asia, are popular delivery systems for consumers who prefer a hygienic and easy-to-use application form. Typical ingredients for aerosols include isopropyl myristate, isopropyl palmitate, volatile silicone, dimethicone, silica, clays, propylene carbonate, and ethanol. Propellants include propane, butane, and isobutane (Table 48.6).

As acidic aqueous ACH solutions lead to corrosion of the aerosol can, current aerosol antiperspirant products are formulated as water-free suspensions. The active is suspended as a powder in an oil phase like cyclomethicone or in a mixture of ester oils/cyclomethicone. Agglomeration of solid particles and settling of actives can be minimized by the usage of suspending agents like fumed silica (amorphous silicon dioxide) or clays (bentonite and hectorite). The clays form a weak gel in the presence of an oil phase that can be destroyed by shaking the aerosol can before usage. The gel structure is reformed on standing, thereby holding the active in suspension. Because the organoclays are agglomerated, shear is needed to deagglomerate the platelets, and a polar activator like propylene carbonate or ethanol is used to disperse them and induce the gelation of the oil phase.

TABLE 48.6
Antiperspirant Aerosol

	Wt%
Volatile silicone	13.4
Quaternium-18 hectorite	0.8
Ethanol	0.8
Antiperspirant powder	10.0
Propellant (butane/propane)	75.0

The steps involved to prepare an aerosol product can be summarized in the following sequence (7):

1. Preparing bentonite or hectorite clay with the emollient in the presence of the polar activator and shearing the mixture
2. Adding the antiperspirant active until a uniform agglomeration-free suspension is obtained
3. Filling the concentrate into the aerosol can and adding the propellant (pressure filling)

Efficacy studies of aerosols, including comparison with other drug delivery systems, have been reported in the literature (30). ZAG complexes are not allowed to be used in aerosols.

Environmental Issues

Aerosols contain volatile organic compounds (VOCs) usually in a weight ratio of propellant to concentrate of 75:25 (54). The environmental impact of VOC, like the reaction with nitric oxides (NOX), in the presence of sunlight causes formation of unwanted ozone in the lower atmosphere. US antiperspirant companies especially were forced to reduce VOC emissions by reformulating hydrocarbon propellants and/or exchanging hydrocarbon propellants with the fluorohydrocarbons, 1,1-difluorethane (propellant 152a) or 1,1,2,2-tetrafluorethane (Propellant 134 a). The water-soluble dimethoxyethane (DME) is another propellant that is thought to have no impact on the damage of the ozone layer (55).

The current trends in the aerosol market can be summarized as follows:

- Higher ratio of concentrate/hydrocarbon propellant
- Higher amount of silicone oils
- Usage of 1,1-difluorethane (propellant 152a)
- Formulations with lower vapor pressure
- Usage of smaller aerosol cans

Aerosols containing 20% to 50% propellants with a concentrate–propellant ratio from 1.0:1.0 to 2.3:1.0 have been patented (56).

FUTURE TRENDS

Some new trends in the antiperspirant field concerning new actives and delivery systems have been described in this chapter. Improvements of current formulations and innovative concepts will need the ongoing investigation and better understanding of the interaction of active/vehicle and vehicle/skin. Improving efficacy and skin compatibility is another major trend in the antiperspirant field. New packaging concepts, like the extrudable gels, the big-ball applicator for roll-ons, and reduced-size aerosol cans with ozone-friendly propellants, are probably, in a few years, the state-of-the-art. The influence of perfume components to the skin and the increasing rate of contact allergies attributable to fragrance ingredients have to be closely monitored (57).

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49 Deodorants

Jörg Schreiber

INTRODUCTION

This chapter intends to give an overview on the current knowledge about the origin of underarm odor and the biology of the underarm microflora and its interaction with deodorizing agents. The contents of this chapter have been arranged in a particular sequence to facilitate the understanding of rational deodorant product development.

BIOLOGY OF THE UNDERARM MICROFLORA

The resident microflora of the human underarm skin consists of up to $10^6/cm^2$ organisms, for example, aerobic cocci, lipophilic diphtheroids, and varying species of gram-negative bacteria [1]. In the axillae, two types of bacterial flora exist—coryneform bacteria and micrococcaceae such as *Staphylococcus epidermidis*. Coryneform- or *S. epidermidis*-dominated populations are characteristic for human beings. The resident microflora is a quite stable population, not varying a lot between both axillae [2]. The organisms are perfectly adapted to their ecological niche with its higher pH value and higher moisture content compared with other skin areas [3]. Hair in the axilla, according to the literature, is not a good substrate for bacterial growth; the bacteria prefer to reside on the underarm skin [2]. Moisture is required for bacterial proliferation and is secreted especially from the eccrine sweat glands [4]. The origin of strong compared with weak underarm odor is associated with a numerical dominance of coryneform bacteria [5]. Components of apocrine secretion, for example, isovaleric acid and androstenone, were proposed to contribute to axillary odor. Hydrolytic exoenzymes of skin bacteria cleave the ester bonds of odorless water-soluble precursors of androstenol to the corresponding volatile steroid [6]. Other studies proposed that the key odorants are branched, straight-chain, and unsaturated C6-C*n* fatty acids [7]. (E)-3-methyl-2-hexenoic acid (E-3M2H) is the most abundant fatty acid compared with the rest of the C6-C*n* fatty acids that contribute to the axillary odor bouquet. Apocrine sweat extracts have been analyzed, and concentrations of 0.5 ng/μL for androstenone and 357 ng/μL for E-3M2H were detected [8]. Volatile odor molecules of E-3M2H found in sweat secretions are transported, according to the authors, in a nonvolatile fashion to the skin surface. Two apocrine secretion odor-binding proteins (ASOB and ASOB2) were identified, carrying 3M2H molecules to the skin surface. Coryneform bacteria liberate the odor molecules from the protein precursor/odorant complex [8].

The reader should be aware that occurrence of these chemical compounds does not mean that all of us can smell them. Individual differences in odor perception for both isomers of 3M2H [9] and for the steroid androstenone are well known [8]. Approximately 50% of the adult population is not able to smell androstenones; this anosmia to androstenone—or to 3M2H—is genetically determined.

DEODORANTS

Deodorants are topically applied products designed to reduce underarm odor. They are considered in the United States as cosmetics, while antiperspirants are treated by the Food and Drug Administration (FDA) as drugs. Deodorants tend to be less irritating than antiperspirants. In Europe, the consumers today prefer deodorants compared to antiperspirants. In the United States, the trend is approximately reversed.

Concepts for Controlling Underarm Odor: State-of-the-Art

The current knowledge of the biology of the underarm microflora and the origin of underarm odor is the basis for developing strategies against odor formation. Numerous patents and literature articles disclose the incorporation of chemical compounds for their deodorizing properties. The intention here is to describe and exemplify major strategies, but not all deodorant actives that were developed in the past.

Strategies to reduce underarm odor include the following:

- Antiperspirant active-containing deodorants
- Odor-masking deodorants
- Odor-neutralizing deodorants
- Odor-quenching deodorants
- Enterase inhibitors
- Antimicrobial active-containing deodorants

Antiperspirant Active–Containing Deodorants

Antiperspirant actives such as aluminum chlorohydrate or the Al–Zr complexes (see Chapter 48) reduce the secretion of eccrine sweat. Their excellent antimicrobial properties against *S. epidermidis* and coryneform bacteria have been published [10]. The acidity of the aluminum salts may be a major factor in bacterial growth inhibition.

Odor-Masking Deodorants

Fragrance compositions (such as perfumes) have been used to mask odors since ancient times. It is conventional to incorporate 0.2% to 1.5% of a perfume in body deodorants [11]. They are designed to blend with the underarm odor and thus act as a masking agent. The perception of a perfume may differ significantly between individuals because of different interactions with the skin, washing habits, and specific underarm odor. The fragrance materials are blended to achieve what is known as "top note," "middle note," and "bottom note" components. The first is the refreshing note upon application, while the last are the olfactoric components, which stay on after application to the underarm skin.

Perfumes with antimicrobial properties have been described in patents and in the literature [12–14]. An additional benefit, especially for emulsion-based products, is that they might also act as a preservative. The increasing rate of contact allergies against fragrance ingredients should be taken into account using this approach to combat underarm odor [15].

Odor-Neutralizing Deodorants

In Chapter 48, it was mentioned that odorous C6-C*n* fatty acids contribute to underarm odor. Chemical neutralization with sodium bicarbonate ($NaHCO_3$) yields the corresponding odorless soaps [16]. This active, however, is not stable for long in aqueous compositions. Patents for deodorant applications and usage of $NaHCO_3$, in the presence of antiperspirant actives, have been filed [17,18]. Zinc carbonate–containing deodorants are also the content of a patent [19].

Odor-Quenching Deodorants

Zinc Ricinoleate

Zinc salts of ricinoleic acid have no bacteriostatic or antiperspirant effect [20]. They strongly bind odorous fatty acids, amines, and mercaptanes. Ligand-exchange reactions of ricinoleic acid for odor molecules are probably the reason for the quenching properties of zinc ricinoleate [21]. Interactions with perfume components in a deodorant formulation may weaken the desired quenching effect of the odor molecules after topical application to the underarm.

Metal Oxides

The oxides of calcium, magnesium, and zinc form in the presence of fatty acids in the corresponding metal soaps [22]. Zinc oxide particles aggregate to form a massive lump. This leads to clogging of aerosol products [23]. Hybrid powders were developed in which the metal oxide covers the surface of a spherical nylon powder [23]. The advantage of this technology is the increased surface area of zinc oxide and, thus, the enhanced odor-quenching efficacy and the reduced particle aggregation in aerosols.

Esterase Inhibitors

Zinc Glycinate

The inhibition of exoenzymes from the underarm bacteria should also result in odor reduction. Zinc glycinate has been described as a suitable active [24]. Antimicrobial tests showed no inhibitory effect against *S. epidermidis* or against the lipophilic diphtheroid bacteria supporting the suggested mechanism against microbial exoenzymes.

Triethylcitrate

The optimal pH value for development of underarm odor caused by coryneform bacteria is approximately about 6 in axillary extracts [25]. Shifting the skin surface pH to the acidic side should decrease the activity of skin esterases, which are proposed to be responsible for degradation of underarm secretions. Triethylcitrate was proposed to form citric acid by an enzymatic process on the underarm skin. In 1991, it was shown that this active has no pH-reducing effect after application to the underarm skin [26]. Nevertheless, deodorants containing this active are still in the market.

Antimicrobial Active-Containing Deodorants

This approach is currently the most commonly used strategy to prevent underarm odor. Ethanol is probably one of the best-known actives for deodorization [27]. Additional efficacy is normally required for a long-term deodorization, and this can be achieved by the additional usage of fragrance, an antiperspirant active, or other antimicrobial actives (famesol, phenoxyethanol, etc.).

Triclosan (2,4,40-Trichloro-20-Hydroxydiphenylether)

This active has a broad-spectrum antimicrobial activity against most gram-positive and gram-negative bacteria, molds, and yeasts. The presence of triclosan in antiperspirant sticks and roll-ons leads to a higher reduction of the bacterial microflora versus the triclosan-free antiperspirant composition [28]. Triclosan is also used in skin care products, hand disinfectants, and household products [29].

Glyceryl Fatty Acid Ester

Monoglyceryl and oligoglyceryl fatty acid esters such as glyceryl monocaprylate, monocaprinate, monolaurate, and diglyceryl monocaprinate are effective deodorizers [30]. Combinations of glyceryl monolaurate with farnesol and phenoxyethanol showed synergistic efficacy effects against coryneform bacteria [31]. The advantage of this ingredient combination over the first-generation deodorant actives such as triclosan is attributed to their higher biodegradability and their selective bacterial action. These actives are all naturally occurring in plants and animal species. In addition, it could be demonstrated that combinations of monoglyceryl and oligoglyceryl fatty acid esters with a variety of natural antimicrobials (e.g., wool wax acids) displayed a synergistic antimicrobial efficacy against underarm bacteria and serve as highly effective deodorant actives [32–35]. Products containing such actives have been successfully marketed for a number of years.

Sucrose Fatty Acid Ester

The fatty acid esters of sucrose are well known as emulsifiers in food products [36]. Sucrose can be substituted on eight hydroxyl

groups with fatty acids. The antimicrobial potential depends strongly on the substitution degree of the sucrose. Sucrose monostearate and sucrose monolaurate have been described as deodorizers in the literature and in patents [37–39].

Glycerolether

2-Ethylhexyl glycerolether (octoxyglycerol) is a clear liquid with good solubility in cosmetic oils, polyols, and alcohol but only moderate solubility in water (0.2%). Synergistic antimicrobial activity with other ingredients has been described [40]. This active has become popular recently in European deodorant formulations.

New Concepts for Controlling Underarm Odor

Ongoing research activities focusing on a better understanding of the interaction between underarm skin/skin microflora and skin microflora/odor formation, in combination with the discovery of highly selective actives, today allow more specific designs for deodorant products. In the next sections, some of the new trends are discussed in detail. New concepts for controlling underarm odor include the following:

- Chitosan
- Bacterial enzyme inhibitors
- Odor-inhibiting precursor mimics
- Product and skin-mediated perfume transformations
- Antiadhesives

Chitosan

Chitin is a naturally occurring polysaccharide (e.g., in insects, lobster, crabs, or fungi) containing *N*-acetylated-d-glucosamine units. Deacetylation of the amino group leads to the slightly water-soluble chitosan. The deodorizing properties of chitosan and the combination of this active with aluminum salts have been the subject of a patent [41].

Bacterial Enzyme Inhibitors

The enzyme amino acid (3-lyase) is, according to a patent filed in 1990, a catalyst for the formation of underarm odor [42]. This enzyme is located in odor-releasing bacterial cells and cleaves the apocrine precursors of sweat components, such as amino acids with the structure unit COOH–CH–(NH_2)–CH_2–S–R, to the corresponding odorous sulfur products. Several classes of enzyme inhibitors such as derivatives of hydroxylamines, 3-substituted amino acids, cycloserine, and pyridoxal were identified.

Odor-Inhibiting Precursor Mimics

Another approach to the inhibition of the above-mentioned enzyme amino acid lyase is to provide an alternative substrate for the bacteria that cleave the structure unit CH(NH_2) CH2–O–C(O)–R instead of the sulfur-containing amino acid sequence [43]. This approach leads to the corresponding nonodorous ingredients, such as benzoic acid, or to pleasant odor-generating substances, such as phenylacetic acid.

Product- and Skin-Mediated Perfume Transformations

The physical and chemical interaction of a perfume with the underarm skin is a very complicated matter. Research activities in this area focused on the question of which components of a perfume stay on and above the skin after topical application [44]. Headspace analysis is one of the techniques to gain more information concerning skin/perfume interactions. It could be demonstrated that the longlastingness of a fragrance can be achieved by using a prodrug (ester, acetal) of a perfume ingredient [45]. The esters or acetals of a fragrance composition hydrolyze on human skin because of the slightly acid pH value. The hydrolysis products (acids, alcohols, and aldehydes) impart a pleasant smell to the underarm skin. These product- and skin-mediated perfume transformations are especially suitable for alkaline formulations such as soap-based deodorant sticks. The advantage of the perfume precursor approach is attributed to a prolonged fragrance impression of a deodorant after topical application to the underarm skin.

Antiadhesives

An alternative concept to reduce the amount of skin bacteria in the underarm skin is the antiadhesion approach. The understanding of the adhesion mechanisms of the resident underarm microflora to the skin surface is the basis for developing strategies against bacterial adhesion. Numerous skin microorganisms adhere preferentially to specific sites on various body surfaces. For example, *Staphylococcus aureus* and *Pseudomonas aeruginosa* adhere to collected nasal epithelial cells [46]. *Corynebacterium xerosis* binds to epidermal cells, whereas yeasts species such as *Candida albicans* bind to corneocytes. Structures of the skin specifically involved in adherence to the underarm bacteria are thought to be proteins, oligosaccharide structures, lipids, and hydrophobic surfaces. Imitation of these adhesion motifs by saccharides, oligosaccharides, polysaccharides, and glycoproteins allows one to inhibit the bacterial adherence to the skin. Additionally, it was discovered recently that among others, sucrose esters such as sucrose myristate and sucrose laurate have antiadhesive properties to various microorganisms, including the typical microflora of the underarm skin [47].

DRUG-DELIVERY SYSTEMS AND APPLICATION FORMS FOR DEODORANT ACTIVES

Products designed to reduce underarm odor can be formulated in a variety of delivery systems such as suspensions, water or hydroalcoholic solutions, and emulsions. Typical

application forms are sticks, roll-ons, creams, pump sprays, aerosols, and gels. Sticks, roll-ons, and aerosols are discussed in detail in the chapter "Antiperspirants." Lowering the amount of an antiperspirant active, such as aluminum chlorohydrate, in an antiperspirant is one option to formulate a deodorant. In this case, the antiperspirant active has only deodorizing properties and nearly no impact on the eccrine sweat glands. Deodorants can be formulated in acidic, neutral, or alkaline environment. Designing a deodorant, the formulator should have in mind the following points:

- Long-term deodorization
- No irritation potential
- Good solubility of the active in the delivery system
- Selection of a stable fragrance
- Viscosity control of the product
- Good skin feeling of the product

Protocols for the in vitro and in vivo evaluation of deodorants have been designed. The reader is referred to the literature [48]. A new method for in vivo evaluation of antimicrobial agents was recently developed, where the underarm bacteria were translocated to the forearm, allowing the simultaneous evaluation of multiple deodorizers in an individual [49].

Deodorant Sticks

Deodorant sticks are solidified by 6% to 8% sodium stearate. The deodorizing agent and a fragrance are dissolved in a hydrophilic carrier. Two stick categories can be differentiated, the ethanol-based and the propylene glycol-based sticks [50].

Transparency is usually achieved by usage of a high polyol content. Clarifying agents for sticks such as PPG-14 butylether, cocamide DEA (Diethanol Amine), lauramide DEA and Steareth-100 have been patented [51,52]. Ethanol-based sticks are preferred if it is the intent of the formulator to create a cooling sensation for the consumer. Shrinkage of the stick has to be taken into account because of evaporation of the alcohol. Propylene glycol-based sticks tend to be more resistant to shrinkage, and solubilization of a fragrance is easier in some instances [53] (Table 49.1).

Deodorant Aerosols

Spray products containing a solution of an antimicrobial active in an ethanol and/or propylene glycol carrier, blended with a liquefied propellant, are typical for deodorant aerosols. The difference from an antiperspirant active containing aerosol is that the deodorizer is solubilized in an alcohol- or polyol-based formulation and not suspended. Deodorant sprays provide a dry-skin feeling to the underarm skin because they are anhydrously formulated.

Typically, 20% to 60% of the sprayable contents of an aerosol reaches the skin, because the liquefied hydrocarbon propellant vaporizes as it is sprayed [54]. Propane, butane, and isobutane are the most commonly used propellants. They condense to form a clear, colorless, and odorless liquid with densities of 0.51 to 0.58 g/mL at 20°C [55]. These propellants are inflammable in the presence of air or oxygen. Labeling of cosmetic aerosols concerning flammability risks of volatile organic compounds and volatile solvent abuse is discussed in detail in a recently published review [56]. Aerosol containers can be fabricated from tin-coated steel, tin-free steel (chromium-coated steel), or aluminum. Numerous types of aerosol can cause corrosion, and testing for it was recently discussed in the literature [57].

The environmental issues of aerosols are explained in greater detail in the chapter "Antiperspirants" (Table 49.2).

The formulator of an aerosol has to optimize the following parameters to get a dry deodorant product:

- Spray rate
- Spray shape
- Particle size, concentrate/propellant ratio
- Fragrance/deodorizer concentration
- Pressure of the aerosol can

Deodorant Pump Sprays

Hydroalcoholic Pump Sprays

An alternative to aerosols is pump sprays. This category is quite popular in Europe, whereas it is of lower interest for the consumers in the United States, because they tend to prefer a dry application form, like the anhydrous sticks. Pump sprays allow a good dosage of the formulation to be delivered to the underarm skin in a hygienic way. They consist of low-viscosity hydroalcoholic solutions of a deodorizer and a

TABLE 49.1
Deodorant Stick

	Wt%		Wt%
Water	16.0	Water	3.0
Ethanol	75.5	Propylene glycol	10.0
Deodorizer	1.0	Deodorizer	1.0
Sodium stearate	6.5	Sodium stearate	8.0
Fragrance	1.0	PPG-3 myristyl ether	77.0
		Fragrance	1.0

TABLE 49.2
Deodorant Aerosol

	Wt%
Alcohol	42.0
Laureth-4	0.5
Deodorizer	1.0
Fragrance	0.5
Isobutane	47.6
Propane	8.4

TABLE 49.3
Pump Spray

	Wt%
Water	35.6
Alcohol	60.0
PEG-40 hydrated	2.0
Castor oil	–
Deodorizer	2.0
Fragrance	0.4

Note: PEG, polyethylene glycol.

perfume. Usually, a solubilizer, such as PEG-40 hydrogenated castor oil, is incorporated into the formulation to maintain a clear and homogeneous solution (Table 49.3).

Phase Inversion Temperature Emulsion Pump Sprays

A disadvantage of hydroalcoholic pump sprays is the alcohol content in the formulation that may contribute to unwanted side reactions, especially in the shaved axilla. Beiersdorf AG in Hamburg, Germany, introduced to the European market under the brand name Nivea a new pump spray on the basis of an emulsion in 1995. The sprayable low-viscous deodorant is based on the phase inversion temperature (PIT) technology. Suitable mixtures of ethoxylated surfactants, oils, and water in the presence of antiperspirant and deodorizing actives are heated to 60°C to 90°C. Cooling the resulting water in oil (W/O) emulsion to room temperature yields, via a PIT process, a finely dispersed bluish-white oil in water (O/W) emulsion [58–60]. The droplet size distribution of such PIT emulsions ranges from 80 to 250 nm. The above-mentioned pump spray contained a skin-friendly deodorizing combination of glyceryl monocaprinate and wool wax acids in an alcohol-free delivery system (Table 49.4).

Microemulsion Pump Sprays

Hydroalcoholic pump sprays are usually transparent, whereas sprayable PIT emulsions are white or bluish-white products. Sprayable alcohol-free and additionally transparent pump sprays were recently introduced into the European market (e.g., Basis pH, Beiersdorf AG, Hamburg, Germany). Transparency of an emulsion is achieved when the size of the droplets is below 100 nm. This O/W microemulsion can be obtained with and without the PIT technology but needs careful selection of ingredients and considerable fine-tuning [61]. The main advantage compared with classical microemulsions is the low surfactant concentration (<10%). Furthermore, it could be demonstrated that, in the presence of hydrophobically modified water-soluble polymers, the above-mentioned technology allows the formulation of gels, sprayable gels, roll-ons, sticks, and aerosol products [62].

TABLE 49.4
PIT Emulsion Pump Spray

	Wt%
Glyceryl stearate, Ceteareth-20, Ceteareth-10, cetearyl alcohol, cetyl palmitate (Emulgade SE)	4.5
Ceteareth-20	1.0
Dioctyl cyclohexane	5.0
Dicaprylylether	5.0
Deodorizer	2.0
Aluminum chlorohydrate	5.0
Water	77.5

Source: Wadle, A. et al., *Parf. Kosmet.,* 77, 1996.

FUTURE TRENDS

The deodorant market has undergone some remarkable changes concerning the principles to reduce underarm odor in the last years. It is expected that the search for effective, skin-friendly actives with a highly selective action against the cutaneous underarm microflora will lead to long-lasting and safe deodorants. Improvements in understanding how microorganisms adhere to human skin should facilitate the development of new strategies to reduce underarm odor. Improvements of aerosols with no/low impact to the environment or aerosol alternatives, such as sprayable emulsions, probably will be, in a few years, in the portfolio of every deodorant-selling company.

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50 Use of Cosmetics in Sports

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INTRODUCTION

Our objective was to search the literature for cosmetic and pharmaceutical products that are frequently used or specially designed around the specific needs of sport activities and athletes. A narrative literature search was performed using the online databases Pubmed and Google Scholar. The authors choose to differentiate between pharmaceuticals and cosmetic products not by following the definition of an "active" or "inert" action on the skin but by restricting to products that may be sold directly to a consumer without a prescription from a health care professional. These products can be classified into five functional groups of cosmetic products with a potential biological effect to skin.

1. The use of aesthetic cosmetic products in sports is well established, especially in aesthetic sports such as gymnastics, ice skating, and synchronized swimming where an attractive appearance supports the self-confidence of the athlete. Not only the individual performance will benefit but also beauty and attractiveness can influence the scores given by the judges committee.
2. During a sport, the skin of an athlete is often exposed to harsh environmental conditions. In order to protect and to prevent damage to the skin, the cosmetic industry has developed special preventive and protective skin care products, meeting the requirements of the individual sportsperson.
3. Cosmeceuticals are cosmetic products that exert a pharmaceutical therapeutic benefit without having a biological effect on living tissue. Cosmeceuticals are effective in the treatment of sport-related dermatological disorders and can be a useful adjunct to prescription medications.
4. Stimulating products especially those with a hyperaemising or cooling capacity are frequently used by athletes in the preparation for sport or to stimulate the body in order to enhance physical performance.
5. After physical activity, adequate personal hygiene is important in maintaining the body healthy and vital. Intensive showering after each training session requires a cosmetic cleansing formulation that supports the natural skin balance with regard to pH value, moisture content, and cleansing capacity.

AESTHETIC SPORT COSMETICS

It is well known that sport and physical activity have a positive effect on medical health and subjective well-being. Sport has a positive effect on our attitude to our own body image and our feelings of strength and fitness. To participate in sports because of "body and appearance" is, through an interaction between age and gender, clearly more important in women than in men, as the women's body image is more closely linked to overall self-esteem than men's [1].

In modern society, the perception of health and beauty and the desire to maintain youthfulness are considered to be of great importance. As people wish to maintain a youthful look for as long as possible, the demand for products designed to treat and reduce the cosmetic effects of aging continues to grow [2]. Attractiveness and beauty have gained importance since the media started to broadcast sporting competition and events on television. Millions of people are watching, the athletes perform, and in the prize-giving ceremonies, the media is highlighting and focusing on the athlete in person [3]. The appropriate use of cosmetics and the feeling of looking good and happiness positively support the self-confidence and self-esteem of an athlete. Happy and attractive personalities attract the interest of media and sponsors; the combination of being successful and media publicity can have a major impact on the career of an athlete. Today, sport equipment companies bring out their own cosmetic line, with colors in line with the clothing, supporting the appearance and attractiveness of the athlete on the track. These sport cosmetics are adapted to the needs of the individual athlete, with waterproof and sweat resistant makeup and deodorant fragrances.

DEPILATORY CREAMS

It is a common practice for professional and amateur road cyclist to remove leg hair for a number of reasons. The absence of hair increases the comfort and effectiveness of a massage, as the therapist can effleurage the skin without irritating the hair follicles. In the case of a crash, the absence of the leg hair reduces friction on the skin during a sliding fall reducing skin damage. "Road rash" and the affected area can be treated more efficiently. Also, professional swimmers remove hair off their legs not to prevent drag with the water from slowing them down as is commonly believed but to remove a dead layer of skin, providing a heightened "feel"

for the water. Depilatory hair removal is an economical and easy method, which can be performed at home. Chemical depilatories function by damaging the hair to the point where it breaks at the skin surface; they contain detergents to remove the protective sebum from the hair and adhesives that aid the depilatory in sticking to the hair shaft. Disulfide bond-breaking chemicals with a high degree of acidity such as sodium thioglycolate, or calcium thioglycolate, react with the keratin structure of the hair and break down and dissolve hair within the follicle. Depending on hair coarseness, the process takes from 5 to 15 min; during that time, the hair is dissolved into a glop, which can then be washed away. As the hair shaft and skin have a similar keratin composition, most chemical depilatories interact with the skin and hold a high irritancy potential if the manufacturer's recommendations are not carefully followed [4]. Adverse effects from the use of thioglycolates include burning, itching sensations, and allergic contact dermatitis [5].

PREVENTIVE AND PROTECTIVE SPORT COSMETICS

Sunscreens and UV Protection

Outdoor sports with sun exposure can cause both local and systemic immunosuppression depending on the area of exposure and the dosage of UV radiation. The immunosuppressive and carcinogenic effects of UV light on the skin are complex, involving a variety of cell types, including antigen-presenting cells, lymphocytes, and cytokines. UV radiation can cause dysregulation of antigen-presenting cells such as Langerhans cells and dermal dendritic cells, which, in turn, can activate regulatory T cells to suppress the immune system [6].

Epidemiological studies show that participating in outdoor sport activities and sun exposure during leisure time activities and outdoor sport in general can increase the risk of developing basal cell carcinoma (BCC) and cutaneous melanoma (CM) [7–9]. In cutaneous photobiology, radiant exposure is often expressed as multiples of "standard erythema dose" (SED); one SED corresponds to 100 J/m^2. In various dosimeter studies, the anatomical distribution of sunlight and UV exposure during physical activity was documented. Playing golf or tennis or participation in sailing was associated with relatively high UV exposure ranging from 3.5 up to 5.4 SED per hour [10]. In the Tour the Swiss cycling race, the daily average personal UV exposure of a professional cyclist was determined to be 20.3 SED [11]. Three triathletes participating at the 1999 Ironman Triathlon World Championships in Hawaii had a mean personal UV exposure of 20.8 SED [12]. The study conducted by Rigel et al. [13] showed that skiers with an average skin type and without sunscreen protection started to get sunburned only after 6 min at an altitude of 11,000 ft. [13]. Sweating induced by physical exercise in warm environmental conditions increases the stratum corneum hydration, which can significantly contribute to UV-related skin damage as it increases the photosensitivity of the skin, facilitating the risk of sunburn [14,15]. Although studies indicate that a single application of sunscreen efficiently reduces sunburn [16–18], it should be considered that despite the use of water-resistant sunscreen preparations, protection might be less effective because of water exposure, sweating, friction, and possible interaction of clothing with the sunscreen formulation. Sport sunscreens specially designed for outdoor sport activities should be very water resistant, have a higher sun protection factor (SPF), and block both UVA and UVB rays.

Since 2002, FDA regulations have required companies to eliminate the use of the words "Sunblock," "Sweat proof," and "Waterproof" when referring to sunscreens as these claims cannot be substantiated. Instead, the label on the front of the package can only read either "water resistant (40 minutes)" or "water resistant (80 minutes)." Also, sunscreens may no longer claim to provide "instant protection" nor can they claim to maintain efficacy for more than 2 h without reapplication [6].

Unfortunately, athletes frequently seem to know little about the risk of sun exposure and do not apply sunscreen, and those who initially apply it do not reapply it after perspiration or water exposure [19]. Therefore, the use of water-resistant sunscreen and the need to reapply it every 2 h, after swimming, or after heavy perspiration still needs to be promoted in the community of an outdoor sportsman [15].

Petroleum Jelly

Petroleum jelly, petrolatum, white petrolatum, soft paraffin, or better known as "Vaseline" (trademarked brand of petroleum jelly) is a semisolid mixture of saturated hydrocarbons, originally promoted as a topical ointment for its healing properties [20]. Petroleum jelly is recognized by the US FDA as an approved over-the-counter (OTC) drug and is widely used in cosmetic skin care as skin protectant.

In sporting activities, athletes use petroleum jelly as a topical agent in the prevention of blisters [21–23], chafing and abrasions [19,24,25], and otitis externa (swimmer's ear) [26] and as protecting ointment to cold environmental conditions [27,28].

Blisters affect athletes who sustain mechanical friction on the sole of the feet in an environment of increased temperature, dryness, or moisture. Horizontal shearing forces cause epidermal splits, leading the separated layers to be filled with tissue transudate or blood [29]. Prevention of blisters should primarily focus on measures reducing the mechanical aspect of friction by the use of well-fitting shoes (with appropriated space around the toes) and moisture-wicking socks. Several studies reported the topical application of petrolatum to decrease the risk of blisters and an acceleration of the healing process [21–23,29–32]. Besides these measures, running athletes can promote the hardening of the skin with 10% tannic acid soaks [21,22,24,31,33].

Chafing is a superficial inflammatory dermatitis appearing on skin surfaces subjected to increased moisture, friction, and maceration [30,34]. Jogger's nipple, a particular form of

chafing, is a common phenomenon in long distance runners as a result of repetitive friction between a runner's shirt and their nipples [19,24,25]. Prevention of chafing is best accomplished by wearing dry, synthetic moisture-wicking clothes. Talcum and alum powders are mildly helpful for drying, and the use of petroleum jelly, patches, or adhesive tape over the nipples is effective in reducing friction [29,30,34].

Cotton wool coated in petroleum jelly was reported to be the most effective method of ear protection and was found to be a comfortable and easy-to-use method in the prevention of otitis externa (swimmer's ear) [26,35]. Long distance swimmers and triathletes coat themselves in petroleum jelly as a protection against the stingers of jellyfish and as a thermal isolation in cold water when doing training or long ocean swims. Some controversial reports were found on the thermal insulation provided by petroleum jelly in cold environmental conditions. The in vivo study from Lehmuskallio et al. [27] showed that subjects with petroleum jelly applied thickly on half of the face cooled at least as quickly as the untreated half; however, white petrolatum often produced a subjectively warming skin sensation. The authors concluded that "protecting" emollients can provoke a false sensation of safety leading to an increased risk of frostbite by neglecting efficient protective measures [27].

Insect Repellents

Besides children and occupational groups such as farmers, the outdoor sport enthusiast is frequently a victim of insect stings and bites [36]. In a prospective study by Dannenberg et al. [37], the most frequent overuse injuries and medical problems in Cycle Across Maryland tour in 1994 were evaluated. Next to common overuse injuries such as knee pain, hand or wrist numbness, and dehydration, this study revealed that insect stings and bites had a high incidence rate among cyclists [37]. Arthropods, notably insects and arachnids, are vectors of potentially serious ailments and remain a major cause of patient morbidity. Measures to curtail the impact of insect bites are important in the worldwide public health effort to protect people and to prevent the spread of disease [38]. The use of a skin-based insect repellent, combined with protective clothing, limiting outdoor time and chance in patterns of activity or behavior, are elementary in the prevention of bites. A variety of formulations of different insect repellents are available including pump sprays, aerosols, lotions, creams, grease sticks, and cloth-impregnating laundry emulsions [39]. Repellents containing DEET (N,N-diethylmetatoluamide) as an active ingredient are considered to be effective broad-spectrum, insect repellents and are recommended by most authorities. Formulations containing less than 35% DEET are recommended and provide adequate protection against mosquitoes, ticks, and other arthropods [40]. Permethrin-containing repellents are recommended for use only on clothing, shoes, bed nets, and camping gear. Permethrin is a highly effective insecticide–acaricide and repellent. Permethrin-treated clothing repels and kills ticks, mosquitoes, and other biting and nuisance arthropods [40,41]. Repellents that are applied according to label instructions may be used with sunscreen with no reduction in repellent activity; however, limited data show a one-third decrease in the SPF of sunscreens when DEET-containing insect repellents are used after a sunscreen is applied. Products that combine sunscreen and repellent are not recommended, because sunscreen may need to be reapplied more often and in larger amounts than needed for the repellent component to provide protection from biting insects. In general, the recommendation is to use separate products, applying sunscreen first and then applying the repellent [40,42,43].

Prevention of Jellyfish Stings

Jellyfish stings are a common occurrence among people swimming, wading, or diving in seawaters [44]. In the United States, 500,000 jellyfish stings are estimated to occur in the Chesapeake Bay and up to 200,000 stings in Florida waters annually [45]. Contact with the tentacles trailing from the jellyfish body can discharge microscopic barbed stingers that release venom into the skin, causing skin irritation and sometimes-severe manifestations [44–46]. In the randomized control trial of Boulware [44], the efficacy of a jellyfish sting inhibitor lotion (Safe Sea) was evaluated. In comparison to the placebo product, the Safe Sea topical barrier cream was effective in preventing >80% jellyfish stings. In the studies of Kimball et al. [47] and Tønseth et al. [46], the prophylactic and protective effects of a jellyfish sting inhibitor formulated in sunscreen lotion versus a conventional sunscreen was investigated. The authors concluded that the prophylactic treatment with jellyfish sting inhibitor did not eliminate but significantly reduced the frequency and severity of stings [46,47].

SPORT-RELATED DERMATOLOGICAL DISORDER

Efficacy of Topical Antifungals in the Treatment of Dermatomycosis

Epidemiological studies show that tinea pedis, formerly known as athlete's foot, tinea corporis gladiatorum, and onychomycosis are common sport-related dermatoses affecting the athletes' skin [24]. Dermatomycosis are fungal infections that are widespread throughout the world, which are an important cause of morbidity [48–50]. Dermatophytosis is the most common, caused by different species of dermatophytes particularly *Trichophyton rubrum* and *Trichophyton mentagrophytes*, followed by *Candida* species and nondermatophytic molds [51]. The prevalence of dermatomycosis is increased in population with avid sport participation. Athletic activities with an increased incidence are wrestling, judo, swimming, gymnastic, cycling, horse riding, and in general sports with occlusive footwear. The athletes are mainly exposed to fungal contamination at places where sports are practiced barefooted such as public swimming or using showers and changing rooms [24,52]. The treatment of these conditions often consists of the use of topical or oral antifungal

agents or a combination of these, depending on the site, extent of infection, and the causative organism [53–56]. There is good evidence for the effectiveness of topical antifungals in the treatment of dermatomycosis. In the systematic review and meta-analysis ($k = 135$) of Rotta et al. [56], the efficacy and safety of topical antifungals versus placebo in the treatment of tinea pedis and onychomycosis were evaluated. The authors concluded that azoles, allylamines, and other antifungals, such as butenafine and ciclopirox olamine, are all efficacious in the management of any dermatomycosis compared to placebo treatment. These results are in line with other published systematic reviews with meta-analysis conducted by Hart et al. [57] and Crawford and Hollis [58] investigating the management of tinea pedis.

STIMULATING AND PERFORMANCE-ENHANCING SPORT COSMETICS

Liquids with Cooling Properties

Our literature search revealed a study from Leite et al. [59], evaluating the therapeutic efficiency of a cooling liquid versus conventional cryotherapy. Liquid Ice is an all-natural liquid cooling solution including menthol and alcohol and is applied soaked in a wrap [59]. The manufacturer claims that the product was designed to cool efficiently and effectively though natural evaporative cooling. The two cryotherapy modalities compared included crushed ice in a room temperature wet towel and Liquid Ice. The crushed ice induced lower skin surface temperatures compared to the Liquid Ice application. The authors concluded that Liquid Ice is not useful as a clinical cryotherapy modality.

Cooling Liquids to Increase Physical Performance

Heat production by intense prolonged exercise induces a decrease in physical performance. Over the last decade, several studies have been conducted to investigate the effects of local cryotherapy on physical performance.

Duffield et al. [60] conducted a study on the effect of cooling the skin with an ice jacket before and between repeated sprint exercises in warm, humid conditions. There was no improvement in physical performance, although the perception of thermal load was reduced [60]. Under warm and humid environmental conditions, evaporation is the primary mechanism for muscle heat dissipation [61].

In our own study, we evaluated the effects of local upper arms cooling, upper body cooling, and combined cooling of the upper arms and upper body on the endurance capacity during cycling in warm (35°C) humid (40%) conditions. For cooling, we used *Energicer* bands and cotton vests saturated with a cooling liquid, based on alcohol and menthol and produced by the Swiss company Liquid Ice Cosmedicals. The manufacturer claims that the use of *Energicer* Bands regulates the body temperature, optimizes the heart rate, reduces the lactate buildup in the muscle, and increases the power during exercise.

In a randomized crossover study design, we conducted a standardized incremental bike ergometer test, where time to exhaustion was determined and used as the independent variable for endurance capacity. At the end of each incremental step, the following variables were measured: blood lactate, heart rate, body temperature, and perceived exhaustion (BORG scale). Mean time to exhaustion did not differ between the four conditions ($p > 0.05$). We observed no significant differences at blood lactate, heart rate, and body temperature during examination between the four conditions. However, all participants mentioned they felt more comfortable when wearing the cooling vest under the used environmental conditions. This effect might aggravate with the airflow when cycling under outdoor conditions, which may lead to psychological advantages for the athlete [62].

TOPICAL MUSCLE AND JOINT ANALGESICS

During recent years, the use of OTC topical muscle and joint analgesics has become increasingly common in sports. Topical analgesics are applied to the skin for temporary treatment and management of musculoskeletal injuries and disorders. Topical OTC analgesic products are available in a variety of formulations, including gels, ointments, creams, lotions, and patches in single-entity or combination formulations. In clinical use, topical analgesics can be divided into four basic groups: nonsteroidal anti-inflammatory drugs (NSAIDs), local anesthetics, counterirritants, and other agents. As this chapter is mainly focusing on cosmetic and cosmeceuticals, we will discuss only revulsive products such as capsaicin and nicotinates.

Revulsive products produce a reddening of the skin. This erythema is due to an increased perfusion of the microcirculation after a vasodilation of the arterial plexus at the different skin levels. Nicotinates act via an endothelium relaxant factor, while capsaicin uses a neurogenic cascade with the involvement of substance P.

Revulsive products (i.e., rubefacients and urticants) are known for several clinical and nonclinical applications. Clinically, they are used in the treatment of neuropathological (diabetic neuropathy, postherpetic neuralgia [PHN]) and/or musculoskeletal disorders (e.g., osteoarthritis, rheumatoid arthritis, muscle soreness, and back pain). Nonclinically, they are used in some sports as passive warming-up products and in the cosmetic industry as an ingredient in skin products [63].

Despite the widespread use of revulsive products in sport ointments, patches, wraps, gels, sprays, and balms, studies reporting on the nonclinical effectiveness of these products are scarce. Clarys et al. [64] reported only significant warming of the superficial skin after application of nicotinate containing revulsive products.

FDA is alerting the public that the use of certain (OTC) topical muscle and joint analgesic products has been reported to cause rare cases of serious skin injuries, ranging from first- to third-degree chemical burns, where the products

were applied. Consumers using an OTC topical muscle and joint pain reliever who experience signs of skin injury where the product was applied, such as pain, swelling, or blistering of the skin, should stop using the product and seek medical attention immediately [65].

CLEANSING PRODUCTS

Skin cleansing is essential for maintaining healthy skin and hygiene. Its primary function is to remove dirt, soil, bacteria, and dead cells from skin. The athletes' skin is more exposed to intense sunlight, dirt, bacteria, and excessive sweat. Showering after each training session prevents dirt and bacteria from clogging the pores and is an important aspect of skin care. Frequently cleansing with commonly used soap-based shower and bath products induces skin dryness and leads to a weakening of the stratum corneum barrier. Over the last decades, the personal cleansing market has evolved greatly manufacturing mild cleansing formulations that remove oils and soil from skin, but without the dryness and irritation that accompanied typical soap-based products. Frequently cleansing with water alone does not prevent the skin from getting dry, as the contact to water only hydrates skin transiently, leaving the skin after evaporation as dry or drier than before [66].

For regular body cleansing, athletes should use mild emollient-rich body washes as they have been shown to be milder and more moisturizing than regular body washes [67].

Also, daily skin care using a moisturizing emollient-rich cream or lotion containing a lipid system is an effective treatment to rehydrate and restore dry skin.

CONCLUSION

The terminology "sport cosmetic" is used by the cosmetic industry to commercialize a wide range of cosmetic products. Some are specially developed for the use in sport and well adapted to needs of sporting people. Others are normal cosmetics using the co-notations associated with the term "sport" intending to provide a subjective feeling associated with being physically active. Some products are widely used without any prove of efficiency. There is a need for further studies concerning the efficacy of different mentioned sport cosmetics. Equally no data are available regarding possible side effects on the skin of repetitive and long-term use of these products.

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51 Cosmetotextiles
A New Aspect of Technical Textiles

Mukesh Kumar Singh

INTRODUCTION

Cosmetotextiles are fast emerging as today's most potential customer's lifestyle. Men and women on both sides of the Atlantic are equally excited by the concept of well-being clothes, especially those worn close to the body and capable of providing cosmetic effects. The textiles that provide cosmetic and biological functions such as pleasant feeling, energizing, slimming, refreshing, vitalizing, skin glowing, antiaging, body care, fitness, and health are categorized as cosmetotextiles [1]. Wellness or health-promoting aspects of textile finishes have become a delighting functional argument in the twenty-first century. Wellness can be defined as a pleasant state free from disease and as a healthy balance of human body and mind. It has become a social determination that symbolizes the wish of eternal youth against aging. Extracts of natural products and selected essential oils are loaded to the textiles, which not only causes healing but also keeps the wearer fresh and vigorous. In wound dressings, where slow release of a drug is essential, drug complexes with natural compounds such as chitosan, hyaluronic acid, and alginates serve purpose of controlled release. Natural moisturizing factor (NMF) is a scale to express the activeness of the skin in terms of the moisture level in the horny layer of skin surface. At 20% moisture level, skin remains lustrous and elastic; at the moment it reduces to 10%, the skin becomes dry and rough. The merging of two apparently different sectors cosmetics and textiles clears the way to climbing the heights of cosmetotextiles.

Cosmetotextiles are capable of imparting skin care benefits, combating aging, and promoting a feeling of wellness or well-being. Cosmetotextile is a consequence of fusion of cosmetics and textile industry through various techniques like microencapsulation. In other words, a cosmetotextile is a textile consumer article containing a durable cosmetic substrate that is released over time [2]. Legally, cosmetotextiles are not accepted as cosmetic products. European Cosmetic Directive has defined the cosmetotextiles as follows: "any textile article containing a substance or preparation that is released over time on different superficial parts of the human body notably on human skin and containing special functionalities such as cleansing, perfuming, changing appearance, protection, keeping in good condition or correction of body odors is called Cosmetotextiles" [3]. Cosmetotextiles can be considered as cosmetic textiles when the cosmetic ingredients grafted on the textiles have to be transferred to the wearer's skin and the transferred amounts have to be enough to ensure that cosmetic benefits are possible.

CHRONICLE DEVELOPMENT IN COSMETOTEXTILES

Innovations targeted to incorporate cosmetic ingredients to textiles started to appear in the late 1980s with Japan leading the way. The European Union further extended this lead in 1995, and Hermes launched a scarf perfumed with encapsulated Calèche, a woman's large folded hooped hood, worn in the eighteenth century. A French company Euracli also registered its remarkable presence in the field of cosmetotextile innovations and catalyzed to other multinational companies such as Cognis in 2001 and Invista in 2003, which launched their branded solutions for cosmetotextiles that they called Skintex and Lycra Body Care. French cosmetotextile brand Lytess surprised its counterparts in 2003 by exhibiting a significant growth in its product sales in the European market.

CLASSIFICATION OF COSMETOTEXTILES

The classification of cosmetotextiles is not available in literature. Authors made an effort to classify the cosmetotextiles on the basis of various concepts.

Classification of Cosmetotextiles on the Basis of Influence on Human Body

In relation to the influence of cosmetotextiles on the human body, the cosmetotextiles can be classified into the following classes:

- For slimming
- For moisturizing
- For energizing
- For perfuming
- For refreshing and relaxing
- For vitalizing
- For UV protection
- For improving firmness and elasticity of skin

Cosmetotextiles for Slimming

Slim body structure is desired by both men and women around the world. The textile structures that work to offer slimming effect by means of yarn properties, fabric structure, and finishes are called cosmetotextiles for slimming. Surgery and exhaustive exercises are two major ways to reduce cellulites. The use of compressional garments has offered a third option for slimming, reducing muscle damage and maintaining muscle function and cellulite reduction. Functional muscles give better appearance and good-looking effect by accelerating the blood flow in veins. Cosmetotextiles for slimming provide rehabilitation to the wearers [6,7] and require a large amount of ingredients on human skin. Skintex slimming has better efficacy in a pair of elastic and tight jeans, which provide an intimate contact between the fabric and the targeted body part. Skintex has adopted the concept of progressive compression in a typical product "Skintex Anti-heavy-legs."

The French cosmetotextile industry has launched more than 20 compression stockings of the Orange Peel line that includes sleeves, shorts, capris, a top, and leggings. These compression garments are made of double-knit fiber of 92% polyamide and 8% spandex. Both polyamide and spandex are engineered to stimulate blood flow and are dosed with ingredients before being spun and dyed. Encapsulated and woven fabric of the Orange Peel line is a blend of propylene glycol diethylhexanoate and *Gelidium cartilagineum* extract of red algae and firming agent created with 1.5% active sterol. These ingredients in addition to the fabric's micromassaging effect help to reduce cellulite presence.

In addition, providing comfort to consumers wearing the garments, moisturizing ingredients are included—elemi resin, for instance, which according to Lahmani also has a firming effect. Sweet almond and mango butter are also said to moisturize while copaiba accelerates moisturization. Other shape-wear garments by the company include caffeine and shea butter, which are claimed to slim and moisturize. The company also offers products such as gloves that moisturize with sweet almond oil, jeans lined with red vine to tone the skin, green tea to mobilize fat, and peach oil to moisturize.

Cosmetotextiles for Moisturizing

Cosmetotextiles for moisturizing are a group of textiles that work to provide moisturizing effect on human skin. Major reasons for skin dryness are exposure to strong harsh wind, low temperature and low humidity, and dry air from furnaces and other heating sources. Squalane, a stable form of squalene and a major component of the lipids, can be extracted from various essential oils like olive oil and shark liver. It can add a layer of oil on the human skin to suppress the water loss from skin to keep it soft and supple. Squalane substance with several hydrophilic groups has affinity to form hydrogen bonds with water molecules on human skin. Squalane is able to reduce presence of wrinkle and fine lines due to its humectant potential. Human skin easily absorbs and spreads squalane with zero oily and greasy marks. Textiles that are able to deliver squalene in a controlled manner can be used as cosmetotextiles for moisturizing [8].

Nanotechnology can lead to the development of hydrophilic textile surfaces. The integration of TiO_2 increases the possibility of moisture absorbance on textile surfaces through a photocatalytic process.

In case of polyethylene fibers, thin film of TiO_2 can be deposited by layer-by-layer deposition method [9]. This approach can be used to develop textiles for quick drying for sports or outdoor clothing. In case of typical pantyhose, 4% skin moisturizing ingredient is required for one single daily dose.

Lytess has improved the comfort index of compression garments by loading elemi resin by microencapsulation on the garment to uplift its moisturizing and firming potential. Sweet almond, copaiba, caffeine, peach oil, and mango butter are also used to moisturize the human skin.

Cosmetotextiles for Energizing

Cosmetotextiles for energizing include some textile articles that are able to uplift the energy level of human beings. CoQ10 is a shortened name of coenzyme Q10. The human body's cells use this coenzyme to enhance movement and energy level. It is a natural antioxidant [10]. Although scientific proofs are not available, authors thought that it can be a useful substance to develop textiles for energizing.

Cosmetotextiles for Perfuming

Cosmetotextiles for perfuming include textile articles that absorb foul odors and offer pleasing perfumes. The growing awareness of protecting the human being from foul body odors and providing pleasant smell are the prime drivers to achieve a good deodorant textile. A variety of synthetic and natural products is used to add the functionality of deodorancy in textiles. Chitin, chitosan, acetyl-glucosamine, D-glucosamide, and various essential oils like clove, jasmine, lavender, hyssop, sandalwood, rose, and frankincense are used to achieve the perfuming effect. The incorporation of deodorant on textile substrate is conducted during polymerization or during dope formation or at finishing stage. Toyobo Co. Japan treated a fabric made of 90:10 acrylonitrile–methylacrylate copolymer fibers with 30% hydrazine solution for 3 h at 98°C to give an absorbing capacity of NH_3 and H_2S [11,56].

Cosmetotextiles for Refreshing and Relaxing

A textile structure that enables to provide refreshing and relaxing effect comes under the class of cosmetotextiles for refreshing and relaxing. During summer, the cool feel gives the refreshing feeling and relaxation that can be achieved either by using phase change materials in the form of microcapsules or by increasing the area of contact between highly moisture transmitting fibrous surfaces and the human body. Skintex Superccol is a typical commercially available cosmetotextile that works on the principle of increasing the large area of body contact with a seamless microdenier polyamide/elastane structure. Skintex supercool can also be produced by encapsulation of menthol along with emollients and highly durable synthetic coolants. Highest cooling is required on the armpits, back, chest, and shoulder because these areas are most prone to sweating [12].

Cosmetotextiles for Vitalizing

A textile structure that is able to release revitalizing aromas synthesized by plants- and fruits-based ingredients like ginger, menthol, orange, or rosemary at a slow rate comes under the category of vitalizing textiles. These ingredients are loaded on textiles by using a microencapsulation technique. The durability of this functionally remains active with textiles even after a number of launderings. Vitalizing cosmetotextiles are suitable to wear clothes for bathrobes and other similar purposes [13]. Whirlpool Personal Valet clothes vitalizing system smooths wrinkles and eliminates odors from clothing within 30 min in perfect conditions.

Cosmetotextiles for UV Protection

Prolonged exposures to ultraviolet radiation can result in skin damage such as sunburn, premature skin aging, allergies, and even skin cancer, which causes an inferiority complex in human beings. Textiles that can provide effective protection against such damages are called cosmetotextiles for UV protection. The fabric cover factor directly decides the protection against UV radiation that indirectly depends on the type of weave, depth of shade, fabric areal density, stretchability, wetness, and washing cycle of the fabric. 1,2-Ethanediol, Zn nanoparticles, iron oxide, zinc oxide, titanium oxide, carbon black, bi-reactive oxalic acid, dianitide derivatives, and various other chemicals are used to improve the UV protection factor (UPF) of textiles [14–19]. Various multinational companies (MNCs) have developed some garments for UV protection and some of them are listed here: non-UV-enhanced fabric—cotton T-shirt fabric TF 437W, tubular, 124 g/m^2 (Test Fabrics Incorporated, Middlesex, New Jersey); and UV-enhanced fabric—cotton T-shirt fabric TF437W treated with three washes in a household washing machine (Miele Deluxe ElectronicW724) at 40°C (cotton program) with a common laundry detergent containing 0.25% of the UV-cutting agent (UVCA) Tinosorb FD (Ciba Specialty Chemicals Incorporated, Basel, Switzerland).

Below are factors that significantly affect the UV protection potential of apparels:

- Fabric color: UPF increases with darker colors.
- UV absorber: UPF increases by UV absorber loading on textiles.
- Fabric material: Polyester provides usually higher UPF, cotton rayon, and linen, and provides lower UPF than nylon, wool, and silk fabrics.
- Washing: UPF increases for cotton after washing.
- Stretch: UPF decreases under stretch.
- Wetness: UPF decreases for cotton under wet conditions.

Cosmetotextiles for Improving Firmness and Elasticity of Skin

A specialized group of textiles dedicated to improve the firmness and elasticity of human skin is classified as cosmetotextiles for firmness and elasticity of skin. These textiles are capable of releasing some natural products that soothe and improve skin firmness and elasticity in a controlled manner [20]. *Padina pavonica* is believed to improve the firmness and elasticity in human skin. It is extracted from the protective coating of brown algae found in the Mediterranean Sea. After a successful collaboration between Cosmetil and Variance, a cosmetically inspired fluid lingerie called Hydrabra to provide moisturizing and firming effects has been launched in the market [21]. The Hydrabra has a specially designed lower cup made of ultrathin cloth impregnated with a lotion formulated with extracts of *P. pavonica* to enhance firmness and elasticity.

Cyclotella

A genus of diatoms, the microscopic algae Cyclotella is found in many saltwater lakes and in the Mediterranean. In suboptimal conditions, it can subsist in a semivegetative state.

However, when its living conditions improve, Cyclotella develops special "messages" to reproduce.

Scientists have isolated and analyzed these "messages." They describe a neurobiochemical composition very similar to that of endorphins, the body's main messengers of sensations of pleasure and relief.

Through its action, Cyclotella acts as a relaxant and helps ease pain. Research has also shown that endorphins, which are associated with pleasure, are produced both in the brain and by the skin, where they have a definite effect on lymph circulation and fat metabolism; the draining and slenderizing action of Cyclotella stimulates the production of endorphins in the skin, which encourage lymph circulation, for pain relief, and action on swelling, edema, water retention, and cellulite.

Classification of Cosmetotextiles on the Basis of Method of Grafting

Various wellness ingredients are loaded on textiles at different stages of manufacturing to achieve cosmetic effect by different techniques. The selections of techniques depend on the nature of the cosmetic ingredient, the nature of textiles, and the amount of the cosmetic ingredient to be loaded. On the basis of the method of incorporation of a wellness substrate on textile, classification can be done as follows.

Cosmetotextiles by Insertion of Dope Additives into Fiber

The active agents are added with fiber forming material at the time of dope preparation before fiber extrusion. For example, manufacturing of inherently conductive, UV-absorbing, and delusturing fibers can be possible by using carbon nanotubes, Zn nanoparticles, and TiO_2, respectively, as dope additives. The inherently functionalized fibers can be used to manufacture textile materials for various purposes [22].

Cosmetotextiles by Use of Grafting Layers

Various cosmetic ingredients are grafted on fiber, yarn, and fabric surfaces to achieve cosmetic effects. Cyclodextrins (CDs) are cyclic structure oligomers of glucose and consist

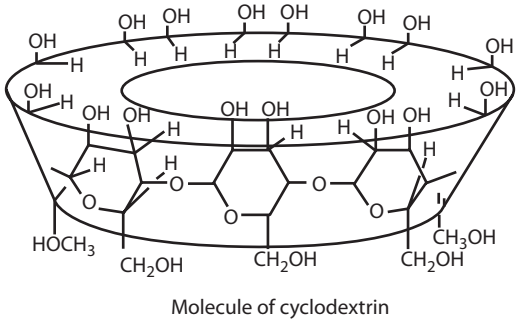


FIGURE 51.1 Chemical structure of CD.

of 6 to 8 glucose units, produced by the starch digests of the bacteria *Bacillus macerans*. The chemical structure of CD is shown in Figure 51.1. The individual glucose units are connected by 1,4 bonds. The polar OH groups of the individual glucose units are on the outside of the cylinder due to their steric arrangement. The outside is hydrophilic, whereas the inside of the cylinder is nonpolar and thus hydrophobic. The cavities of the host CD can take in all those molecules that could fit into cavity and are nonpolar enough to interact with the lipophilic cavity surface and release them again. α-CD, β-CD, and γ-CD have 6, 7, and 8 glucose units, respectively, in which γ-CD has proven a suitable wellness substance. A research group has grafted β-CD molecules permanently on Tencel (cellulosic fiber) fabric surface [23]. After successful grafting, benzoic acid, vanillin, iodine, etc., either by spraying their solutions on CD grafted fabric or by grafting previously prepared inclusion compound on Tencel, a fabric with cosmetic potential is prepared. Treated fabric was found satisfactory after various aroma and antimicrobial finishing tests without any significant modification on the original fabric surface [24].

Textile finishing with CDs significantly enhanced the period of release of perfumes, especially from polyester-made fabrics. γ-CD is the most versatile of the three native CDs tested, while the citric acid group and butanetetracarboxylic acid (BTCA) cross-linking reagents present the same qualities. Fabrics can be functionalized by fragrances by immersion in their water–alcohol solution and can also efficiently capture the volatilized compounds in the atmosphere. It was also observed that the textile finishing with CDs could improve the resistance of the odor to washings with water. The CDs grafted by the intermediate of polycarboxylic acid (PCA) keep most of their original complex-forming properties. However, the fragrance retention phenomenon was not only due to *host–guest* complexation but also dependent on nonspecific interactions of the substrates with the surface-modified fibers.

Direct Coating on Textile Products

Some active agents are coated on fiber, yarn, or fabric surface as per suitability of existing facility and the use of the end product. Bed linen can be made more comfortable and healthier using fiberfill coated with microcapsules having essential oils or antibacterial or antidust and mite chemicals. Fuji Spinning Co. Japan disclosed in a European patent that fiber treated with an emulsion of alpha-tocopherol acetate gets reduced antioxidant function. A host–guest molecule technology is required to prolong the antioxidant function on textile surface [25]. As the CD vitamin E complex does not show any substantivity with textile surfaces, padding, spraying, coating, and printing are the other alternative application techniques. For permanent fixation of the complex, reactive polyurethanes have proven to be advantageous. The fixation of CD vitamin E complex can provide the following effects:

- Free from formaldehyde
- Soft handle
- Good fastness on textile surfaces
- Applicable on all types of fibers

Fixation of the complex is physical particularly on cellulose and wool chemicals (reaction with –OH and $–NH_2$ groups). Fixation of the charged CD with reactant cross-linking agents is also possible but only for application on cellulose fibers.

Through Microencapsulation Technique

Various cosmetic ingredients are susceptible to heat or prone to oxidation, whereas deodorants are volatile. These are the major driving forces to adopt microencapsulation as the major technique to develop cosmetotextiles. Microencapsulation can prolong the self-life of various volatile and nonvolatile cosmetic ingredients by delaying the oxidation and evaporation, respectively. The suitability of microcapsules for cosmetotextile applications depends on the range of diameter, mechanical robustness, and the content release profile of microcapsules to offer the appropriate potential for specific functionality. Microcapsules can be integrated with a textile substrate by two major methods: (1) by covalent grafting through selecting skin-friendly binder and type of binder used, which depends on the textile substrate and must be capable of binding the microcapsules firmly to offer adequate wash fastness; and (2) by an exhaust method that requires very precise control of temperature and pH; this method is suitable to treat the knits and woven garments [26].

CONTROLLED RELEASE PROFILE FROM MICROENCAPSULATION

The controlled release profile of a cosmetic ingredient can be followed by the following routes:

- Burst release.
- Triggered release: This release profile is triggered by means of an attribute like pressure, pH, temperature, moisture, and electronic changes in a typical device.

- Sustained release: Release under this profile is sustained for an extended span of time. This profile can be useful to establish constant ingredient release for a limited period.
- Combined release profile.

Release Mechanisms

The release mechanism of an active ingredient from a microcapsule may be based on one of the following: diffusion, molecular trigger (such as pH), dissolution, thermal, biodegradation, mechanical rupture, osmotic, and biochemical action.

Classification of Cosmetotextiles on the Basis of Fabric Engineering

Fabric engineering aspects are able to generate various cosmetic and wellness functions in textiles. For example, knitted compression garments are able to apply a specific pressure on a localized area of the human body [6]. On the basis of fabric engineering, cosmetotextiles can be classified into three groups:

- Woven fabric-based cosmetotextiles
- Knitted fabric-based cosmetotextiles
- Nonwoven fabric-based cosmetotextiles

COSMETIC INGREDIENTS

Generally, major cosmetic ingredients originated from inorganic and synthetic chemicals, animal derivatives, and plant derivatives. People are consciously avoiding the use of inorganic and synthetic chemicals and animal derivatives for cosmetic applications because their intensions are biased toward using plant derivatives. Various scientific and medical researches prove that plant derivatives are safer than chemicals and animal derivatives as cosmetics.

Synthetic and Inorganic Compounds

Various inorganic and synthetic compounds are used to provide cosmetic benefits to wearers. 1,2-Ethanediol, Zn nanoparticles, iron oxide, zinc oxide, titanium oxide, carbon black, bi-reactive oxalic acid, and dianitide derivatives are used to provide protection against UV radiation. Acetyl-glucosamine and D-glucosamide are used to provide deodorant effect in textiles [11,14–19]. Copper oxide is used to promote the healing and antimicrobial functionality in textiles.

Animal Derivatives

Chitosan

Chitosan is an animal derivative used for wound healing, antibacterial effects, blood clotting, and deodorant effects. It is a natural product derived from chitin, a polysaccharide found in the exoskeleton of shellfish like shrimp or crabs. It improves skin texture, nourishment, and moisture level; stimulates cell regeneration; and forms high molecular film for skin protection [27].

Squalene

Squalene is a fatty compound that is found in a number of vegetable oils, including palm oil and olive oil, but is usually extracted from shark liver where it is found in high concentration. Squalene is a natural antioxidant. Squalane is the saturated form of squalene. It provides additional permeability, nutrients, and water retention capability. Squalane chemically resembles the natural skin lipid called sebum and is well absorbed into the skin to support the skin's ability to regenerate and maintain hydration naturally. Its ability to penetrate into the skin also helps carry other ingredients into deeper skin levels. Squalane along with ascorbyl phosphate, vitamin E, and hyaluronic acid helps to protect the skin against photoaging and the formation of brown age spots. Along with other ingredients, it helps soften the skin to reduce fine lines and wrinkles [28].

Sericin-Based Cosmetotextiles

Silk sericin is a natural macromolecular protein derived from raw silk filament *Bombyx mori* through silk worm and consists of almost 25%–30% silk protein. Sericin covers the fibroin part of silk filament with successive sticky layers. These sericin layers assist in the development of cocoon. In usual practice, most of the sericin is degummed during processing of raw silk at the time of degumming and reeling and discharges in the form of effluent causing water pollution. However, it has been proved scientifically that sericin is a biomolecule of great importance. Sericin has antibacterial, oxidative-resistant, and moisturizing properties. The recovery of sericin from degumming liquor or waste cocoons helps in reducing environmental problems and can be used in various cosmetic purposes and cosmetotextiles. Functional properties of various textiles can be improved by coating with silk sericin protein. Some Japanese scientists have modified polyester fabrics (4% add on) with sericin and the modified polyester fabrics exhibited five times higher hydrophilicity than untreated polyester. Sericin can be applied to fabric by a simple pad–dry–cure method.

Sericin content in finished samples was estimated by dyeing treated fabrics with an acid dye, Supranol Bordeaux B, and determining K/S and L values of the dyed fabrics. The treated fabrics were tested for free formaldehyde content, crease recovery, tensile strength, electrical resistance, water retention, and biocidal activity.

Increasing sericin content in the finishing solution increased the amount of coated sericin, and a greater depth of color in dyed samples and reduced free formaldehyde content in treated samples were observed.

The sericin content in samples was found to have a negligible influence on tensile strength and crease recovery

angle. With increasing sericin content, electrical resistivity of the samples dramatically decreased and water retention increased, indicating that sericin-treated fabrics may be comfortable to wear because of its maintenance of moisture balance with respect to human skin. Because cotton textile coated with sericin exhibited low formaldehyde content and no biocidal activity against *Klebsiella pneumoniae* and *Staphylococcus aureus*, the fabric may reduce skin irritation and disturbance of physiological skin flora arising from textile contact.

In order to take advantage of the specific property of sericin to improve wear property of cotton fabric, cotton fabric was finished with sericin. Sericin was fixed onto the surface of the cotton fabric in the presence of polycarboxylic acids (CA and BTCA) at high temperature using a pad–dry–cure process. The effects of catalyst concentration, pH value, curing temperature, and curing time on the finish were investigated. The optimized finishing conditions for cotton fabric were obtained. The weight gain of the treated fabric with BTCA as a cross-linking agent was higher than CA. The whiteness, breaking strength, moisture regain, permeability to gas, and crease recovery properties of treated fabrics were measured. The results showed that the wrinkle recovery angle evidently increased, and the wrinkle recovery angle of BTCA combined with secrin-treated fabric was higher than CA. The breaking strength, moisture regain, and whiteness of the treated fabric slightly decreased, and the permeability to gas of cotton fabrics was not changed. Sericin protein can be cross-linked, copolymerized, and blended with other macromolecular materials especially with man-made polymers to manufacture materials with improved functional properties. Sericin-coated fibers can be used in diapers and diaper liners to provide a skin touch surface for improving skin integrity and moisturizing level.

Plant Derivatives

The plant-based cosmetic ingredients secured directly from nature are valuable essential oils and extracts. These ingredients are derived from cold press peel of plants, herbs, and fruit carefully by distillation from blossom and leaves.

In another process, algae are combined with clothing fibers, and when the clothing is worn, minerals, proteins, and vitamins are released.

Algae

The cosmetic industry uses algae as a thickening and water-binding agent as well as an antioxidant. It is rich in vitamins and minerals. Algae condition and hydrate the skin while they nourish, rejuvenate, detoxify, and replenish minerals. Algae contain proteins, vitamin A, sugar, starch, vitamin B, iron, sodium, phosphorus, magnesium, copper, and calcium. These are all useful sources for skin care, either as emollients or antioxidants. Different types of algae contain elements that help to firm and moisturize the skin. Algae also help in reducing puffiness and protect against radiation and other agents that cause harm to the skin. Phytomer, a leading spa brand, has studied the effects of algae and their natural properties. They discovered that an active element in algae called coben is the main responsible agent in decreasing and regulating the production of melanin. For this reason, a wide range of whitening products has been based on this ingredient.

Aloe Vera

Aloe vera is a semitropical plant of the lily family. Over 250 different species are available but only four have nutritional value in which Aloe vera Barbadenis Miller group is prominent. The aloe vera leaf contains over 75 nutrients and 200 active components including 20 minerals, 18 amino acids, and 12 vitamins. Aloe vera products lost much of the original beneficial components by overprocessing, which results in 10%–15% of aloe vera at best. Aloe vera should be processed with extra care to minimize the loss of essential vitamins, minerals, and other active constituents. Aloe product should be guaranteed by the International Aloe Science Council. Scientific research on aloe vera has proved that textiles treated with aloe vera are very pleasant to wear. It provides a significant effect on energy levels, which offers a feeling of well-being. Aloe vera is used to get antibacterial, antiviral, antimycotic, wound healing, and anti-inflammatory effects [29]. Evergreen Corporation, China, has rank in the field of development and sale of various aloe vera products commercially in Asia and Europe. Aloe vera conditions the wearer's skin and provides fresh air.

Ginseng

Ginseng is promoted as an adoptogen, and the root of the ginseng plant is the most valued form [30]. The extract of ginseng can be used by microencapsulation technique to protect the skin from cancer and inflammation. The ginseng extract is able to block carcinogens such as 12-O-tetradecanoylphorbol-13-acetate (TPA) and cancer-causing enzyme ornithine decarboxylase and expression of cyclooxygenase-2 (COX-2). Ginseng expression led to the reduction of the production of prostaglandin E-2.

Fruits

Various fruit oils are used to provide aroma to the wearer that is refreshing and relaxing. Various chemicals are extracted and applied as source of aroma on fabric surfaces by different techniques like citral (lemon scent), allyl caproate (rose scent), anillin (apple scent), cinnamaldehyde (Pineaple), prenyl acetate (banana), and heliotropin (cherry) [31].

Essential Oils

There are various essentials oils that found their place in aromatherapy to provide skin with glowing, moisturizing, refreshing, and other wellness effects. These oils are microencapsulated by covering them by means of a polymeric coating and then applying them on cotton, polypropylene,

polyacrylonitrile, and polyamide fiber surfaces. The prominent essential oils are lavender oil, thyme oil, sage oil, peppermint oil, eucalyptus oil, chamomile oil, etc.

Flowers

Some flowers get their way to wellness after extraction of specific chemicals like innone (violet), cedar oil (lilac), hydroxycitronellol (lily), and alpha-hexylcinnamaldehyde/benyl alcohol (jasmine) by using various extraction techniques. Finally, these well-being extracts can be loaded on textiles by a microencapsulation technique to achieve various cosmetic targets [31].

Marigold Flower Petal Extract

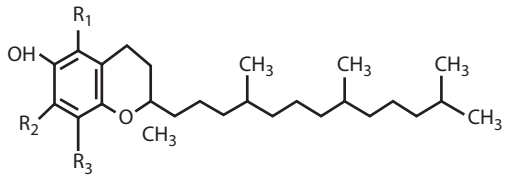
The Marigold flower petal extract is commonly known as Calendula. It has antibacterial, antioxidant, and anti-inflammatory properties. The topical uses of calendula offer healing, soothing, and protection from eczema. The Marigold herb is applicable in some rescue ointment cosmetics with antiseptic property. Calendula extracts also help soothe and repair damage to the scalp and hair shaft, and can be used to help relieve irritated scalps and repair dry hair. It is useful for the hydration of human skin particularly during the winter season. Calendula's great anti-inflammatory properties are most useful in cases where tissue degeneration is an issue, such as in stubborn wounds, sores, acne, ulcers, bed sores, varicose veins, bruises, rashes, and eczema. Marigold flower extract rich in flavonoids and saponins that promote healing and repair acts as an anti-inflammatory, prevents tissue degeneration, heightens the metabolism of proteins and collagen during the healing process, balances the skin, and reduces irritation; its therapeutic benefits are similar to aloe vera. Calendula extract can be applied on textiles by a microencapsulation technique.

Vegetable Glycerin

Vegetable glycerin is also known as vegetable glycerol. It is a carbohydrate that is usually derived from plant oils, for example, coconut and soybean oils. Glycerin is an organic compound composed of three carbon atoms, hydrogen atoms, and three OH groups. These OH groups form hydrogen bonds with water, slowing down its movement and giving liquid glycerin the property of a syrup. It is used as a moisturizer in various cosmetics and has ample scope in cosmetotextiles. Due to its monomeric nature, it is easily absorbed by human skin and moisturizes it.

Padina pavonica

P. pavonica is extracted from the protective coating of a brown algae found in the Mediterranean Sea. It is believed to improve firmness and elasticity of the skin [32]. Cosmetil after collaboration with variance has developed cosmetically inspired fluid lingerie called Hydrabra to provide moisturizing and firming effects. The bra has a specially designed lower cup having an ultrathin cloth impregnated with a lotion formulated with extracts of *P. pavonica*.



Vitamin E or (α-Tocopherol)

FIGURE 51.2 Structure of vitamin E.

Hinokitiol

Hinokitiol is natural wood oil extracted from domestic Hinoki trees. It is effective in keeping away bacteria, molds, and insects. It gives antibacterial effect against various microorganisms like *Staphylococcus aureus*, *Staphylococcus epidermidis* [1,7,8,13], and *Schistosoma mansoni*. It is effective in offering a relaxation effect due to its aromatic nature [33].

Vitamin E

Vitamin E belongs to the group of lipid-soluble vitamins and is available in nature in many vegetable oils. The chemical term for vitamin E is "α-tocopherol," as shown in Figure 51.2. Vitamin E is used as an antioxidant and active substance due to its moisture binding ability in emulsions, creams, lotions, body and face oils, and aliphatic cosmetic for dry skin care as well as for decorative cosmetics like lipsticks. Vitamin E oxygenates the tissues and reduces the need for oxygen intake markedly. It is essential for normal reproductive functions, fertility, and physical vigor. It is helpful in caring for various skin diseases. Vitamin E is a powerful antioxidant. The term "antioxidant" describes the capability of molecules in neutralizing the radicals and in performing as scavengers. Free radicals emerge by the normal cell breathing as side products and try to snatch away an electron from other structures as a means of completing their outer shell [34]. As a consequence, the cell membrane gets damaged. Antioxidants and thus also vitamin E "deactivate" the free radicals by giving off an electron and in this way protect the cells from "oxidative stress."

MECHANISM OF SKIN CARE FINISH

Human skin or dermis is made up of cells, blood vessels, and nerves in an extracellular matrix composed of fibrillated protein formations, collagen, elastin, etc., which provide resilience to stretching, and a colloidal gel substance, which fills up the spaces between all the different dermal components. This gel substance is chiefly composed of water, mineral salts, and glycosaminoglycans [23]. Free radicals are generated on human skin by photosynthesis. The antioxidants are capable of neutralizing the free radicals. These free radicals are atom or molecules that possess an unpaired electron in their outer shell, and they emerge as byproducts while cell breathing tries to snatch the electron from other structures, which causes damage to the cell membrane. The

antioxidant (like vitamin E) protects the cell membrane by giving electron to free radicals.

CHARACTERIZATION OF COSMETOTEXTILES

European Standardization concerning testing of multifunctional textiles is felt to be strongly needed to form test standards for cosmetotextiles. The European Union formed a working group WG-25 to form test standards for cosmetotextiles. WG-25 has identified some areas where standardization is required immediately and formed five subgroups to work on different aspects of cosmetotextiles. Both subjective and objective evaluations of cosmetotextiles are possible to test various cosmetic effects. Various research organizations have developed testing standards for textiles, which are directly applicable in cosmetotextiles, and are given below:

- AS/NZS 4399 (1996): sun protective clothing evaluation and classification
- American Association of Textile Chemists and Colorists 183-2000: transmittance or blocking of erythemally weighted ultraviolet radiation through fabrics
- BS 7914 (1998): method of test for penetration of erythemally weighted solar ultraviolet radiation through clothing fabrics
- EN 13758-1 (2001): textile UV protective properties. Part 1: method of test for apparel fabrics

Chemical Properties

The chemical properties of cosmetotextiles are tested as per existing legal directives and regulations for cosmetics. Presently, 13 relevant directives are identified and listed dealing with both textiles and cosmetics. The European Cosmetic Directive is working on the development of standards for cosmetotextiles.

Toxicity/Innocuousness

This subgroup made a common thought that cosmetotextiles cannot be considered as medicine. The cosmetics have to be first applied on textiles then textiles have to be used close to the skin. Cosmetotextiles should successfully pass EN ISEO 10993 and OECD test methods (OECD 405, 406, 407, and 471). OEKO TEX may also be another option. All members of this group agreed to process their activities in two steps:

Step 1: Test an individual ingredient based on the current testing standards in the cosmetic industry.
Step 2: Test a whole product with a general biological test similar to antimicrobial test.

These test methods should be balanced between costs and security.

Presence of Vitamin E

The testing of vitamin E content on textile surfaces can be performed quantitatively through a color reaction by utilizing the reduction properties of vitamin E as follows:

- The $FeCl_3$ solution is dripped onto the finished textiles. In the presence of vitamin E, the Fe^{3+} ion is reduced to Fe^{2+}.
- The dipyridyl solution is dripped, which forms a red chelate complex in the presence of Fe^{2+} ions.

Efficacy

Efficacy of cosmetotextiles should be tested using same testing tool and testing conditions as cosmetics. WG-25 agreed to set some guidelines to solve the complexity of this problem. ISO/DIS 11930 test may be successful in testing the efficacy of cosmetotextiles, although this test is designed for cosmetics.

Perfume Performance Analysis

This analysis is required to test the performance of various perfumed textiles and odor-combating textiles. Headspace gas chromatography/mass spectrometry (headspace GC/MS) is a specific technique used to analyze volatile compounds. A specimen is placed in an airtight closed sampling vessel and then subjected to different temperatures with known temperature profile. The vapors in vessels are sampled to analyze odor issues, identification of polymer additives, and residual solvent analysis as per various ASTM standards like ASTM D3362, D3452, and D4128 [4].

Durability

The WG-25 has formed a separate subgroup to emphasize the durability aspect of cosmetotextiles. For wash fastness, a lot of testing methodologies are recommended by this subgroup. The efficiency of a binder to bind microcapsules on a textile surface depends on the compatibility of the different interfaces of the products involved in the finishing process. The choice of binder adapted to fix the microcapsules can be finalized by making a comparison of the surface energy components induced by various components in terms of a contact angle. Generally, the adhesion of microcapsules is closely dependent on the chemical nature and structure of the textile substrates [5].

Labeling

In the last meeting of the group WG-25 for studying the feasibility of introducing a standard of labeling in packaging of cosmetotextiles, they presented recommendations in two parts: the first part was regarding the level sewed in the cosmetotextiles, and in the second part, a note was written on packaging. The WG-25 group is still actively involved in finalizing the labeling standards for cosmetotextiles.

Moisture Retaining

The moisture-containing ability is a very essential property of cosmetotextiles. The moisture-holding capacity of cosmetotextiles can be measured by the Check-Line TEM-1 textile moisture meter. The measuring principle of the TEM-1 is based on the electrical conductivity of the material, which always maintains a fixed relation to the moisture. The electrical indicating accuracy of the TEM-1 is ±0.1%, while the reproducibility is ±0.2%, referred to as the absolute readings on the meter dial.

Skin Surface Profilometry

Skin surface profilometry (SSP) is an evaluation process for skin surface profile, which is appropriate in examining the effect of wrinkle treatment, skin moisturization studies, as well as cellulite treatments.

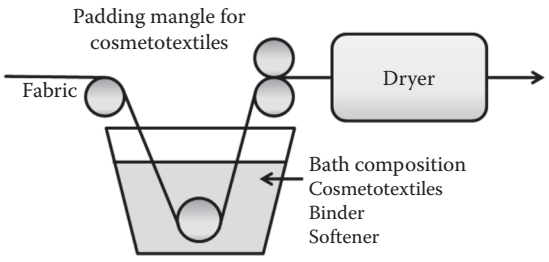
During SSP, the subject is guided to wear the slimming garment typically twice daily: in the morning and in the evening. The specimen is worn for a few months and is tested at intervals of 15 to 30 days. For cellulite studies, the typical test site is located on the posterior outer aspect of the upper thigh, halfway between the hipbone and the knee. For each measurement, a single silicone replica is made of an area on one side of the targeted area and a record is kept of this target. The silicon replica is stored in controlled conditions for comparative analysis. Comparative surface roughness measurement of skin is conducted. The height of the replicated wrinkles is measured using the Miyomoto Surftest profilometer as per Dermatest SOP DESOP 039.

INCORPORATION OF COSMETIC EFFECT INTO TEXTILES

The following two techniques are very popular in incorporating cosmetic ingredient in virgin or in microcapsule form.

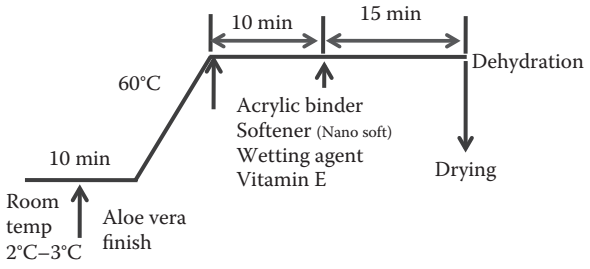
One Bath Dry Method

This is the simplest way to incorporate cosmetic ingredients on textiles. One bath is used to dip the textiles where appropriate solution of cosmetic material in a solvent remains available. Cosmetic transfer from solution to textiles takes place by one dip, one nip mechanism. One nip is used to squeeze the extra amount of cosmetic from textiles. Finally, a dryer is used to fix the cosmetic ingredient on textiles.



Exhaust Method

The exhaust method starts from the preparation of material to liquor ratio of 1:10 and followed by the addition of microcapsules to get the dispersion by gentle stir. The dispersed microcapsules are added to the bath with a small amount of an anionic dispersing agent. The stirring will continue for up to 10 min. The temperature of bath was raised to 50°C–60°C depending on the type of binder used in the recipe. After 15 min of stirring, nonionic softener was added and the machine was run for 15 min. Hydroextraction is followed by drying by keeping the temperature less than 155°C (Table 51.1).



FINISHES FOR COSMETIC EFFECT

A wide variety of natural and man-made materials are used on textiles to incorporate cosmetic functionality as a finish. Different textile and cosmetic industries have developed various cosmetic finishes for textiles commercially. Some of them are discussed here.

Parafine SC-1000

This finish is developed by Ohara Paragium Chem. JP and mainly consists of silk-based amino acids. The amino acids are rich in moisture-retaining properties, which promote skin well-being by enhancing the amount of moisture on skin.

Parafine SC-3000

This finish imparts the fat-burning effect by presence of capsaicin as well as moisture-retaining and skin care effect by raspberry and squalane, respectively.

Parafine SC-5000

This finish contains extracts from rice germ oil (ferulic acid and γ-oryzanol) and vitamin E. This combination offers antioxidation properties that contribute to skin antiaging. This finish promotes the antioxidation, biomembrane stabilization, and blood circulation in human skin.

Evo Care Vital

This finish developed by Dystar Auxiliaries GmbH, Frankfurt, Germany, contains a combination of vitamin E, aloe vera,

TABLE 51.1
Selected Cosmetotextiles with Manufacturer's Product Name, Basic Cosmetic Ingredient, and Product Features

S. No. and Ref.	Manufacturer and Brand Name	Basic Cosmetic Ingredients	Product's Features
1 [49]	Ajinomoto with Mizuno Corp USA with brand name "Amino Veil"	"Arginine" amino acid	Tennis and golf clothes. Amino acid dissolves into a wearer's perspiration, enhancing the material's ability to absorb moisture, keeping the skin's pH level balanced, and regenerating the skin.
2 [50]	Yonex: Sports cloth manufacturer	Xylitol	Tennis and badminton clothes: These fabrics mainly consist of xylitol that absorbs heat when in contact with water and offers cool feel (when the wearer starts sweating).
3 [51]	Fuji Spinning, Japan with brand name V-Up	Pro-vitamin C soluble in sebum	Cosmeto-clothing: pro-vitamin C converts into vitamin C in presence of sebum and is applied on blouses and men's and women's shirts.
4 [35]	Dri-Fit fabrics by Nike	Made of microfibers of nylon, polyester, and spandex	Fine diameter of the fibers plays key role in creating the ideal levels of surface tension and adhesion between the molecules and creates a strong capillary action. This capillary action helps to move the sweat as fast as possible along those fibers.
5 [52]	Outlast Tech. Inc. (Boulder, CO, USA)	Phase-change materials (PCM)	Apply PCM based microcapsules into textiles structures, to enhance wearer comfort and to provide temperature control for consumers in bedding, medical supplies, sportwear, and protective clothing.
6 [53]	Cognis Oleochemicals Corp. with brand name "Skintex"	Distilled oils of plants, fruits, and leaves	Fabric has ability to provide gentle care of tired feet and legs with special effects of invigorating aromas. This functionality lasts up to several launderings.
7 [54]	Quiospheres®	Quiospheres moist and Quiospheres slim	Quiospheres moist microcapsulated fabrics treated up to 120°C able to control the moisture level on skin while Quiospheres slim works to control the cellulite level on human body parts.
8 [55]	LYOSILK® Hefel Textil GmbH, Austria	Tencel and Silk Fibre	Lyosilk consists of microfine Tencel fibers and pure silk; 300–1000 m long individual delicate threads are twisted together to form open and soft silk yarn to be used as weft. The actively breathable, fluffy Tencel fibers become shinier, smoother, and even more refined by the incorporation of pure silk.
9 [55]	SEACELL®ACTIVE	Silver particles works as antimicrobial agent	SeaCell Active fiber in its blending with other fibers like cotton or viscose makes the yarn and fabric antimicrobial fungicidal. Offers good functionality even after 20 washes at 60°C.
10 [39]	Solidea, Italy MicroMassage Magic	80% polyamide; 18% elastin; 2% cotton	MicroMassage collection provides elegant shaping as well as toning and smoothing of the skin. The key is in the special patented three dimensional wavelike knitted process of the fabric that lightly massages the skin by working with natural body movement to promote circulation of skin and fat tissue and stimulate drainage of fluids causing the orange peel effect on skin.
11	Cosmetil and Variance, Hydrabra	Ultrathin cloth with extracts of *P. pavonica*	Cosmetically inspired fluid lingerie "Hydrabra" provides moisturizing and firming effects.
12	Lytess cosmetotextiles, Lytess, France	Caffeine and sea butter based textiles	Underwear with caffeine and shea butter, funky tops with aloe vera, and hosiery with ginger and shea butter.
13	Spanx, MANX Spanx Inc., Atlanta	Body shaping undergarments	Controlled compressive intimate garment made with Elastane fibers.

and jojoba oil that offers antiaging function in textiles [46]. These natural substances are embedded in silicone matrix to enhance the washfastness of finished fabric. Evo Care Vital is applied as the last step in conventional pad and exhaust finishing process. DyStar has also introduced Evo Care BeeWell with beewax, Evo Care AVP and Evo Care AVS with aloe vera, and Evo Care SJO with jojoba oil [47]. Evo Care Vital fulfills the current cosmetic requirement by its antiaging effect and is applicable on broad textile spectrum that comes into human skin contact directly. West and Zhu [48] experienced a consistent improvement in skin integrity by exposing factory workers against dry-coated aloe vera gloves.

Skin Care Finishes

A range of wellness textile finishes have been launched by various finish manufacturers and some of them are described in Table 51.1.

Skinsoft 415 New

This finish is developed by Daiwa Chemical Inc. Japan, which is mainly composed of phospholipid containing 2-methacryloyloxyethyl phosphorylcholine (MPC) with phosphatidylcholine polar groups. Skinsoft 415 New based

on water-soluble polymer exhibits superior moisture-retaining effects. Sweet softener AN is also available for use with Skinsoft 415 New. This finish improves soil release and antibrowning and antistatic effects [44].

Ohara Paragium Chemicals, Kyoto, Japan, have launched a broad spectrum of skin care and antiaging functional finishes for textiles; some of them are available in the market [45].

RECENT AND FUTURE TRENDS IN PRODUCT DEVELOPMENTS

Specific engineering aspects can generate wellness abilities in typical textiles. Invista International, Switzerland [35], suggested that the use of graduated compression in garments for the leg offers many physiological benefits for the wearer, such as reduced fatigue and leg swelling and enhanced athletic performance. Invista developed new Lycra leg care stockings, which are a combination of function and fashion and have the potential to significantly reduce postexercise muscle soreness.

Institute fur Textil- und Verfahrenstechnik (ITV Denkendorf), Germany [23], described the potential of Atmofil polyester yarns to produce wellness effects. Atmofil is a differential shrinkage elongation (DSE) yarn in which a functional partially oriented yarn (POY) component is combined with a core component through air intermingling. After realization of Atmofil yarns into woven or knitted fabric, the respective elongation and shrinkage components are released during fabric finishing. The finished fabric offers viscose-like handle and appearance.

Tejin Co. Ltd, Japan, was the first that manufactured and sold out 2 million of its trademarked "Amino Jeans" within 24 h that are treated with arginine and blew a new potential in the wellness innovation market. Arginine is an amino acid that is said to maintain skin youthfulness [36]. The field of cosmetotextiles is full of potential for the future for those who always work with a positive and an optimistic mind frame. This field feels a strong requirement of clubbing of different types of industries like textile, cosmetic, herbal, pharmaceutical, etc. In a typical research finding, Phaneuf et al. [37] considered a polyester fabric as a control material and exposed to ethylenediamine (C-EDA) to achieve wellness effects.

Skintex technology incorporates active ingredients by microencapsulation. The active ingredients are encapsulated inside the microcapsule and firmly anchored on the fiber within the fabric of a textile without affecting the feel and visual appearance of the textiles. In a typical application, chitosan is encapsulated to save from warmth, drying out, and cold. At the same time, chitosan helps to protect the skin from dehydration and helps skin keep a supple and velvety soft touch. The ingredients are released either by friction during wearing or the chitosan layer is slowly reduced over time through a wearer's enzymes. Each textile structure has a limit to load the extra ingredients. A highly active ingredient in each microcapsule with maximum utilization of capsule interior is required for attaining the long-lasting well-being effect. The skintex well-being ingredients are highly concentrated; so, even when released in very small quantity, an effect can be seen and felt. They are dermatologically tested as per Eco-Tex standard 100. This technology is effective even after 100 washings in terms of traces of vitamin E if cloths are washed as per Skintex recommendations [38].

A clinically proven patented fabric design from Solidea, Italy, has offered cellulite-reducing shorts and hosiery range by micromassaging of body parts. The manufacturer claimed that "MicroMassage Magic" garments are helpful for smoothing and reshaping bottom and legs, and improving the health and appearance of legs and thighs. This patented design of Solidea combined the compression with massage through everyday movement. Solidea range in Australia is going to include Magic Maman Anticellulite maternity shaping shorts to promote blood circulation and to reduce water retention [39]. A typical MicroMassage Magic Shorts contain 80% polyamide, 18% elastane, and 2% cotton fibers.

Active textile-based composite materials are used as a wellness product by Swiss Federal Laboratories for Materials Testing and Research for the improvement of physical performance of multiple sclerosis (MS) patients [40]. Two polymer membranes are laminated along with a textile material to make personal lightweight cooling garments. This composite clothing provides moderate body cooling to patients who are suffering with abnormal hardening or thickening of an artery or other body part along with improved nerve conduction velocity. This garment was manufactured by using 10- and 15-μm-thick polymer membranes from Sympatex, while the textile substrate was a polyester fabric having 100-μm thickness. Wellness finishes with vitamin E have zero affinity with most of the textile fibers, so they first combined with CD before application. Generally, 3%–7% wellness finish with appropriate binder and softener is padded to get 80%–130% pickup. The treated fabric is dried and cured at 140°C–160°C for 2–4 min [41].

USA-based company Cupron Inc. has launched a commercial range of pillows and pillowcases with slogan "Beauty while You Sleep" that helps to reduce wrinkles and liver spots [42]. The polyester filament was treated with a wicking surfactant to maintain the sufficient breathability of pillows and pillow covers. The Cupron used the copper oxide to offer antimicrobial and healing properties. This compound also promotes the healing of wounds because it has the ability to bind amino acids and create collagen. Cupron used copper oxide as a melt additive in melt spun fibers while coated on a fiber surface in case of natural and solution spun fibers. The clinical trials of Cupron fibers improve the skin tone and texture significantly. The Cupron used 4 × 1 twill weave fabric having copper impregnated weft and a Pima cotton warp.

Cognis, Germany, has introduced chemically and technically fine-tuned baby diapers "Caremelts" with maximum dermatological compatibility based on the application of phase change materials. Caremelts works close to room temperature. It has utilized the compositions of cosmetic waxes with fabrics, which melt partially at body temperature. Caremelts is manufactured in a discontinuous way in order

not to disturb the liquid acquisition functionality of diapers or other hygiene potentials [43]. The most commercial successes have been achieved via sales channels like teleshopping or catalogues, which give more detailed information on the concept and the products. The increasing collaboration between traditional cosmetics and fashion brands will further strengthen the credibility of the cosmetotextile concept.

Spanx, Inc., is an Atlanta, Georgia-based hosiery company that is primarily involved in pantyhose and other undergarments. Spanx specializes in "bodyshaping" undergarments and bodysuit shapewear, intended to give the wearer a slim and shapely appearance.

INTERFILIERE Hong Kong got a very high international attendance on day 1, which reaffirmed the importance of Hong Kong as a center for intimates and swimwear.

CONCLUSIONS

In the future, wellness finishes will play a key role to develop value-added products to compete in barrier-free market where the customer's expectations are climbing new heights every day. Optimization of cosmetic ingredient quantity and enhancing durability of cosmetic effects are the two real challenges in this field. Cosmetotextiles is a fast-growing industry in which different types of industries are working together. Worldwide customers have turned toward well-being through natural resources in an ecofriendly health-promoting environment. The development and optimization of cosmetotextiles are in the neonatal stage and require proper attention and adequate funding. Various explored and unexplored natural products are available to feed the cosmetotextile industry that has enough potential to offer wellness effects. Cosmetotextiles have to be designed in such a fashion so that composition and construction of textiles, garment design, and cosmetic finish must all work together to exhibit optimum cosmetic effects.

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52 Measuring Hair

R. Randall Wickett and Janusz Jachowicz

STRUCTURE OF HUMAN HEAD HAIR

Desmond Morris referred to humans as "naked apes" [1]. While the body hair on most humans is vellus, we grow hair on our heads that is far longer than the more abundant coat on most other mammals. Human head hair typically ranges from 20 to more than 100 μm in diameter [2], and some people can grow their hair to lengths of more than 5 ft.

The hair shaft is composed of columnar cortical cells that are surrounded by the overlapping cuticle scales. In some hairs, there may also be a more porous area in the center called the medulla. A transmission electron micrograph (TEM) of a horizontal cross section through the hair follicle at the level of the reticular dermis is shown in Figure 52.1. The various layers of the follicle and the hair can be clearly seen. Working in from the glassy collagen layer of the dermis (D) that surrounds the follicle, we see the outer root sheath (ORS), Henley's (he) and Huxley's (hu) layer of the inner root sheath, the innermost cuticle of the inner root sheath (cl), and the cuticle (CU) and cortical (CO) layers of the hair shaft itself. This particular hair does not show clear evidence of a medulla.

Human head hair has 6–10 layers of cuticle when it emerges from the scalp [3]. A cross section of a hair stained with silver methenamine is shown in Figure 52.2. Each cuticle cell is connected to the cortex, and the cells overlap from the root to the tip at an angle of about 5° [4] causing the well-known directional difference in hair friction [5].

Cuticle cells are flat and approximately square, being about 50 μm on a side and about 0.5 μm thick. Each cuticle cell is composed of a cell membrane complex (CMC) and three distinct internal layers of differing sulfur content. The CMC has two 3-nm-thick β-layers on either side of an 18-nm-thick δ-layer. The upper β-layer that faces out from the hair has an outer surface of 18-methyl eicosanoic acid (18-MEA) [6] that is covalently attached to proteins by thioester bonds [7,8].

Cortical cells are roughly cylindrical being 50–100 μm long and 3–6 μm in diameter. They have longitudinal flutings and may separate into smaller finger-like structures. The cells are closely packed together in the hair shaft so that the fluted surfaces interlock putting their CMCs in contact [9].

The mechanical properties of hair are dominated by the keratin microfibrils in the cortex, while the optical and surface properties are dominated by the cuticle and particularly by the state of the 18-MEA on the surface. For a recent review of hair structure and chemistry, see Swift [10].

DETERMINATION OF HAIR DIMENSIONS

In order to determine tensile properties such as the elastic modulus, it is necessary to accurately measure the cross-sectional dimension. Determining the cross-sectional dimensions of a hair is not always straightforward. Not only is hair a thin fiber, it is also not necessarily uniform in cross section. While Caucasian hair is generally considered elliptical in shape, significant variations from ellipticity can occur. With African-American hair, the problem is compounded by the high elliptical ratio and the presence of many nonuniform shapes. There are now laser micrometers that can be used for this purpose, and Dia-Stron makes an instrument that can be interfaced with their automated tensile testers. Several measurements must be made along the fiber, and the fiber must be rotated to be sure to measure the major and minor axes of the ellipse. Perhaps the most accurate method of determining the cross-sectional area of a hair is to section it and measure the diameters directly from the micrographs [11].

TENSILE PROPERTIES OF HAIR

The mechanical behavior of hair is frequently studied in extension by obtaining a stress/strain curve. Stress/strain curves for hair can be determined using a tensile tester such as the Instron or Dia-Stron.

Figure 52.3 shows stress/strain curves for the adjacent sections from the same hair fiber in extension in water and at 50% RH. The curves can be characterized by three different regions. In the first region (A–B), stress versus strain is approximately linear and a slope can be determined. This part of the curve is often called the Hookean region, and it extends to about 102% of the equilibrium length of the fiber (2% strain). The slope of the Hookean region is considerably higher in dry hair. Between 2% and 4% "strain, the curve 'turns over' " into the yield region (B–C). In the yield region, very little increase in force is required to increase extension. In the postyield region (C–D), which typically begins between 25% and 30% strain, the force again increases markedly with strain. For this hair, under the conditions tested, the slope in the postyield region was about one fifth of that in the Hookean region of the dry fiber. There is little difference in postyield slopes between the wet and dry sections of the hair.

Published reports on the mechanical properties of keratin fibers date back to the 1920s and the work of Speakman [12] who first reported on the effect of water on keratin mechanical properties. Since that time, extensive research on hair and wool has led to an interpretation of each region of the

FIGURE 52.1 TEM of cross section of human hair. (Micrograph courtesy of Raymond Boissy.)

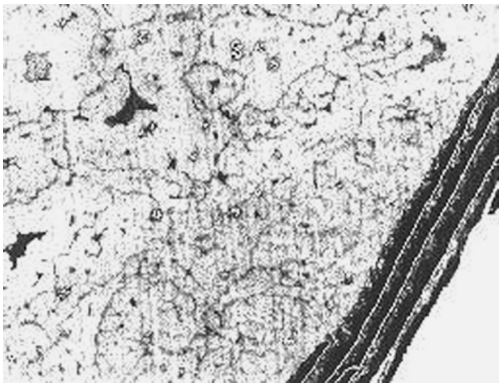

FIGURE 52.2 Cross section of a hair stained with silver methenamine.

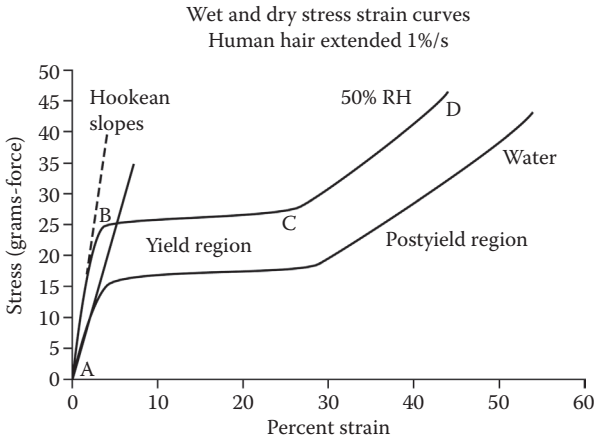

FIGURE 52.3 Stress/strain curve for hair at 50% RH. Data obtained using a Dia-Stron miniature tensile tester.

stress/strain curves of keratin fibers in terms of changes occurring in the molecular structure. Much of the seminal work in this area was carried out by Max Feughelman [13], and his book on the subject gives an excellent and detailed overview of the physical properties of keratin fibers.

The curve in the Hookean region (A–B) can be used to calculate Young's modulus of elasticity, *E*. The elastic modulus is the stress divided by the strain so the cross-sectional dimensions of the fiber must be known accurately. The sample calculation below was done assuming a circular hair with a diameter of 60 μm using typical numbers for a virgin hair at 50% relative humidity.

Sample calculation of the elastic modulus:

- E = stress/strain = $\Delta\ F*L/(\Delta L*A)$.
  - ΔF is the change in force during length change ΔL.
  - L is the length of fiber.
  - A is the cross-sectional area.
- Assume circular hair 60 μm in diameter.
  - $A = 2.83 \times 10^{-5}$ cm^2 = 2.83×10^{-9} m
- A 10-cm length is extended to 10.2 cm.
  - 2% extension
- Force change is 22 grams-force = 0.216 N.
  - 1 gram-force = 980 dynes = 9.8×10^{-3} N
- $E = (0.216 \times 10\ \text{cm})/(0.2\ \text{cm} \times 2.83 \times 10^{-9}\ \text{m}^2)$.
- $E = 3.8 \times 10^9$ N/m^2.
- 1 Pa = 1 N/m^2, 1 N = 105 dynes, 1 m^2 = 10^4 cm^2.
- E = 3.8×10^9 Pa = 3.8×10^9 N/m^2 = 3.8 GPa.
- In older papers, E is called Y and is reported in dynes/cm^2.
- $E = 3.8 \times 10^{10}$ dynes/cm^2.

E is typically about 1.5 to 2.0 GPa for wet hair and 3.5–4.0 GPa for hair at 50%–65% RH. The mechanical properties of hair or wool in the Hookean region and the effect of water on mechanical properties (Figure 52.3) can be explained by the two-phase model proposed by Feughelman [14–16]. Feughelman's model considers the mechanical properties of the fiber to be determined by a water-impenetrable phase, *C*, the microfibrils, and a water-permeable phase, *M*, the matrix. The microfibrils consist of α-helical proteins (keratins) aligned parallel to the fiber axis [17–20], and the matrix is composed of keratin-associated proteins [21], which are packed around the microfibrils. The composite is modeled as a fixed spring in parallel with a spring and viscous dashpot in series; the spring contributes about 1.4 GPa to the Young's modulus and is contained in the water-impenetrable microfibrils. The main resistance to extension of the microfibrils probably comes from the hydrogen bond network in the α-helical proteins [22]. The matrix contributes viscous forces that decay with time causing stress relaxation. The viscosity of the matrix decreases greatly as the water content of the fiber increases. The two-phase model of keratin fibers accounts for the effects of water on the mechanical properties, the effect of strain rate on Young's modulus, the stress relaxation behavior in the Hookean region, and the behavior of wet, dry, and permanently set fibers in torsion [16,22].

YIELD REGION

Somewhere around 2% to 3% strain, the stress/strain curve "turns over" into the yield region. Past this point, the stress

does not increase markedly until about 25%–30% extension. The mechanical properties of a fiber extended into the yield region can be recovered by relaxing the fiber in water for a few hours if the fiber is not held too long in extension and the extension is carefully confined to the yield region. This fact is of great practical importance in designing protocols to measure the effect of treatments on hair strength as we will see. X-ray diffraction results have demonstrated that there is a progressive loss of α-helical content and a concomitant increase in β-sheet as a fiber is extended through the yield region [23]. By the end of the yield region, about 30% of the original α-helix has been unfolded reversibly. Mechanical behavior of keratin in the yield region can be accounted for by application of a Burte–Halsey [24] model. The fiber is considered to contain a continuum of units, which can exist in a short state, A (α-helix), or an extended state, B (β-sheet), with an energy barrier between the states. The yield region corresponds to a phase transition between state A and state B at constant stress. This first-order phase transition, producing a length change at constant stress and temperature, is thermodynamically equivalent to the transformation of water to steam producing a volume change at constant temperature and pressure.

Data from hair strained into the yield region are usually reported as either force at a given extension such as 15% or 20% or the work to extend a hair to a given extension, which is obtained by integrating the area under the stress/strain curve. Hu [25] measured the work to extend hairs to 20% and compared results with Caucasian, Asian, and African-American hair. Results from Caucasian hairs as a function of hair diameter are shown in Figure 52.4.

Table 52.1 shows the correlation equations for the work to extend to 20% the hair from each ethnic group. The work to extend African-American hair by 20% was found to be about two-thirds of that required for either Asian or Caucasian in agreement with other studies showing that hair of African origin is not as strong as either Asian or Caucasian hair [26].

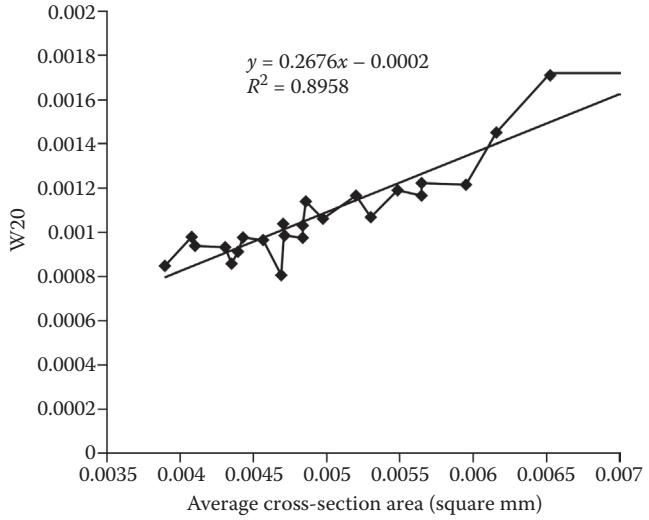

FIGURE 52.4 Work (in J) to extend hair by 20% versus cross-sectional area.

TABLE 52.1
Correlation of Work of Extension to Area for Different Ethnic Groups

Hair Type	Correlation Equation	R^2
Caucasian	W20 = 0.2676A – 0.0002	0.90
African-American	W20 = 0.1844A + 0.0002	0.83
Asian	W20 = 0.2961 – 0.0003	0.91

POST-YIELD REGION

The C–D region in Figure 52.4 is known as the postyield region. Speakman [12] found the postyield slope to be independent of the water content of the fiber. This is born out by the essential equivalence of the slopes of the C–D region shown in Figure 52.4. The increased stiffness in the postyield region apparently results from a covalently bonded network involving cystine. The postyield slope has been shown to be dependent on the disulfide content of the fibers [27–29].

TENSILE TESTERS

In the past, stress/strain measurements on hair were mostly made using one of the models of the Instron Tensile Tester [2,5,30,31]. Instron is a robust and versatile instrument but is more than a bit of overkill for measuring hair mechanics. In recent years, the Dia-Stron Miniature Tensile Tester (MTT) has become a widely used choice. The instrument can be equipped with an automated sample head [32] to allow running of up to 100 hairs in one setup and can be interfaced with a laser micrometer system to automatically measure the dimensions of the hair fiber.

Software with the instrument can automatically record the stress/strain curve and report various parameters to the operator including elastic gradient, work of extension, and breaking load. Evaluation of hair tensile properties has come a long way from the days of tediously measuring data points off a chart recording with a ruler.

TENSILE MEASUREMENTS OF HAIR DAMAGE

The most common use of hair tensile properties is for the evaluation of the effects of treatment on hair strength to determine the level of "damage" produced by a given treatment. The mechanical properties of wet hair are greatly affected by treatments that lead to a reduction in the number of disulfide bonds. Measurement of breaking strength may show differences between treatments if they are large and large numbers of hairs are run, but one must try to select hairs of approximately the same diameter for measurement if possible. For this reason, many workers have relied on the fact that mechanical properties of hair extend into the yield region, but not beyond can be recovered by soaking in water. Beyak et al. [30] extended hair by 20% and measured the force. After recovery, treatments were investigated using each hair as its own control. The average change between

tests for 25 untreated control hairs was only −0.33%. A 5-min "cold wave" treatment led to a 12.6% decline in the force at 20% extension.

Tate et al. [33] and Robbins and Crawford [34] also used mechanical measurements in extension to study hair damage. The study by Robbins and Crawford revealed the interesting fact that significant damage to the cuticle can occur with little or no effect on the tensile properties of hair.

Gamez-Garcia [35] described the use of short-term relaxation measurements from small deformations to assess the effect of oils, emulsions, and solutions of salts, amino acids, and proteins on stress recovery in hair fiber. The author analyzed the relaxation curves and showed that the curves had a short-term (on the order of minutes) component and a long-term (of the order of hours) component. The medium in which the fiber is immersed was found to have a strong effect on short-term relaxation.

Hu [25] investigated the effects of heat and relaxer treatment using both breaking stress and the work to 20% extension (Figure 52.5). Heat treatments were for 5 min. Relaxer treatment was with a commercial relaxer according to label directions. Data are summarized in Table 52.2.

It does appear that breaking stress is more affected by heat at low temperature, and the effect of heat on break stress

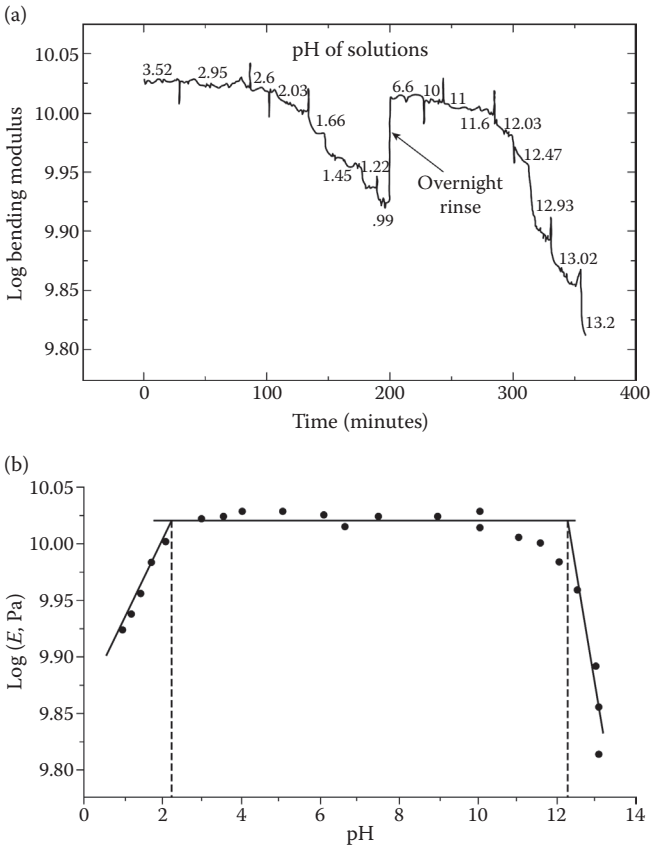

FIGURE 52.5 Dynamic mechanical analysis. (a) DMTA trace obtained by employing an online treatment procedure (with HCl and NaOH solutions) and shown as the logarithm of the bending modulus. (b) Bending modulus of wet intact hair as a function of pH.

TABLE 52.2
Effect of Treatment on W20 and Break Stress

Treatment	% Change W20	% Change Break Stress
60°C	1.9	−5.2
115°C	−0.02	−9.9
130°C	−3.5	−10.6
160°C	−5.9	−10.5
Relaxer	−25.7	−37.5

was significant at 130°C while W20 was not significantly affected. This may be due to loss of disulfide bonds that are not extended before the postyield region is reached. In order to obtain this kind of result for breaking strength, the hairs must be carefully prescreened to be of approximately the same diameter.

MECHANICAL FATIGUE BEHAVIOR

Kamath et al. [26,33] described an apparatus for studying the mechanical fatiguing of hair. The instrument subjects the fibers to an impact-loading mode of fatiguing at a constant load and rate of 1 cycle per second for up to 100,000 cycles. The strain in hair was kept within the Hookean range, and the fatigue data were interpreted in terms of the following equation:

$$F(x) = A(x)^n$$

where $F(x)$ is the cumulative probability of failure, x is the number of cycles to failure, and A is constant. The exponent n and the number of cycles required for half of the specimens to fail (h_f) were employed to quantify the damaging effect of grooming treatments on hair. The exponent n was found to vary from approximately 0.5 ± 0.052 for untreated hair to 0.11 ± 0.098 for hair after three perming treatments. A concomitant change in half-life parameter, h_f, ranged from more than 100,000 cycles to 3000 cycles. This technique was also found to be useful in evaluating fiber damage as a result of bleaching and perming. Evans [36,37] has performed detailed studies on fatigue behavior and hair breakage.

DYNAMIC MECHANICAL ANALYSIS

Dynamic mechanical analysis has not been employed frequently in hair studies. This was probably due to the fact that the old generation instrumentation was difficult to use, and the experiments were very time-consuming.

During the last 20 years, several new instruments were introduced. They include the dynamic mechanical and thermal analyzer (DMTA) (Rheometric Scientific) and the dynamic mechanical analyzer (Perkin Elmer DMA7), characterized by high sensitivity, broad dynamic range, and high force control.

The dynamic mechanical experiment gives information on both storage and loss moduli (or tan delta) and can provide

a complete characterization of viscoelastic properties of hair fibers. The measurements can be performed as a function of time, temperature, or frequency in either stretching or bending mode of fiber deformation. For characterization of hair and hair-care products, the bending mode of operation is of particular interest because it is probably a predominant mode of deformation for in vitro hair on the scalp.

An example of the use of dynamic mechanical measurements in hair studies is the pH dependence of storage modulus shown in Figure 52.5. The data were obtained through the use of the DMTA equipped with a humidity controller and an online treatment attachment (M. Zielinski, unpublished data).

Figure 52.5a presents the actual DMTA trace, presented as the logarithm of bending modulus as a function of the experiment's time, obtained for a 40-fiber assembly mounted in a frame for a single cantilever bending measurement. The active length of fibers was 1 mm with an amplitude and frequency held constant at 128 mm peak to peak and 3 Hz, respectively. The pH was adjusted with HCl and NaOH by using solutions at 22°C continuously flowing over the hair sample.

Figure 52.5b shows the averaged modulus data from Figure 52.5a plotted as a function of pH. The results demonstrate relative constancy of bending modulus in the pH range from 3 to 10 and its decrease in both very acidic (pH < 2) and very basic (pH > 12) solutions. A similar analysis can be performed for hair exposed to chemical treatments, surfactants, or polymer solutions.

FLEXABRASION TESTING

Swift [38,39] has pointed out that the mechanism of hair breaking on the head is probably different from simple breakage in tension. A method called the flexabrasion test may be more relevant to the actual consumer experience. In this test, weighted hairs are pulled back and forth across a fine wire by a reciprocating motor as illustrated in Figure 52.6.

The parameter measured is the number of cycles required to break 50% of the hairs. This number has a very high variance from hair to hair. Swift reported data obtained using adjacent sections of the same hair for treatment and control to reduce the variance. By using three sections from each hair, a control, a damaging treatment, and an intervention could be studied. Some of Swift's [39] data are presented in Table 52.3.

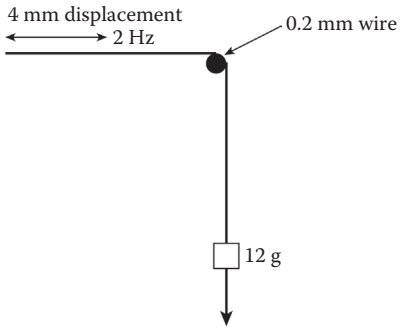

FIGURE 52.6 Illustration of the flexabrasion test.

Data from this method are the basis of some rather extreme sounding claims for large increases in hair strength. It is not the tensile strength that is increased by the treatment but the resistance to fraying under repeated abrasion. This method will obviously reflect the presence of treatments that can reduce the friction between the wire and the hair.

TORSION AND BENDING MEASUREMENTS

Forming a curl from straight hair involves a combination of twisting and bending deformations. Response to torsion or bending tress is highly dependent on hair diameters as both the bending and torsional moments of inertia depend on the fourth power of cross-sectional dimensions. For example, the resistance to bending of an elliptical hair is given by E^*I_b where E is the bending modulus and I_b is the bending moment of inertia and is given by $(\pi/64)ab^3$, where a and b are the major and minor semidiameters of the ellipse, respectively. The torsional moment of inertia is given by $I_t = (\pi/4)(a^3b + b^3a)$; the resistance to twisting is given by $G^*(\pi/4)(a^3b + b^3a)$, where G is the shear modulus. Because of this extreme dependence on cross-sectional dimensions, the cuticle may contribute more to torsion or bending than to extension, especially with very fine hairs.

The shear modulus of a fiber can be determined using a torsion pendulum. In this method, a small cylindrical weight is hung from the hair. Application of torque to the weight causes it to rotate back and forth. If a small white strip or a small mirror is attached to the weight as illustrated in Figure 52.7, the amplitude and period of the torsional deformation as the weight rotates can be easily determined. The shear modulus can be determined from the following equation:

$$G = (Ml/\omega 2)/I_t$$

where l is the fiber length, I_t is $(\pi/4)(a^3b + b^3a)$ as above, and M is the moment of inertia of the weight. Another parameter that can be determined from a torsion pendulum is the log decrement (δ). Log decrement is related to the log of the change in amplitude between one cycle and the next. Log decrement will be increased by treatments or conditions that either increase frictional loss or decrease the storage of elastic potential energy as the fiber twists.

Persaud and Kamath [40] have recently described such a device in detail. Bogaty [41] first pointed out how important the behavior of hair under torsional and bending strains is to formation and maintenance of hair style. He reported that permanent waving decreased the torsional rigidity of hair in the wet state but actually increased it slightly at 65% RH. Harper and Kamath [42] reported similar results for bleached hair. At low RH, the shear modulus of bleached hair was higher than untreated hair, but above 70% RH, the shear modulus of bleached hair was lower than untreated.

Wolfram and Albrecht [43] carried out torsional measurements on hair using a torsion pendulum. They concluded that

TABLE 52.3
Effect of Conditioner of Bleached Hair by Flexabrasion

Conditioner	Bleached	Bleached + Conditioner	Difference	% Increase	*p*
Leave On	1066.5	1621.5	555.1	52.1	0.04
Rinse Off	649.7	1548.9	699.2	82.3	0.01

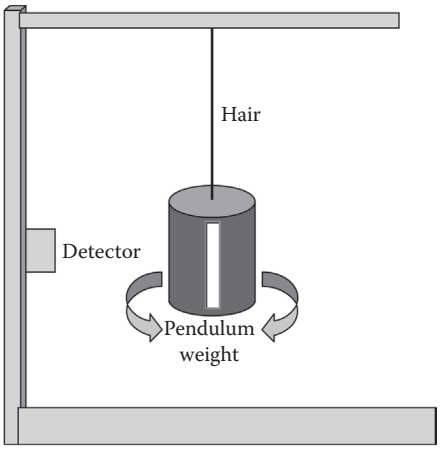

FIGURE 52.7 Schematic of a torsion pendulum for the study of hair.

the cuticle is very stiff in the dry state and may make a significant contribution to the torsional rigidity, especially for fine hairs. However, in the wet state, the cuticle was found to be so plasticized as to make no contribution to mechanical behavior. Harper and Kamath [42] and Yasuda et al. [44] reported that the cuticle makes a significant contribution to the shear modulus of dry hair.

Bending is also a key component of hair style, but bending measurements are generally not simple to perform. Scott and Robbins [45] described a balanced fiber method for measuring the bending stiffness of hair. A long hair is draped over a small wire with small weights attached to each end. The bending stiffness can be calculated from the distance between the two ends. It is also possible to measure bending strength by a three-point beam deflection method. This method has been applied to measuring the stiffness of beard hairs [46]. Another approach is the cantilever beam method as applied by Yasuda et al. [44]. The balanced fiber method has the disadvantage of requiring a relatively long fiber but is simpler to use. Wortman and Kure [47] used the balanced fiber method to study bending relaxation during permanent waving of hair.

For a more detailed analysis of measurement of mechanical properties of hair, see Wickett [48].

SPECTROSCOPY

Fluorescence Spectroscopy

Fluorescence spectroscopy is employed to measure the wavelength dependence of the intensity of emitted light as a function of the excitation wavelength. Hair and skin are characterized by strong fluorescence due to the presence of tryptophan, kynurenine, tyrosine, and phenylalanine amino acids in the structure of keratin. Tryptophan has the strongest absorption (λ_{max} = 280 nm, ε_{max} = 4500 M^{-1} cm^{-1}) and a high quantum yield of fluorescence. Its fluorescence band, excited at 290 nm, has a maximum in the range from 330–350 nm, which is dependent on the extent of hair pigmentation.

The technique has been employed as a sensitive analytical technique to study reactions accompanying hair photo and thermal degradation [49,50]. A fluorescence instrument used to study hair is typically equipped with remote fiber optics, which allow for recording the spectra directly from hair fibers. Experiments described in the literature consisted of irradiating hair with UV/visible light and recording emission spectra in a wavelength range where fluorescence emission occurs. Photodegradation studies have shown that tryptophan undergoes photodecomposition that can be quantified by the measurements of the emission intensity at 300–550 nm for hair before and after exposure (Figure 52.8). The peaks at 350, 420, and 465 nm have been assigned to tryptophan, N-formylkynurenine, and kynurenine, respectively [50,51]. The intensity of emission of all these chromophores is shown to decrease as a result of photoirradiation with tryptophan emission undergoing the largest change. The phenomenon was shown to occur both in natural outdoor conditions and as a result of artificial light irradiations in a weatherometer. This technique can also be used to determine the extent of hair photoprotection, in terms of tryptophan damage, by incorporating photofilters in hair-care formulations such as conditioners, mousses, shampoos, and hairsprays.

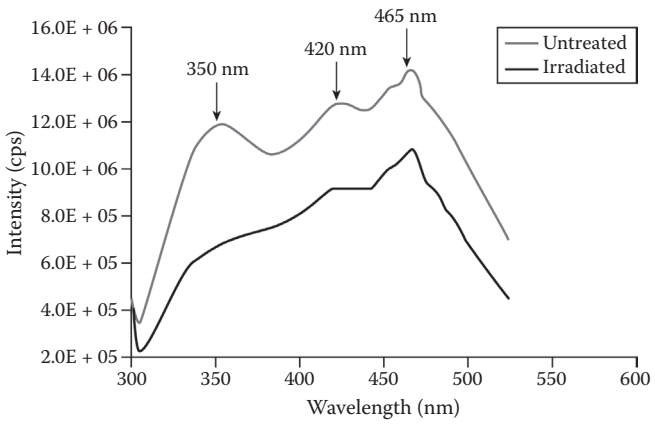

FIGURE 52.8 Comparison of the fluorescence spectra of untreated and piedmont hair irradiated for 48 h in a weatherometer.

IR and Raman Spectroscopy

Raman and infrared (IR) spectroscopies provide alternative ways to detect vibrational states of molecules. While transitions producing IR bands are due to vibrations of groups with a permanent dipole, those corresponding to Raman bands are due to changes in the polarizability of nonpolar groups as a result of nuclear motion.

In recent publications on hair, FT-IR spectroscopy was combined with a microscope (referred to as FT IR microscopy), which allowed researchers to examine hair shaft areas in the size range 10–100 nm. Bramanti et al. [49] examined microregions of hair for anagen, catagen, and telogen hair. The data from bulb and shaft areas were analyzed in terms of relative intensities of amide II (1540 cm^{-1}) and amide III (1238 cm^{-1}) vibrations of the protein component of hair structure and O-P-O vibrations (1080 cm^{-1}) of nucleic acids. It was shown, based on simple spectra and their derivatives, that there was a gradual change in the ratios of absorbance values for nucleic acids/proteins (A_{1080}/A_{1238}) for inferior bulb, central bulb, suprabulbar, and shaft regions for anagen, catagen, and telogen hair. It was suggested that these ratios can be used as reproducible parameters to differentiate the anagen, catagen, and telogen hair phase and to estimate the degree of hair aging.

Other reported applications of FT-IR microscopy included an estimation of the extent of hair oxidation by analyzing the intensity of a peak at 1041 cm^{-1} corresponding to cysteic acid formation in the oxidation of cystine [51].

In a typical Raman experiment, the sample is irradiated with an intense beam of light at a specified frequency u. The emitted light consists of radiation with an unchanged frequency of u (light scattering and refractive index), Raman bands with frequencies at $u + u'$ (Stokes band), and $u - u'$ (anti-Stokes band).

The main advantage of Raman spectroscopy in studies of biological molecules is the low intensity of the water spectrum. A strong IR spectrum of water overlaps the regions where biomolecules have IR absorption bands. The intensity of water spectrum in Raman spectroscopy is relatively weaker, which makes it useful for studies of proteins, etc.

For hair, Raman measurements were reported for unpigmented and bleached hair, employed to minimize the fluorescence effects predominant in more pigmented fibers [52,53]. The spectra of untreated hair show a number of bands not observed in the IR analysis. They correspond to disulfide bonds (510 cm^{-1}), tyrosine (646 and 853/827 cm^{-1}), phenylalanine (1003 cm^{-1}), and tryptophan (1554 cm^{-1}).

Raman spectra can be used to assess the incurred damaged associated with hair bleaching, permanent waving, and photoirradiation:

- For bleaching, a decrease in intensity of the 510-cm^{-1} band with a concomitant increase in the intensity of a band at 1045 cm^{-1} (SO_3, cysteic acid) was observed.
- For perming, reduced hair showed a peak reduction at 510 cm^{-1} and the appearance of a peak at 2568 cm^{-1} (mercaptan).
- Permanent waving was found to produce only a small reduction in the α-helix content of the hair [54].
- For photodamaged hair, analysis showed a decrease in intensity of the disulfide band with the appearance of vibrations corresponding to sulfur in various oxidation states including a thiosulfonate bond. In addition to this, there was an increase in the intensity of a mercaptan band and a change in the amide I region corresponding to a disordered protein.

Near IR Spectroscopy

Near IR (NIR) spectroscopy refers to the portion of the IR spectrum in the wavelength range from 1000 to 2200 nm (10,000 to 4500 cm^{-1}). The observed bands correspond to overtones and combination of characteristic bond vibrations. The technique is typically employed to study bands corresponding to O–H from water, C–H from hydrocarbons, and N–H for proteins. It may also cause transitions of highly delocalized electronic systems, such as those present in the structure of melanin.

Several authors evaluated NIR spectroscopy for studying hair [55,56]. Pande and Yang [56] found the technique well suited for measuring the relative moisture content of hair in situ. The NIR spectra of hair conditioned at 50% RH and dehydrated by heating to 110°C for 90 min are shown in Figure 52.9. It was concluded that peaks at 1450 and 1935 nm are due to water, while peaks at 1740 cm^{-1} are related to methylene C–H stretch; the bands at 2051 and 1984 nm are due to protein. The 1900-nm absorption was further used to quantify the amount of water in hair at 50% RH after drying and as a result of moisture regain. The technique proved to be sensitive to detect small differences in the kinetics of

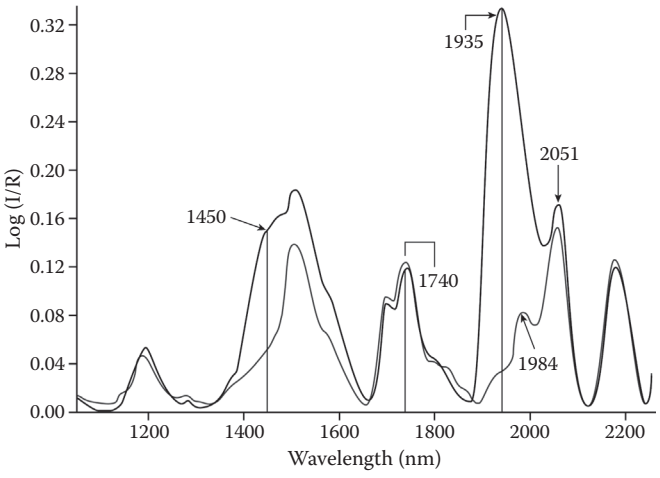

FIGURE 52.9 Near-IR absorption spectrum of unpigmented human hair (fine line) and the effect of water (bold line).

moisture regain observed for hair treated with a hair-care product such as conditioner.

Another application described by Pande and Yang [56] is the measurement of melanin bleaching during oxidative coloring of hair. Such measurements are possible because the synthetic hair dyes have no effect on the reflectance of hair beyond 750 nm, while natural melanin strongly absorbs in the NIR range from 1000 to 1300 nm. The data revealed that the bleaching effect can be quantified in terms of melanin absorption, and even small differences between bleaching products can be detected. It should be added that such measurements cannot be performed with a typical colorimeter due to interference from the synthetic dyes.

Integrating Sphere Spectrophotometry

Integrating sphere spectrophotometry is employed to study the absorbance of light scattering samples such as dispersions of solids and liquids. In cosmetic research, one frequently deals with nontransparent materials or chromophores incorporated in turbid formulations or deposited on nontransparent substrates such as skin or hair. In such cases, one cannot employ routine UV–Vis spectroscopy because the light scattering results in a very weak intensity of transmitted light. In order to include scattered light, a technique referred to as integrating sphere UV–Vis spectroscopy is employed. In this method, the scattered light is focused by reflecting from the walls of a $BaSO_4$-coated sphere. The spectra can be obtained by UV-Vis spectrophotometers equipped with an integrating sphere, which operate in the transmission or reflectance mode (e.g., a Perkin Elmer Model 950). An example of the usage of the instrument in the transmission mode is testing skin care sunscreen formulation to determine a sun protection factor (SPF) value. The tested product, which is typically a turbid formulation, is spread on the surface of a substrate (such as, e.g., artificial skin), and the spectra are collected in the transmission mode and subsequently converted into Koubelka–Munk absorbance units. By using appropriate controls as well as the erythema action spectrum of sunlight, one can calculate the SPF value of a given product. In hair research, the technique is a quantitative tool to study hair coloration. Figure 52.10 presents a plot of absorbance (in Koubelka–Munk units) as a function of wavelength for hair with various levels of natural hair color classified by visual grading on the scale from 1 (black) to 10 (white). The data, such as those presented in this figure, can be further processed by, for example, mathematical spectral subtraction in order to derive information about the hair deposition of sunscreens, artificial hair color, color fading, etc.

X-Ray Spectroscopy

The structure of keratins has been extensively studied in the past by x-ray diffraction [20,23,57–59]. A new approach to this problem has been the use of synchrotron radiation, which can produce high-quality x-ray diagrams in short experimental times [60].

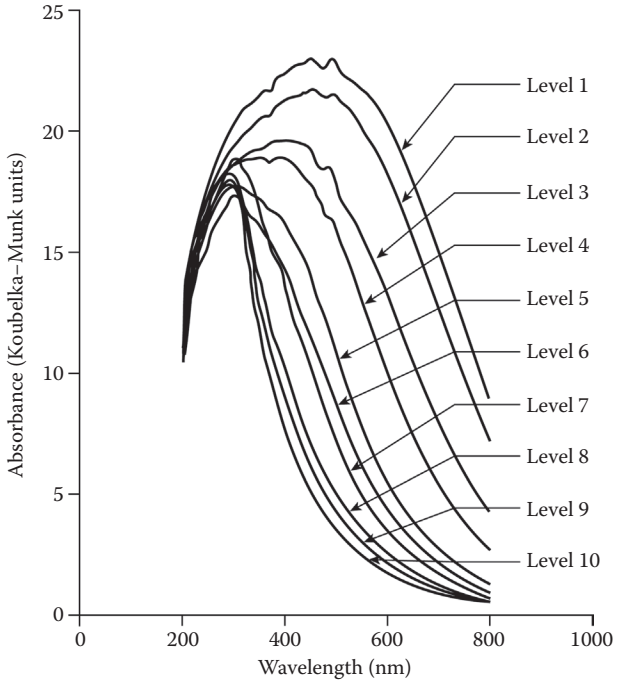

FIGURE 52.10 Absorbance (Koubelka–Munk units) as a function of wavelength for hair with various levels of pigmentation. The data were obtained by using integrating sphere UV–Vis spectrophotometer in the reflectance mode. (Courtesy of Johnson and Johnson Co.)

High-resolution small angle x-ray scattering (SAXS) and wide angle x-ray scattering (WAXS) diffraction patterns were obtained by using high-intensity synchrotron radiation. SAXS diffractograms permitted the calculation of intermacrofibrillar, intermicrofibrillar, and interprotafibrillar distances (88, 67.7, and 40 Å). WAXS gave the distance between individual helices (5.15 and 9.8 Å).

The key result was that cosmetic treatments, including perming, bleaching, or a combination of both, affect not only the distances between supramolecular elements of hair structure but also the distances between individual protein chains [60]. Larger distances between microfibrils, macrofibrils, and protofibrils are reflected in the increased swelling of chemically treated hair, a well-known phenomenon previously described. On the other hand, an increase in interhelical separation, probably as a result of interaction with water, was unexpected due to previously accepted models that assumed that the crystalline phase of hair structure was impenetrable by water or aqueous solutions of hair treatments.

Electron-Spin-Resonance Spectroscopy

Electron-spin-resonance (ESR) spectroscopy measures the transitions of an unpaired electron between energy levels produced by magnetic fields [61]. This is due to the phenomenon that an electron spinning at a given frequency can adopt two spin orientations in a magnetic field with each characterized by a different energy. It is possible to induce transitions between electronic spin energy levels by applying

electromagnetic radiation with the frequency equal to the electron's precessional frequency. A typical magnetic field range employed in an ESR experiment is from zero to a few tesla units. ESR can only be employed for the detection of unpaired electrons such as free radicals and radical ions. The key parameters employed to characterize the ESR spectrum are (1) the electron precessional frequency given by

$$\text{Vprec} = 13.95\ (\text{GHz T}^{-1})\ gB_0$$

where B_0 is the strength of the magnetic field in tesla units (T), and (2) g, which is referred to as the Lande factor. The values of g vary from 2.00220 for the ethylene radical to 2.0091 for the trichloromethane radical. A typical precessional frequency for a free electron at a field strength of 0.34 T is about 9500 MHz. The population difference between electrons in different spin states is larger than in NMR experiment for protons (it can be calculated from Boltzmann distribution), and therefore, ESR spectroscopy is more sensitive than NMR spectroscopy. An ESR spectrum can be obtained for radicals at a concentration as low as 10^{-8} M at room temperature for a volume of a few tenths of a milliliter in both the liquid and solid states. Also, the timescale of an ESR event is about 10^{-9} s; thus, it is faster than in proton NMR so the technique can provide information about processes that are too fast for NMR analysis.

As in NMR, an ESR signal of one electron can be split by a magnetic field of neighboring hydrogens according to Pascal's triangle rule. The separation between lines in a multiplet in the ESR spectrum is termed hyperfine coupling and designated by a symbol a (in Gauss units). For example, for a methyl radical, the ESR signal will be a quadruplet (with the intensity ratio of 1:3:3:1) at $g = 2.00255$ and $a = 23.0$ G.

In cosmetic chemistry, the application of this technique is limited to the studies of oxidation, antioxidants, and melanin chemistry. For melanins, the ESR spectrum consists of a featureless peak with a line width of about 4–6 G and a g value close to 2.004 [62]. There is no hyperfine coupling and the spin concentration is very small, about $4–10 \times 10^{17}$ spins/g. The ESR method of melanin characterization is important because this natural polymer is considered to be a photoprotective and antioxidant agent for skin and hair.

Photoirradiation of hair was also investigated by ESR using spin trapping with 5,5-dimethyl-1-pyrroline N-oxide (DMPO), which forms DMPO-OH adduct with brown, bleached, and red hair [63]. The spectral evidence confirms the formation of oxyradicals during photoirradiation. Bleached and red hairs (pheomelanin) were also found to produce more oxyradicals than black hair melanin (eumelanin).

MICROSCOPY

Optical Microscopy

Optical microscopy is employed for a variety of tasks in a cosmetic laboratory. It is very useful to evaluate hair geometrical shape and dimensions, detect the presence of surface deposits, or assess a degree of fiber damage in terms of cortex integrity, state of cuticles, or the presence of split ends. Traditional light microscopy, however, has limited resolution (approximately half of the wavelength of light) and is characterized by limited depth of field. New instruments address this problem by collecting images of the object at various focal lengths and subsequently computing the reconstructed in-focus image.

Scanning Electron Microscopy

Electron microscopy requires high vacuum and the metal coating of a nonconductive polymer for biological samples. The resolution of this technique is limited to a few nanometers. Scanning electron microscopy (SEM) is usually not sensitive enough to detect adsorbed polymers or a surfactant layer with molecular dimensions. Newer SEM instruments offer ease of use and are capable of producing good resolution images (below a magnification of 5000×) without metal coating. They can be also equipped with energy dispersive x-ray fluorescence detectors, which can display energies characteristic of the elements in the sample. The results are typically presented in the form of a histogram of signal strength as a function of energy (eV) with the detection limits ranging from 0.05% to 2%. Signal strength is related to relative concentration of a given element in the sample. Qualitative, semiquantitative, and quantitative bulk determination of elements for atomic numbers higher than 13 (all elements except for H, He, Li, and Be) is possible. Energy dispersive spectroscopy (EDS) can also provide elemental maps of the sample by identifying the elemental compositions of sample features as small as 1 μm.

Figure 52.11a presents a SEM image of hair (without metal coating) at a magnification of 3000× obtained by a benchtop Hitachi SEM instrument. The image illustrates the presence of granular surface deposits, which concentrate in the areas close to the cuticle edges. The use of EDS detector (Figure 52.11b) indicates a high content of Si and Ca in the structure of the deposited material.

Atomic Force Microscopy

Scanning probe microscopy (SPM) can be used for imaging nonconductive surfaces of materials from the atomic to micron scale. Atomic force microscopy (AFM) and lateral force microscopy (LFM) fall under SPM designation of techniques, which also include scanning tunneling microscopy (STM), chemical force microscopy (CFM), and phase detection microscopy (PDM). These techniques can provide information about the topography and frictional and mechanical properties of a sample from the nanoscale to micron level. In both AFM and LFM, the probe, in the form of a sharp tip attached to a cantilever, scans the surface by using force on the order of 10 to 20 nN in the contact mode and 0.1 nN in the tapping mode. The latter is used to measure the surface characteristics of soft materials such as keratin fibers. In AFM, one obtains a topographical image by measuring the deflection of a soft cantilever, to which the tip is attached, as

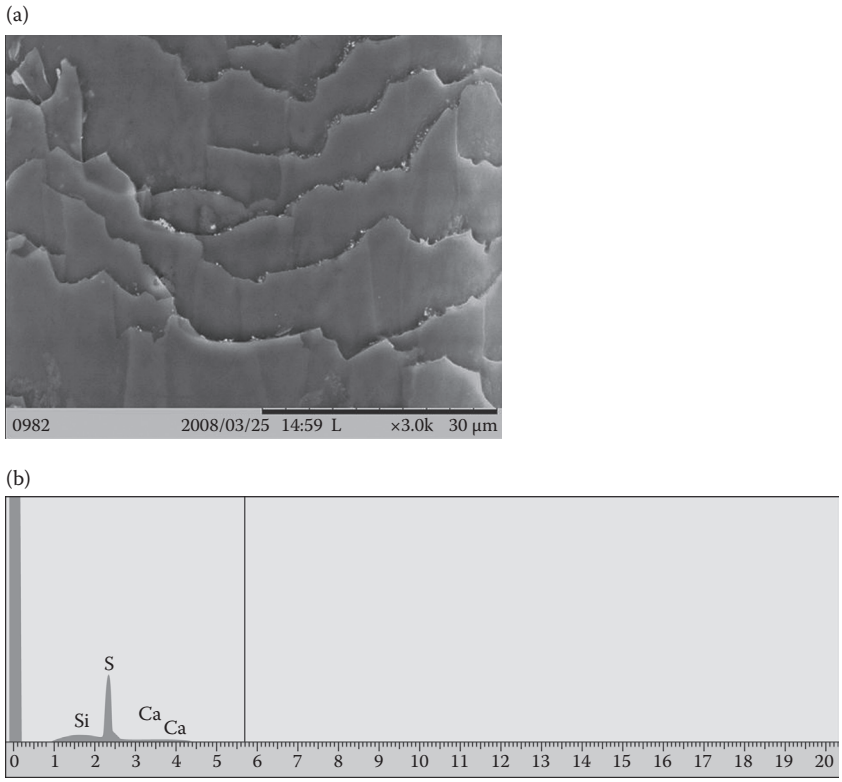

FIGURE 52.11 (a) SEM micrograph of hair at 3000× magnification and (b) results of EDS analysis of surface deposits concentrated near the cuticle edges. (Courtesy of Sunny Chen of Johnson and Johnson Co.)

the tip is rastered over the surface. The cantilever deflections normal to the surface are representative of topographical surface features. In LFM, one measures the torsional twisting of the cantilever as it is rastered over the surface. These lateral cantilever deflections result from drag forces between the tip and the sample surface.

Both AFM and LFM have been used in cosmetic field to image hair fibers and to identify new morphological features under dry and wet conditions [64–71].

O'Connor et al. [67] employed AFM to quantitatively analyze the morphology of hair in air and water, the kinetics of hair hydration, and the effect of pH on hair morphology. Images were analyzed quantitatively by taking line cuts to illustrate height data versus position. They have shown that an average step height in the cuticle sheaths increased from 500 nm for dry hair to 1200 nm for wet fibers. By following the changes in geometrical dimensions of the cuticle height, they were also able to determine the rate constant of hydration and the effect of pH on swelling.

Goddard and Schmitt [66,68] used AFM in both modes (contact and tapping) to collect data on the distribution and configuration of adsorbed cationic polymers on the surfaces of hair and mica. They concluded that the hair surface is not smooth enough for quantitative analysis. Mica, on the other hand, has a model, well-characterized surface. Goddard's study of cationic polymers on mica demonstrated loop-and-train configuration of adsorbed polymer chains, molecular-weight dependence of the layer thickness, and polymer distribution as a function of charge density.

McMullen et al. [64] employed LFM to visualize deposition of cationic polymers on hair. Figure 52.12 presents a comparison of the error signal image of untreated and co(vinylpyrrolidone–methacrylamidopropyl trimethylammonium chloride) treated hair. The surface properties of hair treated with the polymer change significantly with the polymer deposits taking the form of donut-shaped structures. The inside and outside diameters of the polymer deposits were determined to be 70 ± 11 and 202 ± 47 nm, respectively. Since the diameter of hair micropores was found to be 149 nm, it is plausible that the polymer may bind preferentially to the perimeter of the pores whose edges would presumably have a higher electric field than the more homogenous portion of the hair surface.

Confocal Microscopy

Confocal microscopy can be used to obtain specimen images that do not have out-of-focus areas. In a classical light microscope, the light illuminates a large portion of the sample, and if its geometry is not flat, a part of the image is always out of focus. Confocal light microscopy employs a focused beam of light with a reflected light passing through a pinhole in front of a detector, eliminating out-of-focus reflections. A focal plane image is generated by scanning the surface. Internal

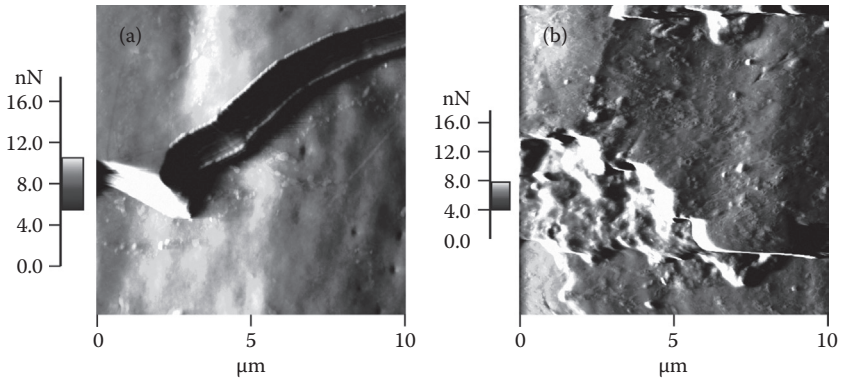

FIGURE 52.12 (a) Untreated hair and (b) hair treated with polyquaternium-28, as imaged by using error signal by LFM [64].

elements of a structure can also be scanned in a similar way, with the limitation being the opacity of the specimen.

Swift and Allen [72] studied the penetration of fluorescently labeled proteins through intact and chemically modified hair fibers. The extent of penetration was assessed by imaging transverse sections of resin-embedded hair with a confocal laser-scanning fluorescence microscope. It was determined that the main sites for peptide deposition were endocuticle, cortex, nuclear remnants, intermicrofibrillar matrix, and cell boundaries that undergo massive swelling by water.

Corcuff et al. [73] used confocal microscopy to study the surface of dry, wet, and chemically modified hair. They claimed resolution of 0.25 mm versus 0.6 mm for conventional light microscopy. Their technique made possible the direct observation of sweat and sebum on hair surface and the quantitative assessment for periodic bulging of cuticles on swelling. They have also performed optical sectioning of hair samples at various depths to provide a three-dimensional reconstruction of the internal structure of hair stained with a fluorescent marker.

Microfluorometry

The instrumental setup consists of a fluorescence illuminator, objective, interference filters, photomultiplier, and a scanning sample stage. A fiber was illuminated by a focused beam of light, and the fluorescence emission is monitored by a photomultiplier as the specimen is moved under the exciting light.

Weigmann et al. [74] employed microfluorometry to study deposition, substantivity, and buildup of various components of cosmetic formulations on hair. They used the sodium salt of fluorescein as a marker and assumed that the deposited film thickness, resulting from the precipitation of polymers, surfactants, and polymer–surfactant complexes, is directly proportional to fluorescence intensity. Various distributions of emitted light intensity were observed, including honey comb patterns, which may be indicative of hair damage. Multiple treatments of hair with shampoos containing cellulose (and fluorescent marker) showed gradual increases in fluorescence intensity and an uneven distribution of surface deposits.

SURFACE ANALYSIS

Dynamic Electrokinetic and Permeability Analysis

Electrokinetic measurements have been applied in wool and hair research for some time. Recent developments, however, have made possible simultaneous measurements of electrokinetic and permeability parameters of fiber plugs in order to obtain information about the interactions of various cosmetic raw materials with hair [75–79]. The technique has been termed "dynamic electrokinetic and permeability analysis" (DEPA).

The DEPA instrument consists of a streaming potential cell, conductivity meter, pressure transducer, test and treatment solution reservoirs, flow interrupter, and electronic balance and several electric and manual valves. The most important features of the design are

- Online positioning of test and treatment solution reservoirs, permitting fiber treatment within the streaming potential cell
- The pulse mode of flow for test and treatment solutions
- Simultaneous measurement of the streaming potential, conductivity, and flow rate (permeability of the plug)
- Special software allowing flexible programming of the experimental procedures, such as the control of pressure and the timing of treatment and test cycles

A typical experiment yields information about the electrokinetic characteristics and permeability of untreated fibers and the kinetics of sorption/desorption of cosmetic actives as a result of one or multiple treatments of hair. Figure 52.13 presents the results of an experiment in which hair was treated with 0.5% solutions of anionic surfactant (SLES-2), cationic surfactant cetryltrimethyl ammonium chloride, and cationic polymer cationic guar gum. The first five data points in each figure correspond to untreated hair. They are followed by a 5-min treatment period, a first measurement period of 30 min, a second 5-min treatment period, and a second measurement period of 30 min.

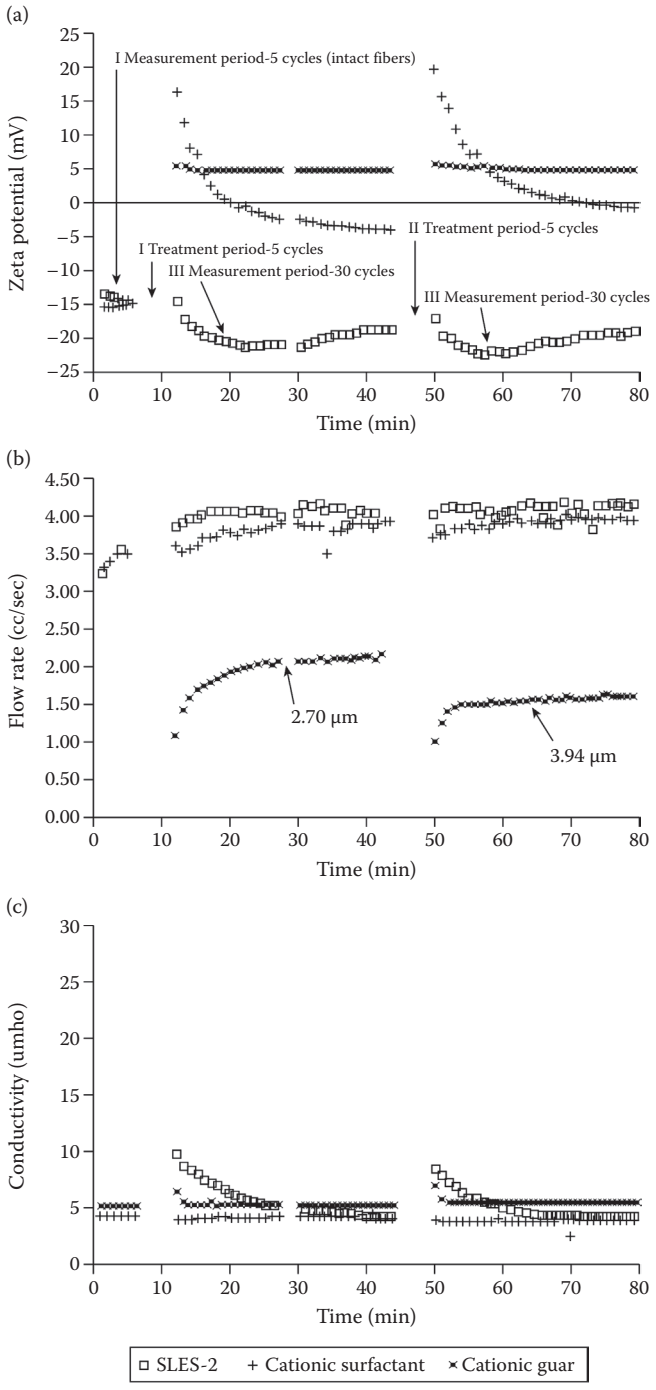

FIGURE 52.13 (a) X-potential, (b) flow rate, and (c) conductivity as function of time for hair treated with different classes of materials such as anionic surfactant (sodium laureth-3 sulfate), cationic surfactant (cetyltrimethylammonium chloride), and cationic polymer (cationic guar gum).

Figure 52.13a presents the time dependence of zeta potential (ζ) and demonstrates an increase in hair negative ζ as a result of binding of SLES-2 to hair. It also shows a reversal of the surface charge for hair treated with quaternary ammonium surfactant and cationic polymer. While surfactants are rinsed off the hair after prolonged treatment with the test solution, a layer of cationic guar gum is stable and imparts a permanent positive ζ to hair.

The flow rate data (Figure 52.13b) show that formation of a thick layer of the polymer (2.7 and 3.94 μm after the first and second treatments, respectively) on the surface of hair occurs only in the case of the cationic guar gum. This is probably due to the presence of microgels of the polysaccharide in the treatment solution.

The hair conductivity is slightly reduced after quat treatment, a behavior typical for all cationics (Figure 52.13c). A small delay in conductivity decrease for the polymer is related to the reduced flow and the resulting prolonged presence of the excess treatment solution in the plug. In contrast to this, SLES-2 slowly desorbs from hair and gives rise to increased conductivity of the plug even after extended rinsing with the test solution.

Similar data can be obtained not only for simple single-component systems but also for complex, multicomponent solutions such as shampoos, conditioners, hair dyes, or any other finished cosmetic product.

The main criteria of product assessment are changes in the zeta potential, permeability, and conductivity relative to an untreated control. Based on these parameters, one can make conclusions about the deposition of cationic, anionic, and nonionic surfactants and polymers on hair. One can also obtain information regarding emulsions, substantivity of various treatments, their removability upon shampooing, buildup on consecutive treatments with the same formulation, and rate of desorption of residual surfactants or polymers [75–79].

The technique can also be employed to quantify the "sealing effects" produced by surfactants, polymers, and oils on dyed hair [75] or on fibers subjected to reactive treatments such as perming or bleaching [78].

A significant advantage of DEPA is that it performs the measurements on fiber assemblies rather than on single fibers, giving an average value of the assessed parameters. Also, the experimental protocols can be planned so that they simulate any sequence of operations performed on real hair, such as a combination of treatments including shampooing, conditioning, or perming.

Wettability

The use of wettability measurements to study the effect of chemical and physical treatments on the surface of hair has been described in detail by Kamath et al. [80]. The authors employed a high sensitivity balance and measured the wettability forces by immersing hair fibers into water or other liquids. An advance in this area, reported by the same authors, was the development of liquid membrane wettability scanning. In this process, a fiber is passed through a liquid membrane. The measured wettability force is calculated from the following equation [81]:

$$F = P\gamma_{LV}(\cos q_a - \cos q_r)$$

where P is the fiber perimeter, γ_{LV} is the surface tension of the membrane liquid, and q_a and q_r are the contact angles in the advancing and receding modes, respectively.

A typical experimental procedure consists of obtaining wettability scans along the length of fibers before and after treatments with solutions of conditioning actives, such as cationic cellulose or protein [74]. Determining the value of q_r in a separate experiment, and assuming that it does not change along the fiber length, allows one to obtain a plot in which $\cos q_a$ is a function of distance. The wettability traces usually show a lot of variation along the length of a fiber so that an average value is usually employed for comparisons between intact and modified hair. It was shown that unoxidized hair, characterized by a value of $\cos q_a = -0.22 \pm 0.17$, becomes more hydrophilic after a single treatment with a solution of a cationic polymer as evidenced by an average value of $\cos q_a = -0.08 \pm 0.15$. This method was also employed to assess the effect of multiple treatments.

In current laboratory practice, automated wettability instruments are employed. They are capable of detecting a point of contact with the liquid during the fiber movement in the advancing direction, reverse the direction of movement to measure receding wettability forces, detect the point of separation from the liquid, average the forces during the fiber scan, and calculate contact angles using previously determined fiber perimeter (which is accomplished by employing hexane wettability data for a given fiber). Figure 52.14 shows wettability traces (wettability force as a function of distance) for virgin hair (a) and for bleached hair (b). Based on these measurements, the advancing contact angles were found to be 101.6° and 41.2° for intact and commercially bleached hair, respectively.

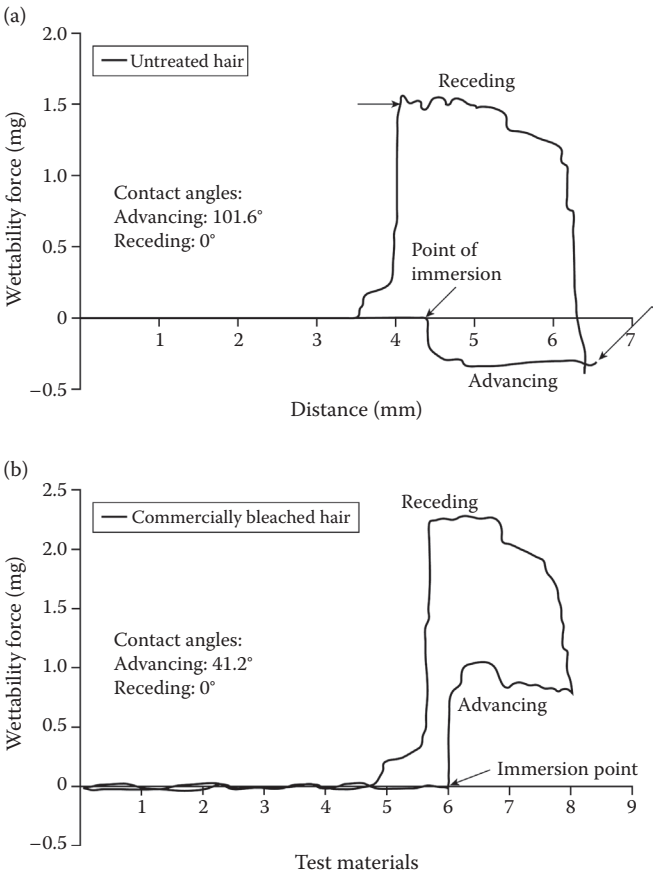

FIGURE 52.14 Wettability trace for (a) intact and (b) bleached hair.

PROFILOMETRY, FT AND FRACTAL ANALYSIS

Profilometry has been employed to study the geometrical properties of hair surface [82,83]. This technique, in which the surface of hair is scanned longitudinally using a wedge-shaped stylus, has a resolution similar to that of optical microscopy.

A typical experimental result is a hair surface profile exhibiting a large number of random peaks and valleys ranging in size from a fraction of a micron to millimeters. The traditional way of handling these data is to calculate roughness parameters such as average roughness depth, average roughness, or geometric average roughness [82]. However, these parameters are not constant and increase with an increase in the scan length, a consequence of self-similarity or fractality of hair surface profile [83].

Hair profilometric traces can be subject to Fourier transform (FT) in which the height versus length dependence is converted into intensity versus frequency spectra, where frequency is termed spatial frequency and is defined as the number of crests per unit length. The analysis of the averaged FT spectra of hair surface showed no preferred frequencies of height variation and allowed for calculation of the fractal dimensions. They were found to be 1.31 for the high-frequency (small spatial dimension) end and 1.63 for the low-frequency (large spatial dimension) end. This leads to the conclusion that hair is "smoother" in the probing scale from 0.5 to 5 mm than in the scale from 5 to 100 mm.

COMBING MEASUREMENTS

The use of quantitative combing measurements has been well established in the characterization of hair-care products. The technique has been developed over the years by Newman et al. [84], Tolgyesi et al. [85], Garcia and Diaz [86], and Kamath and Weigmann [87]. It is widely used in research, development, and claim substantiation.

The method consists of passing a comb through a hair tress, with a well-defined geometry, and measuring force as a function of distance. These measurements can be performed on dry or wet fibers. The parameters used for comparing product performance include the maximum combing force or combing work. The data are typically reproducible within ±20% for wet combing and ±50% for dry combing measurements.

Jachowicz et al. [88,89] reported a modification of the method aimed at increasing its sensitivity. The method, termed spatially resolved combing analysis, employs special frames that allow the application of a treatment to selected areas of the fibers while shielding the remaining portions, thereby providing internal reference sections. The treatments may include thermal exposure, wet applications of cosmetic formulations or raw materials, and physical modification of hair

by photoirradiation. The combing curves of hair treated in such a way, obtained by using a tensile tester such as Instron or Dia-Stron, show positive or negative peaks depending on whether the treatment results in an increase or a decrease in friction of the hair surface.

Figure 52.15 illustrates the application of this method to the analysis of two different conditionings on hair. Their affinity to hair is assessed by performing combing measurements after hair treatment and after two subsequent shampooings. The presented traces are differential combing curves obtained by subtracting a curve for untreated hair from the combing trace obtained after a given treatment.

Figure 52.15a shows the traces of combing curves for hair treated through a two-window frame with a cationic conditioning agent (agent 1), which is characterized by low affinity to hair in terms of its resistance to shampooing. Unlike a conditioning treatment, shampooing is applied to the whole tress including untreated and conditioner-treated portions of a hair. Significant decreases in combing forces are evident in treated sections of hair with the effect nearly completely eliminated by a single shampooing. In contrast to this, Figure 52.15b gives the traces obtained for a high-affinity conditioning agent (agent 2) showing reductions in combing works after treatment in the window areas. The effect persists after one and two shampooings suggesting that the conditioning agent (agent 2) remains adsorbed on hair surface. Note that combing forces corresponding to untreated (shampooed only) portions of hair gradually increase probably as a result of lipid removal or adsorption of anionic surfactants from a shampoo.

Other uses of this technique include the studies of the effect of chemical treatments on hair and the analysis of hair adsorption by cationic polymers, proteins, and complexes.

TEXTURE ANALYSIS

A new tensile meter, referred to as texture analyzer can measure the force in both the compression and tensile modes while monitoring probe displacement in relation to the sample (Texture Technologies Corporation). It was developed primarily for quantitative characterization of food products in terms of texture parameters such as hardness, springiness, tackiness, and resilience.

The instrument was recently adapted for conducting quantitative analysis of hair and hair-care products by employing a procedure referred to as dynamic hairspray analysis (Figure 52.16) [90–92]. It involves the use of hair samples shaped into omega loops by applying a temporary wet set. Both the instrument and the sample are housed in a constant humidity chamber that can maintain relative humidity in the range from 30% to 95% at ambient temperatures. The mechanical measurements of hair loops are carried out by oscillating a plastic probe between the fiber surface and the calibration height of a few centimeters. After touching the surface of hair and sensing a trigger force (1–2 G), the probe produces an additional 1–4 mm deformation (6%–25%) of the loop before rising to the calibration height. One-millimeter deformation is typically within the elastic limit of both untreated and resin-modified hair. On the other hand, 4-mm deformation

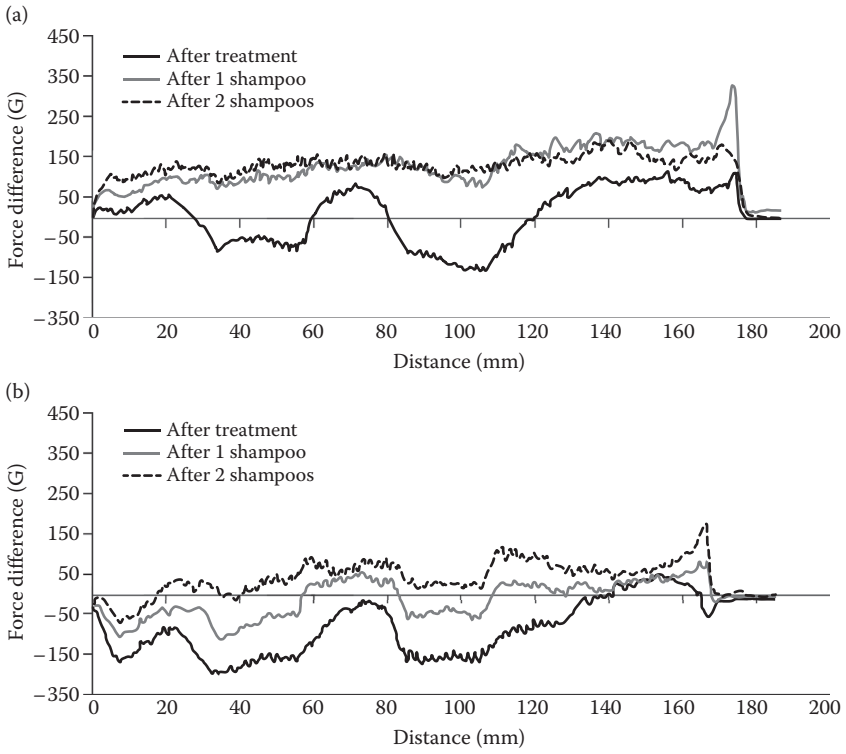

FIGURE 52.15 Differential combing curves for hair treated with (a) conditioning agent 1 and (b) conditioning agent 2 and subsequently subjected to two shampooings.

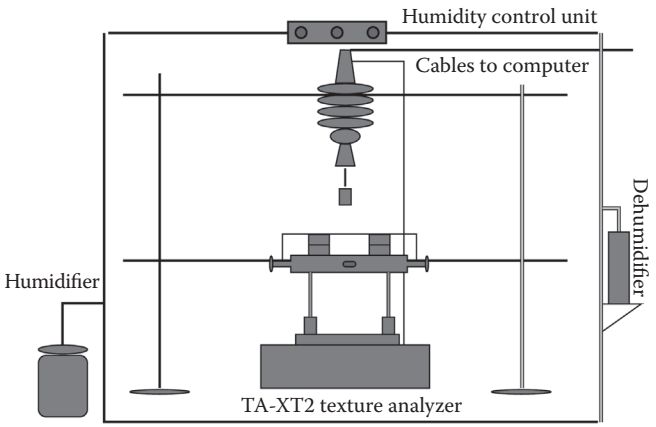

FIGURE 52.16 Experimental setup to study mechanical properties of hair-care polymers. It consists of a texture analyzer and an environmental chamber. Hair sample is in the form of omega loop placed under the probe of the texture analyzer.

(25%) usually results in irreversible breaking of polymer fiber or polymer bonds in polymer-treated hair and is employed to study flexibility of styling products.

Experimental data for polymer-treated hair at high deformation of 25% are presented in a plot of force as a function of distance (Figure 52.17) for the first deformation (a) and the first 10 consecutive deformation cycles (b). The data in the figures correspond to a brittle polymer characterized by an elastic response in the deformation range from 0 to 1 mm. It is in this deformation range that the ratio of moduli, E_{10}/E_1, is calculated (modulus is calculated as the slope of the dependence of force as a function of distance in the linear portion of the curve). E_{10} and E_1 are the moduli of the 10th and the first deformation, respectively. The ratio E_{10}/E_1 can be used as a measure of sample (hair treated with a polymer) flexibility. In order to further characterize the flexibility of the polymer used as a hair treatment, a parameter F_{10}/F_1 can be calculated as the ratio of the maximum force in the 10th deformation F_{10} to the maximum force in the first deformation F_1. As illustrated by the curves in Figure 52.17, at a deformation of about 2 mm in the first cycle, the polymer bonds between fibers break, resulting in a reduction of maximum force (F) in subsequent deformations. The measurements such as those presented in Figure 52.17 can be also used to calculate the plasticity parameter of treated hair.

By using a setup shown in Figure 52.16, the drying of a fixative was also investigated by applying low (1%) intermittent deformations to omega-loop-shaped hair tress. First, the instrument determined the properties of untreated hair. Then, the fibers were treated with a fixative and the instrument measured the changes in both tackiness of a fixative solution on the hair surface as well as mechanical stiffness of the fiber assembly as a function of drying time. The experimental procedure yields parameters such as the stiffness of untreated and resin-modified hair, duration of tack, maximum value of tack force, and time of drying. The kinetic measurements of the stiffness change can also be performed at 90% RH, resulting in information about the resistance of fixative resins to high humidity.

Other applications of this instrument include the characterization of hair (especially ethnic hair) in terms of textural parameters, analysis of skin softness, and the measurements of tactile properties of skin products. This new tensile meter can also be employed for the characterization of the textural (rheological) behavior of cosmetic formulations such as shampoos, creams, waxes, and pomades.

OPTICAL PROPERTIES AND LUSTER MEASUREMENTS

Hair luster is an important property readily assessed by a visual observation and frequently invoked in claim substantiation and advertising. It is largely dependent on the cleanliness, uniformity, and extent of damage in the hair surface.

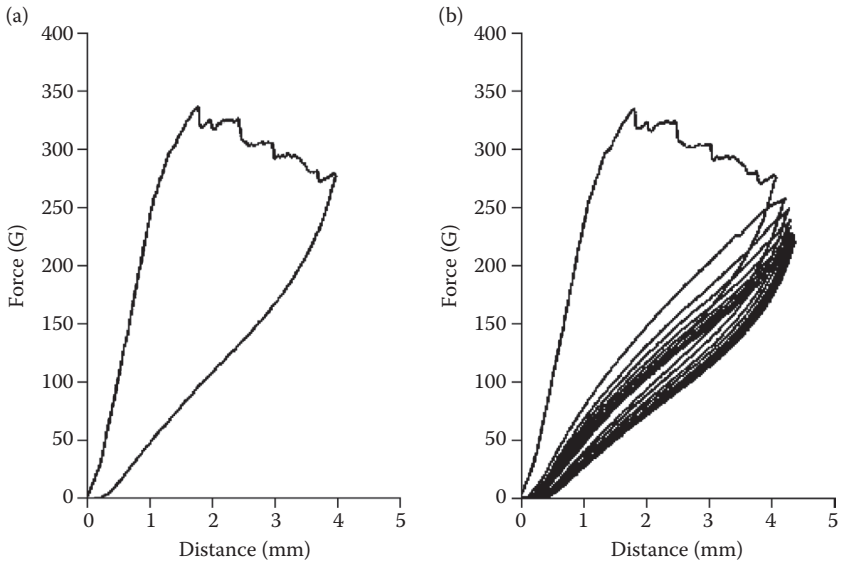

FIGURE 52.17 Force as a function of deformation for hair in the form of omega loops treated with a fixative polymer.

Hair luster can be affected by chemical treatments that reduce hair gloss by damaging cuticles, dissolving lipids, or changing hair color. It can be also modified by application of shampoos, hair conditioners, or special shiner formulations.

The key papers in this area were published more than 30 years ago by Stamm et al. [93,94]. Recent developments include the use of computerized goniophotometers to quantify light-scattering effects produced by single fibers [95–100] or aligned fiber tresses [96,98]. The principle behind these measurements is the same as in earlier work: a light source illuminates the sample at an incidence angle, and the light intensity is recorded for different receptor angles, providing a light-scattering curve. Rotating light-scattering photometers or optical multichannel analyzers can be employed for luster measurements.

The usual criterion of gloss is the sharpness of specular reflection, which can also be quantified by defining various luster parameters given by the following formula [98]:

$$L = S/D(W_{1/2})$$

where L is the luster or shine, D is the integrated reflectance, S is the integrated specular reflectance, and $W_{1/2}$ is the width of a specular peak at half height.

By employing these methodologies, researchers demonstrated small variations in shine as a result of the application of shampoos with and without substantive ingredients on untreated dark brown Asian hair [95]. Other researchers showed the effect of special shine formulations on damaged hair. Luster measurements were also employed to demonstrate a cuticle-abrading effect of multiple combings resulting in a shift of maximum in a light scattering curve. Other investigations based on luster measurements documented gloss variation between root and tip sections of hair and the effect of humidity. Lim et al. [101] measured hair luster directly on heads with a fast polarimetric video camera called SAMBA with a high polarization contrast capable of separating specular and diffuse reflected light. Correlation to consume evaluation of luster was good. Nagase et al. [102–104] have emphasized the importance of internal structure of hair to optical properties and demonstrated the presence of "glittering" patches on the hair surface caused by internal damage due to blow drying.

A different experimental approach to luster measurements was taken by Maeda et al. [105] who obtained pictures of illuminated natural hair wigs on model heads and analyzed them by using a color image processor. The data, obtained by scanning across highlighted and dark areas, could be presented in a format similar to a photogoniometric scattering curve with the ability for resolving reflected light into three color signals R, G, and B (red, green, and blue) or L, a, and b parameters.

A similar approach was taken by McMullen and Jachowicz [106,107] who employed Image Analysis to measure luster of hair simulated by light reflected from a curved hair tress. Hair samples were mounted side by side in a special sample holder in the form of a cylinder and illuminated by a uniform beam of white light. Digital images of hair tresses were captured with a high-resolution camera and were analyzed by scanning across highlighted and dark areas of the resultant image using Image Analysis software. Plots, similar to goniophotometric scattering curves, were used to calculate luster values according to previously published work [93,98].

The procedure was employed to assess the luster of natural white, light blonde, light brown, medium brown, and dark brown hair and revealed an increase in luster indices in proportion to an increase in fiber pigmentation. Figure 52.18 presents images of reflected light from natural white, light blonde, light brown, and dark brown hair. These images were obtained by selecting the exposure values in such a way to visualize the details of the specular reflection band. The light distribution curves are presented in Figure 52.19, and they are consistent with the visual representation of the images shown in Figure 52.18. For example, one can clearly see two specular reflection bands for natural white and light blonde hair, which are evident by two peaks in the light distribution curves. The peak at 16 mm gets progressively smaller with an increase in the extent of fiber pigmentation, which indicates that it is due to reflection from the back-face of the hair fibers. The narrowest light distribution curve was obtained,

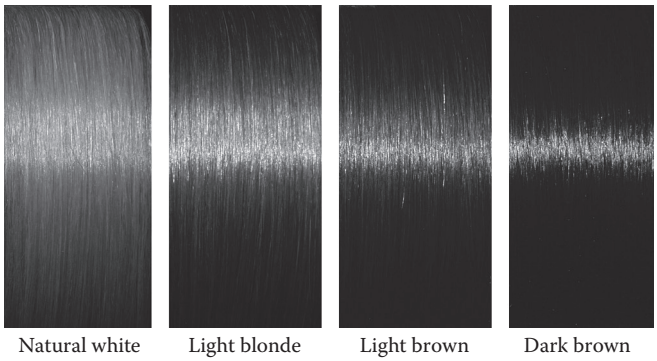

FIGURE 52.18 Images of reflected light from natural white, light blonde, light brown, and dark brown hair.

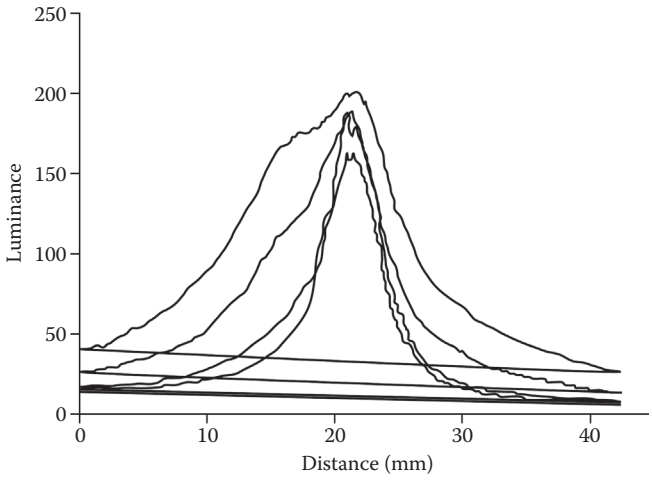

FIGURE 52.19 Light distribution curves for images of hair presented in Figure 52.18.

TABLE 52.4
Luster Parameters for Various Hair Types

Hair Type	$W_{1/2}$ (mm)	L_{Stamm}	$L_{Reich–Robbins}$
Dark brown	5.28 ± 0.44	0.72 ± 0.020	0.67 ± 0.006
Medium brown	5.44 ± 0.16	0.72 ± 0.006	0.67 ± 0.005
Light brown	10.46 ± 0.30	0.70 ± 0.001	0.32 ± 0.008
Light blonde	14.91 ± 0.23	0.65 ± 0.005	0.19 ± 0.006
Natural white	22.78 ± 0.24	0.32 ± 0.013	0.06 ± 0.002

as expected, for dark brown hair. Hair luster parameters, calculated according to equations published by Stamm et al. [93] and Reich and Robbins [98], are presented in Table 52.4.

The calculations carried out by both formulas indicate lower luster values for fibers containing less melanin pigment, that is, the highest luster for dark brown hair and the lowest for natural white hair. A similar result was previously reported by Stamm et al. [94]. Also, $W_{1/2}$ follows the same trend, consistent with visual perception, pointing to an increase in the width of reflected light distribution for less pigmented fibers.

Cosmetic oils such as phenyl trimethicone, amodimethicone, and castor oil were also found to increase luster of hair as a result of change in contrast between the specular and diffuse reflection. Styling resins such as butyl ester of poly(methyl vinyl ether/maleic acid) (PVM/MA) copolymer, vinyl caprolactam/polyvinylpyrrolidone (PVP)/dimethylaminoethyl methacrylate copolymer, and isobutylene/ethylmaleimide/hydroxyethylmaleimide copolymer were shown to increase hair gloss by a similar mechanism as evidenced by calculated higher values of Stamm and Reich–Robbins luster parameters. On the other hand, an effect of hair dulling by deposition of micronized ZnO at various concentrations as well as by synthetic sebum was also discussed.

African hair provided an interesting substrate for optical analysis because the curls that are naturally present provide multiple reflection patterns. It is important to note that unlike straight hair, which exhibits one specular reflection band that coincides with the band on the cylinder mount, homogenous illumination of African hair with a collimated light beam results in many reflection centers of equal intensity in all regions of the sample [107]. In order to quantify the multiple reflection patterns, the authors utilized the Image Analysis software, which allowed them to tally the number of reflection sites as well as to characterize the shape of the reflection. Figure 52.20 provides images obtained for untreated hair along with hair treated with 17 and 61 mg of artificial sebum per gram of hair. Visual inspection of the images reveals a perceived decrease in luster with increasing concentrations of sebum. Further, a decrease in luster is coupled with a decrease in the number of reflection sites. Figure 52.20 includes corresponding images in which all of the reflection sites have been isolated on a black background using an image threshold technique. This is accomplished by looking at a histogram corresponding to the colors present in the image and isolating the bright white light that corresponds to the reflection centers. Image file types usually have

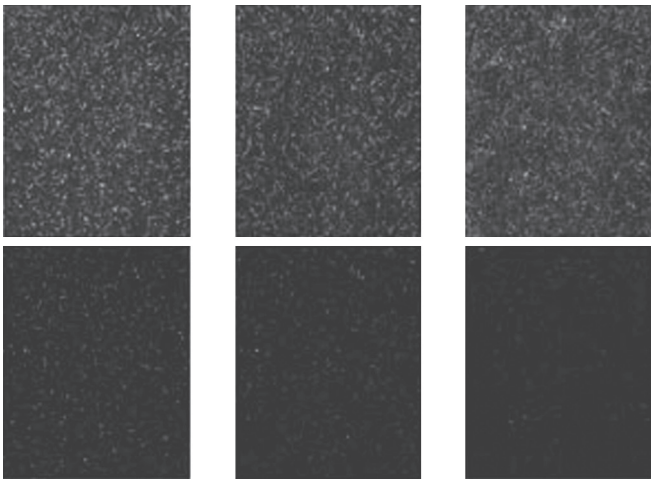

FIGURE 52.20 Effect of sebum on the luster of African hair. First row shows unprocessed images, while the second row presents corresponding images after image thresholding.

TABLE 52.5
Quantification of Reflection Sites on African Hair Treated with Sebum

	# of Reflections	% Black	% White
Untreated	742	98.34	1.66
17 mg/g	536	99.02	0.98
61 mg/g	273	99.67	0.33

a scale from 0 to 255 to represent the colors in the image with 0 representing the darkest colors (black) and 255 the brightest (white). By isolating values that fall in the range from 225 to 250, we can look at the brightest reflections on an entirely black background (Figure 52.19) allowing us to count the total number of reflections.

As shown in Table 52.5, the number of reflections decreases with increasing concentrations of sebum, which was also clearly evident after visual inspection of Figure 52.20. It should also be added that the reflection shapes could be also characterized in terms of perimeter, roundness, and compactness by using the tools of Image Analysis.

EVALUATION OF PERMANENT WAVING

Permanent waving involves breaking disulfide bonds in hair with a reducing agent followed by reformation with a neutralizer [108–110]. Evaluation of permanent waving chemistry can be carried out either by study of reduction and reoxidation rates or by measuring the permanent set achieved in the hair [109]. Reduction rates in hair can be determined either by amino acid analysis [111,112] or by methods based on chemical stress relaxation [112–115]. In chemical stress relaxation methods, a hair is stress relaxed in buffer at fixed extension until a constant level of force is reached. Addition of a reducing agent causes the stress supported by the hair to decrease as disulfide bonds are

broken by reduction. Kinetics of the reaction can be followed, and some information on reaction mechanisms can be deduced. Wickett [114] introduced the term "single fiber tensile kinetics" (SFTK) to describe chemical stress relaxation.

The effect of temperature and pH on reduction with sodium thioglycolate (NaTGA) at pH 9.0 is shown in Figure 52.21. The curves from pH 9.0 at 39°C and pH 10.0 at 22°C clearly show the two shapes of SFTK curves typically observed with this method. At pH 9.0, TGA follows pseudo first-order kinetics [114,116,117]. In this model, one assumes that the reagent is in large excess, that the reaction is slow compared with diffusion, and that all stress supporting S–S are equally reactive. Then, the rate of change in S–S bonds is given by

$$d(S–S)/dt = –kC_0(S–S)$$

where C_0 is the concentration of the reducing agent, and k is the reaction rate constant. If each S–S bond is assumed to support and equal the amount of stress, then the force $F(t)$ at any time t is given by

$$F(t) = F(0)\exp(–kC_0t)$$

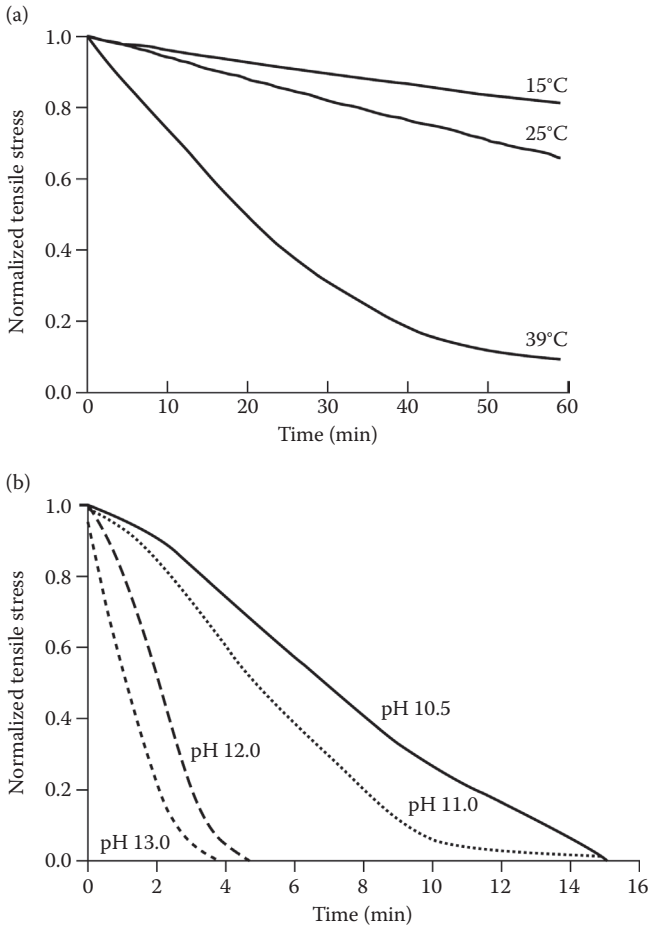

FIGURE 52.21 (a) Effect of temperature on SFTK reaction kinetics with NaTGA at pH 9.0. (b) Effect of pH on NaTGA SFTK curves at 228°C.

FIGURE 52.22 Pegboard for permanent wave evaluation.

Plots of $–\ln(F(t)/F(0))$ versus t will be linear with a slope of kC_0.

The model that fits curves shaped like those for reduction at pH 10.5 (Figure 52.21b) is more complex. It assumes that diffusion is slower than reaction until some reaction has occurred greatly speeding up diffusion. In this model, there is a sharp front or moving boundary of reducing agent working its way into the hair. The moving boundary model for hair reduction has been discussed in detail in several other papers [109,110,114,116,117].

An effective way to measure the efficacy of permanent waves is the pegboard method [118], which is based on a uniform pegboard made of plastic that is 5.5 cm long and 1 cm wide containing 14 removable pegs at a height of approximately 2 cm. The distance between each peg is about 3/10 cm. Two grams of hair is interlaced between the two peg rows without tension and secured at each end with rubber bands (Figure 52.22). The hair must be wound evenly and smoothly without any twisting. After winding, the hair is thoroughly saturated with waving lotion, and the pegboard is covered and placed in a constant temperature bath at 25°C for a prescribed time. The pegboard is then removed from the bottle, rinsed thoroughly with water for 30 s, and then saturated fully with a neutralizer again for a prescribed time and finally rinsed with water. The rubber bands are removed from each end, and the pegs are carefully removed. The curled hair is then immersed in water for at least 5 min, and the length of the waved swatch is determined. Waving efficiency is calculated as given below:

$$100\% – [100 \times (B – A)/C – A] = \%\ \text{waving efficiency}$$

where A is the distance between the first and sixth pegs (2.7 cm), C is the length of straight hair (14.8 cm), and B is the length of the curled hair swatch. Substituting these constants, the equation becomes

$$100\% – [100 \times (B – 2.7)/12.1] = \%\ \text{waving efficiency}$$

For a recent review of permanent waving, see Wickett and Saviades [109], and for a review that includes more detailed

analysis of reduction kinetics and methods for measuring both temporary permanent sets in hair, see Wickett [112].

CONCLUSION

There are a great many methods for evaluation of hair and hair-care products in the hair cosmetics laboratory. We have reviewed several of them, but there are many that we could not review in this chapter. The authors hope that the reader will find this review useful and informative, and apologize for the fact that some other methods were not included.

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53 Hair Conditioners*

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INTRODUCTION

Despite myriad claimed benefits, the primary purpose of a hair conditioner is to reduce the magnitude of the forces associated with combing or brushing hair [1], especially when wet [2,3]. This is generally accomplished by the deposition of conditioning agents that lubricate the hair fiber, diminishing surface friction and, therefore, combing forces [4].

In general, deposition of a conditioning agent also causes the hair to feel softer and more moisturized. Another secondary benefit is the reduction or prevention of flyaway hair [5], especially by cationic conditioners [6]. Besides making the hair more manageable, increasing the ease of combing also improves the ability to align the hair fibers in a more parallel configuration, which can result in an increase in hair shine, even if the shine of the individual fibers is not increased [7]. Some ingredients can also form a film on the hair surface that provides color retention benefits for color-treated hair [8].

A number of other benefits have sometimes been claimed or implied for conditioners including repair of damaged hair, strengthening of hair, repair of split ends, vitamin therapy, etc. In this chapter, we will confine ourselves to a discussion of only the observable conditioner benefits presented above. The chapter will begin with a discussion of the relationship between hair damage, conditioning, and the state of the hair surface. This will be followed by a discussion of the major classes of conditioning agents currently in use. Finally, we will end with a brief discussion of the auxiliary ingredients necessary for the production of a commercial conditioning product.

CONDITIONING AND THE HAIR FIBER SURFACE

Hair Damage

In previous chapters, it has been shown that hair fibers consist of a central cortex that comprises the major portion of the fiber, surrounded by 8–10 layers of overlapping cells termed the cuticle. The cortex is responsible for the tensile properties of the hair [8,9], while the state of the cuticle affects a variety of consumer perceivable properties including hair feel, shine, combability, etc.

A major function of conditioners is to protect the hair's structural elements, especially the cuticle, from grooming damage. This type of stress, characterized by chipping, fragmenting, and wearing away of cuticle cells, is probably the single most important source of damage to the hair surface [10–12].

A rather extreme example of combing damage can be seen in Figure 53.1, which shows the results of an experiment in which a tress of virgin hair was washed with a cleaning shampoo and then combed 700 times while wet. Since hair is more fragile when wet [3] and combing forces are higher [2], combing under this condition incurs maximum damage. It can be seen that damage to the cuticle was extensive with many cuticle cells lifted from the surface, while others were completely torn away by the combing process.

The ability of conditioning agents to protect the hair from the above type of damage can be seen in Figure 53.2, which shows the results of an experiment in which a tress was washed with a high conditioning 2-in-1 shampoo and then combed 700 times while wet. In this case, because the conditioning agents in the shampoo reduced combing forces, the hair surface is seen to be intact with evidence of only minor chipping and fragmenting of cuticle cells. This demonstrates the important role conditioners can play in maintaining the integrity of the hair fiber.

Heat produced by the use of appliances can also cause hair damage [13]. Many styles require the use of blow dryers and/or curling irons, which can produce temperatures of 200°F to 400°F [14]. Steam can be released from the hair fiber causing bubbling and buckling of the cuticles, especially if the hair is not completely dry while being curled. In addition to minimizing combing forces, to protect the hair from this type of damage, certain conditioning polymers can provide added protection in the presence of heat resulting in increased characteristic life of the hair fiber [13].

Hair Damage and the Cuticle Surface

The susceptibility of a hair fiber to grooming damage and the type of conditioner most effective in preventing this damage is affected to a large degree by the nature and state of the hair surface. It is therefore helpful to precede a discussion of conditioning agents with a presentation on the hair surface and how it affects conditioner requirements and deposition.

Virgin Hair Surfaces

Hair that has not been chemically treated is termed virgin hair. The cuticle surface of virgin hair in good condition is hydrophobic [15,16], in large part as a result of a layer of

* This chapter is based on chapters from previous editions of this text written by Charles Reich, Dean Su, and Zhi Lu.

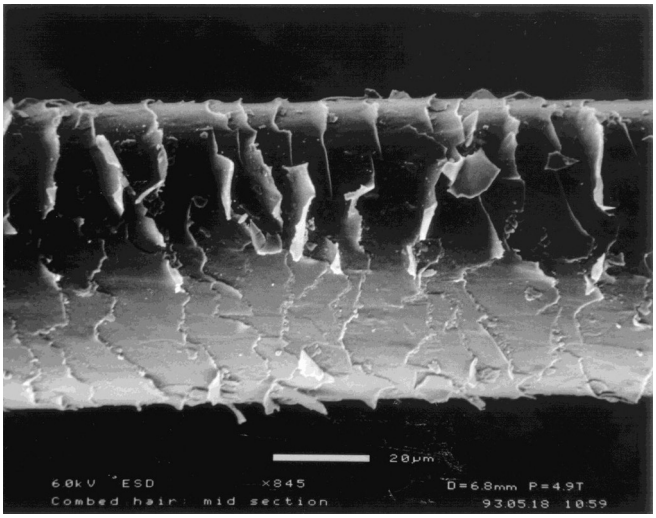


FIGURE 53.1 Typical scanning electron micrograph (SEM) of hair taken from a tress washed with a cleaning shampoo and then combed 700 times while wet. Note raised and chipped cuticle cells, and areas where cells have been completely torn away.

fatty acids covalently bound to the outermost surface of the cuticle (epicuticle) [17,18]. As a result of its protein structure, however, the hair surface has an isoelectric point near 3.67 [19], which ensures that the surface will contain negatively charged hydrophilic sites at the ordinary pH levels of hair care products. This mix of hydrophobicity and hydrophilicity affects, of course, the types of conditioning agents that will bind to the virgin hair surface.

The situation is further complicated by the fact that the negative charge density on virgin hair increases from root to tip. This is primarily a result of oxidation of cystine in the hair to cystine S-sulfonate and cysteic acid as a result of

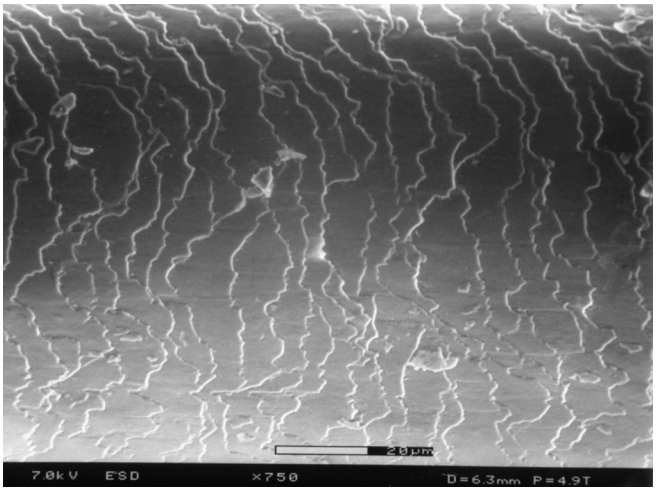


FIGURE 53.2 Typical SEM photo of hair taken from a tress washed with a high conditioning 2-in-1 shampoo and then combed 700 times while wet. Note the minimal damage compared to Figure 53.1.

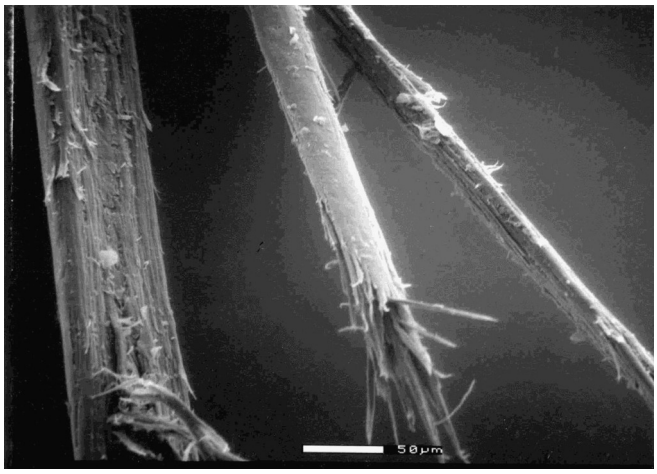


FIGURE 53.3 SEM photo of a split end. Note the exposed cortex and the complete loss of cuticle cells on the fiber surface.

exposure to UV radiation in sunlight [20,21]. The tip portions of the hair, being older than the root portions, will have been exposed to damaging [10] UV radiation for a longer period of time and will therefore be more hydrophilic, again affecting the nature of species that can bind to these sites.

In addition to greater UV damage, the tips of hair are also subject to greater combing damage. One reason for this is simply that, being older, the tip portions will have been exposed to more combing. In addition, the surface friction of hair tips is higher (C. Reich, unpublished data) so that combing forces increase as one moves from root to tip. Finally, the ends of hair are subject to unusually high combing stress as a result of entangling during the combing process [2]. This eventually results in destruction of the covalently bound lipid layer and a feeling of dryness at the tips. Because of this damage, the tip ends of hair require more conditioning than the rest of the fiber. Without sufficient conditioning, the cuticle layer is eventually lost, resulting in a split end. An example is seen in Figure 53.3, which clearly shows the exposed cortical cells.

Chemically Treated Hair Surfaces

Chemical treatments, perming, bleaching, and permanent dyeing, can all cause significant damage to the hair fiber [3,10,22–24]. In addition to causing tensile damage, all of these treatments, which include oxidative steps, modify the surface of the hair, introducing negative charges as a result of oxidation of cystine to cysteic acid. It appears that most of the damage of hair is related to the chemistry of cysteine [3,10,22–24]. This can result in transformation of the entire fiber surface from a hydrophobic to a hydrophilic character. This characteristic change can be used to detect and determine the degree of damage of the hair through dynamic contact angle measurements [25].

All of the above treatments also increase surface friction considerably [3,4,26,27] resulting in a significant increase in

combing forces. The result is hair that feels rough and dry and is subject to extensive grooming damage. Because of this damage, treated hair generally requires significantly more conditioning than is required by virgin hair. By using a conditioner, one can prolong the health of the hair fiber as perceived by consumers. It has been found that cuticle cells on damaged, chemically processed hair are in better condition when a conditioner is used as part of the grooming process [28]. Therefore, using conditioner is particularly meaningful in improving the condition and health of chemically processed hair fibers.

COMMERCIAL CONDITIONERS

The commercial hair conditioners produced to deal with the above problems have appeared in almost every conceivable form, including thick Vaseline pomades, creams, gels, mousses, lotions, and spray mists.

Categorizing by application method, conditioners have been marketed as regular rinse-off conditioners, intensive treatment conditioners, and leave-in products. The first, regular rinse-off conditioners, are normally applied after shampooing, followed by a rinsing step. This is the most common form of conditioner sold.

Intensive treatment conditioners are used similarly to the above products but are not meant for daily application. They are used, instead, for intensive treatment and a higher degree of conditioning. These products generally contain a higher level of active ingredients that are kept on the hair for a longer period of time prior to rinsing. Intensive conditioners are typically sold as thicker creams to provide the perception of higher conditioning.

Leave-in products usually are lighter and can potentially provide more significant benefits than the above rinse-off products since everything applied stays on the hair until the next shampoo. Leave-in conditioners come in various forms, such as detanglers, leave-in lotions, and sprays. They are marketed either for single application or multiple applications during the day.

Despite the wide variety of forms available, most commercial conditioners are oil-in-water emulsions based on cationic surfactants and fatty alcohols in multilamellar vesicles, in lotion form and have viscosities in a range of 3000 and 12,000 centipoise. In addition, despite the different forms and positioning, most commercial conditioners contain the same general classes of conditioning agents with differences mainly in concentrations, numbers of different agents, and the particular members of a conditioning class employed. These conditioner materials are considered to adsorb in a hydrophilic head-down, hydrophobic tail-up conformation that confers hydrophobicity on damaged hydrophilic hair surfaces [29].

The major classes of conditioning agents used in commercial products are surveyed in the following sections. Example formulae taken from the patent literature are listed below for some of the various forms of conditioning products.

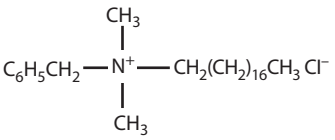
Ingredients	Weight%
Hair Conditioner [30]	
Water	q.s. to 100
Stearyl alcohol	2.50
Stearamidopropyl dimethylamine	1.00
Mineral oil	0.50
Cyclomethicone	0.25
Propylene glycol	0.50
Distearyldimonium chloride	0.75
Hydroxyethylcellulose	1.00
Citric acid	0.20
Polyvinylpyrrolidone	0.10
Formalin (preservative)	0.10
Fragrance	0.20
Deep Hair Conditioner [30]	
Water	q.s. to 100
Cetyl alcohol	6.00
Stearamidopropyl dimethylamine	1.50
Mineral oil, heavy	0.50
Propylene glycol	1.00
Distearyldimonium chloride	1.00
Citric acid	0.20
Germaben II (preservative)	0.50
Fragrance	0.40
Conditioning Spray [31]	
Trimethylolpropane triisostearate	1.00
Methyl myristate	1.00
Cetyl alcohol	1.00
Monoalkyl trimethyl ammonium chloride	0.2
Preservative	0.1
Perfume	0.1
Denatured ethyl alcohol	q.s. to 100
Conditioning Styling Gel [32]	
Sodium PCA (50% aqueous solution)	2.00
Glycerin	1.50
Hydrolyzed collagen	0.50
Carbomer 940	0.35
SD alcohol 40	25.00
Nonionic surfactant	0.50
Fragrance	0.10
Water	q.s. to 100

KEY INGREDIENTS OF HAIR CONDITIONERS

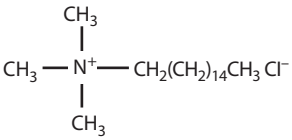
Cationic Surfactants

Cationic surfactants in the form of quaternary ammonium compounds are the most widely used conditioning agents in commercial products [33–35]. Among the reasons for this are their effectiveness, versatility, availability, and low cost.

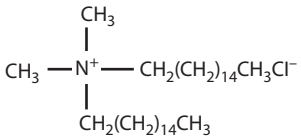
Important examples of these quats include stearalkonium chloride, cetrimonium chloride, and dicetyldimonium chloride. Different counter anions, such as chloride, bromide, and methosulfate, have been used with these materials.



Stearalkonium chloride



Cetrimonium chloride



Dicetydimonium chloride

Because of the positive charge on quaternary ammonium compounds such as the ones described above, they are substantive to hair and bind to negative sites on the hair surface. Treatment with these quats results, therefore, in a hydrophobic coating on the fiber that renders the hair softer and easier to comb [36]. Buildup of static charge (flyaway) is also greatly reduced as a result of this surface modification [6].

Another consequence of the positive charge on quats is that deposition increases with increasing negative charge on the hair surface. This is seen in Table 53.1, which shows the results of an experiment in which hair tresses were treated with 1% stearalkonium chloride and then rinsed. Compared to the roots, 22% more quat was found to bind to the tips of virgin hair, while deposition of stearalkonium chloride on bleached hair was found to be more than twice that on untreated fibers.

This result is important since, as was discussed above, damaged portions of the hair, which generally carry a greater amount of negative charge from either environmental damage or chemical treatment, require a greater amount of conditioning. The fact that cationic surfactants can supply this increased conditioning makes them effective on a wide variety of hair surfaces. This is a major factor in the widespread use of these types of conditioning agents.

Research conducted at TRI/Princeton has shown that the type of deposition and degree of penetration into the hair fiber depends on the size or molecular weight of the compound [86]. The interaction between cationic conditioners and the hair fiber mainly occurs at the surface; however, low molecular weight materials may penetrate into the interior via intercellular diffusion. Cetrimonium bromide (CETAB), for example, is able to absorb into the cuticular sheath as well as the cortex [37].

TABLE 53.1
Binding of Stearalkonium Chloride to Human Hair

Type of Hair	Quat Depn. at Roots (mg/g hair)	Quat Depn. at Tips (mg/g hair)
Virgin hair	0.649	0.789
Bleached hair	1.62	1.83

Source: Data taken from Reich C, Hair cleansers. In: Rieger MM, Rhein LD, eds. *Surfactants in Cosmetics*. 2nd ed. Surfactant Science Series, vol. 68. New York: Marcel Dekker, 1997:373.

Conditioner Properties and Hydrophobicity

Many important properties of quaternary ammonium conditioners are related to the degree of hydrophobicity of the lipophilic portion of the surfactant. Thus, increasing the length of the alkyl chain of a monoalkyl quat and, therefore, making it more hydrophobic lead to increased deposition [38–42] on hair. Cetrimonium chloride, as a result, deposits on hair to a greater extent than does laurtrimonium chloride. Increasing the number of alkyl chains also increases deposition so that tricetylmonium chloride exhibits greater deposition than does dicetyldimonium chloride, which, in turn, is more substantive than the monocetyl quat.

This dependence of deposition on degree of hydrophobicity indicates that van der Waals forces play an important role in deposition of quaternary ammonium conditioners [43]. This conclusion is consistent with the entropy-driven deposition demonstrated by Ohbu et al. [44] and Stapleton [45] for a monoalkyl quat and a protonated long-chain amine.

Increased hydrophobicity also correlates with increased conditioning by quaternary ammonium compounds [38–41,46]. Thus, cetrimonium chloride provides light to medium conditioning, while dicetyldimonium and tricetylmonium chlorides provide heavier conditioning. Detangling and wet combing, in particular, improve significantly from monocetyl to dicetyl to tricetyl quats; differences in dry combing and static charge among these compounds are not as significant.

Increased conditioning with increased hydrophobicity is probably due, in part, simply to increased deposition of quat on hair. Data from Garcia and Diaz [47], however, indicate greater improvements in wet combing from heavier conditioning quats even when present on the hair in much lower amounts than less hydrophobic species. The degree of hydrophobicity of a quat must, therefore, play a direct role in the conditioning efficacy of these compounds [36].

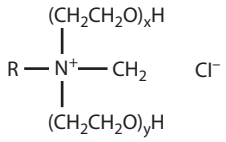
Note that on some types of hair, the greater substantivity of higher conditioning quats can lead to buildup with repeated use and result in limp, unmanageable hair. This is especially true, for example, for untreated, fine hair. Different quats or mixtures of conditioning agents are, therefore, suitable for different uses or different types of hair. A tricetyl quat might be used, for example, in an intensive conditioner meant only for occasional use.

The length and number of alkyl chains of quats also determine water solubility of these compounds. Monoalkyl quaternaries up to cetrimonium chloride are water soluble; for example, distearyldimonium chloride is water dispersible, while tricetylmonium chloride is insoluble in water [41].

Compatibility with Anionics

The quaternium compounds normally used in commercial conditioners are not generally found in shampoos because of incompatibility with common anionic detergents [42]. Introducing hydrophilic groups into the quat can increase

compatibility with anionics. An example is the class of ethoxylated quaternaries, termed ethoquats. Typical members of this class are PEG-2 cocomonium chloride, where $x + y$ equal 2 and R is a C12 alkyl chain, and PEG-15 stearmonium chloride where $x + y$ equals 15 and R is a C18 chain.



Ethoxylated quaternary

Both of these quats are compatible with typical anionic detergents. As would be expected from the above discussion, however, introducing hydrophilic groups decreases the conditioning efficacy of these materials [38,41]. They are, therefore, suitable only in light conditioning formulations. Furthermore, conditioning shampoos based on ethoquats would not be expected to be very effective as a result of low deposition of the detergent-soluble ethoquat complex.

Other detergent-soluble quats have been produced. These include alkylamidopropyl dihydroxypropyl dimonium chlorides [48], lauryl methyl gluceth-10 hydroxypropyl dimonium chloride [49], and even a hydrolyzed ginseng-saponin quaternary derived from Korean ginseng saponin [50]. Although certain advantages have been claimed for these surfactants, particularly low irritation, they all suffer from much the same conditioning limitations as the ethoquats.

Other Cationic Surfactants

In addition to the above examples, numerous other cationic surfactants are in use or have been proposed for commercial products. One example of a compound that has been receiving increasing use is the behentrimonium (C22) quat. This quat exhibits significantly reduced eye and skin irritation compared to the corresponding C18 conditioner due to the longer fatty chain length. In addition, superior conditioning and thickening properties have been claimed [51].

Another interesting example is hydrogenated tallow octyl dimonium chloride [52]. This material is quite substantive and provides high conditioning as a result of its two hydrophobic chains. Unlike conventional dialkyl quats, however, this particular conditioner is soluble in water as a result of branching (2-ethylhexyl) in the octyl moiety. This solubility makes the compound much easier to formulate into a commercial product.

Several patents [53–59] have disclosed imidazoline-based quats containing the imidazoline ring and fatty chains. Some patents have claimed a softening effect on fabrics or hair. Conditioner compositions utilizing these types of quats have also been disclosed [60,61].

Concern for the environment has led to the synthesis of ester quats that exhibit increased biodegradability and environmental safety. One such example is dipalmitoylethyl hydroxyethylmonium methosulfate, an ester quat based on a partially hydrogenated palm radical [62].

Other cationic surfactants used in conditioners include quats derived from Guerbet alcohols [46] (low to high conditioning depending on the length of the main and side alkyl chains), distearyldimonium chloride (high conditioning), and the quaternized ammonium compounds of hydrolyzed milk protein, soy and wheat protein, and hydrolyzed keratin (varying conditioning efficacy depending on the alkyl chain length).

Amines

Amines with fatty chains, such as stearamidopropyl dimethylamine, can also be found in many commercial conditioners. These types of materials become cationic after protonation at the low pH normally employed in conditioning products and therefore act as both cationic emulsifiers and conditioning agents. Neutralization is normally required to decrease the pH and convert the neutral compounds to cationic. Different acids may have different effect on the viscosity of the final product.

Lipophilic Conditioners

Quaternary ammonium surfactants in commercial products are almost never used alone. Instead, they are employed in combination with long-chain fatty conditioners, especially cetyl and stearyl alcohols [35]. These fatty materials are added to boost the conditioning effects of the quaternary compounds [49]. In one study, for example, addition of cetyl alcohol to CETAB nearly doubled the observed reduction in wet combing forces on hair [63]. In another study, using a novel hydrodynamic technique, Fukuchi et al. [64] found that addition of cetyl alcohol to a behentrimonium chloride formulation resulted in significantly reduced surface friction.

Several workers have studied combinations of cationic surfactants and fatty alcohols. Under the right conditions, these mixtures have been found to form lamellar liquid crystal mesophases and gel networks [65–69] that can greatly increase viscosity by converting the micellar structure into the lamellar one capable of forming vesicles and, at the same time, confer stability upon emulsions. As a result of reduced repulsion between cationic head groups when long chain alcohols are interposed, liquid crystal formation has been observed even at low concentrations [68,69]. The combination of cationic surfactants and long chain alcohols has long been the foundation of conditioners for many years, despite that these formulas can leave hair with a slippery feel, which may sometimes be perceived by some consumers as a greasy or unclean feel. The ready formation of these extended structures between quats and cetyl and stearyl alcohols, along with the low cost, stability, and compatibility with cosmetic ingredients, are important reasons why these alcohols are so ubiquitous in conditioning formulations.

Long chain fatty compounds are generally solids at room temperature, requiring heating to incorporate into a product. Care should be taken in manufacturing formulations that the cooling rate is not too rapid to interfere with liquid crystal formation. In addition, it has been claimed that improved

freeze–thaw stability is conferred upon conditioners when using certain combinations of ethoxylated branched-chain fatty alcohol ethers or esters as stabilizers [70].

Cosmetic conditioning treatments can contain both natural and synthetic hair lipids. Some lipids found in commercial products include glycol distearate, triglycerides, fatty esters and acids, waxes of triglycerides, liquid paraffin, cholesterol, and ceramides. Research has found that as hair emerges from scalp, it is coated with a layer of 18-methyleicanosoic acid (18-MEA) that has been shown to confer hydrophobicity and lubrication on hair fibers [29,75–77]. This discovery has influenced the study of the molecule and its use as product formulations. Additionally, several studies have been conducted to determine the benefits of natural oils used as traditional conditioners. Coconut oil used in Southeast Asian cultures was studied to measure its effect on properties like combability, suppleness, softness, tensile strength, shine, and protein loss [71]. Similarly, mineral, sunflower, sesame, mustard, and olive oils have also been investigated [72–74].

Polymers

Cationic Polymers

There are numerous cationic polymers that provide conditioning benefits, especially improved wet combing and reduced static charge. Important examples of these polymers are polyquaternium-10 (PQ-10), a quaternized hydroxyethylcellulose polymer; polyquaternium-7 (PQ-7), a copolymer of diallyldimethylammonium chloride and acrylamide; polyquaternium-11, a copolymer of vinylpyrrolidone and dimethylaminoethyl methacrylate quaternized with dimethyl sulfate; polyquaternium-16, a copolymer of vinylpyrrolidone and quaternized vinylimidazole; and polyquaternium-6, a homopolymer of diallyldimethylammonium chloride.

By virtue of their cationic nature, the above polymers are substantive to hair. The particular conditioning effectiveness of any of these materials depends upon the polymer structure. In one set of studies, deposition on hair was found to be inversely proportional, roughly, to cationic charge density [78,79]. This has been explained by the observation that the higher the charge density, the lower the weight of polymer needed to neutralize all of the negative charge on the hair. Once deposited, however, multiple points of electrostatic attachment make these polymers harder to remove, especially if charge density is high [80,81]. Care must be taken, therefore, in formulating conditioners containing these materials to avoid overconditioning as a result of buildup with continued use.

As with the preceding monofunctional cationics, deposition of polyquaterniums increases on treated or damaged hair [80–82]. Unlike common monofunctional quats, however, the first four of the above polymers are compatible to varying degrees with anionic surfactants [81–85]. As a result, such polymers are used more often in shampoos than in stand-alone conditioners, although they find some use in leave-in conditioners.

PQ-10 and PQ-7 are two of the most frequently used polymers in commercial shampoos. Both of these polymers form negatively charged complexes [78,82] with excess anionic surfactant, resulting in reduced deposition because of repulsion by the negatively charged hair surface. The magnitude of this effect depends on the particular anionic employed and on the anionic surfactant/polymer ratio. In all cases, however, conditioning from shampoos is significantly less than from stand-alone conditioners.

Despite reduced deposition, Hannah et al. [37] has reported that polyquaternium association complexes formed with sodium lauryl sulfate resist removal from hair. Buildup and a heavy, coated feel on the hair can therefore result from conditioning shampoos containing polyquats unless they are carefully formulated.

In addition to providing conditioning benefits, some polyquaternium materials have been shown to improve adhesion of the cuticle scales thereby increasing resistance to scale uplift when the hair is stressed. The same effects were observed for at least one quat—CETAB [86].

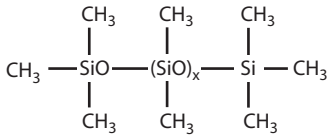
Other Polymers

In recent studies, other polymeric materials, including amphiphilic polymers [87–89], amphoteric polymers [90], block copolymers [91–93], graft polymers [94,95], and dendrimers [96], have been investigated for use as conditioning agents, stabilizers, and deposition agents. In part probably because of cost, commercial products containing those novel polymers are rare. However, these research activities may indicate a future trend toward the use of polymers with more complicated structures in personal care products.

Silicones

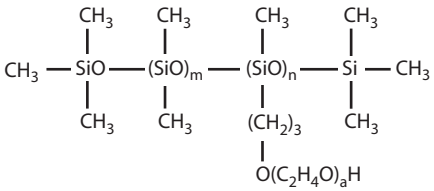
The use of silicones in hair care products has increased considerably in the past two decades, although their first incorporation into commercial products dates back to the 1950s. Different types of silicones find use as conditioning agents in a wide variety of products including conditioners, shampoos, hair sprays, mousses, and gels [97]. The fundamental raw material for the preparation of silicone compounds is SiO_2. Pure silicon (being silicon the 14th element in the periodic table) rarely occurs naturally in its free state, but it accounts in a combined form for about 25% of the earth's crust. The commercial process for making silicone compounds converts inorganic quartz into the silicone compounds that are used in many formulations. It can be divided into silicone homopolymers by only having methyl groups, oxygen, and silicon atoms, and heteropolymers that include other functionalities that make them surface active agents. Both types of silicones possess different surface tension properties than both oils and water. Their unique surface tension properties make them interesting materials for personal care products as the surface tension has a direct impact on the product's

spreadability and wettability characteristics [98]. One of the most widely used silicones is dimethicone, which is a polydimethylsiloxane.

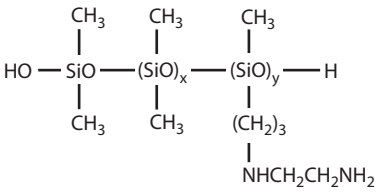


Dimethicone

Other important silicones are dimethiconol, which is a dimethylsiloxane terminated with hydroxyl groups; dimethicone copolyol, which is a dimethylsiloxane containing polyoxyethylene and/or polyoxypropylene side chains; and amodimethicone, which is an amino-substituted silicone and silicone quats, which contain permanently quaternized ammonium groups. In general, amodimethicones and silicone quats condition better than dimethicones, which condition better than dimethicone copolyols [99]. Presumably this is due to differences in substantivity from rinse-off products. Because of the increased substantivity, care should be taken with amodimethicones and silicone quats to make sure they do not build up over time. Likewise, many dimethicone copolyols are soluble in water and therefore may not be as effective in rinse-off products.



Dimethicone copolyol



Amodimethicone

Many silicones used in hair care products are insoluble and must therefore be emulsified. To increase ease of product manufacture, many suppliers offer silicones as preformed emulsions in addition to the pure material. Emulsions can vary in charge (anionic, cationic, or nonionic), size (microemulsion or macroemulsion), and how they are made (mechanical or emulsion polymerization). The factors affecting deposition of silicones from such emulsions have been reported by Jachowicz and Berthiaume [100,101] and Hoag et al. [102].

Silicone emulsions can also vary in particle size. Typically, the smaller the size of the silicone particles, the more stable the product emulsion. Additionally, it has been claimed that reducing the particle size also improves conditioning performance [103]. If a preformed silicone emulsion is not used, particle size of the silicone droplets can be controlled by combining the correct amount of heat and shear when making the product.

Conditioning Properties of Silicones

Silicones used in hair care products possess a range of unique properties including spreadability, wettability, lubricity, low intermolecular forces, water insolubility, and low surface tension. These properties permit the silicones to spread easily on the hair surface, thereby forming a hydrophobic film that provides ease of combing and imparts a smooth, soft feel to the hair without greasiness.

The relative conditioning efficacy of silicones compared to other conditioners was demonstrated by Yahagi [104] who found that dimethicone lowered frictional coefficients and surface energy of virgin hair to a greater extent than did a series of cationic surfactants, including distearyldimonium chloride, a very effective conditioning agent. Dimethicones with molecular weights greater than 20,000 were found to be most effective in reducing surface tension.

Nanavati and Hami [105] measured conditioning on slightly bleached European hair treated with dimethicone fluids and dimethiconol gums. Both types of silicones were found to significantly reduce combing forces on hair. Ease of wet combing was roughly the same for the two silicone treatments, while dimethiconol was found to be more effective in reducing dry combing forces.

Interestingly, under the treatment conditions employed (exposure to silicone solutions for 30 s followed by drying without rinsing), deposition of all silicones studied was found to nearly double if tricetyldimonium chloride was present in the treatment solution. Reduction in combing forces was also roughly doubled when silicones were deposited in the presence of quat. This latter effect was found to be synergistic, that is, it depended on deposition of both silicone and quat, and its magnitude was greater than the sum of the individual conditioner contributions.

Wendel and Disapio [106] used electron spectroscopy for chemical analysis (ESCA) to demonstrate that the presence of amino groups in silicones considerably increases substantivity of these materials. This is a result of the positive charge developed by these groups at the pH commonly found in commercial products.

Comparison of conditioning effects of a series of silicone emulsions on bleached and virgin hair was carried out by Hoag et al. [102]. Most of the silicones were dimethicones or amodimethicones, while emulsions were anionic, neutral, or cationic in nature. Diluted emulsions were applied directly to the hair and combing forces measured both before and after rinsing. Prior to rinsing, reduction of combing forces by most emulsions was greater than 80%. This number was decreased after rinsing as a result of partial removal of deposited silicone. Unsurprisingly, the least change in ease of combing was found for cationic emulsions, especially those containing amodimethicone. Combing forces on virgin hair increased less than on bleached hair after rinsing, indicating that the silicones were more substantive to this type of hair. This

is also unsurprising considering the hydrophobic nature of these conditioning agents.

Further effects of amodimethicones can be seen in work reported by Berthiaume et al. [107], who studied a series of amodimethicone emulsions in a prototype conditioner formulation. Deposition on hair from the conditioner was found to increase with increasing amine content in the silicone. This increased deposition was found, in half-head tests, to correlate with conditioning efficacy, including wet and dry combing, softness, and detangling. A microemulsion in the test series that provided high conditioning was also shown to significantly reduce the color fading caused by shampooing of temporarily dyed hair.

Other Silicones

One silicone that is widely used in conditioners to help improve wet combing is cyclomethicone, which refers to a class of cyclic dimethyl polysiloxanes ranging from trimer to hexamer. Cyclomethicone is volatile and will not remain on dry hair, especially after blow drying. It helps other conditioning agents disperse, however, and form films on hair. It also helps improve wet combing and provides transient shine. In addition, cyclomethicone is widely used as a solvent to reduce the viscosity of silicone gums with much higher molecular weights.

Because of its high refractive index, close to that of hair, phenyl trimethicone is commonly used in leave-in conditioners to enhance the shine of hair fibers. Some silicones have been reported to increase the shine of hair fibers up to 70% [108]. To improve substantivity, higher molecular weight versions (Si-Tec PTM 1000, International Specialty Products) and versions that incorporate amino groups (DC 2-2078 fluid, Dow Corning) have been produced.

Newer silicones include dimethicone copolyol phosphates, which are anionic functional silicones and fluorocarbon-modified organosilicones. The copolyol phosphates are able to complex with tertiary amines of cationic hair conditioners and form effective emulsifiers and conditioners [109]. The fluorocarbon-modified silicones are very hydrophobic like dimethicone; however, they are claimed to have a lighter more lubricious feel [109].

Interesting block copolymers with silicone blocks and organic segments have been developed for personal care application [102]. DC CE8401 from Dow Corning Co. is a commercially available example. This material has a unique structure. In contrast to traditional silicone copolyols that have a rake structure, it is a block copolymer containing silicone and polyether segments in its backbone.

Other examples of silicones include blend of these materials, having different molecular weights [110,111], different functional groups [112,113], and silicones with other hydrophobic oils [114]. These silicone blends have been reported to improve overall conditioning benefits.

2-in-1 Shampoos

Many of today's shampoos are considered to be 2-in-1 conditioning shampoos as the market has evolved from simple cleansing products to a more demanding market in which consumers expect better wet and dry attributes of hair softness, moisturizing, detangling, and general hair manageability. The development of the first 2-in-1 shampoos introduced the concept of a complex made by a cationic polymer and anionic surfactant coacervate that separates and deposits on the hair fiber during the rinsing process.

Silicones find important application as the primary conditioning agents in 2-in-1 conditioning shampoos for the smoothness and lubricity that they confer. Upon their introduction in the latter part of the 1980s, these shampoos represented a major advance in hair care technology, providing a significantly higher degree of conditioning than was then the norm for conditioning shampoos and, at the same time, leaving a desirable soft and silky feel on the hair. Today, formulation of 2-in-1 shampoos optimizes the type and amount of silicone used and the quantity to be deposited on hair based on the complex cationic polymer/anionic surfactant interactions used to increase the efficacy of the 2-in-1 shampoo.

Conditioning from 2-in-1 shampoos is expected to occur primarily at the rinsing stage during which time the shampoo emulsion breaks, releasing the silicone for deposition on hair. This separation of cleaning and conditioning stages permits the shampoo to perform both functions efficiently.

The conditioning agent used most frequently in 2-in-1 shampoos is dimethicone. This silicone can provide good performance in shampoo formulations without excessive buildup on the hair [115]. In addition to dimethicone, volatile siloxanes are known to help on wet detangling and silky effect but are more challenging to formulate. With advances in technology, newer formulations are now employing easier to process silicones, such as dimethicone emulsions, amodimethicones, dimethiconols, and copolyols as well as combinations of these different types to deliver the desired level of conditioning as well as improved product aesthetics.

The level of conditioning from 2-in-1 shampoos is lower than that from stand-alone conditioners. This is especially true for treated hair since the greater the degree of negative charge on the hair surface, the lower the substantivity of a hydrophobic material like dimethicone. Many 2-in-1 products contain polyquats, which might be expected to increase conditioning on damaged hair. In shampoos with high levels of anionic detergent, however, polyquat performance on treated hair may be no better than dimethicone as a result of formation of the negatively charged polymer complexes discussed in "Cationic Polymers."

Yahagi [104] studied the performance of dimethicone, amodimethicone, and dimethicone copolyols in 2-in-1 shampoos. Ease of combing was found to be similar for hair treated with shampoos containing dimethicone or amodimethicone. Unsurprisingly, soluble dimethicone copolyols did not perform well; insolubility, or at least dispersibility, was required for adequate silicone deposition. In the latter case, dimethicone copolyols were found to provide a somewhat lower level of conditioning than the other two silicones studied, especially once blow-drying had begun. Yahagi also studied silicone effects on foam volume. In these studies,

dimethicone was found to significantly reduce foam volume in a model shampoo formulation, while amodimethicone and dimethicone copolyol had a minimal effect on foam.

Auxiliary Ingredients

A number of ingredients besides conditioning actives are added to commercial conditioners for functional, esthetic, and marketing purposes [116]. These include fragrances, dyes, preservatives, thickeners, emulsifying agents, pearlizers, herbal extracts, humectants, and vitamins. Some of these are discussed in the following sections. The literature also contains many examples of such additives [35,117–121].

Preservatives

Preservatives are necessary to ensure the microbiological integrity of a conditioning product. If the product contains high concentrations of ethyl alcohol (generally 20% or above), additional preservatives are not needed and the product is described as self-preserving.

For other products, a wide variety of preservatives are available; in general, combinations of different preservatives provide the broadest possible protection. Every commercial product that is not self-preserving must be carefully tested over time for adequacy of preservation. Most of the preservatives used in personal care products are described in the *Cosmetic Preservatives Encyclopedia* [119]. Special attention must be focused on the nature of preservative, as the micellar–lamellar nature of conditioners may be modified not only by the use of preservatives that disrupt the bacterial cell membrane but potentially also by the cationic/anionic nature, solubility, and/or inactivators (as ethoxylated molecules). A point of interest on preservatives is that some of the most commonly used cationic surfactants used in conditioners (cetrimmonium chloride, behentromonium chloride) are also classified as "miscellaneous" preservatives [122].

Thickeners

"Lipophilic Conditioners" described thickening as a result of liquid crystal formation in those products containing common quaternary ammonium compounds and fatty alcohols. Cationic conditioning polymers ("Polymers") can also act as thickeners. Many formulations may require additional thickening agents. Hydroxyethylcellulose, a nonionic cellulose ether compatible with cationic surfactants and stable over a wide pH range, is the most common thickening agent added to conditioning products [35]. In addition to providing increased viscosity, this material stabilizes viscosity over time. An alternate thickener is cetyl hydroxyethylcellulose, which imparts structure to cationic emulsions with a different rheology that can provide a different consumer product experience. Polyamides may also be used to thicken formulations. A commercial product, Sepigel (which contains polyamide, laureth-7, and isoparaffin), can be used to emulsify and thicken lotion or cream conditioners. Other thickeners are described in reference [120].

Polyacrylate-based thickeners such as carbopol have been widely used in personal care products. However, the majority of those polyacrylate thickeners do not function with cationic surfactants and therefore are not suitable to be used in conditioner formulas. Recently, new thickeners based on polyacrylate chemistry have been commercialized to address this issue. Structure Plus polymer (National Starch & Chemical Company) and Carbopol Aqua CC polymer (Noveon Inc.) are examples that are used at low pH and show great cationic surfactant compatibility. Thickener selection is ultimately a matter of product stability, thickening, and consumer rheology perception requirements.

Humectants

Many conditioners contain humectants whose purpose is to attract moisture. Examples are propylene glycol, glycerine, honey, chitosan, and hyaluronic acid. These materials are not expected to be very effective in rinse-off products; nevertheless, improving the hydrophobic nature of hair may be perceived by consumers as hair moisturization and can potentially support moisturizing claims.

Emulsifiers

As discussed in "Lipophilic Conditioners," the fatty alcohol/quat combinations found in common conditioners confer stability on product emulsions. If necessary, other emulsifiers may be added to improve stability. Information on emulsions and emulsifiers may be found in the literature [121,122], as well as from manufacturers' technical bulletins. Most emulsifiers utilized in conditioners are nonionic, including ethoxylated fatty alcohols, ethoxylated fatty esters, and ethoxylated sorbitan fatty esters.

CONCLUSION

The foregoing sections have surveyed the action and properties of a diverse assortment of commercially available conditioning agents. The availability of a large selection of conditioning materials enables the formulator to tailor products to a wide variety of people having differing conditioning needs and preferences. Thus, a person having short, straight hair in good condition might desire a conditioner primarily to control flyaway. Such a need could be satisfied by one of the ethoquats, which provide light conditioning benefits together with very good static control. A person having long, heavily bleached hair, on the other hand, would require improved hair feel, ease of combing, and manageability. These benefits could best be provided by a trialkyl quat.

Those people sensitive to hair feel might prefer a product containing a silicone as a secondary conditioner. Other people might prefer the convenience of a 2-in-1 shampoo. In many cases, both 2-in-1 shampoos and stand-alone conditioners are used to condition the hair.

There are a number of ways in which one might satisfy the conditioning needs of a target population. It is anticipated that the information in this chapter will help the formulator to quickly choose the best conditioning system for a given

purpose. It is also hoped that the material in this chapter will help the formulator to effectively evaluate new conditioning agents and even to work with synthetic chemists as well as suppliers to design new conditioning compounds to solve particular problems.

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54 Oral Cosmetics
A General Overview

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INTRODUCTION

As a living tissue, the skin requires nourishment to stay healthy. This nourishment cannot be provided by topical preparations only, but it largely comes from foods that are rich in vitamins, minerals, essential fatty acids, and other nutrients that are crucial for optimal skin health and wellness. Skin functioning relies on specific nutritional needs, as evidenced by the development of skin disorders in response to various nutritional deficiencies [1,2]. Supplementation with vitamins, minerals, and other dietary constituents were shown to improve skin conditions [3–6].

Cosmetics are preparations for topical use, that is, products intended to be rubbed, poured, sprinkled, or sprayed on, introduced into or otherwise applied to the human body for cleansing, beautifying, promoting attractiveness, or altering the appearance. Among the products included in this definition are skin moisturizers, perfumes, lipsticks, fingernail polishes, eye and facial makeup preparations, shampoos, permanent waves, hair colors, toothpastes, and deodorants, as well as any material intended for use as a component of a cosmetic product [7]. Cosmeceuticals are topical preparations specifically designed to improve the appearance of aging skin [8]. The term "oral cosmetics" is used in the present chapter with respect to dietary supplements, which claim to have a beneficial, physiological effect on skin, hair, or nails, that is, these are preparations for oral use only, such as capsules, tablets, liquids, or granulate. In this chapter, we will focus on oral antiaging preparations.

AIM OF ORAL COSMETICS

Intrinsic and Extrinsic Aging

Human skin represents the body's barrier to the external environment preventing it from mechanical damage, noxious substances, invading microorganisms, and radiation. Moreover, the skin plays an important role in homeostatic regulation, controlling water retention, sensory perception, and immune surveillance [9,10]. Aging leads to several changes in the skin and its appendages (i.e., hair and nails). These changes can be broadly categorized as either intrinsic aging (chronobiological) or photoaging (actinic aging). Oral cosmetics can be formulated to slow down this aging process.

Intrinsic aging is, by definition, inevitable and thus not subject to manipulation through changes in human behavior. Skin that ages intrinsically is smooth and unblemished and characterized by some deepening of skin surface markings (small wrinkles). Histologically, such skin manifests epidermal and dermal atrophy; reduced numbers of melanocytes, Langerhans cells, and fibroblasts; and increased cell architecture disorders [11,12]. Telomere shortening combined with metabolic oxidative damage is believed to play a major role in the intrinsic aging process [12,13].

Conversely, extrinsic aging is engendered by factors that originate externally and are introduced to the human body, such as smoking, excessive alcohol consumption, poor nutrition, and chronic exposure to the sun [14]. Sun exposure is considered to be most significantly deleterious to the skin; 80% of facial aging is believed to be due to chronic sun exposure [12]. Leathery surface of the skin with blotches, yellowing, and deep wrinkles comprises the clinical presentation of photoaged skin. A marked decrease in collagen, glycosaminoglycans, and proteoglycans is observed. Losses in tone and elasticity, epidermal atrophy, and distinct alterations in collagen and elastic fibers are also associated with photoaged skin. In aged skin, collagen is characterized by thickened fibrils, organized in ropelike bundles that appear to be in disarray in comparison to the pattern observed in younger skin. Alterations in elastic fibers are so strongly associated with photoaged skin that "elastosis," an accumulation of amorphous elastin material, is considered pathognomonic of photoaged skin [10,12,15–18]. Compounds that stimulate the synthesis or inhibit the degradation of connective tissue components (e.g., collagen, keratin, and glycosaminoglycans) may slow down the aging process or even juvenize the dermis and its appendages. Such compounds are potential candidates to be used in oral cosmetics.

Photoaging

Extensive research in the area of photoaging over the past decade has resulted in an improved understanding of the molecular mechanism of the aging process. Ultraviolet (UV) light penetrates into the skin; depending on its wavelength, it interacts with different cells that are located at different depths. UV light of the shorter wavelengths (UVB, 280–320 nm) is mostly absorbed in the epidermis and predominantly affects epidermal cells, that is, keratinocytes, while longer-wavelength UV light (UVA, 320–400 nm) penetrates deeper and can interact with both epidermal keratinocytes and

TABLE 54.1
Primary Components of the Human Antioxidant Defense System

Enzymatic	Nonenzymatic
Direct antioxidant function: Superoxide dismutase (SOD), glutathione peroxidase (GSHPx), catalase	Endogenous antioxidants: α-Lipoic acid, glutathione, melatonin, coenzyme Q
Indirect antioxidant function: Glutathione-S-transferase, GSSG reductase	Dietary antioxidants: Vitamins C and E, polyphenolic compounds

dermal fibroblasts. Melanin pigmentation of the skin absorbs UV light and thus protects skin cells from the detrimental effects of UV exposure. Once UV light has reached the cells of the skin, the different wavelengths exert their specific effects. UVA light mostly acts indirectly through generation of reactive oxygen species (ROS). "ROS" or "pro-oxidants" is a collective term for oxygen-derived species, that is, oxygen radicals (e.g., superoxide anion, hydroxyl radical) and certain nonradicals (e.g., peroxides) that easily convert into radicals [19].

ROS exert a multitude of effects such as lipid peroxidation, activation of transcription factors, and generation of DNA damage. While UVB light can also generate ROS, its main mechanism of action is the direct interaction with DNA via induction of DNA damage [13,20–22].

The skin's enzymatic antioxidant defense includes an enzymatic and a nonenzymatic system (Table 54.1). Copper–zinc superoxide dismutase (SOD), manganese SOD, catalase (CAT), and the seleno-enzyme glutathione peroxide (GPX) have a direct antioxidant function, that is, SOD converts superoxide anion into hydrogen peroxide, whereas CAT and GPX degrade hydrogen peroxide into water. Nonenzymatic antioxidants are classified into two groups, namely, endogenous (e.g., glutathione, α-lipoic acid) and dietary antioxidants such as vitamins and polyphenolic compounds (e.g., flavonoids). Increased ROS generation and/or a depletion of the antioxidant levels will cause oxidative stress defined as a disturbance in the balance favoring ROS generation and leading to potential tissue damage [22,23]. The use of oral supplements, which contain antioxidants (e.g., polyphenols, vitamins E and A), or compounds, which stimulate the enzymatic antioxidant system (e.g., selenium compounds to stimulate GPX activity), may protect the dermis against oxidative stress thereby preventing tissue damage.

Immune Function and Inflammation

In the last two decades, it has become clear that skin is an essential part of the immune system [24]. Reduced immune function and inflammation can alter skin condition and functioning. Sunburn is a well-known acute effect of sun exposure and is clinically visible as erythema triggered by inflammation. After a certain threshold of UV exposure is reached, delayed and prolonged vasodilatation allows the passage of lymphocytes and macrophages into the tissue, which induces inflammation. Increased dietary intake of antioxidants or oral anti-inflammatory compounds was suggested to reduce UV irradiation-induced erythema [3], that is, these compounds are useful in the formulation of oral cosmetics claiming specifically photoprotection.

ACTIVE COMPOUNDS IN ORAL COSMETICS

Screening of Active Compounds

Clearly, the oral route of administration requires other product characteristics compared to a classical, topical cosmetic, and key issues in the development of an oral cosmetic are toxicology, bioavailability (absorption, distribution), and metabolization of its components. In vitro studies and animal studies are useful to study the mechanism of action, but studies in humans are required to document the efficacy of oral cosmetics to validate product claims. Observational studies do not have control over product exposure (e.g., longitudinal study) and are therefore limited to identifying associations between a dietary ingredient and skin benefits; that is, such studies cannot provide a sufficient basis to determine whether a significant correlation between a product and a benefit reflects an underlying rather than a chance relationship. Intervention studies are more reliable since the investigator can control exposure of the study population to the investigated product. Nevertheless, these clinical trials should be randomized, placebo-controlled, and double-blinded to minimize bias of the study results. In addition, any change in dietary habits should be avoided, and the intake of other food supplements should be controlled during the complete study. The use of validated bioengineering methods combined with both clinical observations by the investigator and observations by the participant using standardized questionnaires are highly recommended to evaluate the efficacy of the treatment. Intervention studies to study the effect on the dermis are lengthy (5 to 12 months), and the use of a placebo with an identical appearance (e.g., galenic form, color, taste, and odor) is essential to determine the seasonal influence and the subjective effect of both the investigator and the participant on the efficacy parameters. A parallel study design (i.e., separate groups of volunteers administered different products) is recommended since seasonal influence may bias the results from crossover studies (i.e., one group of volunteers administered different products).

Vitamins

Vitamins A (retinol and β-carotene derivatives), C (ascorbic acid), and E (tocopherols) are dietary antioxidants of particular interest when formulating oral cosmetics. Combined oral supplementation with vitamins C and E as well as carotenoids provides significant antioxidant activity in human skin with demonstrated UV protection and enhancement of cutaneous immune response [9,25].

In a randomized, placebo-controlled double-blind study, a food supplement (Seresis, Pharmaton, Switzerland) containing a combination of carotenoids (β-carotene and lycopene), vitamins C and E, selenium, and proanthocyanidins was administered for 3 months in healthy females, and the effect on UV-induced matrix metalloproteinases (MMP-1, MMP-9) was investigated as a marker of photoprotection [25]. In fact, the expressions of MMP-1 and MMP-9 are known as markers of the activity of the endogenous defense mechanism and are mainly induced by UVB irradiation. After supplementation and following UV irradiation, a significant difference was found between active treatment and placebo for MMP-1 with an increased level in the placebo group compared to a decreased level in the active-treated group. The changes in MMP-9 showed a similar but nonsignificant trend.

La Ruche and Césarini [26] investigated in a double-blind, parallel, placebo-controlled trial the photoprotective effect on the skin of combined intake of α-tocopherol (14 mg) and retinol (2700 μg) for 3 weeks in 16 healthy subjects. Partial protection was observed compared with placebo at a low irradiation dose (suberythemal) against the formation of sunburn cells. No difference between placebo and active treatment was observed for the minimal erythema (MED) of UV, which was needed to induce erythema [26].

Combined oral intake of α-tocopherol, β-carotene, lycopene, and selenium for 7 weeks was found to improve the epidermal defense against UV-induced damage in an open, single-arm study in 25 healthy volunteers [27]. The individual UV sensitivity was measured as the actinic erythema threshold, and skin biopsies were collected to quantify lipoperoxides and to evaluate melanogenesis. After treatment with the antioxidant complex, a significant elevation of the actinic threshold, a general reduction of UV-induced erythema, and a reduction of lipoperoxide levels were observed, respectively. Combined intake for 50 days of high doses of α-tocopherol (2 g/day) and ascorbate (3 g/day) were found to increase the MED compared to placebo in 40 healthy volunteers [28]. These observations were confirmed with lower dosages, that is, after 8 days supplementation with 671 mg vitamin E and 2 g vitamin C daily, the MED increased compared to baseline in eight of 10 subjects, whereas in the placebo group, the MED was unchanged in six of 10 subjects and decreased in four subjects. These studies indicate that combined supplementation of moderate to high doses of vitamins E and C exerts a photoprotective effect.

Several studies indicate that combined intake of a carotene mix such as β-carotene, α-carotene, cryptoxanthin, zeaxanthin, and lutein lowers the degree of erythema after exposure to UV irradiation and that this photoprotective effect is more pronounced when carotenes are combined with vitamin E [29,30]. A recent study observed a decrease in sensitivity toward UV-induced erythema after about 10 to 12 weeks of dietary intervention with carotenoids and flavonoids [31]. Overall, β-carotene exhibits some photoprotection, but carotenoids like lycopene still need more investigation. Because of concern about possible negative effects from large doses, most experts agree that getting carotenoids from foods is the safest.

Vitamin C is an essential cofactor of prolyl-4-hydroxylase, an enzyme that hydroxylates prolyl residues in procollagen, elastin, and other proteins with collagenous domains prior to triple helix formation [32]. Hydroxyproline in elastine has no known function, but prolyl hydroxylation is essential for efficient collagen production. However, to our knowledge, no clear benefit of oral vitamin C supplementation was demonstrated in humans on collagen synthesis in the skin or in a collagen-related process such as wound healing [33,34]. Biotin is an essential cofactor for several carboxylases, which catalyze vital steps in intermediary metabolism in the skin. Deficiency of biotin is known to manifest in various skin disorders, including dermatitis, scaling, and alopecia [35]. Supplementation with large doses of biotin (2.5 mg) for 6 to 9 months in subjects with documented brittle nails resulted in an increase in nail thickness and in a reduction of splitting of the nails [36].

Minerals

A few studies have been published that investigate the effects of oral supplementation of minerals on aged skin. Zinc is an essential element of more than 200 metalloenzymes, including the antioxidative enzyme SOD, and has anti-inflammatory actions. Zinc is a component of enzymes required for DNA replication, gene transcription, and RNA and protein synthesis [37,38]. Roughened skin and impaired wound healing have been reported in association with a mild zinc deficiency, implicating changes in skin [39]. There is a popular belief that zinc deficiency can cause hair loss, but no such correlation is found in published data for alopecia areata [40] or telogen effluvium [41].

Césarini et al. [27] demonstrated that significant photoprotection can be provided by 4- to 7-week supplementation with a specific antioxidant combination of vitamins, lycopene, and selenium. Selenium is present in the form of selenocysteine in the active center of the antioxidative enzyme GPX. Selenomethionine was shown to protect skin cells from UV-induced damage, DNA oxidation, and lipid peroxidation [42]. The effect on skin health and skin aging of supplements containing a combination of lycopene, lutein, β-carotene, vitamin E, and selenium was investigated in a placebo-controlled study in 39 healthy volunteers. Roughness and scaling significantly improved after 12 weeks supplementation in the active group compared with that in the placebo group [43].

Lassus [44] published in 1993 an open study concerning the effect of silicon supplementation on the skin and hair in 50 women with biologically aged skin and fragile hair or brittle nails. The study showed that combined treatment of oral and topical colloidal silicic acid had a beneficial effect on biologically aged skin structure and on the condition of hair and nails. The dermal thickness increased significantly after 90 days of supplementation. In addition, the hair was significantly thicker and less fragile, and nail brittleness had improved. However, no evidence was presented to support the fact that the colloidal silica was actually absorbed in the gastrointestinal tract; that is, it is not clear if the observed

effects on the skin are the result of the oral or the topical treatment with colloidal silicic acid. In fact, it was clearly demonstrated in other studies that polymerized forms of orthosilicic acid, such as colloidal silica, are not bioavailable [45]. Furthermore, seasonal changes may have biased the observed effects on the hair, skin, and nails since no placebo control was used in this study.

Polyphenolic Compounds

Polyphenolic compounds are widely distributed in higher plants and are an integral part of the human diet. In the last decade, the antioxidant activity of flavonoids and other polyphenols such as proanthocyanidins have been studied in detail [46,47].

Silymarin is a mixture of polyphenolic flavonoid derived from the seeds of the milk thistle plant *Silybum marianum* and has been shown in several animal studies to exhibit antioxidant, anti-inflammatory, and immunomodulatory properties, which may contribute to preventing or reducing photoaging [48], especially since silybin (most active component) was demonstrated to be available in skin after oral intake.

Pycnogenol® is the registered trademark of a standardized extract obtained from the bark of French maritime pine, which contains a mixture of procyanidins, also called proanthocyanidins, and phenolic acids, which are potent radical scavengers [49,50]. Proanthocyanidins can also be found in grape seed, grape skin, bilberry, cranberry, black currant, green tea, black tea, blueberry, blackberry, strawberry, black cherry, red wine, and red cabbage. Pycnogenol® has been suggested to support collagen skin density as it displays physical affinity to collagen and elastin and protects it against proteolytic degradation [51,52]. Oral administration with Flavangenol®, another registered trademark of French maritime pine bark, for 12 weeks significantly decreased clinical grading of skin photoaging scores and reduced the pigmentation of age spots in an open, nonplacebo-controlled study [53].

The efficacy of an oral supplement containing vitamins C and E, carotenoids, selenium, zinc, amino acids and glycosaminoglycans, and blueberry extract and Pycnogenol®, respectively, was tested in a double-blind placebo-controlled study in 62 women. After a 6-week supplementation, skin elasticity and skin roughness improved in the active group compared with that in the placebo [9].

The antiaging efficacy of a resveratrol–procyanidin blend (Revidox®, GMC Pharma, Milan, Actafarma Laboratories, Madrid) was evaluated in 25 males and females [54] in a placebo-controlled, double-blind study. After 60 days of treatment, skin moisturization and elasticity had improved, while skin roughness and depth of wrinkles had diminished. Additionally, intensity of age spots had significantly decreased.

Phytoestrogens

Phytoestrogens are polyphenolic nonsteroidal plant compounds with estrogen-like biological activity, which are classified in four main groups based on their chemical structure, that is, isoflavonoids, flavonoids, stilbenes, and lignans [55]. Since phytoestrogens are structurally similar to estrogen 17-β-estradiol, they may exhibit selective estrogen-modulating activities.

For women, particularly in the postmenopausal years, acceleration of chronologic aging is enhanced by the loss of estrogen, which causes a rapid loss of collagen during the first 5 years after menopause. It is assumed that phytoestrogens such as soy isoflavones may mimic the effects of estrogen in skin and reduce skin changes in postmenopausal women. A double-blind study in 26, middle-aged women indicated that oral intake of 40 mg soy isoflavone aglycones per day improved the extent of fine wrinkles at the lateral angle of the eyes after a 12-week supplementation compared to baseline. However, no significant differences in fine wrinkles were found between the active treatment group and the placebo group after supplementation, which may indicate that the observed improvement in the active group was biased by seasonal changes [56].

It is well documented that systemic hormonal therapy (HRT) with estrogens, or combinations of estrogen–glucocorticoid, in postmenopausal women improves the gross appearance of their skin, resulting in decreased slackness, wrinkling, and roughness. At the microscopic level, HRT seems to affect mostly dermal collagen, increasing its content and augmenting dermal thickness [9,57,58]. It should be noted, however, that HRT has been correlated with increased cancer risk [59,60]. Furthermore, HRT is a drug therapy and certainly cannot be categorized as an oral cosmetic.

Glycosaminoglycans

It was suggested that extracts derived from marine fish cartilage have a repairing effect on photodamaged skin. In an open study, 10 females with sun-damaged skin were treated with 0.5 g/day glycosaminoglycans derivatives (Imedeen®, Soeberg, Denmark) for 90 days [61]. After 90 days of treatment, all signs of sun damage had improved, and brittleness of hair and nails was normalized in all cases. These clinical observations were confirmed by changes in skin thickness and elasticity; however, the obtained results may have been biased by seasonal changes since a control group was missing in the study. In a second double-blind, placebo-controlled study, 30 females of the same age range and with similar signs of sun damage were treated with 0.5 g/day glycosaminoglycans derivatives or placebo for 90 days. The results in the glycosaminoglycan-treated group corresponded to those in the first study, whereas no response to treatment was observed in the placebo treatment group [61]. Kieffer and Efsen [62] showed that after 3 months of treatment with glycosaminoglycans derivatives (Imedeen®), there was no significant improvement in photoaging of the skin compared with placebo or baseline. The study was continued for another 9 months in an open design, that is, without the use of a control group. After 1 year of treatment, a significant improvement was found compared with baseline in the

investigator's evaluation of fine lines and overall photoaging, and, respectively, in density measurements by ultrasound, transepidermal water loss, and skin smoothness [62].

Eskelinen and Santalahti [63] studied the effect of oral intake of natural cartilage polysaccharides (Vivida®, Helsinki, Finland) on sun-damaged skin in 15 women aged 40 to 60 years. After 90 days of treatment, significant improvements compared with baseline were found in the active group, respectively, for the clinical evaluation of skin condition (e.g., dryness, thinning, and wrinkles), epidermal and dermal thickness by ultrasound, and the erythemal index, whereas no changes were observed in the placebo group.

Choline-Stabilized Orthosilicic Acid

Choline-stabilized orthosilicic acid (ch-OSA) is a specific complex of orthosilicic acid with choline. The effect of ch-OSA on connective tissue (e.g., bone, skin) was investigated in several animal studies and in randomized, placebo-controlled clinical studies. Physiological concentrations of orthosilicic acid were found to stimulate collagen type I synthesis in skin fibroblasts in vitro [64]. Choline is a precursor of phospholipids such as phosphatidyl choline, which is an essential component of cellular membranes. A cause-and-effect relationship has been confirmed by the European Food Safety Authority between the consumption of choline and a normal homocysteine metabolism [65]. High levels of homocysteine were reported to have a negative impact on collagen synthesis [66–68] resulting in connective tissue–related health problems [69,70].

Supplementation of young animals with low doses of ch-OSA resulted in a significant higher hydroxyproline content in the dermis [71] and increased femoral density [72,73]. Oral intake of ch-OSA for 20 weeks in 50 women with photoaged skin resulted in a significant positive effect on skin microrelief and skin mechanical properties compared with that in placebo group, suggesting a regeneration or de novo synthesis of collagen fibers in the dermis. Assessment of hair and nail brittleness on a visual analogue scale indicated a significant improvement in the ch-OSA group whereas no change was observed in the placebo group [74].

The effect of ch-OSA on hair was further investigated in a randomized, double-blind, placebo-controlled study in 48 women with fine hair. Hair morphology and tensile properties were evaluated before and after treatment with validated methods. Oral intake of ch-OSA had a positive effect on tensile strength, including elasticity and break load, and resulted in thicker hair [75]. The authors suggested that the observed increase in the cross-sectional area of the hair shaft after ch-OSA supplementation may be explained by a stimulation of the collagen synthesis by fibroblasts in the dermal papilla, which determine the volume of the hair follicle.

Polyunsaturated Fatty Acids

Common food sources of n-3 polyunsaturated fatty acids (PUFAs) are cod liver oil, fish oil, and marine animals with a high amount of fat, such as mackerel, salmon, and menhaden. A few studies have assessed the photoprotective effects of dietary intakes of fish oil. Orengo et al. [76] observed a small but statistically significant increase in the MED after intake of a fish oil-enriched diet for 4 weeks, showing that a relatively low dose (2.8 g eicosapentaenoic acid and 1.2 g docosahexaenoic acid) of fish oil is photoprotective. In another study [77], fish oil consumption (10 g daily) was also found to reduce UV irradiation-induced erythema, but the susceptibility of the skin to lipid peroxidation increased because of the unstable nature of n-3 fatty acids.

The effect of an oral preparation rich in omega-3 fatty acids (Eskimo® Skin Care, Cardinova, Uppsala, Sweden) was investigated in a single-blind randomized trial [78] and revealed a 10% increase in skin elasticity after 3 months of treatment. However, no effect on skin roughness was observed.

PUFAs such as linoleic acid (LA), α-linolenic acid (ALA), and γ-linolenic acid (GLA) are widely distributed in plant oils such as flaxseed. Neukam et al. [79] showed that oral supplementation with flaxseed oil led to significant decreases in skin sensitivity, TEWL, skin roughness, and scaling, while smoothness and hydration were increased. The observed effect was correlated with a shift in the plasma FA pattern (increase in ALA concentration).

Dietary fatty acids were reported to be capable of changing the fatty acid composition of membrane phospholipids of immune cells, which may modulate the function of these cells. A few studies are published, which evaluate the effect of PUFAs in delayed-type hypersensitivity (DTH) skin tests using a panel of antigens, which have generally been accepted as an important means to monitor the cell-mediated immunity in vivo. However, variable results were obtained in these studies with respect to the effect on DTH skin reactivity, which illustrates that more research is needed to document the immunomodulating activity of PUFAs [80–82].

The most important benefit of oral n-3 PUFA intakes from fish oil may be ascribed to their anti-inflammatory effects. These effects of n-3 PUFAs have been reported to be the result of their competition with n-6 PUFAs as a substrate for cyclooxygenase and lipoxygenase, resulting in the formation of less-active prostaglandins and leukotrienes. Interference with inflammatory cascades in the skin may occur through reductions in the synthesis of proinflammatory lipid mediators or through reductions in the production of cytokines. Moreover, n-3 PUFAs are unstable and may preferably be damaged by free radicals, thereby protecting other structures from attack by free radicals. Nevertheless, to protect against excessive formation of free radicals and lipid peroxidation, appropriate amounts of antioxidants should also be consumed.

Multicomponent Supplements

Murad and Tabibian [83] conducted a randomized, single-blind study in 72 women to evaluate the effect on skin roughness of a 5-week supplementation with a combination of glucosamine, amino acids, minerals, and various antioxidant

compounds. Women without supplementation were used as a control group, that is, placebo supplementation was missing in this study. A statistically significant reduction in the number of visible wrinkles and fine lines was observed in the active group but not in the control group. There was no significant change in epidermal hydration in either the control or the active study group [83].

Skovgaard et al. [84] investigated in a placebo-controlled study the effects of a novel dietary supplement (Imedeen Prime Renewal®, Soeberg, Denmark) on skin in 100 postmenopausal women. The supplement contained soy extract, fish protein polysaccharides, white tea extracts, grape seed and tomato extract, vitamins C and E, as well as zinc and chamomile extract. The clinical grading of skin condition and the density of the skin measured by ultrasound structure improved after 6 months supplementation in the active group but not in the placebo group [84].

The effect of oral intake of a combination of marine proteins with zinc, copper, vitamins C, E, B3, and B5, α-lipoic acid, pine bark extract, red clover extract, tomato extract, and soya extract (DermaVite®, Florida, USA), respectively, was tested in a placebo-controlled study in 40 women with aged skin [85]. There was a significant increase in skin thickness and elasticity after 6 months supplementation with the active preparation but not in the placebo group. A significant improvement in global evaluation of skin condition using a visual analogue scale was also observed in the active group after 6 months supplementation, whereas no change was observed in the placebo group.

Béguin [86] tested the safety and efficacy of the supplement Estime® (Internal beauty System, Swiss Pharmaceutical Industries SA, Neuchätel, Switzerland) in a 4-month randomized double-blind controlled study including 40 healthy Caucasian females (aged 35–54 years). Efficacy measurements included skin surface evaluation, ultrasound measurement of sun-exposed and protected areas of the skin, and photographic assessment. All these investigated parameters showed a continuous and significant improvement in the active group compared to the placebo: an increase in dermis density of up to 78% in the active group and a visible improvement of the overall skin appearance and reduction of fine lines.

Sixty-two women aged 45 to 73 years participated in a double-blind, placebo-controlled trial to evaluate the efficacy of a proprietary combination of vitamins C and E, carotenoids, selenium, zinc, amino acids, glycosaminoglycans, blueberry extract, and Pycnogenol® (see description in "Polyphenolic Compounds"), respectively. Compared with that of placebo, it was found that skin elasticity and skin roughness improved significantly after 6 and 12 weeks of supplementation [9].

In 2009, Udompataikul et al. [87] investigated the effectiveness of an oral nutraceutical (Radiance Marine Q10, Blackmores Ltd., Warriewood, NSW, Australia) containing antioxidants (i.e., coenzyme Q10, α-tocopherylacetate, beta-carotene, grape seed extract, French maritime pine bark extract, green tea extract), minerals (zinc, selenium), and glycosaminoglycans on cutaneous aging. In this double-blind, placebo-controlled trial, 60 women aged 35–60 years were randomized to receive oral dietary supplement or placebo, once daily for 12 weeks. At the end of the study, the depth of skin roughness and fine wrinkles in the treatment groups showed a 21.2% improvement, whereas improvement in the control group was 1.7%. This difference was statistically significant.

LEGISLATION CONCERNING ORAL COSMETICS

Oral cosmetics are dietary supplements (food supplements), that is, the legislation on food supplements should be followed. Considerable differences in such legislation exist between different countries with respect to the maximal doses, the chemical forms of vitamins and minerals, plant species, and product claims, which are allowed to be used in and for dietary supplements. In the European Community, former national legislation of member states on both the ingredients that can be used in food supplements and the health claims on food supplements is harmonized with the European Commission (EC) Directive on Food Supplements (i.e., Directive 2002/46/EC) and the Regulation on Nutrition and Health Claims (i.e., Regulation 1924/2006).

CONCLUSION

Sun avoidance and the use of sunscreens are well established as primary components in antiaging regimens, although these are still underappreciated by many people. Clearly, sun avoidance is not easy to manage and is often impossible. Consumer-driven demand has led to the development of products to counteract the signs of aging skin. Functional foods positioned as beauty enhancers are a recent concept in Western countries. Bearing this in mind, the development of novel or more active cosmetics (cosmeceuticals) or dietary supplements, which specifically target the skin, hair, and nails (oral cosmetics), is one of the most exciting and promising ways in which the future of cosmetology and dietetics may address human health needs and well-being [88]. Considerable research and firsthand experience of physicians have shown that using topical creams in conjunction with dietary supplements leads to superior results than using either skin care or supplements alone. It may come as a surprise to many consumers that only a few ingredients in topical skin care products have the capacity to penetrate far enough into the dermis to ameliorate deep wrinkles. Therefore, using a combination of topical and oral cosmetics will likely be the favored recommendation in the near future to develop efficient antiaging therapies.

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55 Use of Food Supplements as Nutricosmetics in Health and Fitness
A Review

Jan Taeymans, Peter Clarys, and André O. Barel

INTRODUCTION

Nutrition has been defined as the biological process in animals and plants of food intake and its subsequent assimilation into the tissues. Nutritional (food) supplements are concentrated sources of nutrients (or other substances) with a nutritional or physiological effect that supplements the normal diet [1].

Nutricosmetics can be described as a recent result of a convergence phenomenon between cosmetics and food industries, still unfamiliar to many consumers and sometimes even to foods and cosmetics experts [2]. Nutricosmetics advertisements describe such oral supplementation of nutrients sometimes as "beauty pills," "beauty from within," "oral cosmetics," or "eat yourself beautiful." The nutricosmetics market was valued at $1.5 billion in 2007, but it was projected that it will reach $4 billion in 2015, with Europe as the most important market before Japan and the United States [3,4]. Nutricosmetics can be defined as ingestible products (pills or capsules, tablets, liquids, granulates, or foods) that are formulated and marketed specifically for beauty purposes [3]. Such products are at the intersection between nutrition and personal care, and they should not be confused with cosmeceuticals or nutraceuticals.

Cosmeceuticals are at the intersection between cosmetics (products that simply clean and beautify) and health care (products that cure and heal) [6] and are defined as topical cosmetic products that claim to have medical- or pharmaceutical-like benefits, while nutriceuticals are at the intersection between nutrition and health care and are foods or beverages providing health benefits including the prevention or treatment of disease (also called "functional foods") [2,3]. Figure 55.1 depicts that there is also a common intersection between the three types of care products, indicating that some nutricosmetic ingredients may not only act on skin, hair, and nails but also show more systemic (side) effects resulting in improved health and fitness. Nutricosmetics mostly claim an antiaging effect, for instance, by reducing wrinkles by fighting free radicals generated by solar radiation. Different conditions, such as ultraviolet (UV) radiation or exercise, contribute to reactive oxygen species (ROS) production in different human body tissues, which then can react with DNA, proteins, and fatty acids leading to oxidative damage and antioxidant system impairment. Therefore, antioxidants represent the most crucial among the nutricosmetical ingredients. The best-known antioxidants are carotenoids (beta-carotene, lycopene, lutein, zeaxanthin, and astaxanthin) and polyphenols (anthocyanidins, catechins, flavonoids, tannins, and procyanidins) [2,7]. Such antioxidants may also have important systemic effects on tissues other than skin. For example, they can scavenge singlet molecular oxygen ($^1O_2$) and peroxyl radicals or may influence signaling and gene expression at the muscle cell level during and after exercise.

The market for food supplements and nutricosmetics (French: "alicaments") is characterized by an important annual growth because today, consumers are aware of nutritional products that contribute to disease prevention, fitness, general health, and skin health. During the past decennium, industry developed many new nutritional applications to satisfy people's needs and demands such as protection of the skin from photo-oxidative (UV-induced) damage, which can occur, for example, after years of outdoor work or prolonged outdoor leisure and sports activities. This protection is recognized as beneficial to human health by the European Food Safety Authority (EFSA) Panel on Dietetic Products, Nutrition and Allergies [8].

While the nutricosmetic market is tiny in comparison to other personal care markets, it is estimated to be the fastest growing market (over 12% growth in 2007) [3]. This observed important growth in nutricosmetics is driven by different factors such as a shift toward less invasive treatments and beauty procedures, increasing consumer awareness (especially by the younger generation), rise of the spa and beauty culture, and environmental and societal factors. Furthermore, the increased life expectancy of the baby boomer generation in the industrialized countries demands new strategies to quality of life improvement including the need to appear youthful, fit, and healthy. In this context, nutricosmetics, besides cosmeceuticals and nutraceuticals, have emerged as a new strategy to prevent disease and to sustain general health and fitness while supporting skin health and beauty.

This chapter tries to present an overview about (i) nutricosmetic ingredients that may not only act on skin, hair, and nails but also have a more systemic effect resulting in improved health and fitness; (ii) the possible working mechanisms of such health and fitness enhancing nutricosmetics, focusing on the carotenoid and phenolic ingredients; and (iii) the concept of combined nutricosmetical–cosmeceutical applications (i.e., combined oral and topical applications) and combined nutricosmetical–physiotherapeutical interventions (i.e., combined oral and exercise or massage applications).

METHODS

The bibliography started with a systematic search in PubMed (search algorithm can be provided by the corresponding author on request). However, because of the high specificity of the topic "nutricosmetics as used in health and fitness" (Figure 55.1), the limited number of clinical trials in humans, and the exclusion of many papers reporting on care products such as mouthwash or toothpaste or on supplementation under extreme pathologic conditions such as HIV and AIDS or terminal cancer patients, the number of publications that could be retrieved meeting the criteria for this review question was very limited. Breaking down the combined search in more sensitive parts such as "antioxidant supplementation" yielded, on the other hand, a large amount of data available on such topics. Therefore, this review is not intended to be exhaustive but aims at giving an overview of the status quo on the evidence for the use of nutricosmetics in health and fitness.

HISTORY OF COSMECEUTICALS, NUTRACEUTICALS, AND NUTRICOSMETICS

Historically, the cosmeceutical concept was created in 1961 by Reed, a founding member of the US Society of Cosmetic Chemists. The term "cosmeceutical" was, however, first used about 20 years later by Kligman [9]. Examples of such topical applied drug-like products are antiaging products, skin whiteners or brighteners, acne aids, whitening toothpastes, anticellulite products, or antiperspirants. The global cosmeceutical market is estimated to amount at $55 billion per year. DeFelice [10] first coined the term "nutraceuticals" in 1989. Nutraceuticals became a major focus of food and pharmaceutical companies in the 1990s. Examples of such oral drug-like products are cholesterol lowering, diabetes management, tartar control, digestive aid, and energizing and fitness-enhancing products. In 2012, the US manufacturer Wild Flavors Inc. launched and registered the trademark Vegiceuticals as a series of fruit and vegetable extract-based nutraceuticals [11]. Finally, nutricosmetics emerged as a segment of neutraceuticals, which first gained popularity in Japan and Europe. The development and market are dominated by beauty firms with only a few pharma or food entrants. The Swedish biochemist Dahlgren is one of the pioneers who invented the world's first nutricosmetic product in the late 1980s, called Imedeen [12].

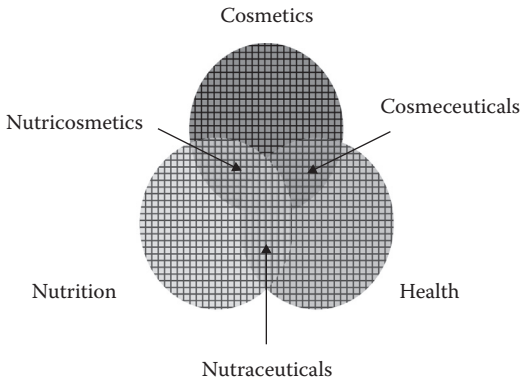

FIGURE 55.1 Intersections between cosmetics, nutrition, and health. (Adapted from Carrie, M., Nutricosmetics: Decoding the convergence of beauty and healthcare, in *In-Costmetics*, Amsterdam, 2008.)

Differences in culture, legislation, and commercial climate between Europe, Japan, and the United States explain that the nutricosmetics market has developed independently in these geographic areas and thus has been tackled differently by the industry. Table 55.1 depicts different characteristics of the nutricosmetics markets in these three areas [5].

Industry believes that it will be very difficult for the US nutricosmetics market to reach the same level of penetration as Japan and Europe. Some top brands have already attempted to penetrate the market; however, they decided to exit again after minimal success. This may be partially explained by the observation that American consumers are less patient and generally less health conscious than their European and Japanese counterparts. Also, nutricosmetics lack good channel fit (e.g., pharmacies in the United States do not have the same influence as they do in Europe). Therefore, industry regards the US nutricosmetics market to be a nice add-on for professional skin care brands; however, it is predicted to remain a niche opportunity.

In the United States, especially consumers dislike the idea of buying ingestible products from cosmetic brands. Therefore, food and beverage manufacturers believed nutricosmetics to be a good opportunity and have tried to enter the nutricosmetic market over the last few years. Not all examples of food suppliers penetrating the nutricosmetic market were successful. For instance, Danone stopped the production of Essensis, which claimed to have skin hydrating properties above those of traditional moisturizing crèmes only 2 years after its appearance on the market. A French consumer organization disproved Danone's marketing claim through a clinical trial [13]. Another example illustrating that penetrating the nutricosmetics market can be difficult is Lumaé. In 2004, a US federal trademark registration was filed for Lumaé by Nestlé. Four years later, Coca Cola and L'Oréal launched the green tea-based health-and-beauty beverage called Lumaé, which claimed to address skin care needs. However, the current federal status of this trademark filing is "abandoned—no statement of use filed" [14]. In the context of

TABLE 55.1
Characteristics of Nutricosmetics Markets in Europe, Japan, and United States

	Europe	Japan	United States
Application form	Tablets	Liquids	Tablets
Price	High	Low	Moderate
Distribution channel	Pharmacies	Mass mainstream	Specialty
Brands	Unique to Europe	Unique to Japan	Unique to United States
Benefits	Skin, hair	Beauty support, skin	Skin
Product claims	Specific	Vague	Vague to specific

Source: Adapted from Carrie, M., Nutricosmetics: decoding the convergence of beauty and healthcare, in *In-Costmetics* 2008: Amsterdam.

"nutriceuticals," however, Danone's probiotic yogurt Activia enriched with *Bifidobacterium lactis* DN-173-010 claims to support digestion of food, to produce certain vitamins, and to provide unfavorable conditions for some harmful bacteria, leading to improved digestive comfort and hence well-being, fitness, and health ("Feel the rhythm everyday with Activia"). According to the World Health Organization, probiotics are "live microorganisms which, when administered in adequate amounts, confer a health benefit on the host" [15]. Activia was backed by Danone Research with 17 published clinical studies, including seven on the strain survival and 10 on the benefit (6 related to transit and 4 to digestive comfort), and has been more successful than Essensis [16,17].

Hence, these examples may suggest that at least in European and US context, scientific investigation is a prerequisite to be successful when entering the nutricosmetics market, while Japanese consumers seem more willing to accept a rather holistic approach and to take such products mainstream without critically questioning their specific claims. In Europe, the legal framework exists under both food and medicinal law, but decisions as to which applies to nutricosmetics vary by country [5].

PROPOSED WORKING MECHANISMS OF NUTRICOSMETICS AS USED IN HEALTH AND FITNESS

Nutrition can modulate the physiology and condition of different tissues and organic systems in the human body including the skin, cardiovascular system, the lungs, and the musculoskeletal system. Therefore, the understanding of the possible working mechanisms is partially derived from pathophysiological observations of organic or skin alterations under malnutrition conditions [1]. Nutricosmetics primarily aim at skin (antiaging, repair and prevention, sun protection, pigmentation, whitening, slimming), hair (retention and growth, restoration, nourishment, volumizing), and nails (strengthening). The ingredients should be safe and ingestible (often derived from food), and in general, they mostly offer an antioxidant, anti-inflammatory, or slimming function [3]. However, many products are being introduced in daily skin care antiaging cosmeceuticals or cosmetic products based on hypothetical in vitro mechanisms of action, without confirmation by controlled clinical trials [18]. The same may apply for nutricosmetics.

Nutricosmetics are usually based on combinations of the following ingredients: carotenoids, polyphenols, several vitamins, soy extracts (e.g., polyphenolic isoflavones), micronutrients, glycopolyglycans, amino acids, other plant-based elements (e.g., herbs), and polyunsaturated fatty acids (e.g., fish oils). The top nutricosmetic ingredients are collagen, co-Q10, grape-seed extract, green tea, lutein, lycopene, Marine Complex (deep sea fish ingredient), omega-3, superfruits (e.g., acai), vitamins A, C, and E, and zinc [4,12]. In the following section, the use of most of the aforementioned ingredients in (commercial) nutricosmetics and their suggested working mechanisms on skin (adnexes), health, and fitness together with scientific evidence will be discussed. However, while Chapter 54 discussed mainly the effects on skin, the following section has the intention of focusing on the (possible) nutricosmetic effects on health and fitness.

CAROTENOIDS

In the following section, evidence based on simple solution studies, cell studies, and clinical trials will be discussed.

Aging seems to be associated with an increased oxidative stress resulting from an imbalance between free radical production and antioxidant defense [19]. Carotenoids (CARs) comprise a class of natural fat-soluble pigments (e.g., beta-carotene, lycopene, lutein, zeaxanthin, and astaxanthin), which are found in numerous fruits and vegetables. These C_{40} carotenoids and their oxygenated derivates, the xanthophylls, are often used as antioxidant ingredients in nutricosmetics.

Furthermore, carotenoids may influence signaling pathways and gene expression at the cellular level of different tissues or enhance the intercellular gap junction communication [20].

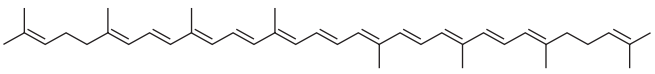

FIGURE 55.2 Chemical structure of lycopene.

As a nutricosmetic, there is convincing evidence that carotenoids are important components of the antioxidant network involved in the pathobiochemistry of several diseases affecting the skin and the eye. Within the retinal macula lutea, lutein and zeaxanthin are the predominant carotenoids acting as photoprotectors preventing its degeneration [21]. Their physicochemical properties make them suitable candidates for photoprotection of the retina against UV light-induced oxidative damage. While lutein and zeaxanthin accumulate mainly in the macula lutea, beta-carotene (provitamin A) accumulates preferentially in the skin providing it a "golden yellow" color. Beta-carotene is used as an oral sun protector preventing sunburns and has been shown to be effective either alone or in combination with other carotenoids or antioxidant vitamins.

The mechanisms observed in simple solvent and cell membrane model studies were supported by several cell-based studies. Based on the findings from clinical trials, there is evidence for carotenoid protection against sunlight-induced (long and short term) skin damage with treatment length as an important efficacy-influencing factor. Many other clinical trials on the effects of carotenoids (in general) and lycopene (in special) on different chronic diseases, such as the well-known prostate cancer studies, and on exercise capacity have been conducted.

Inneov Anti-Age Firming was launched as a nutricosmetic based on ingredients such as lacto-lycopene (similar to tomato lycopene), soy isoflavones, and vitamin C. The manufacturer recommends an intake of 2 capsules per day during 90 days. In 2010, researchers of the Inneov laboratory published a study that aimed to assess the effects of a nutricosmetic combining the probiotic *Lactobacillus johnsonii* (La1; has been reported to protect skin immune system homeostasis following UV exposure) and nutritional doses of carotenoids on early UV-induced skin damage. Three clinical trials (CT1, CT2, and CT3) were performed using different UV sources: nonextreme UV with a high UVA irradiance (UV-DL, CT1), extreme simulated solar radiation (UV-SSR, CT2), and natural sunlight (CT3). All three clinical trials were carried out in 139 healthy women over 18 years of age with skin type II–IV. In CT1, early markers of UV-induced skin damage were assessed using histology and immunohistochemistry. In CT2, the minimal erythemal dose (MED) was determined by clinical evaluation and by chromametry. Chromametry was also used to evaluate skin color. Dermatologists' and participants' assessments were compiled in CT3. The researchers found that a 10-week nutricosmetic intake prevented the UV-DL-induced decrease in Langerhans cell density and the increase in factor XIIIa+ type I dermal dendrocytes, while it reduced dermal inflammatory cells. Clinical and instrumental MED rose by 20% and 19%, respectively, and skin color was intensified, as shown by the increase in the DeltaE* parameter. The efficacy of the nutricosmetic was confirmed by dermatologists and participants under real conditions of use. Therefore, the researchers concluded that nutricosmetics combining the probiotic (La1) and nutritional doses of carotenoids reduced early UV-induced skin damage caused by simulated or natural sun exposure in a large panel of female participants (n = 139). The authors further concluded that "this latter result might suggest that nutricosmetic intake could have a beneficial influence on the long-term effects of UV exposure and more specifically on skin photoaging" [22].

In athletes involved in sports with a high esthetic component such as dancers, rhythmic gymnasts, synchronized swimmers, body builders, and more recently, athletic pole dancers, a slightly tanned skin appearance may be wanted without UV radiation or the risk of smear of topical applied tanning sprays. The French nutricosmetic, Oenobiol Solaire, contains ingredients such as lycopene, luteine, selenium, and vitamin E, which support the melanin synthesis. Therefore, this nutricosmetic claims to have a natural photoprotective effect while supporting the tanning of the skin. The manufacturer recommends an intake of 1 capsule per day 1 month before, during, and 1 month after sun exposure. The manufacturer pretends that Oenobiol Solaire was backed with clinical trial validation. On the brand's Web site, an internal study is mentioned evaluating the effect of Oenobiol Solaire versus placebo in 50 participants followed up by dermatologists during 10 weeks showing 18.7% increase in tanning effect. Unfortunately, most of this research was internal and hence not published [23]. One such Oenobiol Laboratory study was found in Medline. The aim of this study was to demonstrate that modification of the cellular redox equilibrium occurs as a consequence of antioxidant nutrient intake (carotenoids, vitamin E, and vitamin C) and that these nutrients play a role in the pigmentation of the skin without any UV exposure. The researchers conducted a randomized, double-blind study in 20 healthy subjects to evaluate and compare the efficacy of two mixtures of dietary antioxidants with regard to direct determination of melanin and carotenes by chromametry at selected skin sites and multiple reflection spectrometry from a 1-cm^2 region of skin of different parts of the body. Efficacy was assessed by a significant improvement of these parameters, in comparison with measurements performed before nutricosmetic intake. The formulations per capsule of nutricosmetics under investigation were: 13 mg of beta-carotene, 2 mg of lycopene, 5 mg of vitamin E, and 30 mg of vitamin C (B13/L2) or 3 mg of beta-carotene, 3 mg of lycopene, 5 mg of vitamin E, and 30 mg of vitamin C (B3/L3). An 8-week B13/L2 supplementation led to a detectable carotenodermia, whereas the B3/L3 supplementation did not. An increase in melanin concentrations in skin was found after 4, 5, 6, and 8 weeks of dietary antioxidant intake in both groups ($p < 0.05$). The authors discussed these results with regard to the redox control theory of melanocytes, which regulate the tyrosinase activity [24].

Recent studies suggested that combined oral (nutricosmetic) and topical (cosmeceutical) treatments with the carotenoids lutein and zeaxanthin may increase skin elasticity, cutaneous hydration, and antioxidant protection and that the latter two effects were more pronounced in the combined oral and topical treatments compared to the isolated applications, hence suggesting a synergistic effect of nutricosmetic and cosmeceutical approaches [25].

While most knowledge about the carotenoid anti-oxidant working mechanisms comes from UV-induced skin damage

studies, some information has also been derived from nutritional studies. Carotenoid-rich diets have been epidemiologically correlated with a lower risk for several diseases indicating that such antioxidants may also have important effects on several biologic tissues. Therefore, oral intake of nutricosmetics based on carotenoids may have "side effects" on other tissues than on the skin or eye leading to improved general health or physical (and mental) fitness status. For example, nutricosmetic carotenoids may help to interrupt the ROS damaging effects such as lipid peroxidation of the polyunsaturated fatty acids in biological membranes and blood (leading to impaired cell functioning) during and after strenuous exercise. Protective effects are also achieved with the tomato-derived lycopene (i.e., an exogenous carotenoid), also showing efficient singlet oxygen quencher characteristics. Following 10 to 12 weeks ingestion of lycopene or tomato-derived products rich in lycopene, a decrease in the sensitivity toward UV-induced erythema was observed in volunteers. Dietary carotenoids such as lycopene may contribute to lifelong photoprotective effects against harmful UV radiation [26,27]. Naturally derived lycopene seems to have a 25% higher effect compared to synthetic lycopene [28].

One study determined the relationship between concentrations of lycopene in human serum and other body tissues after tomato lycopene oleoresin supplementation (30 mg/day) or placebo administered for 1 to 7 weeks to 75 volunteers undergoing elective hemorrhoidectomy or perianal fistulotomy. Carotenoid concentration in blood and in the surgically removed skin and adipose tissues was measured by high-performance liquid chromatography (HPLC). The serum concentration of lycopene increased after supplementation from 0.26 ± 0.12 to 0.52 ± 0.25 μmol/l ($n = 35$; $p < 0.0001$), while in the placebo group ($n = 40$), lycopene serum concentration remained unchanged. Serum lycopene concentration after treatment was 2.2-fold greater in the lycopene group than in the placebo group, a slightly higher ratio than that found in skin and adipose tissue (1.6- and 1.4-fold higher than the placebo, respectively). These results show that tomato–oleoresin supplementation increases lycopene concentrations in serum, adipose tissue, and skin [29].

The observed ability to increase lycopene levels in tissues after supplementation is one of the prerequisites for using lycopene as an ingredient in a nutricosmetic with health and fitness benefits. Cardiovascular disease is associated with oxidative stress, inflammatory processes, and vascular dysfunction. As an antioxidant, lycopene has been suggested to protect against atherosclerosis based on its protective effect on lipid peroxidation; however, the exact mechanism of such protection is not yet clear [30].

One study on cell cultures investigated whether lycopene is able to counteract oxysterol-induced proinflammatory cytokine cascade in human macrophages, limiting the formation of atherosclerotic plaque. THP-1 macrophages were exposed to two different oxysterols, such as 7-keto-cholesterol (4–16 μM) and 25-hydroxycholesterol (2–4 μM), alone and in combination with lycopene (0.5–2 μM). Both oxysterols enhanced proinflammatory cytokine (interleukin [IL]-1beta, IL-6, IL-8, tumor necrosis factor alpha) secretion and mRNA levels in a dose-dependent manner, although at a different extent. These effects were associated with an increased ROS production through an enhanced expression of NAD(P)H oxidase. Moreover, a net increment of phosphorylation of extracellular regulated kinase 1/2, p-38, and Jun N-terminal kinase and of nuclear factor κB (NFκB) nuclear binding was observed. Lycopene prevented oxysterol-induced increase in proinflammatory cytokine secretion and expression, which was accompanied by an inhibition of oxysterol-induced ROS production, mitogen-activated protein kinase phosphorylation, and NF-κB activation (Figure 55.3). The inhibition of oxysterol-induced cytokine stimulation was also mimicked by the specific NF-κB inhibitor pyrrolidine dithiocarbamate. Moreover, the carotenoid increased peroxisome proliferator-activated receptor γ levels in THP-1 macrophages [31].

The protective effect of lycopene on atherosclerosis was supported by a random-effects model meta-analysis, summarizing the current evidence on the effect of lycopene supplementation of at least 2 weeks duration on serum lipid concentrations and blood pressure. Twelve studies (13 trial arms) investigated the effect of lycopene on serum lipids, and four studies examined its effect on blood pressure. A meta-analysis on serum lipids revealed a cholesterol-lowering effect of lycopene for total serum cholesterol (mean difference ± SE: −7.55 ± 6.15 mg/dl; $p = 0.02$) and low-density-lipoprotein (LDL) cholesterol (mean difference ± SE: −10.35 ± 5.64 mg/dl, $p = 0.0003$) in the subgroup of trials using lycopene dosages of ≥25 mg daily, whereas subgroup meta-analysis of trials using lower lycopene dosages was not significant. Meta-analysis of the effect of lycopene on systolic blood pressure of all trials suggested a significant blood pressure reducing effect (mean difference ± SE: −5.60 ± 5.26 mmHg, $p = 0.04$). This meta-analysis suggested that lycopene intake of ≥25 mg daily is effective in reducing LDL cholesterol by about 10%, which is a similar effect then of low statin doses in slightly hypercholesterolemia patients. More high-quality and large-scale studies are needed to confirm the suggested beneficial effects on total serum cholesterol and systolic blood pressure [32]. In commercial nutricosmetics (primarily aiming at the skin), lycopene concentration per capsule ranges from 2 [23] to 6 mg [33] per tablet or capsule. According to epidemiologic studies, tomato lycopene may also reduce the risk of prostate and several other cancer types. It was suggested that lycopene may have immunosuppressive properties, which

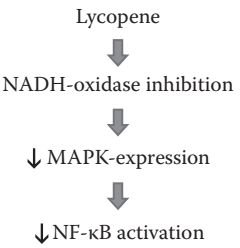

FIGURE 55.3 Proposed pathway of the lycopene antioxidant and anti-inflammatory protective mechanism.

may be therapeutically useful in controlling chronic immune and inflammatory diseases [31]. Positive effects are also hypothesized in case of other diseases such as osteoporosis, neurodegenerative diseases, and hypertension, while adverse effects upon lycopene supplementation or lycopene toxicity have not been reported [34].

Based on its physicochemical properties, lycopene may also exert positive effects during and after exercise. In order to investigate the effects of lycopene on human ROS metabolism after high-intensity endurance exercises (a 4000-m run), a Chinese study randomly divided 70 college students into two groups: the intervention group (lycopene drink) and the placebo group (physiological saline drink). The students of each group were respectively given lycopene solution or physiological saline 45 min before running, and then each group took a lycopene solution or physiological saline drink at 1500-, 2500-, and 3500-m race distances, respectively. The blood samples were drawn before running and immediately after running to analyze proxies of ROS activity and oxidative tissue damage (such as malondialdehyde [MDA], a byproduct of lipid peroxidation), and total antioxidative capacity (TAC) levels were determined instantly. The results of this study indicated that the MDA level of the lycopene-supplemented group was significantly lower compared to the values observed in the control group ($p < 0.05$), while the TAC levels of the lycopene solution group were increased ($p < 0.05$). The authors concluded that lycopene supplementation can effectively enhance the person's antioxidation ability [35]. In another Chinese-controlled study, lycopene-supplemented athletes showed a delayed occurrence of exercise-induced fatigue and an improved immune function [36].

POLYPHENOLS

Polyphenols represent a wide range of naturally occurring plant products, for example. anthocyanidins (berries), catechins (tea, apples, red wine), flavonols (green tea), flavavones (citrus fruits), isoflavones (soy), tannins (red grape), and proanthocyanidins (cacao, grape seeds), and contribute to the beneficial health and fitness effects of a diet rich in fruits and vegetables. Most evidence comes from in vitro as well as from in vivo observations from polyphenols such as flavonols, proanthocyanidins, silymarin, genistein, and resveratrol using both animal and human systems. Keratinocytes typically react to an acute and chronic UVB radiation with cyclooxygenase-2 (COX-2) expression and a subsequent prostaglandin (PG) metabolite secretion. COX-2 expression can be used as a proxy for inflammation and carcinogenesis.

UVB-induced COX-2 expression and PG metabolite synthesis can be inhibited by green tea (*Camellia sinensis*) polyphenol intake in mice experiments (Figure 55.4) [37].

The same pathway was proposed for the other phenols such as proanthocyanidins, silymarin, genistein, and resveratrol in mice studies, and therefore, the authors concluded that although more clinical studies are needed, supplementation of skin care products with green tea may have a profound impact on various skin disorders in the years to come [38].

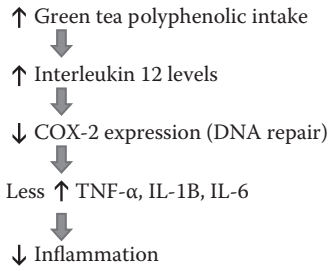

FIGURE 55.4 Proposed pathway of the green tea phenolic anticarcinogenetic protective mechanism.

This evidence is furthered with some clinical trials in humans. For example, tackling cellulite to increase a healthy and fit appearance is one of the most important aims of nutricosmetics. Inneov was launched as a green tea-based nutricosmetic aimed at decreasing cellulite. The manufacturer claims that based on internal studies in humans, "efficacy is proven by dermatological research and confirmed by female consumers" [39].

Also, in the context of health and fitness, the effects of polyphenolic antioxidants on exercise-induced oxidative stress have been studied in 30 male cyclists (23.6 ± 0.9 years) [40]. Based on the knowledge that intensive exercise may cause the perturbation of the physiological balance between oxidative reactions and antioxidant capacity in humans (i.e., oxidative stress; Figure 55.5), and that polyphenol-supplemented beverages are able to transfer their antioxidant capacity to body fluids [41], this controlled study (double-blinded randomized crossover design) investigated the effect of the flavonoid contents as the only antioxidant agent in a replacement drink designed for sportsmen on various oxidative stress biomarkers after two identical trials of submaximal aerobic exercise. The intervention trial participants consumed the antioxidant supplement (i.e., 2.3 g polyphenols/trial), while those in the control group consumed a placebo. Both at rest and after exercise (90 min at 70% VO_{2max} bike ergometry) immediately and 45 min later, blood samples were collected to analyze plasma proxies of oxidative stress such as lipid oxidation (thiobarbituric acid reactive substances [TBARS]), total antioxidant status (TAS), protein oxidation, and the lactate dehydrogenase (LDH) and creatine kinase (CK) enzymes for each trial. All values were adjusted for changes in plasma volume.

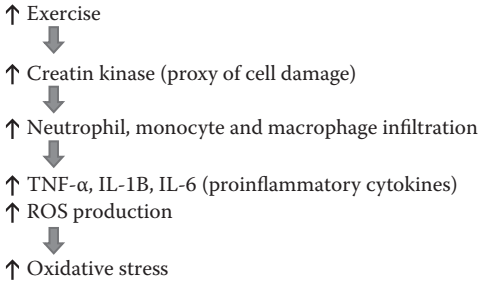

FIGURE 55.5 Proposed pathway of the strenuous exercise–induced oxidative stress in muscle tissue. Flavonoids show capacity to interrupt this chain.

The results showed no changes in plasma TAS and LDH after exercise or after the polyphenolic supplement. CK and TBARS increased after exercise in both tests. However, in response to strenuous exercise, the polyphenol-supplemented test showed a smaller increase in plasma TBARS and CK than the placebo test. CO increased by 12% in response to the placebo test, whereas it decreased by 23% in the polyphenol-supplement test. The authors concluded that this may indicate that the antioxidant supplement offered protection against exercise-induced oxidative stress [40].

SOYBEAN ISOFLAVONES

Soy isoflavones are often used in nutricosmetics for their antioxidant and phytoestrogenic properties. For example, Imedeen Prime Renewal claims to "combat the effects of hormonal aging" and focuses on postmenopausal women. This nutricosmetic is based on ingredients such as MarineComplex (fish proteins and polysaccharides), soy extract, zinc, white tea, lycopene, grape-seed extract, chamomile, vitamin C, and vitamin E. The manufacturer recommends an intake of two tablets twice a day (in the morning and in the evening) and claims visible effects (e.g., reduced skin wrinkles) within 12 to 24 weeks. On the brand's Web site, links are uploaded to three reports of clinical studies on Imedeen Prime Renewal effects. Unfortunately, most of this research was presented only at congresses [12]. One study was found in evaluating whether MarineComplex affects skin morphogenesis differently in female and male human skin equivalents (HSEs). HSEs were established with cells obtained from female or male donors between 30 and 45 years of age and cultured for 7 or 11 weeks in the presence or absence of MarineComplex. Using immunohistochemistry, the researchers examined early differentiation by keratin 10 expression, (hyper)proliferation by keratin 17 and Ki67, and basement membrane composition by laminin 332 and collagen type VII. In addition, the expression of collagen type I and the secretion of pro-collagen I were measured. MarineComplex strongly increased the number of Ki67-positive epidermal cells in female HSEs. In the dermis, MarineComplex significantly stimulated the amount of secreted procollagen I and increased the deposition of laminin 332 and collagen type VII. Furthermore, MarineComplex prolonged the viable phase of HSEs by slowing down its natural degradation. After 11 weeks of culturing, the MarineComplex-treated HSEs showed higher numbers of viable epidermal cell layers and a thicker dermal extracellular matrix compared with controls. In contrast, these effects were less pronounced in male HSEs. The authors concluded that the MarineComplex nutrient positively stimulated overall HSE tissue formation and prolonged the longevity of both female and male HSEs. The ability of MarineComplex to stimulate the deposition of basement membrane and dermal components can be used to combat human skin aging in vivo [42].

Soy intake may have positive effects on bone and cardiovascular health. Based on a Bayesian meta-analysis including 17 randomized control trials (RCTs; humans), it was concluded that exposure to soy isoflavones can modestly, but significantly, improve endothelial function as measured by flow-mediated dilation. Therefore, exposure to isoflavone supplements may beneficially influence vascular health [43]. Another meta-analysis of 19 randomized placebo-controlled trials of at least 12 weeks duration concluded that soy intake (dietary, extract, or concentrate) as a phytoestrogen to prevent climacterium-attributed flashes in postmenopausal women showed a significant tendency in favor of soy, but that it is still difficult to establish conclusive results given the high heterogeneity found in the studies [44].

One meta-analysis was conducted to evaluate the effect of ingesting soy isoflavone extracts (not soy protein or foods containing isoflavones) on bone mineral density (BMD) in menopausal women. Eleven, seven, five, and five RCTs were selected for estimation of the effects on spine, femoral neck, hip total, and trochanter BMD, respectively. Meta-analyzing data from 1240 menopausal women showed that an average of 82 (47–150) mg soy isoflavones (aglycone equivalent) intake per day for 6 to 12 months significantly increased spine BMD by 22.25 mg/cm^2 (95% CI: 7.62 to 32.89 mg/cm^2; $p = 0.002$) or by 2.38% (95% CI: 0.93% to 3.83%; $p = 0.001$) compared with controls (random-effects model), while no significant effects on femoral neck, hip total, and trochanter BMD were found [45]. In the context of fitness, one study determined changes in body composition (BC), physical performance, metabolic and hormonal parameters induced by lifestyle counseling, resistance training, and resistance training with soy protein-based supplementation in 40 middle-aged males (50–65 years, BMI 25–29.9 kg/m^2). This RCT consisted of resistance training without or with a soy protein-based supplement and a control group with lifestyle education only. Changes in body weight (BW) and waist circumference (WC) were measured, and BC, fat mass (FM), and lean body mass (LBM) were measured by skin fold anthropometry at baseline and after 12 weeks of intervention. In addition, changes in physical fitness and metabolic and hormonal parameters (lipids, glucose, fructosamines, insulin, insulin-like growth factor-1, leptin, human growth hormone, dehydroepiandrosterone, testosterone, hs-CRP, Il-6) were evaluated. Thirty-five participants completed the 12-week study. No significant changes in BW were noted, although RM and WC dropped and LBM increased after training, particularly in the soy-supplemented group (FM: 22.6 ± 5.5 to 21.2 ± 4.7 kg; LBM: 68.5 ± 7.2 kg to 70.1 ± 7.4; $p < 0.01$). Subjects in the soy-supplemented group experienced more pronounced improvements in the strength measurements than the nonsupplemented resistance training group. After the training intervention, there were significant changes in hormonal and metabolic parameters as well as in glycemic control, particularly in the soy-supplemented group. The authors concluded that resistance training, particularly in combination with a soy protein-based supplement, improves BC and metabolic function in middle-aged untrained and moderately overweight males [46]. Soy isoflavones are also of interest to protect against exercise-induced ROS damaging of muscle cells [47].

Another study was aimed to quantify the effects of Imedeen Prime Renewal on skin in postmenopausal women. It was a 6-month double-blind, placebo-controlled, randomized study on healthy postmenopausal females. Two tablets of Imedeen Prime Renewal or placebo were given twice daily for 6 months. Thirty-eight (active group) and 42 (placebo group) subjects completed the study out of 100. Clinical grading showed that the active group had a greater improvement ($p < 0.05$) compared to placebo for the face after 6 months treatment for forehead, periocular and perioral wrinkles, mottled pigmentation, laxity, sagging, under-eye dark circles, and overall appearance; skin on the décolletage after 2, 3, and 6 months of treatment; and skin on the hand after 3 and 6 months of treatment. Photo evaluation showed that the active group had a greater improvement ($p < 0.05$) on the face after 3 and 6 months for several parameters. Ultrasound measurements showed that the active group had a greater improvement ($p < 0.0001$) for density measurements after 6 months of treatment. Therefore, the researchers concluded that Imedeen Prime Renewal provides improved condition, structure, and firmness of the skin in postmenopausal women after 6 months [48].

OTHER PLANT-BASED FLAVONOIDS

Examples of other bioflavonoids used in nutricosmetics are ginseng or ginkgo. Chronic supplementation of Panax ginseng (PG) (a deciduous perennial plant belonging to the Araliaceae family) enhances physical performance. In areas nearby the equator, athletes need to perform in a hot and humid environment due to the climatic characteristics of such regions. A Malaysian study evaluated the ergogenic effect of acute supplementation of PG on endurance performance in a hot and humid condition. Nine heat-adapted recreational runners (age: 25.4 ± 6.9 years, body mass: 57.6 ± 8.4 kg; body height: 168.3 ± 7.6 cm) were recruited in this placebo-controlled double-blind randomized study. Subjects ingested 200 mg of PG 1 h before the exercise test on treadmill at 70% VO_{2max} in a laboratory environment of 31°C and 70% relative humidity. They drank 3 ml/kg BW of cool water every 20 min during the exercise to prevent adverse effects of dehydration. Blood samples were drawn every 20 min for the analysis of glucose, lactate, insulin, and free fatty acid levels. Oxygen uptake was determined every 20 min, while heart rate, body and skin temperatures, and ratings of perceived exertion (RPE) were recorded every 10 min during the trials. The researchers found that endurance running time to exhaustion did not differ between PG and placebo trials. Heart rate, skin temperature, core body temperature, oxygen uptake, RPE, plasma insulin, glucose, free fatty acid, and lactate levels during the endurance exercise did not show any significant differences between the trials. Therefore, the authors concluded that acute supplementation of 200 mg of PG did not affect the endurance running performance of the heat-adapted male recreational runners in the heat [49].

Over the last decennium, many in vitro and in vivo studies have suggested health and fitness enhancing effects of the phenolic compounds (mainly flavonoids such as catechin and epicatechin) of cocoa. For example, these bioactive molecules were found to have vasoactive properties and therefore may enhance endothelial function. In a double-blinded randomized crossover trial, 20 subjects received a 100 ml drink with high (176 to 185 mg) and low doses (<11 mg) of cocoa-rich flavan-3-ol (a flavonoid subclass). Upon ingestion, an increased flow-mediated dilution of conduit arteries was observed [50]. This finding was supported in a similar study with 11 smokers [51]. The mechanisms underlying the observed vasodilating effects are not yet well described, but there is evidence that the circulating NO pools are increased after flavonol cocoa consumption [51,52].

Among the other health-enhancing effects of cocoa are suppressed development of atherosclerotic lesions [53], decreased platelet function, increased skin microcirculation [54], inhibited proliferation of cancer cells in breast cancer patients [55], and hypoglycemic properties [56]. The main compounds are the procyanidin monomers (catechin and epicathechin), which showed antioxidant capacities [57–59]. In addition to these phenolic compounds, cocoa contains also methylxanthine (caffeine and theobromine), which was suggested to decrease the insulin-mediated glucose uptake and disposal as well as reduced lipid profiles in hypercholesterolemic animals [60]. A recent in vitro study supported the observation that the suggested antioxidant properties of cocoa are mainly based on the presence of the phenolic compounds. The methylxanthines, however, showed only low antioxidative capacities. Moreover, the presence of methylxanthines could even reduce the flavonoids' antioxidant properties [61].

Bark extract of *Pinus pinaster* has a long history of ethnomedicinal use and is available commercially as herbal dietary supplement with proprietary name Pycnogenol. It is used as a food supplement to overcome many degenerative disorders [62]. French maritime pine bark extract (*Pinus maritima*; Flavangenol) has gained popularity as a dietary supplement in the treatment of various diseases due to its polyphenol-rich ingredients. Oligometric proanthocyanidins (OPCs), a class of bioflavonoid complexes, are enriched in French maritime pine bark and have antioxidant and anti-inflammatory activity and a wide range of cardiovascular benefits such as lowered blood pressure, improved glycogemic control, lipid profile, fatty acid synthesis, and peripheral circulation [62,63]. A recent review described that the underlying mechanisms of these health- and fitness-enhancing maritime pine bark extract properties may be explained by studies showing evidence for their free radical scavenging activity, synergism with synthetic antioxidants, protecting biomolecules against oxidative damage, androgen synthesis stimulation, protective effect against I/R-induced oxidation, anti-inflammatory activities, and antimicrobial and antiviral activity [62].

One of the so-called "superfruit" ingredients of the nutricosmetics is based on extracts of the Amazonian palm berry, *Euterpe oleracea* (acai fruit). One animal cell-based study on mice peritoneal macrophages revealed the strong velutin (a flavone isolated from the pulp of the acai fruit) effects in

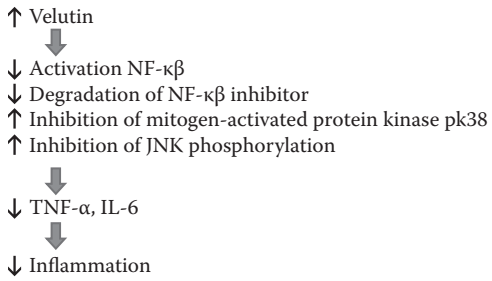

FIGURE 55.6 Proposed pathway of the anti-inflammatory effects of velutin.

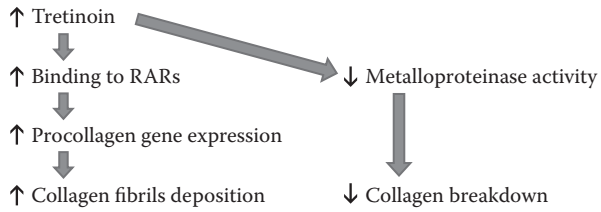

FIGURE 55.7 Proposed pathway of the vitamin A effect on collagen fibers of the connective tissue of the skin (RAR = retinoic acid receptors).

reducing lipopolysaccharide-induced proinflammatory cytokines TNF-α and IL-6 production (Figure 55.6) [64].

Age-related diseases of the brain compromise memory, learning, and movement and are directly linked with increases in oxidative stress and inflammation. Because of their high polyphenolic content, fruit pulp fractions of acai were explored for their protective effect on BV-2 mouse microglial cells. Studies were conducted to investigate the mitigating effects of acai pulp extracts on lipopolysaccharide (LPS, 100 ng/ml) induced oxidative stress and inflammation. Treatment of BV-2 cells with acai fractions resulted in significant ($p < 0.05$) decreases in nitrite production, accompanied by a reduction in inducible nitric oxide synthase (iNOS) expression. The protection of microglial cells by acai pulp extracts can be explained by the pathway described in Figure 55.7. The current study offers valuable insights into the protective effects of acai pulp fractions on brain cells, which could have implications for improved cognitive and motor functions [65].

Taken together, the above-mentioned effects may play a significant role in the prevention of chronic inflammatory diseases, and hence, acai-based nutricosmetics may show promising effects for health and (mental) fitness. However, more human well-designed and large RCTs are needed to further verify these promising effects.

VITAMINS

In commercial nutricosmetics, vitamins are nearly always present as a part of the formulation. Vitamin A is more frequently used in pharmaceutical products than in cosmeceuticals or nutricosmetics. Vitamin A, present in the human skin, cannot be synthesized; hence, it must be obtained through dietary means. The ingestion of vitamin A depends on the presence of retinoids (animal sources) and carotenoids (vegetable sources) in the diet. In the body, a small percentage of retinol is converted to its biologically active form, all-trans retinoic acid (tretinoin), through an intermediary, retinaldehyde. Most of retinol is converted to retinyl ester, its storage form. Topical retinoids have successfully been used to treat acne for nearly four decades [18]. Variations of this molecule have resulted in three generations of topical and systemic retinoids: the nonaromatics (retinol, tretinoin, and isotretinoin), the monoaromatics (etretinate and acitretin), and the polyaromatics (arotinoid, adapalene, and tazarotene). The efficacy of topical use of tretinoin in the treatment of photoaged and intrinsically aged skin is sufficiently evidence based. Figure 55.7 depicts the possible pathways resulting in an improved collagen deposition in connective tissue of the skin.

An overall thickening is observed in the epidermis: a compaction of the stratum corneum and deposition of a mucinous material (glycosaminoglycans) in the stratum corneum and intercellular spaces. The effects result in an improvement in the clinical and histologic skin appearance. However, the benefits of retinol and retinyl cosmeceutical products marketed as "antiaging" formulations are doubtful. They have varying low concentrations, and usually, there are a few clinical trials demonstrating efficacy. It seems that the useful concentration of topical retinol should range from 0.3% to 1%. Most of the over-the-counter products available usually contain lower levels of retinol (about 0.08% or less) [18].

Vitamin B_3 (nicotinamide or niacinamide) is a derivative of niacin obtained through diet from meat, fish, milk, egg, and nuts. Niacin has cholesterol-lowering properties. Nicotinamide is part of the coenzymes nicotinamide adenine dinucleotide (NAD) and NAD phosphate (NADP), and the reduced forms (NADH and NADPH) may act as antioxidants. NAD and NADP are important in many cellular metabolic enzyme reactions. Nicotinamide is involved in the synthesis of sphingolipids, free fatty acids, cholesterol, and ceramides (decreasing transepidermal water loss), in the suppression of melanosome transfer from melanocytes to keratinocytes, and it increases collagen production as was observed in a fibroblast culture study. All of these effects may help to reverse some of the aging (skin) signs, and for this purpose, it has been used in cosmeceutical products in concentrations ranging from 3.5% to 5%. There is certainly opportunity and interest to optimize use of this agent to achieve a higher performance [18].

The role of vitamin C as a free radical scavenger has been researched extensively. The water-soluble molecule functions in the aqueous compartment of the cell by donating electrons, neutralizing free radicals, and protecting intracellular structures from oxidative stress. L-ascorbic acid is essential for collagen biosynthesis. It serves as a cofactor for prolyl and lysyl hydroxylases, enzymes that hydroxylate proline and lysine in collagen, stabilizing its triple helical structure. Recent studies have demonstrated that vitamin C also influences collagen synthesis independently of hydroxylation by

activating its transcription and stabilizing procollagen messenger ribonucleic acid [18]. Because of these antioxidant and collagen synthesis supporting mechanisms, vitamin C is often used in nutricosmetic formulations.

Throughout evolution, vitamin D (the sunshine vitamin) has been critically important for health. It has hormone properties and is produced in the skin or ingested from the diet and converted sequentially in the liver and kidneys to 1,25 dyhydroxyvitamin D. Vitamin D receptors are present in nearly every tissue cell in the human body. Therefore, vitamin D deficiency has been linked to increased risk for preeclampsia, requiring a cesarean section for birthing, multiple sclerosis, rheumatoid arthritis, types I and II diabetes, heart disease, dementia, deadly cancers, and infectious diseases. Therefore, sensible sun exposure along with vitamin D supplementation of at least 2000 IU/day for adults and 1000 IU/day for children is essential to maximize their health [66].

A Swiss review suggested strong evidence that higher 25-hydroxyvitamin D (25(OH)D) levels are protective against fractures and falls, while promising epidemiologic and mechanistic studies suggest a key role of vitamin D in the preservation of cardiovascular health and the prevention of cancer and other common chronic diseases. Lower extremity function, fall prevention, hip bone density, and fracture prevention optimal benefits are observed with 25(OH)D levels of at least 75 to 100 nmol/l. This threshold may be reached in 50% of adults with 800 to 1000 IU vitamin D per day [67].

It can be questioned, however, if the commercial nutricosmetics, available on the market today, reach the proposed health- and physical fitness-enhancing concentrations (1000 to 2000 IU vitamin D per day) in their formulations. Furthermore, the findings from observational and clinical studies on the association between vitamin D and physical performance remain controversial. A systematic review on the effects of low serum vitamin D concentration and vitamin D supplementation on muscle strength, balance, and gait performance among people aged 65 years and older was performed based on 16 studies (8 observational and 8 interventional studies) including 24 to 33,067 participants. Observational studies and clinical trials yielded divergent results, which highlights the complex and to date still poorly understood association between serum vitamin D concentration or vitamin D supplementation and physical performance [68].

Vitamin E is the major lipid-soluble antioxidant in the human body and, like vitamin A, is present in the skin. The probable physiologic function of epidermal vitamin E is to contribute to the antioxidant defense of the skin. Owing to its physical properties, vitamin E absorbs UV light in the solar spectrum. α-Tocopherol is the most active (from the eight existing vitamin E forms) and is important in protecting cellular membranes from lipid peroxidation by free radicals. Once oxidized, vitamin E can be regenerated back to its reduced form by vitamin C or L-ascorbic acid. Vitamin E has also an immunostimulatory effect, which has been shown to be associated with resistance to infections. Nutritional status is an important determinant of immune function. A double-blind, placebo-controlled trial determined the effect of 1-year supplementation with 200 IU/day vitamin E on the incidence and duration of respiratory infections in 617 elderly nursing home residents. The results of this clinical trial showed that vitamin E supplementation significantly reduces the incidence rate of common colds and the number of subjects who acquire a cold among elderly nursing home residents. Because of the high rate and more severe morbidity associated with common colds in this age group, these findings have important implications for the well-being of the elderly as well as for the economic burden associated with their care [69]. The effects may be partially explained by an increased enhancement of Th1, IL-2, and IFN-γ production [70,71]. However, the immunostimulatory effect of vitamin E may be reduced when ingested in combination with other supplements (such as fatty acids) as is usually the case in the context of nutricosmetics. For example, one study determined if concomitant consumption of fish oil and vitamin E would modify the vitamin E level needed for improving T cell-mediated function in elderly. This double-blinded RCT was conducted using 40 healthy male and female elderly subjects (>65 years) who were randomly assigned to one of four groups (n = 10/group). All the subjects received 5 g of fish oil daily containing 1.5 g eicosapentaenoic acid (EPA) and 1 g docosahexaenoic acid (DHA), and a capsule containing different doses of dl-alpha-tocopherol (0, 100, 200, or 400 mg/day) for 3 months. Plasma vitamin E and fatty acid levels and in vivo (delayed-type hypersensitivity skin response [DTH] and T cell subpopulation analysis) and ex vivo (mitogen-stimulated peripheral blood mononuclear cell [PBMC] proliferation and IL-2 production) immune functions were determined at baseline and after supplementation. The authors concluded that the immuno-enhancing effect of vitamin E in the elderly is dampened when it is concomitantly consumed with fish oil. This may be due to the smaller increase in plasma concentrations of vitamin E in the presence of fish oil [72].

The available literature concerning the efficacy of systemic antioxidant substances such as vitamins, carotenoids, and vitamins, specifically C and E, is very extensive, but the results are often contradictory. For vitamin E, promising photoprotective effects were reported, specifically when it is combined with other antioxidants. However, more controlled studies especially on oral applied vitamin E in humans are needed before it can be recommended as an effective nutricosmetic agent for improved health and fitness.

MICRONUTRIENTS

Zinc is one of the most used ingredients in nutricosmetics. Besides its described effects on the skin, a meta-analysis including four RCTS suggested potential benefits of zinc supplementation as a stand-alone intervention or as an adjunct to conventional antidepressant drug therapy for depression. However, there are methodological limitations in existing studies, and so further well-designed, adequately powered research is required [73].

Selenium is another commonly used nutricosmetic ingredient, mostly claimed to have photoprotective effects. In a

large RCT, 974 men were randomized to either a daily supplement of 200 μg of selenium or a placebo. Patients were treated for a mean of 4.5 years and followed for a mean of 6.5 years. Selenium treatment was associated with a significant (63%) reduction of prostate cancer (relative risk, RR = 0.37, $p = 0.002$). There were significant health benefits also for total cancer mortality and the incidence of total, lung, and colorectal cancer; however, no protective effects against the squamous and basal cell carcinomas of the skin could be observed [74].

The effects of a choline-stabilized orthosilicic acid on the skin and hair in humans was investigated, and the results are described in Chapter 54.

Recently, the Panel on Dietetic Products, Nutrition and Allergies concluded that a cause-and-effect relationship has not been established between the consumption of a combination of lycopene, vitamin E, lutein, and selenium and protection of the skin from UV-induced (including photooxidative) damage [8].

FATTY ACIDS

Omega-3 fatty acids [75] are micronutrients with known antiaging effects such as reduced wrinkle formation by protecting the skin against the damaging effects of UV light exposure. Oenobiol ("La beauté qui vient de l'intérieur") has been on the market since 1985 with its first product called "Fortepa 500," an essential fatty acids-based hydrating capsule for female consumers.

COLLAGEN, GLUCOSAMINE, AND CHONDROITIN

One study assessed the incidence of total joint replacement (TJR) during the long-term follow-up of patients with knee osteoarthritis (OA) formerly receiving treatment with glucosamine sulfate or placebo. Knee OA patients participating in two previous randomized, placebo-controlled, double-blind, 3-year trials of glucosamine sulfate and receiving treatment for at least 12 months were systematically contacted to participate in a long-term follow-up retrospective assessment of the incidence of total knee replacement. Out of 340 patients with at least 12 months of treatment, 275 (81%) could be retrieved and interviewed for the present evaluation: 131 formerly on placebo and 144 on glucosamine sulfate. There were no differences in baseline disease characteristics between groups or with the patients lost to follow-up. The mean duration of follow-up was approximately 5 years after trial termination and treatment discontinuation, making up a total of 2178 patient-years of observation (including treatment and follow-up). Total knee replacement had occurred in over twice as many patients from the placebo group, 19/131 (14.5%), than in those formerly receiving glucosamine sulfate, 9/144 (6.3%) ($p = 0.024$, chi-square test), with a relative risk that was 0.43 (95% CI: 0.20 to 0.92), that is, a 57% decrease compared with placebo. The Kaplan–Meier log-rank test survival analysis confirmed a significantly decreased ($p = 0.026$) cumulative incidence of total knee replacements in patients who had received glucosamine sulfate. A pharmacoeconomic analysis in a subgroup of subjects suggested that patients formerly on glucosamine sulfate had recurred to less symptomatic medications and use of other health resources than those from the placebo group during the last year of follow-up. The authors concluded that treatment of knee OA with glucosamine sulfate for at least 12 months and up to 3 years may prevent TJR in an average follow-up of 5 years after drug discontinuation [76]. An extensive indirect comparison meta-analysis determined the effect of glucosamine, or chondroitin, or both combined joint pain and on radiological progression of disease in OA of the hip or knee. Direct comparisons within trials were combined with indirect evidence from other trials by using a Bayesian model that allowed the synthesis of multiple time points. Ten trials in 3803 patients were included. On a 10-cm visual analogue scale (VAS), the overall difference in pain intensity compared with placebo was −0.4 cm (95% CI: −0.7 to −0.1 cm) for glucosamine, −0.3 cm (95% CI: −0.7 to 0.0 cm) for chondroitin, and −0.5 cm (95% CI: −0.9 to 0.0 cm) for the combination. For none of the estimates did the 95% credible intervals cross the boundary of the minimal clinically important difference. Industry-independent trials showed smaller effects than commercially funded trials ($p = 0.02$ for interaction). The differences in changes in minimal width of joint space were all minute, with 95% credible intervals overlapping zero. Therefore, the authors concluded that compared with placebo, glucosamine, chondroitin, and their combination do not reduce joint pain or have an impact on narrowing of joint space. Health authorities and health insurers should not cover the costs of these preparations, and new prescriptions to patients who have not received treatment should be discouraged [77].

Glucosamine appears to be the rate-limiting substrate for hyaluronic acid production in the wound. Adequate dietary protein is absolutely essential for proper wound healing, and tissue levels of the amino acids arginine and glutamine may influence wound repair and immune function. The botanical medicines *Centella asiatica* and aloe vera have been used for decades, both topically and internally, to enhance wound repair, and scientific studies are now beginning to validate efficacy and explore mechanisms of action for these botanicals. To promote wound healing in the shortest time possible, with minimal pain, discomfort, and scarring to the patient, it is important to explore nutritional and botanical influences on wound outcome [78].

BioCell Technology launched its BioCell Collagen as a nutricosmetic that promotes both active joints and younger looking skin. It is a chicken sternal cartilage derived, low molecular weight extract consisting of a matrix of hydrolyzed collagen type II and low-molecular-weight hyaluronic acid and chondroitin sulfate [79]. One pilot open-label study investigated the effect of BioCell Collagen in 26 healthy females who displayed visible signs of natural and photoaging in the face. Daily supplementation with 1 g BioCell Collagen for 12 weeks led not only to significantly increased skin properties but also to a significantly increased hemoglobin content (15%, $p = 0.008$) [80].

OA is an important source of pain and disability. One randomized, double-blind, placebo-controlled trial has investigated the efficacy of BioCell Collagen, in the treatment of OA symptoms. Patients ($n = 80$) had physician-verified evidence of progressive OA in their hip and/or knee joint. Joint pain had been present for 3 months or longer at enrolment. Subjects were divided into two groups and administered either 2 g of BioCell Collagen or placebo for 70 days. Outcome measurements included VAS for pain and Western Ontario and McMaster Universities Arthritis Index (WOMAC) scores taken on days 1, 35, and 70. Intention-to-treat analysis showed that the treatment group, as compared to placebo, had a significant reduction of VAS pain on day 70 ($p < 0.001$) and of WOMAC scores on both days 35 ($p = 0.017$) and 70 ($p < 0.001$). The BioCell Collagen-supplemented group experienced a significant improvement in physical activities compared to the placebo group on days 35 ($p = 0.007$) and 70 ($p < 0.001$). The authors concluded that BioCell Collagen can be considered a potential complement to current OA therapies [81].

CONCLUSIONS

In the literature, the results from clinical trials on the benefits (or harms) of nutricosmetics on skin, health, and fitness are still confusing. This can be partially explained by the diversity of the used research methods. For instance, in clinical trials on the effects of carotenoids, many different carotenoid concentrations are studied, with many different end points, trial durations, and seasonal differences, different types of subjects and patients, and different measurement instruments. Some studies were not controlled (i.e. single arm prepost study design), while in other studies, it was not clearly described if it was a randomized clinical trial and/or if allocation was concealed or if participants, researchers, or observers (e.g., statisticians) were blinded. Most studies were characterized by small sample sizes and hence may be underpowered. Furthermore, in the carotenoid trials, the role of oxidative metabolites and the isomerization status of the carotenoids may increase the heterogeneity of the study results. Another real threat is the so-called "internal research" of manufacturer laboratories, not willing to publish the trial results in peer-reviewed journals. Together with the fact that small nonsignificant trials tend to be less published, this may lead to publication bias. Therefore, large and high-quality (randomized) clinical trials with low risk of bias are needed to further the evidence for the use of nutricosmetics in health and fitness. Industry itself realized that clearly documented and independent scientific research findings should be published [5]. This is also of crucial importance to be successful as a manufacturer of nutricosmetics, especially in the European market with well-informed critical consumers.

It is important to understand the science behind nutricosmetics as they are increasingly becoming popular as skin care products in Europe and Japan. Many compounds of commercial nutricosmetics also show promising health- and fitness-enhancing properties. Phenolic compounds and vitamins, for example, are strong antioxidants. However, depending on their concentrations, promising antioxidants may behave as prooxidants. Also, the low antioxidant methylxanthines may reduce the antioxidant effects of flavonoids in mixed formulations or even start to behave as prooxidants. This may induce unintended side effects. It can be argued that the concentrations of some of the bioactive compounds (such as the vitamins) in commercial nutricosmetics may be too low to have an effect on health and fitness. Marketing offices of manufacturers are ahead of their scientific departments. Nutricosmetic specific studies are lacking. Most studies are in vitro or in vivo animal studies on oral supplements. High-quality, large randomized controlled trials in humans and meta-analyses including such high-quality RCTs are needed before nutricosmetics can be recommended to be used for health and fitness.

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56 Normal Nail and Use of Nail Cosmetics and Treatments

Josette André, Christel Scheers, and Robert Baran

THE NORMAL NAIL

Anatomy

The nail plate, also abbreviated as ''nail,'' is a hard keratin plate slightly convex in the longitudinal and the transverse axes. It is set in the soft tissues of the dorsal digital extremity, from which it is separated by the periungual grooves (proximal, lateral, and distal; Figure 56.1) [1]. It stems from the nail matrix located in the proximal part of the nail apparatus. The nail plate and matrix are partly covered by a skin fold called the proximal nail fold (PNF). The lunula, also known as ''half-moon,'' is a whitish crescent, visible at the proximal part of some nails and more specifically those of the thumbs and big toes. It corresponds to the distal part of the matrix. From the latter, the nail plate grows toward the distal region sliding along the nail bed to which it adheres closely and from which it only separates at the distal part, called hyponychium.

Two other structures deserve our attention:

1. The cuticle, which is the transparent horny layer of the proximal nail groove. It adheres to the nail surface and acts as a seal between the nail plate and the PNF. Its disruption favors paronychia (periungual inflammation).
2. The onychodermal band or onychocorneal band, which is ''orangey,'' is located in the distal region of the nail. It provides a zone of rugged attachment of the nail-to-nail bed. As for the cuticle, disruption of the onychocorneal attachment will severely affect the nail function, leading to onycholysis (detachment of the nail from its bed).

The upper surface of the nail plate is smooth while the under surface is corrugated with parallel longitudinal grooves that interdigitate with opposite ones of the nail bed surface, enhancing the adhesion of the nail plate to the nail bed.

Physicochemistry

The nail is highly rich in keratins, especially in hard keratins, which are close to those of hair and have a high content of disulfide linkage (cystine) [1].

Sulfur represents 10% of the nail's dry weight; calcium represents 0.1% to 0.2%. The latter, contrary to conventional wisdom, does not intervene in the nail toughness. Lipid content (particularly cholesterol) is low in nails: from 0.1% to 1% compared with 10% in the stratum corneum of the skin. Water concentration varies from 7% to 12% (15%–25% in the stratum corneum), but the nail is highly permeable to water: when its hydration level increases, it becomes soft and opaque, and when its hydration level drops, it becomes dry and brittle.

Studies carried on nail permeability are important for the development of cosmetic and pharmaceutical products specifically devoted to nails. As a permeation barrier, it has been shown that the nail plate reacts like a hydrogel membrane, unlike the epidermis that reacts like a lipophilic membrane. Molecular size also influences nail penetration.

The normal nail is hard, flexible, and elastic, which gives its good resistance to the microtraumatisms it undergoes daily. Those properties are attributable to the following factors: the regular arrangement and important adhesion of onychocytes, the high sulfur-containing keratins and their regular orientation, and the hydration level of the nail.

Physiology

The nail growth is continuous. In a month, fingernails grow about 3 mm and toenails grow about 1 mm [1].

The origin of the nail plate production is still a debatable topic. Most studies agree and show that at least 80% of the nail plate is produced by the matrix. It should be added that the main source of nail plate production is the proximal part of the matrix.

The nail plays an important role in everyday life. It protects the distal phalanx from the risks of traumatisms. It provides counter-pressure to the pulp, which is essential to the tactile sensation involving the fingers. The nail allows scratching in case of itching and can be used as means of attack or defense. Finally, the esthetic importance of the nail should not be forgotten.

THE MANICURE

The art of manicure is very ancient as can be testified by the Egyptian tomb of Niankhkhnum and Khnumhotep, dated approximately 2400 years BC. They were "Manicurist and Overseer of the Manicurists in the Palace, King's Acquaintance and Royal Confidant" [2].

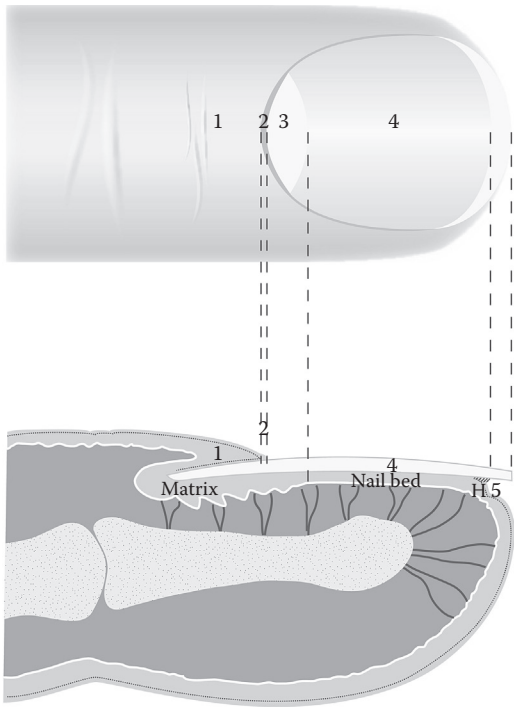

FIGURE 56.1 Normal nail: 1. Proximal nail fold; 2. cuticle; 3. lunula; 4. nail plate; 5. distal nail groove. H: hyponychium. The small dots (…..) represent the stratum granulosum.

Currently, a professional manicure is made up of several steps: the first one consists of removing any nail enamel remaining on the nail plate. A cotton ball soaked with nail polish remover is used. *Nail enamel removers* dissolve nitrocellulose and remove lipids from the nail plate. They mainly contain a mixture of organic solvents, with small amounts of oils added to counteract the drying effect of the solvents.

Typical formula [3]:

Solvents (~98%); examples are acetone, butyl or ethyl acetate, and ethoxyethanol.
Lipids (~2%); examples are castor oil and lanolin oil.

Other additives can also be found such as dyes, fragrances, preservatives, vitamins, and ultraviolet (UV) absorbers. Nail polish removers should not be used more than once a week and should not be left too long in contact with the nails and the skin. Correctly used, they most probably do not cause nail brittleness [4]. Nail polish removers specifically for brittle nails are also marketed. These are mainly nail polish removers without acetone. Actually, they are not better but are odorless. They often contain alpha butyrolactone [5]. Next, the nail is given the desired length, shape, and surface. Therefore, the nail is cut and filed. Trimming should be performed after showers in order to decrease mechanical trauma [6].

The cuticle is then covered with *cuticle removers*, which can be a liquid, gel, or cream [7]. They usually contain sodium hydroxide and potassium hydroxide, in a 2% to 5% concentration. α-Hydroxy acids (1%–5% lactic acid, pH 3–3.7) are also used. They attack keratin by disruption of the disulfide bonds of cystine. Cuticle removers contain various additives: emollients (lanolin) or humectants (glycerin, propylene glycol) whose purpose is to decrease evaporation, increase viscosity, and reduce irritation. Preservatives, perfumes, and thickening agents can also be added.

Typical formula [3]:

Water (~90%).
Softening agent (1%–5%); an example is potassium hydroxide.
Thickener (0.5% to 1%); examples are sorbitol and magnesium aluminum silicate.
Perfume (0.1%).

The cuticle remover increases the softening of the cuticle and of the cuticle remnants, which adhere to the nail plate surface. These are then gently pushed back, with an orangewood stick covered with cotton or a rubber-ended stick (Figure 56.2). The distal and lateral nail grooves are cleaned. It is most important to avoid overaggressive cleaning beneath the free edge of the nail. Finally, hang nails are cut with a special nipper.

Cuticle softeners are simply emollients to which quaternary ammonium compounds or urea are sometimes added to promote softening of the cuticles. These latter can then be gently reversed with the fingers.

Creams for brittle nails are best applied after moistening the nail plate. Indeed, when a nail has been hydrated by immersion, they prevent dehydration, maintaining and increasing nail flexibility, especially if they contain phospholipids [8].

Unwanted Effects of Manicure

Excessive pumicing or buffing can cause thinning of the nail plate, and repeated traumatisms on the nail matrix area can cause leukonychia striata (transverse white streaks) (Figure 56.3) or Beau's lines (superficial transverse grooves) [9]. The destruction of the cuticle leads to chronic paronychia [6]. The caring of the distal nail groove can cause onycholysis (Figure 56.4), and the caring of the periungual fold can cause small sores [10]. The damage to the cuticle, the onycholysis, and the periungual sores favor microbial and mycotic infections. The risk of transmitting infections in nail salons should not be neglected. Mycobacterial furunculosis were repeatedly reported with footbath spas in nail salons [11,12],

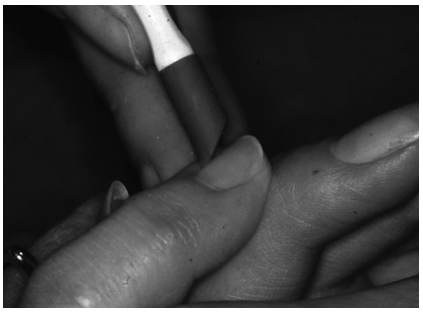

FIGURE 56.2 Rubber-ended stick to push back the cuticle remnants.

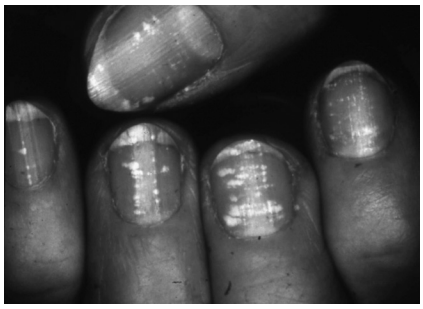

FIGURE 56.3 Leukonychia striata caused by overzealous manicure.

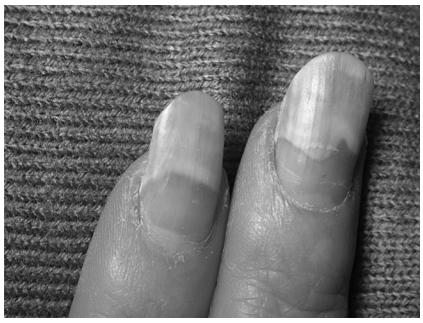

FIGURE 56.4 Onycholysis caused by excessive caring of the distal nail groove.

and one case of fatal infective endocarditis was observed in a patient with prosthetic valve, after pedicure [13]. Abrasive files can spread verruca [12]. Some studies incriminate professional but also intrafamilial manicure as a risk factor for hepatitis C virus infections [14,15]. Precise standards of disinfection should be observed [10]. After contact with polish removers, the skin may have a white scaly appearance caused by stratum corneum dehydration. Repeated application leads to irritant dermatitis, brittle nails, and onychoschizia [9].

NAIL POLISH (SYNONYMS: NAIL VARNISH, NAIL ENAMEL, NAIL LACQUERS)

The existence of present nail polishes is linked to the discovery of nitrocellulose properties and to the progress made in the early 1920s in the automobile paint industry [16].

The perfect nail polish has to be easy to apply, and it should dry quickly. As well, it should leave a shiny, smooth, even, hard, and flexible film, able to last 5 days. Moreover, this polish should be removed without leaving any trace and should not have any side effects. Finally, it should be stable in the bottle and should offer a wide range of colors enabling one to get the expected esthetic effect.

Composition

The main constituent of the film that remains on the nail after drying (evaporation) is *nitrocellulose* [10]. The nitrocellulose film has a lot of qualities. In particular, it is hard, tough, stable, and waterproof but is not sufficiently adherent nor glossy or flexible.

To improve adherence and gloss, *film modifiers* are added. The most common one is Santolite or toluene sulfonamide/formaldehyde resin (TSFR), best designed under its International Nomenclature of Cosmetic Ingredients (INCI) name: tosylamide/formaldehyde resin. As TSFR is a potent sensitizer and contains formaldehyde, it tends to be replaced, for example, by phthalic anhydride/trimellitic anhydride/glycols copolymer, especially in hypoallergenic varnishes (PTGC) [17]. Other film modifiers are alkyd resins, acrylates, vinyls, or polyesters [18]. *Plasticizers* are added to increase flexibility. Dibutyl phthalate and camphor were the most frequently used, but dibutyl phthalate, although declared "safe as used" in the United States by the Cosmetic Ingredient Review Expert Panel, tends to be eliminated by nail polish manufacturers it is classified as carcinogenic, mutagenic or toxic for reproduction (CMR) Category 2 on the EU Dangerous Substance List, and is no longer used in European cosmetics [10]. Other examples are castor oil, tricresyl phosphate, and glyceryl tribenzoate [10,18].

In the nail polish bottle, there are also solvents and thinners, pigments and dyes, thixotropic agents, as well as other additives such as sunscreens to protect the nail varnish from discoloration, that is, benzophenone-1 and octocrylene. The most commonly used *solvents* are alkyl esters (ethyl, n-butyl acetate) and glycol ethers (propylene glycol monomethyl ether). These molecules, which have different boiling points and evaporation rates, allow the regulation of drying time. They also allow to lower viscosity, which improves brushability. *Thinners* are not real nitrocellulose solvents, but they are miscible with them. This allows reduction of the nail varnish price. Thinners also help regulate the evaporation rates and stabilize viscosity. Indeed, a polish low in viscosity is easy to apply and leaves a homogeneous film. If viscosity is too low, the coverage of the film will be insufficient. If viscosity is too high, the film will be thick and streaky. Thinners essentially are aliphatic alcohols such as ethanol, isopropanol, butanol, and aromatic hydrocarbures such as toluene. The latter, which is now suspected to be cancerogenous, teratogenous, and an air contaminant, tends to be replaced in new nail lacquer formulations [10]. However, in Europe, toluene is considered "safe from a general toxicological view" in nail cosmetics, up to an amount of 25%, for adult use only (CMR category 3). It is recommended to keep it out of children's reach. *Pigments and colorants* also need to draw the attention. Some are soluble in nitrocellulose and originate transparent polishes, very slightly colored. Most of them are not soluble and originate the most used polishes: the nail enamels [16]. Coloring agents such as organic colors can be selected from a US FDA-approved list of certified colors. Inorganic colors and pigments may also be used but must conform to low heavy metal content standards [9].

Example:

Mineral pigments: ferric ferrocyanide (Prussian blue), titanium dioxide (TiO_2)
Organic pigments: D&C Red 6,7,34/FD&C Yellow 5

Prussian blue is used in small amounts to enhance blues and alter other shades. Titanium dioxide allows the attainment of

pastel shades. D&C Red 6 is a barium lake, D&C Red 7 and 34 are calcium lakes, and D&C Yellow 5 is a zirconium lake, a lake being formed by precipitating a particular pigment with aluminum hydroxide to form a salt complex [10]. In pearlescent nail varnishes, there are guanine crystals, derived from scales of Atlantic herring and other fish, bismuth oxychloride, or mica coated with TiO_2. They are all used to enhance light reflection.

Since a lot of pigments are not soluble, manufacturers had to cope with major problems of precipitation until *thixotropic agents* were discovered in the 1960s [16]. Thixotropic agents increase nail enamel viscosity at rest, thus preventing pigment precipitation. They, however, become fluid as soon as a mechanical constraint is exerted by shaking either the brush or the bottle itself. This can be further improved by adding small metallic (Ni) beads or preferably plastic ones in the bottle. Stearalkonium hectorite is the most frequently used thixotropic agent.

Theoretical composition of a nail varnish with 15% nitrocellulose, 7% resin, and 7% plasticizers is shown in Table 56.1.

Besides classic nail polishes, base coats, topcoats, hardeners, varnishes for brittle nails, varnishes for ridged nails, and hypoallergenic nail polishes are also marketed. All are nail varnishes with a slightly modified formulation.

The *base coats* contain more resin because they must increase the adherence of the varnish to the nail (Table 56.1). They contain no colorant to act as a protective antistain barrier between the nail plate and the shaded varnish [10].

On the contrary, the *topcoats* contain more nitrocellulose and plasticizers because they must be tough and flexible to improve nail varnish resistance (Table 56.1). Cobalt may be added in the topcoat to give a glitter color and may be responsible for contact dermatitis [19]. Base coats and topcoats also contain different proportions of thinners and solvents to ease application and to speed up drying.

TABLE 56.1
Nail Polish: Typical Formula

Ingredients (Approximate Concentration, in%)	Nail Polish	Base Coat	Topcoat
Nitrocellulose	~15%	↘	↗
TSFR	~7%	↗	↘
Plasticizer	~7%	↘	↗
Solvents–diluents	~70%	#	#
Butyl acetate	1.5%–52%		
Ethyl acetate	4%–42%		
Isopropanol	2.7%–7.7%		
Toluene	20%		
Suspending agent	~1%		
Color pigment mixture	0.1%		

Notes: Comparison with base coat and topcoat. TSFR, toluene sulfonamide formaldehyde resin; ↘, lower concentration; ↗, higher concentration; #, different concentrations.

Sources: Reprinted from *Unwanted Effects of Cosmetics and Drugs Used in Dermatology*, 3rd ed., de Groot AC et al., Nail cosmetics, pp. 524–529, Copyright 1994, with permission from Elsevier. Adapted from Sainio EL et al., Engström K, Henriks-Eckerman ML, Kanerva L. Allergenic ingredients in nail polishes. *Contact Dermatitis*, 37(4), 155–162, 1997.

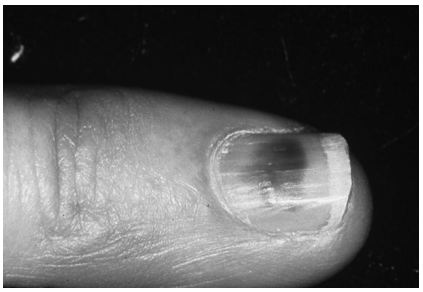

FIGURE 56.5 Subungual hemorrhage caused by formaldehyde-based hardener.

Hardeners, varnishes for brittle nails, varnishes for ridged nails, etc., are generally base coats to which nylon fibers, acrylic resins, or formaldehyde is added. Nail lacquers containing 10% urea lead to subjective improvement in the nail cosmetic appearance. Nevertheless, no relevant changes were detected in nail thickness nor water loss [20]. In hardeners, formaldehyde is in its free state, which is not to be confused with formaldehyde in the form of the TSFR in nail polishes [21]. Formaldehyde is believed to cross-link protein in the nail plate, which increases surface hardness but decreases flexibility. In the long term, it may eventually be the cause of nail rigidity and cracks. Severe nail damage such as paronychia, nail bed involvement with subungual hyperkeratosis, onycholysis, or hemorrhages have been attributed to formaldehyde-based hardeners (Figure 56.5). Lip hemorrhages are also possible in nail biters [21]. Moreover, the presence of formaldehyde, which acts as an irritative agent, would favor TSFR sensitization [22]. The presence of formaldehyde is now limited to 5%, and formaldehyde-containing hardeners are meant to be applied only to the free edge of the nail [21]. Calcium, vitamins, sulfured amino acid, and collagen can be added to the treating nail varnishes. These have probably no value.

Use

After the manicure, the nail is degreased, dried, and covered with one layer of base coat, two layers of the colored nail enamel, and one layer of topcoat. The layers have to be thin and as uniform as possible. The wearing of varnish is recommended 5 out of 7 days. The "French nail manicure" is a popular technique, which consists of applying a pink color nail varnish to the nail plate and a white color to the free edge to give the nail a natural and healthy aspect.

Nail Polish Advantages

There are many advantages of nail polishes. They have an esthetic advantage and play a protective role by forming a film on the nail surface. They also maintain a more constant degree of nail hydration. In pathology, nail lacquers allow chromonychia and onycholysis to be hidden.

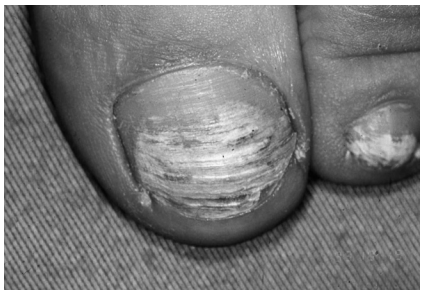

FIGURE 56.6 Keratin granulations. (Courtesy of B. Richert, Belgium.)

Unwanted Effects of Nail Polishes

They do exist but are relatively rare. The nail polishes can cause an *orange staining of the nail plate*, prominent at the distal part [10]. It can be prevented by the former application of a base coat. Nail lacquers can cause *keratin granulations* (Figure 56.6), presenting as superficial friability [9]. This happens when individuals apply fresh coats of enamels on top of old ones for several weeks. Nail varnishes can also cause *allergic contact dermatitis*, more rarely contact urticaria [3]. Eczema breaks out especially on the eyelids, on the lower half of the face, on the sides of the neck, and on the upper chest. Allergic airborne contact dermatitis should be suspected when lesions on the face, neck, and ears are symmetrical. Periungual dermatitis or widespread dermatitis are also possible [23,24]. In a recent study conducted by Warshaw et al. [25], allergy to nail products was found in 10% of all females allergic to cosmetics. In this study, TSFR was responsible for 6.1% of cosmetic contact allergy in females. More than half of women allergic to nail products reacted to TSFR, followed by methyl methacrylate and ethyl acrylate [25].

Allergy to TSFR may be suspected in case of nonelucidated lips and tongue swelling mimicking angioedema or in case of worsening of eczema in atopic children [26,27].

ARTIFICIAL NAILS

Bringing very long nails into fashion may have originated from China [28]. The first artificial nails appeared in the United States, around 1935. Since then, thanks to their improving quality, they have become more and more popular. In 2004, annual business in nail salons was estimated at $6.84 billion in the United States [6]. Artificial nails were almost exclusively applied by professionally trained manicurists in nail salons. However, professional nail applications are expensive, and artificial nail kits designed for home use are now available over the counter [29].

Composition and Techniques

To make things easier, two different kinds of artificial nails can be considered: the sculptured artificial nails and the preformed artificial nails. In the *sculptured nail technique*, the nail technician will really sculpture an artificial nail on the client's nail. This artificial nail is made of an acrylic resin obtained by blending a methyl, ethyl, or isobutyl methacrylate monomer, which comes in a liquid form, and a polymethyl or ethyl methacrylate polymer, which is powder. The methyl methacrylate monomer has been banned by the FDA since 1974 because of its side effects, but it is still used especially in discount price salons. Indeed, this monomer, which smells terrible, is cheap and allows one to work quickly [30]. The monomer also contains a stabilizer such as hydroquinone and N,N-dimethyl-p-toluidine as an accelerator. The polymer also contains benzoylperoxide as a polymerization initiator.

Typical formula [3]:

Liquid: acryl-type monomer (~99%); an example is methyl methacrylate monomer.
Stabilizer (~1%); an example is hydroquinone.
Powder: acryl-type polymer (~97%); an example is polymethyl methacrylate.
Polymerization initiator (~3%); an example is benzoylperoxide.

Other components such as plasticizers, solvents, accelerators, pigments, sunscreens, and titanium dioxide may be included [31].

The nail plate surface is pumiced. After disinfection, a metallized paperboard template is placed to frame the new nail, and a primer is painted on the nail (Figure 56.7). The latter is a highly acidic solution, most commonly of methacrylic acid with a pH as low as 2. It acts as a double-sided sticky tape. To avoid its toxicity, new acid-free primers, which are not corrosive, have appeared as well as newer artificial nail formulation, which no longer requires a nail primer [32]. Next, several layers of the acrylic paste that has just been blended are applied (Figures 56.8 and 56.9). After hardening at room temperature, which occurs rapidly, the template is removed and the nail is pumiced (Figure 56.10), filed, and buffed, producing a long, smooth, attractive nail. This sculptured nail technique is rather difficult to perform. However, it can be used on a seriously damaged nail surface. Sculptured nails can be dissolved in acetone.

The second technique uses *preformed plastic tips*, which are packaged in several shapes and sizes, adapted to the different fingernails (Figure 56.11). The nail plate surface is

FIGURE 56.7 A metallized paperboard template is placed. Then a primer is painted on the nail.

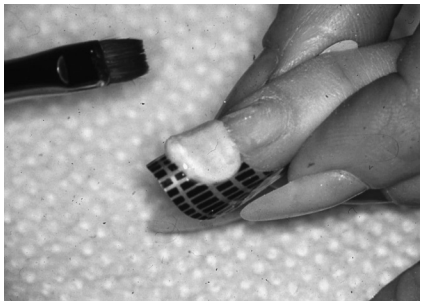

FIGURE 56.8 A white paste is first applied on the template.

FIGURE 56.9 A pinker paste is applied on the natural nail and the template.

FIGURE 56.10 The sculptured nail is pumiced.

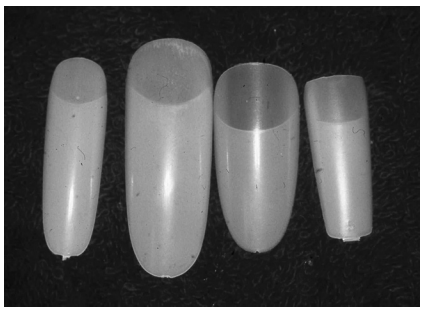

FIGURE 56.11 Preformed plastic tips.

buffed. After disinfection, the preformed plastic tip is simply fixed with cyanoacrylate glue, on the distal part of the nail (Figure 56.12).

Typical formula of cyanoacrylate glue [33]:

Ethyl cyanoacrylate (ECA): 90.6%
Polymethyl methacrylate: 9%
Hydroquinone: 0.4%
Stabilizer (organic sulfonic acid): trace

Plasticizers and thickening agents may be added.

Then, the artificial nail, which is too long, is cut with a special "guillotine clipper" (Figure 56.13). It is also filed and buffed to give it the desired shape and length. At this stage, the nail surface is not smooth; it presents a bump between the proximal natural nail and the distal artificial nail (Figure 56.14). An acrylic gel is therefore painted on the nail, like a nail polish (Figure 56.15). It will harden, in other words, polymerize, after UV exposure. This technique is much easier to learn than the sculptured nail technique but can only be applied on a nail surface that is almost normal. Entire colored or decorated plastic tips may be fixed on the whole nail plate with cyanoacrylate glue. The system is cheap

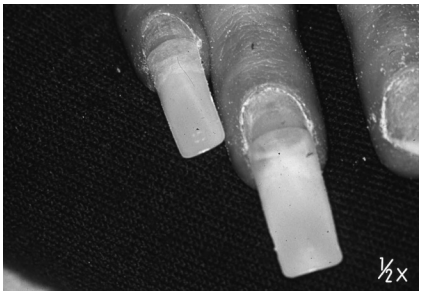

FIGURE 56.12 Preformed plastic tips glued on the distal part of the nail.

FIGURE 56.13 The artificial nail is cut with a special "guillotine clipper."

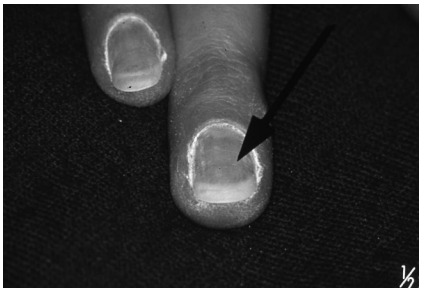

FIGURE 56.14 Bump (arrow) between the proximal natural nail and the distal artificial nail.

FIGURE 56.15 An acrylic gel is painted on the nail.

and easy to use (do-it-yourself kit) for special occasions but has to be removed after a maximum of 2 or 3 days [32].

Nail Gels

Gels are a variant of sculptured nails. They are made of a mixture of acrylic monomers and polymers directly provided by the manufacturer. They are more and more popular because they are odorless, give a more natural aspect to the nails, and do not require irritant (meth)acrylic acid as a primer.

New gel formulations are regularly marketed, but there are mainly two types of gels:

1. Acrylic light-cured gels in which the polymerization or hardening is obtained by exposure of the successive gel layers to light (most often to UV). These gels may contain (meth)acrylated urethanes, triethyleneglycol dimethacrylate, methacrylated epoxy resin or hydroxyfunctional methacrylates, and a photoinitiator [34]. The bonding is similar to "restorative dental bonding" commonly used by many dentists worldwide [33]. It should be pointed out that acetone will have no effect on UV gels. Waiting nail growth is the best solution to remove UV gel nails. If a patient becomes allergic, the artificial nails must be grinded off with heavy abrasive [32]. "Gel polishes" partly removable with acetone have recently appeared (Shellac CND, Axxium by OPI). They are applied in nail salons. Their marketed benefit is that they dry quickly, give a 2-week perfect manicure with a large panel of colors, and then can be removed with acetone and manual peeling [35].
2. Cyanoacrylate gels in which the polymerization is obtained by spraying or brushing an activator. An example is ethyl-cyanoacrylate gel, with N,N-dimethyl para-toluidine as activator.

There are gels with different consistencies, designed for different uses, with the consistency being determined by the resin-to-monomer ratio. There are also colored gels. According to their composition, the gels can be used for different purposes [32]:

- For the sculptured nail technique. However, they do not allow producing as long and resistant nails than the classical liquid-powder technique.
- Over plastic tips as we have just seen.
- To protect a natural or polished nail. This procedure, which is actually the most frequent, is known as nail capping.
- Capping with fabric (silk, linen, fiberglass) allows repairing broken nails and is known as nail wrapping.

Other Nail Adornments

Stick-on nail dressings (press-on polish, synthetic nail covers, nail wraps) are thin colored or decorated synthetic films coated on one side with a heat-sensitive adhesive backing that fixes them firmly to the nails [10].

Artificial Nail Advantages

Artificial nails are much more resistant than natural ones; nail polishes easily remain on them for 3 weeks. They allow refinement and fantasy, which are much superior to those obtained with nail enamels. They can be decorated with jewels. Genuine work of art can be realized with special air brushes (nail art) [29] (Figure 56.16).

Artificial nails are mainly used for brittle or broken nails and for onychophagia. However, artificial nails should not be recommended in onychophagia. Indeed, periungual sores so frequent in nail biters could favor acrylic sensitization. In pathology, they allow to hide more serious dystrophies than the nail polishes, such as posttraumatic permanent nail dystrophy (Figures 56.17 and 56.18) or racket nails. After big toe nail shedding in a tennis player or a skier, an artificial nail

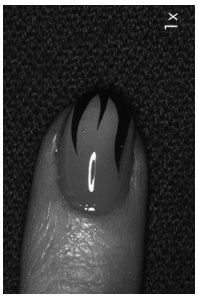

FIGURE 56.16 Air brushes made "nail art."

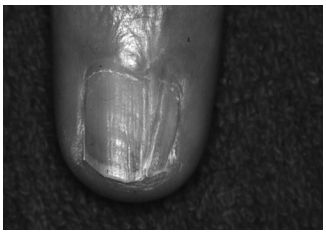

FIGURE 56.17 Posttraumatic permanent nail dystrophy.

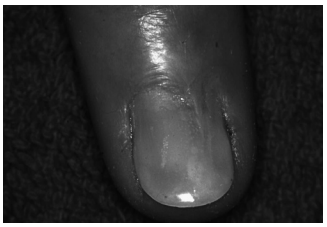

FIGURE 56.18 Posttraumatic permanent nail dystrophy as seen in Figure 56.17, after application of an artificial nail. Note the small sore in the lateral nail groove.

can prevent the hypertrophy of the periungual soft tissues, allowing a painless nail regrowth. Artificial nails should not be recommended in case of nail psoriasis or lichen planus because they can worsen the condition by Koebner phenomenon [32].

Unwanted Effects of Artificial Nails

Artificial nails are expensive and time consuming. It takes about 1 h to put a set of 10 artificial nails in place, and they must be taken care of every 2 to 3 weeks. As the natural nail continues to grow, the proximal part of the artificial nail must be refilled. The adhesion between the natural and the artificial nail must also be checked: it must remain watertight.

Side effects of artificial nails can be classified as nonallergic reactions and allergic or toxic reactions [7].

Nonallergic Reactions

Technical errors or bad care causes brittleness of the natural nail by excessive filing or pumicing and inadequate use of the primer. After 2 or 4 months of wear, it is not unusual for a sculptured nail to damage the underlying natural nail. The problem may well not be the acrylic nail materials but rather the thinning of the nail due to overfiling with heavy abrasives. Small periungual sores (Figure 56.18) are frequently observed. They could favor acrylic sensitization.

The penetration of water between the natural and the artificial nail is a frequent complication in artificial nails, which shows up as superficial nail plate discoloration (Figure 56.19). This side effect is well known by nail technicians who remove the artificial nail, buff the nail surface to make the discoloration disappear, and sculpture a new, tightly adherent, artificial nail.

Irritant reactions to monomers are possible. They lead to subungual hyperkeratosis or onycholysis. Sensation of nail bed tightness may be related to excessive shrinkage of gel enhancement products [9].

Wearing of too long nails favors onycholysis as well as nail fracture. It impairs finger and hand performance [36].

Infections

The wearing of too long nails, the onycholysis, the periungual sores, and the penetration of water favor mycotic and microbial infections. These can be severe, especially in diabetics and immunocompromised people [37]. Three cases of *Pseudomonas* corneal ulcers after artificial fingernail injuries have been described [38]. In health care workers with artificial nails, it has been shown that there is an increased amount of carriage of pathogen [39,40], and this is observed both before and after washing the nails [41]. Nosocomial infectious outbreaks have been described where nurses wearing artificial nails could have played an important role in the transmission of potentially lethal infections caused by *Serratia marcescens* [42,43] or *Pseudomonas aeruginosa* [44,45]. It should be added that individuals wearing artificial nails tend to wear their nails longer [41] and are more careful about their nails when washing their hands.

There is a recent concern that repeated exposure to UV nail light might favor *nonmelanoma skin cancers* on the hands [46], but the risk seems very low [47,48] and patients may probably be reassured regarding the safety of the devices [49].

Allergic or Toxic Reactions

Contact dermatitis to artificial nails can affect the client and less frequently the manicurist [34,50]. Here, unlike nail-lacquer dermatitis, paronychia accompanied by onycholysis or subungual dermatitis is more likely to be present. Eyelid dermatitis is frequently associated. Women are usually not aware that artificial nails are a possible cause of allergy, and diagnosis is often delayed [51,52]. When the artificial nail use is discontinued, it is worth noting that it takes several months for the nails to return to normal.

Sculptured Nails

The most frequent allergen is the (meth)acrylate monomer, whereas the polymer is considered to be a weak sensitizer. Sensitization seems to be primarily caused by the monomer, which remains unpolymerized, in the final sculptured nail and in the filing dust, produced when the sculptured nail is trimmed [34]. This is specially observed with self-curing resins, but, even in photo-bonded acrylic nails, monomer also persists. The nail technicians should apply thin successive gel layers and should expose each layer to UV, which is rarely done. Moreover, UV bulbs are not changed frequently enough [32]. Allergic contact-type reactions were first described with methyl methacrylate monomer, but other monomers (e.g., ethyl and butyl methacrylates) can induce sensitization and cross-reactions also occur [33,53]. They may be even stronger sensitizers than methyl methacrylate [54]. The allergic reaction usually starts 2 to 4 months, and even as long as 16 months, after the first application [29]. The first symptom

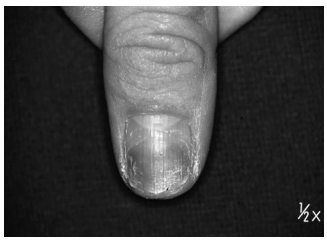

FIGURE 56.19 Superficial nail plate discoloration due to bad adherence between the normal and the artificial nail.

is an itch in the nail bed, followed by painful paronychia, which can be associated with paresthesia. Nail bed hyperkeratosis or onycholysis is frequently observed. Distant allergic contact dermatitis may affect the eyelids and the face, but more widespread lesions are also possible [55]. Six cases of occupational asthma due to ethyl methacrylate have been reported in cosmetologists working with artificial nails [29]. Rhinitis has also been described [56]. Exceptional cases of severe paresthesia evolving for several years were described [57]. These were accompanied by Raynaud's-like syndrome and permanent nail loss [53]. They could result from a direct, toxic effect on the cutaneous nerves, with patch tests remaining negative in one case [58]. Prolonged paresthesia of the finger tips was also observed with "photo-bonded" acrylic sculptured nails [33], and two natural nails had to be surgically removed because of resistant superinfections [34].

Cases of allergy to dental or orthopedic acrylates may be explained by previous sensitization to acrylates in artificial nails. History of allergy to artificial nails should be looked for in patients before dental treatments or orthopedic surgery [59–61]. In contrast to the manufacturers' declarations, all "hypoallergenic" products continue to include acrylate functional monomers and therefore continue to cause allergic sensitization [34].

Sculptured nails and UV gels share many ingredients but are sufficiently different that they will not necessarily cross-react [32].

Cyanoacrylate Nail Preparations

Cyanoacrylates do not usually cross-react with the (meth) acrylate monomers used in nail preparations [31,50,53], although in a study performed by Koppula et al. [62], ECA did cross-react with several acrylates. Patients allergic to ECA can usually wear acrylic artificial nails without problems [63].

The cyanoacrylate glue, used either for nail wrapping or with plastic tips, can be responsible for eczema of the fingertips, with nail involvement [51,64] (Figure 56.20). Eyelid dermatitis can be present as well as a nummular eczema particularly over the dorsal hand [65]. More widespread eruption was also described [51]; one case mimicked a small plaque parapsoriasis eruption [66]. Persistence of the dermatitis until the nails grow out is frequently observed and is probably caused by retained adhesive [51].

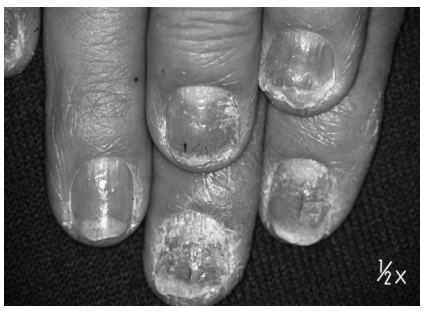

FIGURE 56.20 Severe psoriasiform alterations of the nail plates caused by artificial nail allergy.

Patch Testing

Constandt et al. [67] demonstrated that two allergens, that is, 2-hydroxyethyl methacrylate (2-HEMA) and ethyl-2-cyanoacrylate (ECA), could be sufficient to screen contact allergy but proposed a series of 10 acrylates to advise patients. Other authors suggest a screening with three allergens: 2-HEMA, ethylene glycol dimethacrylate (EGDMA), and triethylene glycol diacrylate (TREGDA) [30,68,69]. Latex or vinyl gloves are not protective for occupational allergic contact dermatitis. Nitrile gloves may protect but only for a short time and have to be changed many times a day [67].

Methacrylic Acid-Containing Primers

Nail care products are a common cause of accidental poisoning in children. Such products accounted for 198,084 exposures (16% of exposures to cosmetics and personal care household products) reported to the American Association of Poison Control Centers in 1997 [70]. Methacrylic acid-containing primers are particularly involved. *Dermal burns* were mainly reported, but burns of the airway and gastrointestinal tract with residual esophageal dysfunction were also described [71]. Of the 759 exposures, 84.9% had occurred at home, expressing the recent trend toward home use of artificial nail products, previously restricted to professional cosmeticians in nail salons.

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57 Impact of Formula Structure to Skin Delivery

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INTRODUCTION

In the past 50 years, despite the availability of intensive literature in the field of the impact of formula structure to skin delivery, there is still no satisfactory conclusion, with many contradictory findings and views. A rational treatment of the impact of formula structure to skin delivery should consider the following three aspects: the structure and activity of the active substance, the formula and the structure of the skin, and the interaction.[1]

It is critical to deliver the right active ingredient to the right location at the exact concentration for the correct period of time. The extent (and therefore the concentration) of this delivery depends on the formulation. Proper formulation to ensure the efficacy of the active ingredient delivered became more important with the legislation in the United States and the European Community, requiring experimental evidence for any claimed cosmetic activity.

In this chapter, we will cover the impact of formula structure, including delivery systems to the skin and the noninvasive methodologies in measuring real skin delivery and efficacy.

IMPACT OF FORMULA STRUCTURE TO SKIN DELIVERY

Cosmetic formulations are generally quite complex mixtures in different forms and with different structures. More and more forms of cosmetic products are developed and introduced into the market. The basic cosmetic product forms are listed in Table 57.1.

Structure

Based on Table 57.1, one can expect that the formulation structure varies greatly in different formats. A useful concept into cosmetic formulation structure was found in traditional Chinese medicine, which has proven its usefulness for more than thousands of years. The formula is systemically classified into the following four roles based on the functionality: leader, minister, assistant, and deliverer (Table 57.2), regardless of the format (aerosols, liquids, or solids). The "leader" indicates the active to be delivered, the "minister" is the main structure (body) of the formula including but not limited to emollient and emulsifier, the "assistant" indicates necessary components assisting the body structure-like preservative, and the "deliverer" guides the leader (active) to the target site. The leader can only be delivered to the target site under the help of the minister and the assistant via the deliverer.

With the clear systematic structure in mind, the following questions are generally asked before starting to design a cosmetic formula:

- What do you want to deliver and where do you want to deliver (leader)?
- What are the physicochemical (e.g., polarity [hydrophilic or lipophilic]) properties of the active and formula (leader and minister)?
- What is the impact of the emulsifier and emollient (minister)?
- Are other ingredients present, such as preservatives and fragrance (assistants)?
- What is the stability of the delivery system in the formula (minister, deliverer)?
- Is there a difference in delivery amount vs. delivery speed (leader, deliverer)?
- How are efficacy and tolerance of formulation related to delivery profile (deliverer)?

It then becomes easier to discuss the impact of individual function parts in the formula to skin delivery.

Leader

Active ingredients have been around in cosmetics for a long period of time, but whether they actually resulted in active cosmetic products remains in question. In order to achieve this, the right active needs to be delivered to the right location at the exact concentration for the correct period of time.

The physicochemical characteristics of the penetrating active ingredient are the most important factors that determine the rate of the skin delivery. Common characteristics of an ideal active of skin delivery are an octanol/water partition coefficient of 1–2, a molecular weight <500 μm, and nonionized species at skin pH. Ionized molecules do not penetrate membranes.[3,4]

Minister: Emulsifier and Emollient Effects

The extent (and therefore the concentration) of skin delivery depends on the formulation base. You can design the formula to achieve a lipophilic or hydrophilic product, and some

TABLE 57.1
Formulation Types and Structure

Forms	Characters	Key Components	Product Examples
Solutions	Homogeneous mixture of soluble ingredients	Water-soluble ingredients	Baby shampoo
Gel	Clear thick products, "shear thinning" property	Gelling agent: acrylic polymer, a natural gum, or a cellulosic thickener	Body washes, shaving products
Suspensions	Solutions with visible particles	Water-soluble ingredients and visible particles	Shower gel with beads
Serum/concentrates	Usually low-viscosity lotion with highly concentrated active	Water, oil as any other cream	Eye treatment serum, night repair serum
Lotion/cream	Pseudo-stable mixture of immiscible liquids dispersed in another liquid; lotion is thinner than cream	Including an oil phase, aqueous phase, and an emulsifier(s)	Facial moisturizers, leave-in hair conditioners, and moisturizing cleansers
Ointments	Super thick products without water	Usually contain petrolatum, lanolin, and dimethicone	Hairdressing and medicated skin products
Films/stick	Mostly materials are solid at room temperature, blended, and pressed into shape	Film-forming agent: PVP, acrylates, acrylamides, and copolymers	Water-dissolvable, preservative-free antiwrinkle film
Powders	Fine powder mixture	Talc, silicates, and starch	Baby powder, makeup powder
Aerosols	Spray the product evenly as fine particles	Pressurized can, propellant, and nozzle setup	Volatile organic compounds; regulations have reduced the use of aerosols in cosmetic products
Soft capsules	You will need special equipment to create these products. They are generally more expensive	Served as second packaging to protect/isolate the content; could be serum or gel	Antiaging eye treatment

Source: Romanowski P., The 10 different types of cosmetic formulas you must know. Available at http://chemistscorner.com/the-10-different-types-of-cosmetic-formulas-you-must-know/. Accessed May 16, 2013.

Note: PVP, polyvinylpyrrolidone.

extreme examples are water solution and ointment. Both formats are relatively simple.[3]

Cosmetic formulations are generally quite complex mixtures. Emulsions are a common format in cosmetic products because of the application acceptability and excellent solubilizing capacities for lipophilic and hydrophilic active ingredients. This will be used as an example to explain the emulsifier and emollient effects.

One can regulate the delivery of an active molecule (and therefore the efficacy of a cosmetic formulation) by selection and control of the emollient system. Using different emulsifier systems could also have an impact on steering the active ingredient to the right layer of the skin. The emulsifier determines the distribution profile of the active ingredient within the skin.[5] Emulsion constituents, such as emollients and emulsifiers, should be selected carefully for optimal efficiency of the formulation.

TABLE 57.2
LMAD of Formula Structure

Roles	Ingredient Example
Leader	Active
Minister	Emulsifier, emollients
Assistant	Preservative, sensory component (fragrance, skin feel component)
Deliverer	Delivery system, enhancer

Note: LMAD: leader, minister, assistant, and deliverer.

A two-step process could improve skin delivery of active ingredients. The first step involves selecting a primary emollient with a polarity similar to that of the active ingredient, which will have a high solubility. The second step is to reduce the solubility of the active ingredient in the primary emollient via the addition of a secondary emollient with the opposite polarity (lower solubility for the active ingredient). This approach produced a three- to fourfold increase in skin penetration without increasing the amount of active ingredient in the formulation.[6]

Furthermore, skin delivery can be influenced by the interactions that may occur between the vehicle and the skin or the interactions between the active ingredient and the skin.

To better understand the influence of emulsion on skin delivery, the physicochemical properties of the formulation after application should also be considered. When emulsifiers interact with the lipid crystallinity of skin lipids and influence the skin membrane structure, this results in a higher permeability to penetrating molecules.[6] This should be considered carefully, while most of the studies focus on the characteristics of the formulation before application.

Emulsifiers arranging in liquid crystalline structures in the water phase of the emulsion enhance the skin penetration of the active ingredients (hydroquinone [HQ] and octadecenedioic acid [DIOIC]), which could be attributed to an

increased partitioning of the actives into the skin. It was hypothesized that the interaction between the different emulsifiers and active ingredients in the formulations varied, and therefore, the solubilizing capacities of the various emulsifiers and their association structures did the same.[7,8]

Droplet

It has been recognized that the vehicle in which a permeant is applied to the skin has a distinctive effect on the skin delivery of active ingredients. The skin absorption may be enhanced, for example, by an increase in thermodynamic activity, supersaturation, and penetration modifiers. The type of emulsion (water in oil [W/O] vs. oil in water [O/W]), the droplet size, and the surfactant organization (micelles, lyotropic liquid crystals) in the emulsion may affect the skin delivery.[9]

A majority of emulsions, especially those in skin care, do not conform to the traditional definition of emulsions as consisting of two liquids only. In reality, most emulsions, in addition to the drops of a second liquid, contain dispersed material such as liquid crystals and solids.[10] Also, even the simplest emulsions undergo radical structural changes during evaporation, and these changes have a decisive bearing on the effect on the skin.

Many reports showed that the smaller the droplet size, the better the skin delivery. In contrast to this, when the surfactant concentration in the aqueous phase was kept constant, there is no influence of emulsion droplet size on the skin penetration within the droplet size range studied (1 μm–100 nm) for some actives.[11] It should be noted that many reports on the influence of emulsion droplet size on the skin delivery were based on emulsions that, apart from droplet size, also differed in composition and/or system components. A more common and practical approach in the cosmetic industry is to reduce the droplet size to enable the formula to deliver the active into a deep layer of skin. Table 57.3 outlines particle size variations for different consumer products with varied results as end points.

Assistant

Assistants either help to preserve the product during the whole shelf life or provide desire for sensory feeling, such as a pleasant odor or skin feeling "silky" after application. Generally, we should not expect the impact to skin delivery from the assistant.

One exception that is interesting to mention here is that some essential oils (EOs) and their terpene constituents are potential penetration enhancers (PEs). There is currently little control on the topical use of most terpenes, and many aromatherapy oils and formulations contain appreciable quantities of these enhancers. Their excessive use offers potential for permeation of hazardous compounds from the same formulations into the skin; some terpenes also have pharmacological activity.[12] Okabe et al.[13] reported in 1989 that eucalyptus oil and camphor increase the total flux of nicotine. The percutaneous absorption of indomethacin has been promoted by the use of limonene and related compounds.

The role of lemon EO was investigated in enhancing the penetration of alpha-tocopherol (vitamin E) and retinyl acetate (vitamin A), and pyridoxine (vitamin B6) and ascorbic acid (vitamin C), released from O/W or W/O emulsions.

D-limonene, alpha-pinene, and p-cymene when incorporated in a W/O (or O/W) emulsion enhanced the flux of vitamins B6, C, and A through skin by a factor of 4.1, 3.4, and 5.8, respectively. In the presence of lemon EO, the penetration of vitamin E was enhanced by ninefold. Lemon EO produced only reversible modification of transepidermal water loss (TEWL), indicating that it is a safe and effective PE for topical administration of lipid- and water-soluble vitamins.[14]

Deliverer

The literature contains reports describing various elegant formulations that may contain materials that have penetration-enhancing activity. For example, vesicles are often prepared from phospholipids; phospholipids themselves have some penetration-enhancing activity. Oleic acid has been shown to be effective for increasing the flux of salicylic acid (SA) 28-fold.[4,8]

Before moving to the delivery system part, it has to be pointed out that the effect of penetration modifiers could be multidirectional. For example, the effect of dimethyl isosorbide and diethylene glycol monoethyl ether on the solubility of the active ingredients (HQ, SA, and DIOIC) in the skin was counteracted by a simultaneous reduction in the thermodynamic activity in the formulation.[7,15]

However, the main obstacle in the deliverer's path would be the barrier properties of skin to control entry of microbes and foreign particles. In order to overcome the barrier properties and enhance delivery of topical actives, a variety of enhancement strategies have been extensively researched over the last two decades. Penetration enhancement has been achieved by both passive and active techniques. PEs are defined as agents capable of modifying the skin barrier, thereby influencing topical penetration of actives. Modification of the barrier could be a simple result of hydration by occlusion influencing penetration or chemical/physical interactions, thereby altering barrier properties. Over the last decade, numerous chemicals have been screened for their penetration enhancement properties, and only a handful have successfully been incorporated into formulations.[16]

TABLE 57.3
Classification of Products with Different Particle/ Droplet Size

Size	Product Examples
1000 nm	Cleansers, sun protection, color cosmetics, exfoliators
100 nm	Sunless tanning, moisturizers
100 nm	Hair growth modulators
10 nm	Antiaging
1–10 nm	Nerve system modulation, pain relief, prescription systemic drugs

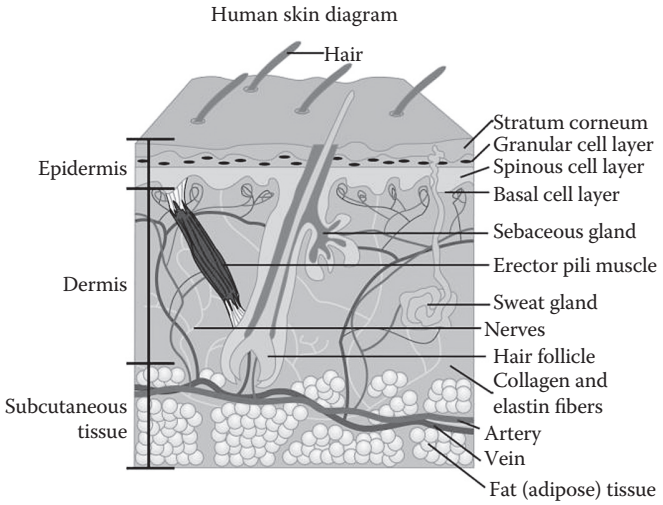

FIGURE 57.1 Skin cross section indicating different areas for topical delivery. (Courtesy of Shutterstock, Skin Image—© Anita Potter.)

Apart from including chemicals as PEs within formulations, delivery systems have been employed to act as carriers for actives into the skin. In 2011, Kline estimated that the market of delivery systems in personal care is EUR 81 million ($105 million). The US market for delivery systems is growing rapidly, as it has increased from $19 billion in 2000 to over $41 billion in 2007 and still growing.[17]

Figure 57.2 shows a basic classification of commonly employed delivery systems for topical actives with emphasis on cosmetic actives. Apart from classification, this section will outline the pros and cons of cosmetically relevant delivery systems, as well as listing market products as examples.

Liposomes

Liposomes are small, spherical lipid pockets encompassing the active of interest. They are differentiated as unilamellar or multilamellar based on the number of lamellae and size. They can further be classified into different forms based on the structure of the vesicle (niosomes, transferosomes, sphingosomes, ethosomes, etc.).

Liposomes enable encapsulation of a wide range of actives, as well as flexibility, to be controlled for their size and morphology. Actives can be encapsulated within the aqueous space or intercalated in the lipid bilayer, depending on its polarity and the composition of lipids. Liposomes have shown great potential as drug delivery systems. An assortment of molecules, including peptides and proteins, has been incorporated in liposomes, which may then be administered by different routes.[18] Resulting from the use of biocompatible lipids, liposomes found wide acceptance for intravenous delivery but have shown promising results for other routes of application. Use of liposomes in skin gels or creams is widely pursued in the field of cosmetics, as they favor disposition of encapsulated active ingredients in the epidermis and dermis while controlling systemic permeation. Also, compatibility of phospholipids from liposomes highly minimizes erythema and irritation. Vegetable phospholipids are widely used for topical applications in cosmetics and dermatology, since they have a high content of esterified essential fatty acids, especially linoleic acid, which is believed to increase the barrier function of the skin and decrease water loss within a short period of time after application. Soya bean phospholipids or other vegetable phospholipids, because of their surface activity and their ability to form liposomes, are also an ideal source for possible transport of linoleic acid into the skin.

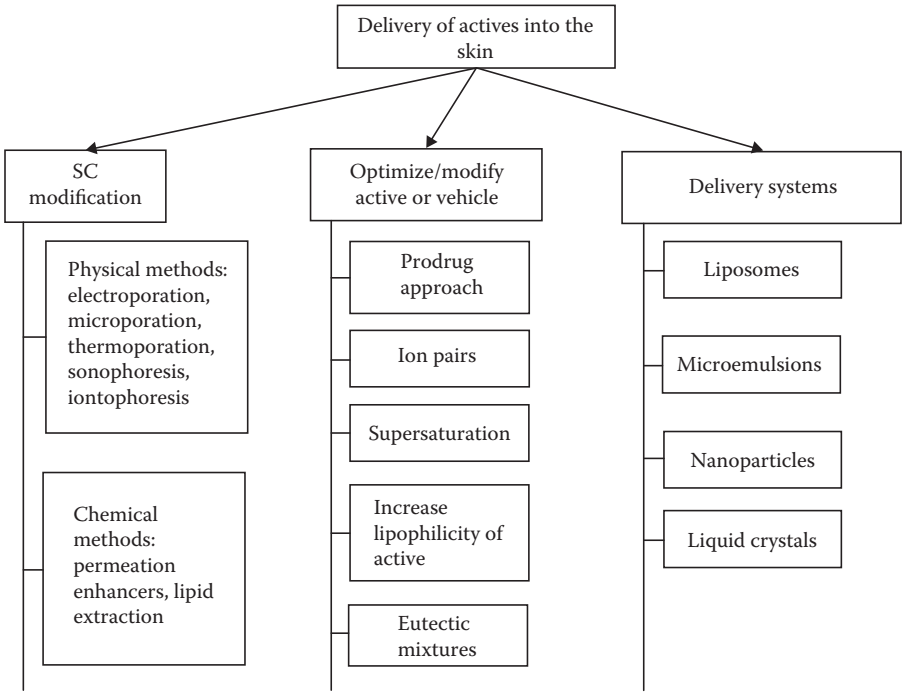

FIGURE 57.2 Classification of delivery systems for enhanced penetration of actives into skin. Abbreviation: SC, stratum corneum.

Egbaria and Weiner[19] discussed the potential use of liposomes derived from soya bean phospholipids in cosmetics.

Some companies have extensively researched nonionic lipid-/surfactant-containing formulations as enhancing agents for a variety of antiaging, antiacne, antidandruff, hair growth, depilatory, and depigmentation actives for delivery into the skin, as well as hair follicles and sebaceous glands. Delivery enhancement of these systems was manifested with minoxidil as the active and hair regrowth in mice as the efficacy result. Also measured, was the amount of minoxidil deposited in the pilosebaceous units of mice posttreatment with and without the delivery systems. In vivo studies performed on mice were accounted for hair regrowth stimulation/inhibition during the dosing period with further confirmation from histology. Results proved that formulations with one or more nonionic lipids generated long anagen follicles (i.e., follicles in the growth phase of the hair growth cycle), whereas the mice treated with commercial minoxidil formulations demonstrated follicles only in the telogen phase similar to the untreated mice. Table 57.4 lists the number of days required for the formulations to initiate hair regrowth in mice.

As shown in Table 57.4, formulation with minoxidil in the novel nonionic lipid vesicles demonstrated a rapid onset of hair growth and performed substantially better in stimulating hair growth compared with commercial solutions of 2% or 5% minoxidil.

Table 57.5 depicts the results from in vitro studies of formulations containing vesicles with one or two nonionic lipids, which deliver over three times the amount of minoxidil into sebaceous glands compared with commercial formulations containing minoxidil vehicle solution, indicating that the delivery systems presented in the related patent are significantly effective.[20]

Microparticles

Microparticles/microspheres have been conventionally described as powders consisting of biodegradable proteins or synthetic polymers <200 μm in size. Critical ingredients constituting microspheres are polymers (synthetic/natural) into which the active ingredient is loaded by means of physical entrapment, chemical linkage, and surface adsorption. Most commonly used synthetic polymers are polyalkylcyanoacrylates, polylactic acid, polyadipic anhydride, and natural polymers including albumin, starch, and gelatin. Porous microspheres intended for topical use are commonly referred to as "microsponges," comprising interconnected voids ranging from 5 to 300 μm in size. Microsponges can entrap a wide variety of ingredients (actives, fragrance, EOs, etc.), and can be postadded into commonly employed skin care formulations, such as creams, lotions, and powders, and release them into the skin over time in response to the precise trigger (rubbing, temperature, pH, etc.).[21]

Microsponge-containing products technically exhibit enhanced performance in different ways, such as greater oil absorption or reduced skin irritation as a result of their sustained release ability. They also improve formulation flexibility by improving physical and thermal stability of the active and aid in converting liquid actives into powders enabling material processing and enhancing the final product esthetic/quality.

Examples of marketed products comprising microparticles/microspheres as gels or microsponges are Retin-A-Micro (Valeant Pharmaceuticals, Bridgewater, NJ) for the treatment of acne vulgaris, Carac fluorouracil cream (Valeant

TABLE 57.4
Number of Days to Initiate Hair Regrowth by Different Formulations In Vivo

Formulation	Black Skin	Spotty Hair	Full Coat
Commercial 2% minoxidil solution, mean (SD)	25.6 (0.5)	>26	>26
Commercial 5% minoxidil solution, mean (SD)	11.5 (1.0)	17.0 (4.8)	23.0 (2.4)
Untreated	>26	>26	>26
2% minoxidil in lipid, mean (SD)	9.2 (1.8)	12.8 (1.1)	16.0 (1.4)
Commercial 2% minoxidil in 2% ketoconazole, mean (SD)	9.6 (0.7)	12.4 (2.6)	16.0 (3.2)
Positive control, mean (SD)	12.0 (2.0)	14.7 (1.2)	18.7 (1.2)

Note: SD, standard deviation.

TABLE 57.5
In Vitro Results from Pig Ear

Ear Strata (% Radiolabelled Formulation [μg])	2% Minoxidil in Commercial Solution, Mean (SD)	2% Minoxidil in One Nonionic Lipid Formulation, Mean (SD)	2% Minoxidil in Two Nonionic Lipid Formulations, Mean (SD)
Sebaceous glands	0.18 (0.04)	0.12 (0.02)	0.13 (0.05)
Ventral dermis	0.0 (0.0)	0.02 (0.0)	0.02 (0.0)
Cartilage	0.02 (0.01)	0.05 (0.01)	0.05 (0.02)
Dorsal ear	0.01 (0.0)	0.04 (0.01)	0.04 (0.01)

Pharmaceuticals) for the treatment of actinic keratosis, and antiperspirants (Aramis Fragrances, London, UK) for a controlled release of fragrance with moisture and temperature.[22]

Nanoparticles

Lately, delivery by nanoparticles has been explored by newer structures such as solid lipid nanoparticles (SLNs) and nanostructured lipid carriers (NLCs). Several studies have demonstrated that these carriers were significantly better performers compared with liposomes. The ability of NLCs to provide enhanced skin hydration and bioavailability and stabilize the active with controlled occlusion enables them to be identified as potential next-generation cosmetic delivery vehicles. Apart from NLCs, nanocrystals and nanoemulsions are also being investigated for cosmetic applications.

SLNs and NLCs are typically composed of biodegradable lipids with a particle size <500 nm, which ensures close contact to the SC. Better hydration of the SC is observed because of the occlusion caused by the lipids employed in these carrier systems, thereby enhancing topical delivery of encapsulated actives. Higher occlusive factors for SLN in comparison with NLC of the same lipid content have been identified by Souto and Müller.[23] Comparing NLCs with different oil content showed that an increase in oil content leads to a decrease in the occlusive factor. Skin hydration after applying SLNs or NLCs leads to a reduction of corneocyte packing and an increase in the size of the corneocyte gaps facilitating the percutaneous absorption and drug penetration to deeper layers of skin.[23] Shah et al.[24] studied tretinoin-loaded SLN with respect to skin irritation, such as erythema, peeling, and burning, as well as increased sensitivity to sunlight, contributing to decreased patient compliance. In the in vitro permeation studies, through rat skin, they found that SLN-based tretinoin gel has a permeation profile comparable with that of the marketed tretinoin cream. But on the other hand, a Draize patch test showed that SLN-based tretinoin gel resulted in a remarkably less erythema response compared with the currently marketed tretinoin cream. Conclusively, applying SLN or NLC may enhance skin penetration of incorporated actives, promote the epidermal targeting, and minimize the systemic side effects, resulting in better patient acceptance.

A few commercially available products with SLNs or NLCs include Allure perfume (Chanel Fragrance) for extended fragrance release, Nanorepair cream and lotion (Dr Rimpler, GmbH, Germany), and Super Vital products (Amorepacific).[25]

However, dermal toxicity of nanoparticles is currently the subject of attention by safety organizations. Nanoparticles with smaller particle size often pose a risk for higher absorption, especially in cosmetic products such as powders, foundations, and concealers. Significant research is being performed by both academia and industry for materials to be deemed as safe to use. This research, along with better regulation and reporting, will enable consumers to choose products with confidence, in turn, allowing companies to benefit from these novel technologies while retaining customer confidence.[26]

Microemulsions/Nanoemulsions

Microemulsions are defined as clear, isotropic oil and water combinations that are thermodynamically stable. The key difference between emulsions and microemulsions is that the former are thermodynamically unstable with a larger particle size and are highly prone to phase separation over time. Physical appearance is another contrasting feature with emulsions appearing cloudy, whereas microemulsions are clear and translucent. Significant buzz has been generated over the past decade for microemulsions as a potential delivery system because of their optical clarity, as well as ease of preparation.[23] Microemulsions as cutaneous delivery vehicles are becoming popular for their ability to solubilize hydrophilic and lipophilic actives. Review by Kogan and Garti[27] includes data from studies demonstrating better permeation of actives from microemulsions compared with conventional emulsions. Strong interactions between surfactants that occur in the interfacial membrane film limit the mobility of the drug between the internal and external phases within conventional emulsions. In addition, emulsions are not stable formulations, and strong fluctuations in bioavailability were detected. However, in microemulsions, the cosurfactant assists in lowering the interfacial tension of the surfactant film, resulting in spontaneous formation of a microemulsion, thereby promoting its thermodynamic stability. This process of drug diffusion across the flexible interfacial surfactant film between the phases of the microemulsion can increase partitioning and diffusion into the SC.[27]

NONINVASIVE AND MINIMALLY INVASIVE MONITORING OF TRANSCUTANEOUS DELIVERY OF ACTIVE AGENTS

It is highly desirable to monitor noninvasively active agents in the skin tissues during and after topical delivery in order to evaluate delivery efficiency and to establish a correlation with efficacy. It is also valuable to know the precise delivery pathways, particularly for certain topical treatments, such as acne and postinflammatory hyperpigmentation (e.g., acne mark), with which follicular delivery is intended. Therefore, there has been a continuous search on the methods to quantify the active agents delivered transcutaneously with minimal disruption or disturbance to the skin.

The noninvasive monitoring methods can be divided into the following two groups based on the techniques of acquiring samples or signals: (1) mechanical sampling and chemical analysis, as represented by the tape stripping method, and (2) spectromicroscopy/spectrophotometry-based methods.

Tape Stripping

Tape stripping refers to a method in which the SC is consecutively removed in predetermined procedures and times using a chosen adhesive tape, and the removed tissues on the adhesive tapes are then submitted to chemical analysis for quantification for the actives. This is a useful and minimally

invasive technique to study the penetration, the distribution, and the disappearance profile of topically applied cosmetic products and drugs within the SC. Jacobi et al.[28] investigated a mechanical method by estimating the relative SC amount removed by tape stripping. It was found that the total amount of SC removed by tape stripping was dependent on a variety of parameters, such as the type of adhesive tape used and the vehicle used for application, as well as the number of tape strips. Interpatient and interseasonal differences in the SC structure also play an important role. Because of these influencing parameters, different amounts of the SC may be removed with single tape stripping from the same patient, in spite of following a standard protocol. Using an adhesive film on the flexor forearm skin of human volunteers, 60 to 100 tape strips completely removed the SC based on the amount of corneocytes on each moved tape strip determined by the corneocyte's pseudo-absorption by ultraviolet (UV)/visible spectrometry. Stepwise, 10 tape strips removed about 35% of the total SC of the forearm skin, another 10 tape strips (a total of 20) removed an additional 30% (mean [SD], total SC = 66% [12%]), and 10 more tape strips (a total of 30) further removed about 10% (total SC = 84% [11%]). Approximately 90% of the SC was removed with a total of 40 strips, and 95% with 50 strips. Therefore, the complete removal of the SC may be necessary if one wants to correlate the removed amounts of topically applied substances to their position within the SC.

This tape stripping protocol was used to evaluate the skin penetration of a UV filter, butyl methoxydibenzoylmentane, in two emulsion formulations. Tape stripping started 1 h after the application of the emulsions. Approximately 53 μm/cm^2 of the compound was recovered in the first 10 tape strips, only about 1/10 of the compound in the first batch was found in the second 10 tape strips, and about 1/5 of that in the second batch in the next 10 tape strips; this trend continued for the next two batches.

Lademann et al.[29] summarized the critical parameters for the tape stripping method, including site of application, type of tape, application of the tape strip, application pressure, and velocity of removal. The following methods for quantification of SC removal were discussed: weighing, protein content, optical spectroscopy, microscopy, and TEWL. Different formulations may strongly influence the amount of SC removed. Penetration profiles of a screen active, Parsol 1789, and a corticosteroid, clobetasol propionate, were evaluated with the tape stripping method 1 h after topical application. Because the sunscreen is meant to cover the skin surface for protection from the sun, the formulation should minimize the skin penetration of the active and retain the sunscreen in the upper part of the SC. On the other hand, since the corticosteroid is intended to reach the target living tissues, the formulation should enable the active to penetrate across the SC layers. The results presented in the paper demonstrate the effects of formulations for the intended penetration profiles. For the sunscreen, >60% of the total recovered drug from tape stripping was found in the first 3% of the SC on the tape strips, 90% cumulative drug from 12% SC, 97% cumulative drug from 21% SC, and no sunscreen detected beyond 32% SC. In contrast, the corticosteroid formulation showed a much deeper drug penetration profile across the barrier membrane with a progressive decrease in the following amounts of drug recovered: 18% cumulative drug from the first 2% SC, 51% cumulative drug from 10% SC, 75% cumulative drug from 20% SC, 88% cumulative drug from 40% SC, 94% cumulative drug from 60% SC, and 98% cumulative drug from 80% SC. The result confirms the value of tape stripping for the assessment of skin penetration of topical formulations.

A recent paper by García Ortiz et al.[30] evaluated bioequivalence of three marketed topical metronidazole creams with using the tape stripping method in comparison with an invasive method of in vivo dermal microdialysis sampling in healthy human volunteers. Nine microdialysis probes were inserted in the volar aspect of the left forearm of 14 healthy volunteers, and following application of the three metronidazole creams, microdialysis samples were collected for 5 h. On the right forearm, tape strip sampling was performed 30 and 120 min after product application. There was no statistical difference in penetration of the three topical formulations as determined by microdialysis. However, their bioequivalence could not be determined because of interpatient variability exceeding the criteria for bioequivalence evaluation. Tape strip sampling established a bioequivalence between two of the creams but rejected any bioequivalence between these two formulations and the third. The third formulation was a generic formulation approved despite containing a lower concentration of metronidazole (0.75%) than the innovator formulation (1.0%). It was concluded that the result of the bioequivalence evaluation would depend on the methodology employed. The fact that a rather simple and noninvasive method of tape stripping could yield as meaningful results as those from much more complex and invasive methods of dermal microdialysis indicates the value of the tape stripping method for evaluation of skin permeation of active agents.

Spectromicroscopy/Spectrophotometry Methods

Various spectromicroscopy/spectrophotometry methods have been reported to track and visualize skin penetration of compounds, including spectrofluoremistry,[31,32] diffuse reflectance spectroscopy (DRS),[33] confocal laser scanning microscopy,[34] infrared,[35] attenuated total reflectance Fourier transform infrared spectroscopy,[36] and confocal Raman microscopy.[37–40]

For the active agents that are either fluorescent themselves or can be fluorescently labeled, spectrofluorimetry methods may readily be used to monitor penetration and deposition of test compounds into various skin tissues. Stamatas et al.[31] demonstrated that fluorescence spectroscopy could be used to quantify the accumulation of a fluorescent compound on a biologic tissue in vivo. The limitation is that interference from native skin fluorescence should be minimal at the wavelengths of interest. Using SA as a model compound, the authors investigated its skin deposition after topical application using a fluorescence method in comparison with the

tape stripping method. In this study, a total of three sets for experiments were performed. In the first set of experiments, SA solutions of selected concentrations were applied to the skin of volunteer's forearms, and fluorescence measurements were taken to establish the quantitative relationship between the amount of applied compound and fluorescence signal intensity. A mathematic model based on a diffusion approximation theory was utilized to correct fluorescence measurements for the attenuation caused by endogenous skin chromophore absorption. In the second set of experiments, a known concentration of SA solution was applied to a volunteer's skin, followed by recovering the dosed compound with tape stripping of 20 times for chemical analysis by high-performance liquid chromatography (HPLC). Similarly, the skin sites of volunteers were dosed with five known concentrations of SA solutions first, followed by one tape strip to recover the compound for HPLC analysis. It was found that the SA quantities determined by the spectrofluorimetry method correlated well with the tape stripping HPLC method ($R^2 = 0.967$). Although for the same SA concentrations, the HPLC method resulted in significantly lower values than the fluorescence measurement. This method was also used to obtain SA profiles in the epidermis of the human skin after topical application with two different concentrations over a period of 3 days. The conclusions for the study on the major advantages of the spectrofluoremistry method include the following: (1) it is sensitive (low detection limit); (2) it is noninvasive; (3) it shows good reproducibility; and (4) it is fast and simple to implement compared with the methods available.

Recently, Ra et al.[32] reported an evaluation of a handheld dual-axis confocal (DAC) microscope developed by the authors' team that is capable of in vivo fluorescence imaging of skin, using both mouse models and human skin. The capacity of the DAC system was demonstrated to be able to visualize cellular architecture in mouse skin, to evaluate anatomic differences in human skin at different places in the body after topical delivery of near-infrared (NIR) dyes, and, most importantly, to demonstrate the ability of NIR dye-conjugated small interfering RNA (siRNA) to penetrate the SC barrier when applied topically to mouse skin in vivo. The value of this technology lies in its ability to noninvasively evaluate the active agent delivery and effectiveness in patient skin.

Gillies et al.[33] reported noninvasive in vivo determination of UVA efficacy of sunscreens using DRS. The DRS method essentially treats the sunscreen as a filter on the skin. The transmission spectrum of the sunscreen is obtained by measuring the change in absorption of the skin with and without the product. These measurements effectively uncouple the optical properties of the sunscreen from the biological responses of the skin. The study compared measurements of UVA efficacy of oxybenzone and avobenzone at different concentrations using DRS, human phototest, and an in vitro technique on human patients. All three techniques showed a linear response between calculated UVA efficacy and product concentration. It was concluded that DRS is a rapid and reproducible method to calculate UVA efficacy of sunscreen materials, and that its results correlate closely with those obtained by human phototesting.

Confocal Raman spectroscopy enables nondestructive depth scanning of skin tissues for individual changes of skin components or exogenous chemicals. As such, it is an ideal method to track a chemical agent's penetration or to monitor the bioconversion in the skin.[37–40]

Mélot et al.[39] investigated the use of confocal microscopy for in vivo skin penetration of trans-retinol with four different PEs in human patients. Raman measurements were performed up to 6 h after application of the test materials. Significant penetration enhancements were observed with formulations with or without enhancers. The differences between enhancers could also be detected.

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58 Natural Ingredients and Sustainability

Claude Saliou

INTRODUCTION

"Natural" is a "mega-trend" around the world across most consumer markets such as personal care, home care, and nutrition, but also in construction, transportation, and automotive. Consumers expect "natural products" or natural-based products to be safer, more effective, and better for the environment. In other words, "naturals" carry more trust from the consumers. Under this "mega-trend," several other trends or movements have sprouted; these include organic and biodynamic agriculture, fair trade, locally sourced, lifestyle of health and sustainability (LOHAS), recycling, etc.

Altogether, the global natural personal care market is estimated to be worth about $25 billion annually. This is a little less than 10% of the total global personal care market but still a very significant portion.

Defining "natural" in the context of cosmetic ingredients, and even more so for cosmetic products, is challenging, as there are nearly as many definitions on what constitutes a natural ingredient as there are brands marketing "naturals" or standards defining "naturals."

Technically speaking, there are two key criteria to consider: the origin of the feedstock, whether it is from nature or it is man-made, and the process used to produce the ingredient, whether it modifies the natural chemistry in such a way nature is incapable of. For instance, the tea leaves making black tea (*Camellia sinensis*) undergo a process of oxidation meant to release the catechins, which is followed by heating to stop that reaction. While in the course of this process there is a chemical modification of a fresh leaf composition, it is, however, one that would happen in nature without human influence. Black tea can duly be considered a natural ingredient.

Other considerations are also used to categorize an ingredient as natural or not: the renewability of its feedstock, its environmental friendliness, its safety profile, or even the potential abuse for marketing purpose (e.g., water is excluded from many natural standards to prevent intentional product dilutions, resulting in higher natural percentage—a practice known as "green-washing").

This chapter will start with definitions of the various terms used around naturals and an overview of some standards commonly used for personal care products. The main natural feedstocks used to make cosmetic ingredients will be presented, leading to sustainability opportunities and challenges in cosmetics.

DEFINITIONS

"Natural" means coming from or created by nature. From this perspective, botanicals, animals, minerals, and water are all products of nature and form the basis of natural feedstocks. Botanical means any terrestrial or aquatic flora, including algae and fungi. Minerals are substances occurring via geological processes and found in solid or solubilized form with a specific crystalline structure. Although petroleum (or tar), natural gases, mineral oil, and petrolatum are also products of nature, given their consistent exclusion in the various standards, they will also be excluded for the purpose of this chapter. So, the natural feedstocks discussed in this chapter will be those originated from either renewable materials (botanicals, microorganisms, and animals) or minerals. In considering how natural an ingredient is, one will need to assess the process to manufacture the ingredient, and whether this process alters intentionally or not the natural chemistry.

"Naturally derived" ingredients are raw materials made using a natural feedstock (Figure 58.1), which is intentionally modified using either another natural feedstock or one derived from petroleum to yield a chemical identical (or not) to one found in nature. An example of such ingredients is sodium lauryl sulfate (Figure 58.2), whereby the lauryl chain comes from hydrolyzed triglycerides from coconut oil or palm kernel oil, the sodium from sea salt converted into sodium carbonate or sodium hydroxide, and the sulfate from sulfuric acid or sulfur trioxide, an oxidized form of sulfur [1]. While sulfur can be obtained from natural sources, most of its production today comes as a by-product of petroleum or natural gas refining. Sodium laureth sulfate (Figure 58.3) is similar to sodium lauryl sulfate with the exception of the ethoxylate coming from ethylene, a petrochemical derivative.

These examples point to the complexity of defining a naturally derived ingredient and its natural portion. In fact, several formulas can be used to calculate the natural portion of a molecule: one is to strictly account for the carbons coming from a natural feedstock over the total carbons (this method will be reviewed in the discussion on "biobased" below); another one is to consider the portion of the molecular weight of the ingredient attributable to atoms coming exclusively from a renewable feedstock such as a plant, microorganism, or animal over the total molecule's molecular weight; last, one could consider every atom coming from nature, including minerals [2]. These various methods yield different natural

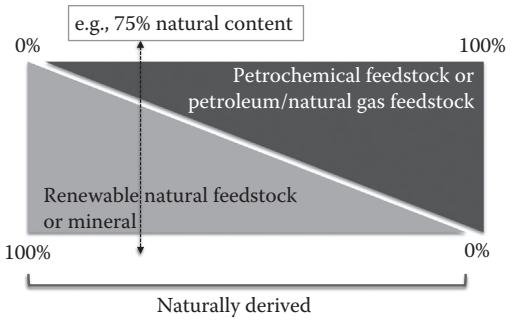

FIGURE 58.1 Naturally derived ingredients are ingredients containing up to 100% of one or more renewable feedstocks and some part of petrochemical materials. In the example presented, the naturally derived ingredient contained 75% natural feedstock and 25% petrochemical feedstock.

percentages (see Table 58.1). Efforts in accompanying any such numbers with their respective method are critical to keep the public informed and avoid any misleading labeling.

"Renewable" means nondepletable or easily replenishable by nature. Of course, there is a certain element of time in this concept. The renewability of an ingredient is therefore not simply a "yes or no" attribute but should be weighted based on how easy and fast is for nature to replenish. For instance, an oak tree takes 50–100 years before it reaches maturity and can be harvested for timber. In contrast, acorns from the oak tree can be harvested every year without impact to the originating tree and are consequently more easily renewable than the tree itself. Cereal crops are renewable resources and often used to produce cosmetic ingredients (e.g., colloidal oatmeal). For slow growing plants or endangered plant species, plant cell culture techniques have been developed enabling an unlimited, fully renewable supply of material. This type of ingredients will be discussed in more details in "Biotechnologies."

"Biodegradable" means the chemical alteration of a material by biological ways such as it lost its functions and it is partially or fully converted into chemical building blocks usable by nature. Biodegradability can be measured using a variety of methods [3]. Certain naturally derived surfactants are used in personal care products; these include surfactants from the alkyl polyglucoside family (e.g., coco-glucoside and lauryl-glucoside), alkyl sulfonates (e.g., sodium C13-17 alkane sulfonate), and isethionates (e.g., sodium cocoyl isethionate).

"Biobased" means biologically made either by a microorganism, plant, or animal (i.e., a renewable biological resource). The biobased content of an ingredient or product can be assessed by quantifying the amount of carbon isotope 14 (^{14}C) over the total amount of carbons. Biobased materials will contain higher amount of ^{14}C than petrochemical-based materials, which will contain exclusively ^{12}C. The percent biobased carbon of an ingredient can consequently be determined by comparing its $^{14}C/^{12}C$ ratio to the atmospheric one [4]. This method is also at the basis of the United States Department of Agriculture (USDA) Bio-Preferred program launched in 2011 [5]. At the end of 2012, among the 89 product categories included in the USDA biobased program, 10 categories are relevant to personal care: hand cleaners and sanitizers; lip care products; cuts, burns, and abrasions ointments; hair care conditioners; shampoos; deodorants; lotions and moisturizers; shaving products; and sun care products. Unfortunately, as of the end of 2012, the program is on hold due to the absence of funding ("American Taxpayer Relief Act of 2012").

Bioplastics have gained popularity in the last decade, with more options available. While most bioplastics are

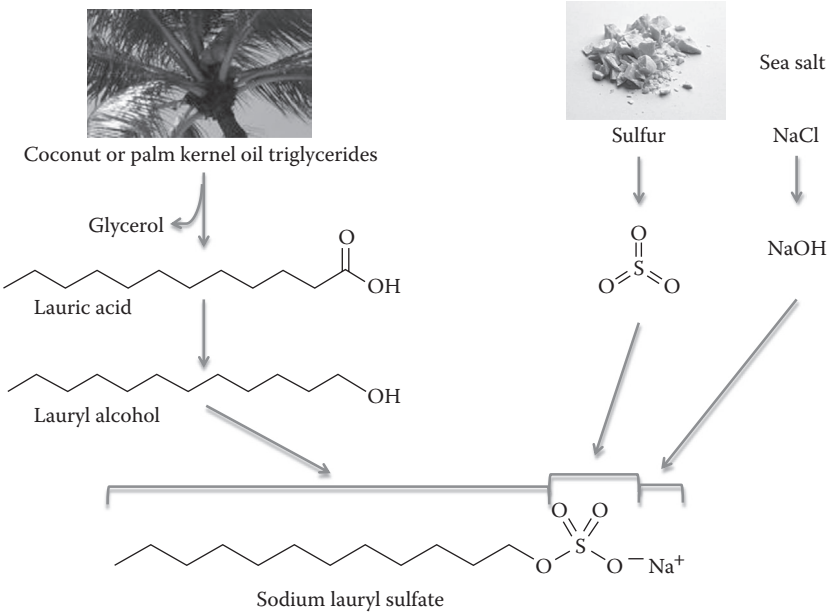

FIGURE 58.2 Origin of sodium lauryl sulfate (SLS), a naturally derived ingredient. In this example, all the original feedstocks are natural.

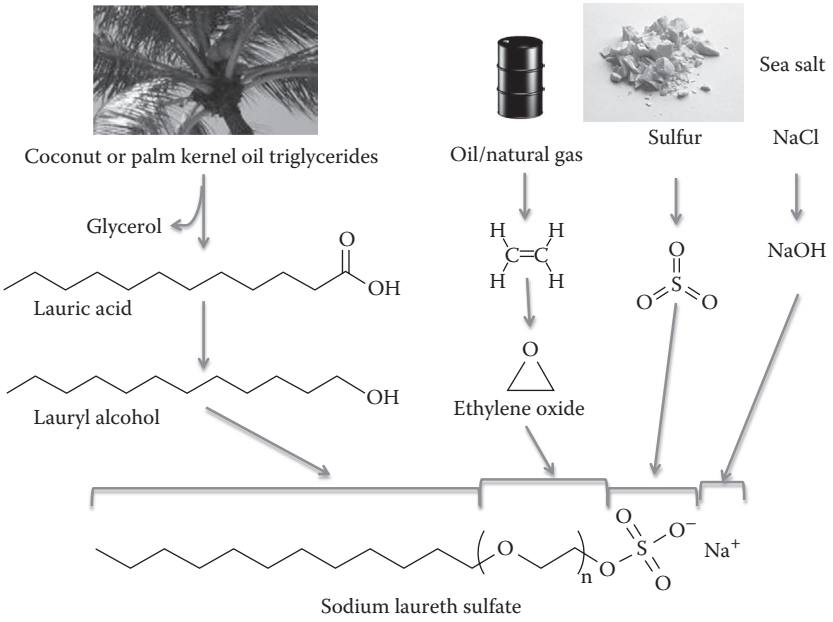

FIGURE 58.3 Origin of sodium laureth (-1) sulfate (SLES), a naturally derived ingredient. In this example, most of the molecule has a natural origin, except the ethoxylate, coming from petroleum.

TABLE 58.1
Comparison of Calculation Methods to Determine the Natural Content Percentage of Naturally Derived Ingredients

	% Biobased Carbon Content	% Renewable Molecular Weight	% Natural Molecular Weight
Sodium lauryl sulphate	100%	64%	100%
Sodium laureth-1 sulfate	86%	51%	87%

Notes: The "% biobased carbon content" method is based solely on the carbon atoms. The "% renewable molecular weight" uses the portion of the molecule that can be traced to a renewable feedstock (e.g., lauryl). The "% natural molecular weight" includes all portions of the molecule that can be traced to any natural feedstock irrespective of its renewability (e.g., sulfur, sodium).

biodegradable (e.g., starch-based, polylactic acid-based), this is not true for all of them. For instance, polyethylene-based bioplastics made from cane sugars are not biodegradable but are recyclable. Biobased ingredients are typically botanicals or biotechnology products, such as fermentation products.

"Organic" relates to a set of stringent farming principles used to produce the raw material at the origin of the ingredient. Specific guidelines on the farming practices and the postfarming processes apply to "organic" ingredients (as well as products). The first broad regulation on organic ingredients and products (also called "bio" in French) came from the European Union in 1991, later revised into the Council Regulation (EEC) No. 834/2007(6) on organic production and labeling of organic products. The USDA National Organic Program (NOP) was first published in 2000. In 2001, the Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO) issued the "Codex Alimentarius—Organically Produced Foods" to bring uniformity globally. It is important to note that these regulations and guidelines were primarily established for food and later used for cosmetics.

While the guidelines are well adapted to organic foods, it is a lot more challenging for cosmetic products due to their complex composition. The range of organic cosmetic ingredients is quite limited, particularly in the emulsifier, cleansing agent (limited to soap), and preservative categories. Although organic farming is expected to have a lower impact on the environment due to use of natural pesticides and fertilizers, the low yield and sometimes high water consumption of certain organic cultures have been questioned as not being that sustainable [7].

These different terms are interrelated with each other; however, they are not interchangeable (Figure 58.4). Most of the terms are ambivalent. For instance, not all botanicals are sustainable, which is dependent on farming or harvest practices. Likewise, a biodegradable ingredient may not be natural but will consistently contribute to its sustainability.

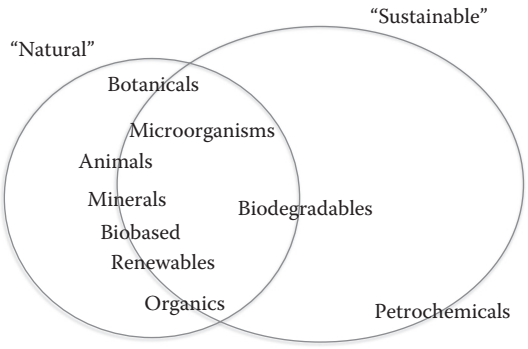

FIGURE 58.4 Natural-related terminologies and their relationships. Most terms are ambivalent, hence their presence on the lines to indicate their dual status.

FORMULATING FOR SUSTAINABILITY

Sustainability is the ability to sustain or endure (in French, sustainability is translated as "développement durable") over time. Sustainability is mainly defined by three components: the most often associated to sustainability is the environment—not depleting it nor damaging it—leaving it intact for the future generations; the second one is our societies or communities and the dynamics between populations; and finally, the third one is the economy, which includes the equitable sharing of resources and knowledge. Sustainable development is expected to impact humans positively and our aspiration for wellness and a longer, healthier life.

As discussed previously in this chapter, natural does not necessarily mean sustainable, and conversely, sustainable does not only equate to natural. Some naturals, like minerals, are not renewable, which makes their use over time nonsustainable.

Beyond natural ingredients, sustainable ingredients are becoming more important on the market place, as the consumers are asking corporations to take more responsibilities to assure sustainability on all fronts: environment, communities, and economy.

Sustainability for a petrochemical-based ingredient is about green chemistry principles [8]. Particular attention is paid to waste prevention, yield optimization (atom economy), and use of renewable feedstocks in order to reduce costs [9] and gain on sustainability. It is also about where the ingredient is produced and the impact of the manufacturing process on the local environment and its biodiversity, and on the local communities from a safety and economic point of view. Fully synthetized natural-identical ingredients are often seen as more sustainable than their natural counterparts mostly for an economic reason but also for the fact that the natural version may require altering the environment to satisfy the demand. A recent press release from Symrise AG announced that it was stopping the production of natural bisabolol, a sesquiterpene isolated from the oil of candeia tree (*Eremanthus erythropappus*) wood to concentrate on the production of natural–identical bisabolol. This press release cited supply concerns and the impact of the production on the rainforest in Brazil [10] as the reasons for the stoppage.

Sustainable naturals are a hot topic. This started perhaps with the concerns over deforestation to make place for palm plantations, consequently endangering biodiversity. The Roundtable on Sustainable Palm Oil (RSPO) was formed in response to the issue with task to identify some sustainable alternatives and bring the current plantations to sustainability standards. In order to promote these conversions, the Green Palm Certificates were created. While sustainable palm oil is still in short supply, these certificates bought together with the conventional palm oil help to establish a larger supply of sustainable palm oil. Several corporations (L'Oreal, Johnson & Johnson, Avon, and Unilever, to name a few) using large volumes of palm oil or its derivatives (in personal care) in their products have already announced their commitment to Green Palm to cover 100% of their palm oil supplies or to use sustainable sources of palm oil.

But beyond palm, it is also any naturals being produced in large quantities that could have a variety of effects on the environment such as deforestation, food source displacement, labor right issues, and pollution.

In October 2010, The Nagoya Protocol on Access to Genetic Resources and the Fair and Equitable Sharing of Benefits Arising from Their Utilization to the Convention on Biological Diversity [11] was agreed upon and later signed by over 90 nations. Its ratification is still pending; once ratified, the protocol will become legally binding for the parties. While ambiguous on several areas, such as the negotiating parties, what materials are in scope, and also the checkpoints, it is an important step in assuring a fair and equitable sharing of genetic resources, particularly from regions economically poor but biodiversity rich. This brings, however, extracomplexity for manufacturers or ingredients and products. Before using ingredients from specific nations or regions, one must assure that the raw material was obtained in compliance with all regulations, including biodiversity; and if the material is in the scope of such biodiversity regulation, mutually agreed terms should be established. This should be the responsibility of the first party procuring the raw material. However, there are expectations that this responsibility carries over to the manufacturers of finished goods. The patenting or marketing of a product with such raw material is sometime seen as an act of biopiracy (reviewed by Robinson [12]).

Taken together, formulating for sustainability is an incredible opportunity open beyond the field of naturals with a potential cost reduction, but it is also more and more challenging as new regulations appear and developing sustainably is an integral exercise, not limited to improving some environmental measures alone.

STANDARDS

As reviewed above, the terminology associated with the various categories of natural ingredients is complex and still controversial. The International Organization for Standardization (ISO) started working in 2009 on an international standard in the form of guidelines on technical definitions and criteria for natural and organic cosmetic ingredients and products

TABLE 58.2
Comparison of Main Natural and Organic Standards Used in Cosmetics

Standards	Ingredients	Products	Natural	Organic	GMOs	Water	Positive Lists	Negative Lists
Cosmos	X	X	X	X	Excluded	Neutral or included	Processes Minerals Petrochemicals	Processes
Natrue	X	X	X	X	Excluded		Naturally derived and nature-identical Processes	
NPA		X	X		No restriction	Excluded	"Synthetic" ingredients	Ingredient classes Specific ingredients
UNITIS	X		X	X	Excluded	Included	Processes	No
NSF 305		X		X	Excluded	Neutral	Preservatives Minerals Processes	Yes (classes—no specific ingredient)
USDA-NOP	X	X		X	Excluded	Neutral	Nonagricultural ingredients Processes	Yes (classes–processes—no specific ingredient)
OASIS	X	X		X	Excluded		Preservatives Processes	Yes (classes—no specific ingredient)

(ISO 16128—at the draft stage). This ISO standard might provide a much-needed uniform terminology framework and natural content calculation methods that could be further adapted according to national regulations or other standards (i.e., setting thresholds or additional criteria).

In the meantime, many standards coexist, each with its distinct set of definitions, exceptions, positive and negative ingredients, and process lists. Some are specific to organic products (USDA-NOP [13]; NSF305 and OASIS [14]). Others are covering both organic and natural products at large (COSMOS [15], NATRUE [16], and UNITIS [2]), while NPA [17] covers natural products only (Table 58.2). As of the end of 2012, the OASIS standard is still yet to be finalized.

Again, the naturally derived ingredient category is covered in different ways according to the standard. In the case of NPA and Natrue, a positive list captures these ingredients, while Cosmos uses a set of criteria including green chemistry principles, renewable resources, nonpersistence (measured as the ability to biodegrade and not accumulate in nature), and allowed processes as listed.

NATURAL RAW MATERIALS

Natural raw materials are not restricted to plant extracts. In this section, the main categories of natural raw materials or feedstocks will be reviewed, starting with botanicals, expanding to biotechnologies, and ending with other plant fractions used as intermediate in the manufacturing of common cosmetic ingredients.

The manufacturing of natural or naturally derived ingredients differs from a petrochemical mainly because the natural feedstock can vary dramatically in quality. In order to limit such variation, thorough documentation and a chain of custody for the natural raw material must be preserved.

Botanicals

Botanicals belong to a very diverse class of raw materials. They vary greatly biologically and ecologically (plants, fungi, or algae). Botanicals contain a wealth of phytochemicals that help them to adapt to their specific environment, fight pests and diseases, and simply differentiate and propagate themselves. They have been at the basis of most modern medicines, either isolated like digoxin (*Digitallis purpura*) and galanthamine (*Narcissus pseudonarcissus*) [18,19] or modified through medicinal chemistry to yield potent drugs such as the statins. Botanicals have also been quite useful for skin health.

Most botanicals used in cosmetics are extracts of the whole plant or individual parts. Extractions are carried out using different solvents according to the chemical profile expected. Supercritical CO_2 fluid extraction can also be used to maximize the extraction, particularly for nonpolar compounds. It is also a sustainable method of extraction.

The quality of a botanical extract can vary greatly from batch to batch or year to year. For this reason, extracts should be standardized to the main constituents. A good chain of custody on the botanical is also necessary to assure the identity of the botanical, its origin, and the knowledge of the processes the fresh matter has gone through prior to extraction. Adulteration of botanical extracts is not uncommon.

Biotechnologies

Biotechnologies offer a set of tools particularly adapted to manufacture cosmetic ingredients. There are two distinct categories of biotechnologies used in cosmetics: one with plant cell cultures and the other with prokaryotic cells (bacteria, yeasts, or microalgae) to ferment a specific substrate and/or to yield specific molecule.

Plant Cell Cultures

The most common method of plant cell culture involves taking a biopsy of a plant tissue and culturing it to first obtain a callus (a mass of de-differentiated cells) and then a cell suspension. The medium conditions of the cell suspension could impact greatly the production of secondary metabolite, in a similar fashion as in plant exposed to a stressor, like a fungi, for instance. This method has been used successfully to make taxol from *Taxis brevifolia* cells [20].

Another technique that is also common is to take meristem tissues, like adventitious roots, and culture them in vitro to propagate them.

The advantages of these methods are multiple: to avoid the batch-to-batch variability due to weather; to utilize endangered or slow growing plants; and to be able to manipulate the culture conditions to yield specific enriched fractions. There are, however, also inconveniences: these techniques require very large vessels and constant mixing to collect isolated compounds. However, if the objective is to make a crude extract, then this limitation does not apply.

Prokaryotic Cultures

Prokaryotic cultures can be used to either ferment a substrate (e.g., a plant material like soybean flour or tea leaves) or convert a defined substrate (e.g., sugarcane) into a specific and defined oil or compound. The former is most common in Asia. The latter has developed in the last decade, and some new ingredients have started to appear (e.g., squalane made from yeast grown on sugarcane—Amyris). Other companies have also developed microorganisms to yield specific molecules. For instance, Allylix produces some plant-identical sesquiterpenes from yeast that can be used as flavors and fragrances at a lower cost than the plant extracts [21].

These prokaryotic cultures are also being used to produce oils that have the potential to replace less sustainable oils currently used in the manufacture of surfactants.

Other Natural Feedstocks

Oils (triglycerides) extracted from palm, coconut, sunflower, safflower, soybean, or corn are used in the manufacture of surfactants. Polysaccharides such as xanthan gum (secreted by the bacteria Xanthomonas campestris) and guar gum (galactomannan from guar) are used as thickeners.

CONCLUSION—PERSPECTIVES

Natural ingredients are very popular with consumers due to the sense of trust they bring. However, natural ingredients are not always safe for use on skin. They could cause allergies (essential oils, some sesquiterpenes, etc.). Naturals are also very challenging to formulate into cosmetics. Many botanicals are not stable and will cause a browning of the product. With progresses in the manufacturing of naturally derived ingredients, like emulsifiers, it is becoming easier to provide the consumer solutions that are made with renewable feedstocks, moving also toward more sustainable products.

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59 Zinc Pyrithione
Critical Pharmacological Factors in Achieving Efficacious Dandruff Treatment Products

James R. Schwartz, Eric S. Johnson, and Thomas L. Dawson, Jr.

BACKGROUND

Overview of Dandruff

Dandruff (and the more severe form, seborrheic dermatitis) is a common affliction of the scalp, affecting approximately 50% of adults, independent of gender or ethnicity [1,2]. The primary symptoms are large skin flakes, itch, and dryness [3]. The condition is chronic and relapsing, leading to reduced quality of life if regular, effective treatment is not used [4]. The symptoms are indicative of an abnormal and unhealthy epidermal physiology, showing morphological and molecular evidence of inflammation, hyperproliferation, and impaired barrier integrity [5,6]. Causality appears to be multifactorial, simultaneously requiring three conditions: sebaceous lipids, commensal scalp fungi of the *Malassezia* genus, and an innate susceptibility [7].

Malassezia

Malassezia globosa and *restricta* are commensal scalp organisms with no known human benefit. They occur on the scalp at fairly high density, approximately 10^4–10^5/cm^2. The *Malassezia globosa* genome has recently been sequenced in its entirety and decoded to reveal the key metabolic elements that explain their presence and adaptation to the scalp as well as their likely dandruff pathogenic mechanism [8]. *Malassezia* use sebaceous lipid components as a necessary food source and tend to reside both on the outer scalp surface as well as within the upper hair follicle (the infundibulum) [9]. The infundibular environment is physically protected, provides ready access to fresh sebum, and is in close proximity to the viable epidermis where the skin barrier function is minimal.

The generally accepted and primary hypothesis of dandruff etiology involves *Malassezia*-derived extracellular lipase activity causing the hydrolytic release of fatty acids from the scalp sebaceous triglycerides [7,10–12]. *Malassezia* lipases are promiscuous, releasing all fatty acids from sebaceous triglycerides. *M. globosa* lacks a delta-9 desaturase and therefore can only utilize saturated fatty acids. The unsaturated fatty acids, either directly or via oxidized species, penetrate the stratum corneum and initiate an inflammatory defense response in susceptible individuals. This inflammation then leads to hyperproliferation and barrier impairment in an overall attempt to repair the damaged epidermis [2]. The common symptoms of itch, flakes, and dryness result from these etiological steps, respectively.

Treatment Strategies

Based on the above etiology, there are a number of possible intervention points. In practice, the most common approach that has been proven effective over the last 50+ years has been to reduce the population of *Malassezia* via the topical delivery of antifungal active materials [13]. Other approaches that target the skin's responsive reaction with anti-inflammatory materials such as topical corticosteroids can be therapeutically successful, but only for a limited time as long-term use can cause epidermal atrophy (thinning). Given the chronic nature of dandruff, the best long-term solution has been proven to be the routine control of the scalp fungal population, which requires regular topical product usage as the fungus readily recolonizes if treatment frequency is insufficient or is initially sufficient and then terminated [14].

Because regular and long-term treatment strategies are required, a convenient, cost-effective [15], and cosmetically pleasant product strategy is required to encourage compliance [16]. For these reasons, shampoos have evolved to be the most common form of dandruff treatment. Since shampoo is generally a part of the regular personal hygiene regimen, simply substituting a dandruff shampoo for a cosmetic shampoo is convenient for the consumer, so long as there are no perceived sacrifices in cosmetic properties or a significant cost increase. Increasingly, however, other product forms are becoming more common either as part of a regimen with shampoo or as separate products—rinse-off conditioners and leave-on tonics. Our focus will be on the shampoo form, as there are much more data available and many of the relevant pharmacological factors are independent of the product form.

Antifungal Active Materials

There are a number of antifungal materials used commercially for topical control of dandruff. While not intending to be

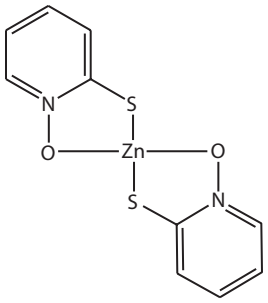

FIGURE 59.1 Structure of ZPT.

exhaustive, the most common of these are selenium sulfide, the imidazoles ketoconazole and climbazole, piroctone olamine, and zinc pyrithione (ZPT). Selenium sulfide can have cosmetic trade-offs (odor); ketoconazole is extremely expensive and it, along with climbazole and piroctone olamine, has limited regulatory approvals for use globally; for these reasons, ZPT has become the most widely used antidandruff active and will therefore be the focus of this review.

ZINC PYRITHIONE

History

ZPT can be represented as the 2:1 metal complex of bidentate pyrithione and zinc (Figure 59.1). The sodium salt was originally developed in the early 1950s by Squibb Co., a subsidiary of Mathieson Chemical Co. (later merging with Olin Chemical Co.), and investigated as an agricultural fungicide [17,18]. It was modeled after the naturally occurring antibiotic aspergillic acid and eventually supplied as the zinc complex in the later 1950s. ZPT was one of thousands of materials being screened at the Procter & Gamble Co. for potential utility as actives for antidandruff shampoo; its anti-*Malassezia* activity, favorable safety profile, compatibility with shampoos, and affordability led to its commercialization [19]. Clinical and safety studies submitted to the US Food & Drug Administration (FDA) led to its approval in 1964 and its first commercial use in Head & Shoulders shampoo [20]. Since that time, it has been accepted by global regulatory bodies.

Chemistry and Physical Properties

The combination of zinc ion (2+) with two molecules of pyrithione (1-) results in an electronically neutral complex with low aqueous solubility. In the solid form, it crystallizes as a dimer [21], which helps explain the low solubility. The solution chemistry of ZPT has been summarized [22,23], which includes aspects related to the metal complex as well as the ligand. As with all metal complexes, there is an equilibrium between the intact molecule and free zinc and pyrithione ions. The metal complex can undergo transchelation, in which another metal ion such as copper or iron replaces zinc. Finally, the pyrithione moiety can be oxidized to a disulfide species.

The low aqueous solubility of ZPT [22], even in the presence of surfactants, means that the majority of material incorporated in shampoos remains as dispersed particles and is likewise delivered to the scalp surface in a particulate form. This nature of ZPT as a solid active material is one source of the pharmacological complexity of this material—the particle size and shape become important attributes affecting both the product dispersion physical stability and the scalp surface coverage efficiency [24]. The most common commercial form of ZPT is a roughly cuboidal particle of approximate average diameter 0.8 μm. Another particle morphology is commercially used in which particle crystal growth is intentionally controlled during material manufacture to produce a platelet morphology in which growth in the length and width dimensions are favored over thickness.

The molecular mechanism of action of ZPT as an antifungal has recently been elucidated [25]. Transchelation with adventitious copper ion appears to occur prior to cellular penetration. The primary target of action appears to be certain mitochondrial proteins that contain iron–sulfur clusters as their active centers. Aconitase is one such protein target that plays a role in amino acid synthesis.

MEASUREMENT OF ANTIDANDRUFF EFFICACY

Clinical Protocol

The most common and definitive approach for assessing antidandruff product efficacy utilizes a double-blind clinical trial to evaluate the reduction in visual flaking via the adherent scalp flake score (ASFS) by an expert grader [26]. A typical protocol involves preselection of likely dandruff sufferers, a washout period where no antidandruff product is used, followed by a baseline assessment of ASFS to meet a minimum threshold. Test products are then used for several weeks with ASFS assessments at various intervals. The most common study involves a 3-week treatment period, but longer studies are sometimes performed to assess particular technical hypotheses. Recent advances have enabled the simultaneous

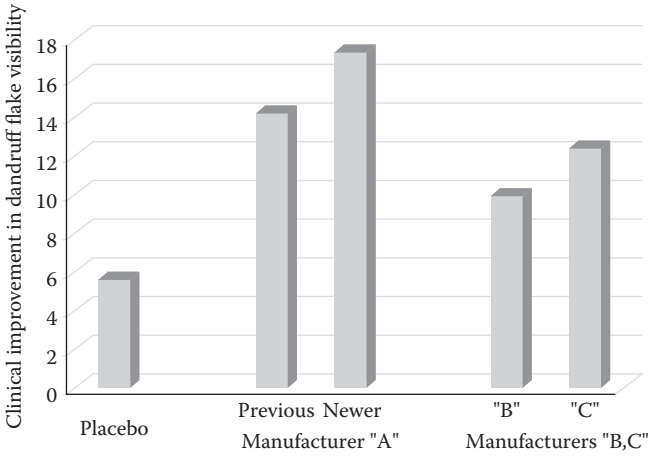

FIGURE 59.2 Antidandruff efficacy of a range of commercial shampoo products, all with 1% ZPT as active. Both differences between products made by different manufacturers as well as improvements over time with a single manufacturer are represented.

accumulation of molecular information indicative of epidermal health and repair [27].

Using this clinical methodology, variation in therapeutic efficacy can be observed for a range of commercial ZPT shampoos, all containing 1% ZPT as the active (see Figure 59.2). Also evident in this dataset is the variation in efficacy over time for a given manufacturer. The observed variation in efficacy for products that all contain the same level of the same chemical active is indicative of the importance of the product pharmacology in modulating the magnitude of achievable benefit. The impact of the product pharmacology is especially critical from a rinse-off product due to the challenges associated with active retention.

SCALP AS A RELATIVELY INACCESSIBLE PHARMACOLOGICAL TARGET

Scalp skin is similar physiologically to other nonpalmoplantar skin with the primary exception being the high density of terminal hair follicles, approximately 150–250 hair fibers/cm^2. Since there is a sebaceous gland as part of each follicular unit, sebum production on the scalp is high (approximately 100 μg/cm^2/h). Further, the presence of a dense fabric of hair on the scalp (averaging approximately 100,000–150,000 hairs and 50,000 cm^2 of hair surface area) makes it very difficult to target the delivery of actives to the scalp surface. This is especially difficult using shampoo as a delivery vehicle since the primary objective is to remove materials from the relevant surfaces, which conflicts with the secondary goal to retain certain shampoo ingredients. Together, these two factors place a great emphasis on product design criteria to be able to penetrate the hair mat and deliver active to the scalp surface.

RELEVANT PHARMACOLOGICAL FACTORS IN ACHIEVING EFFICACY IN ZPT-BASED PRODUCTS

Overview of Relevant Factors

Three groups of parameters are relevant to consider in developing an efficacious ZPT-based shampoo: retention, distribution, and bioavailability (Figure 59.3). The first is the total amount of ZPT retained on the scalp after rinsing; the short exposure during lathering is not believed to be the primary origin of benefits; the residual material likely has the greatest impact over time. The deposited ZPT particles have a relatively limited spatial effect area due to their low solubility, placing an emphasis on the physical distribution of deposited particles, both surface and infundibular regions, to achieve widespread fungal growth inhibition. Finally, the particles must release ZPT molecules in a biologically active form on the scalp surfaces to interact with *Malassezia* cells and result in the antifungal activity that is the origin of symptom resolution.

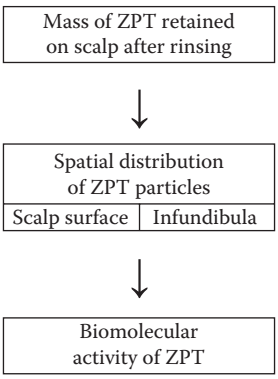

FIGURE 59.3 Overview of the factors involved in realizing antidandruff activity from ZPT-based shampoos.

Retention of ZPT

Modern shampoo formulation technology utilizes coacervation as the primary mechanism to retain ZPT particles on the scalp after rinsing. Coacervate technology relies on the formation of an electrostatic association complex between anionic surfactants and cationic polymers that is most often triggered by product dilution, especially during rinsing. The association complex results in the phase separation of an aqueous gel called coacervate that entraps or adheres dispersed particles such as ZPT with the potential of depositing them on the scalp surface. The phase separation/deposition technology cannot specifically target only the scalp, so the dominant hair surface area becomes involved as well. This becomes important both for achieving desired hair cosmetic feel as well as not impeding scalp delivery of coacervate.

The factors controlling coacervate formation are the type and amount of surfactants, the nature of the cationic polymer (molecular weight, charge density, backbone rigidity, polymer chemistry), and the electrolyte level. The separated coacervate material forms discreet particulate entities, called flocs. Properties of the flocs, such as size and viscosity, affect both ZPT delivery to the scalp as well as cosmetic acceptability of the product [28]. While large floc size can increase the amount of ZPT deposited in certain situations, in others (such as long hair), it can impede delivery. Large floc size also tends to have negative impacts on the spatial distribution of the ZPT that reaches the scalp surface and result in a coated, unclean hair feel from that which gets deposited on the hair surface. Thus, it can be appreciated that utilization of coacervate deposition technology to deliver ZPT effectively in a cosmetically acceptable way represents a delicate balancing of approaches and properties.

As ZPT retention is critical for efficacy [29], a dependable method for its quantification is required. Because of the physical impact that hair can have, the most dependable means of assessing ZPT scalp delivery is to use an in vivo protocol [28], which will include a realistic lathering/rinsing process, incorporating any hair filtering effect that could be missed in an in vitro protocol that does not realistically mimic hair effects. Using such techniques, ZPT deposition on the scalp is generally in the range of

approximately 1–5 μg/cm^2 [28]. While this amount of deposition may not seem substantial, it translates into hundreds of thousands of particles per square centimeter, which, combined with the low minimum inhibitory concentration of ZPT, is sufficient material to effectively inhibit *Malassezia* growth.

Spatial Distribution of ZPT Particles

A physical model for ZPT activity on the scalp involves the indirect interaction of ZPT particles with *Malassezia* yeast cells mediated by the continuous liquid medium provided by sebaceous lipids. ZPT particles and fungal cells must be in close spatial proximity for the antifungal activity of ZPT to be manifested because the relatively low solubility of ZPT results in a rapidly decreasing concentration gradient of soluble ZPT levels at short distances from the particle surface. Thus, to achieve broad antifungal activity, it is critically important that ZPT particles are spread uniformly over all relevant scalp surfaces [24].

There are two predominant factors that impact spatial distribution: particle morphology and coacervate characteristics. ZPT particle morphologies generally consist of either the standard cuboidal shape or an optimized platelet geometry. The advantage of using the particles with a platelet morphology has previously been covered [24]. Briefly, the advantage that platelet ZPT particles provide over the standard cuboidal particles is that, for a given mass, the platelets more efficiently cover the scalp surface than those that are three-dimensionally symmetric (Figure 59.4). While the primary focus of this review is on shampoo-specific ZPT delivery, the benefits of the specialized platelet morphology would be expected to result for other forms of topical delivery (e.g., conditioners, leave-ons).

As discussed above, coacervate formation is a delicate balancing act of ZPT entrainment to result in deposition without resulting in formation of a large floc that impedes uniform spatial dispersion. This can be represented as in Figure 59.5, which shows 25 ZPT particles delivered to a 100 × 100 μm^2 scalp surface area. The benefits of uniform particle distribution are evident over the situation in which highly aggregated particles are delivered as a result of coacervate entrainment.

Recently, it has been appreciated that uniformity of ZPT particle delivery must also include infundibular regions of the scalp surface to access and impact the *Malassezia* residing in these physically protected environments [9]. Utilizing confocal microscopy in vivo, not only can the depth profile of ZPT delivery be quantified, but also an aggregation metric can be obtained, which addresses the impact of coacervate type on the ZPT state of agglomeration [30]. ZPT particles can be delivered to the follicular infundibulum with relatively high efficiency. However, previous data have shown that high levels of agglomeration physically impede efficient ZPT delivery to the lower infundibular regions [30]. It is likely this same agglomeration yields situations similar to those represented in Figure 59.5b.

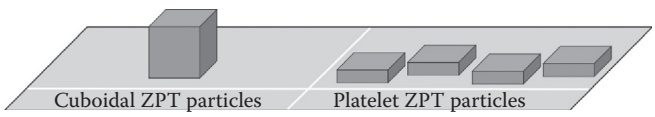

FIGURE 59.4 Conceptual model of the benefits of platelet ZPT particle morphology to increase scalp surface coverage efficiency. The same mass of ZPT is equivalent in both cases; the cuboidal form is a less efficient form for optimization of delivery.

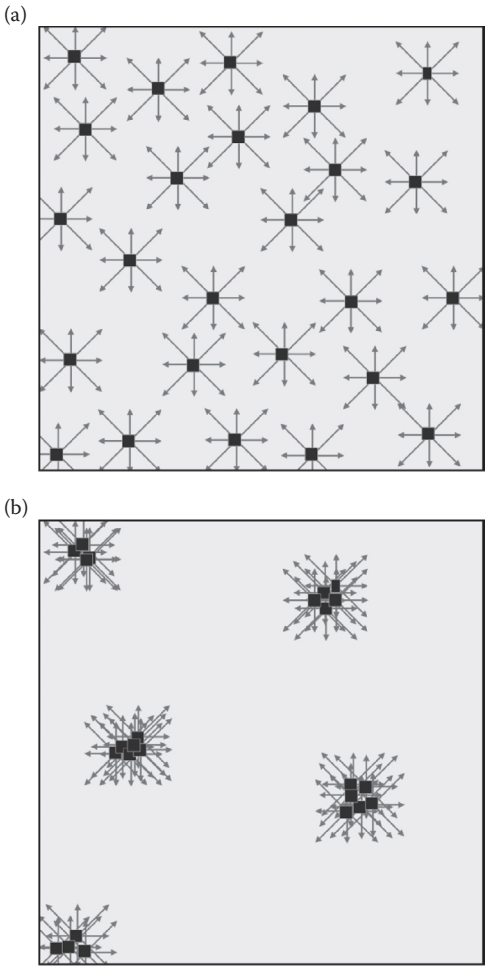

FIGURE 59.5 Comparison of the effect of aggregated ZPT scalp delivery on the efficiency of scalp surface coverage. Twenty-five ZPT particles are represented in a 100 × 100 μm^2 surface area. The arrows represent ZPT molecular diffusion from particle surfaces. In (a) of the representation, uniform particle distribution results in efficient surface coverage, whereas in (b), coacervate entrainment impedes efficient particle distribution and hinders efficient surface coverage.

Bioactivity of Deposited ZPT

Since the ZPT molecule is a noncovalent coordination complex, an equilibrium exists between the intact molecule and dissociated zinc and pyrithione ions. Neither of these latter

species are effective anti-*Malassezia* agents, and thus, this dissociative equilibrium interferes with maintaining ZPT in the intact bioactively antifungal form. A novel technology is employed in some ZPT-based formulations, which exploit Lechâtelier's principle to maintain ZPT in its intact active form [24]. The incorporation of zinc carbonate in some ZPT formulas provides a labile zinc ion pool, which reverses the dissociative equilibrium to increase the bioactivity of ZPT. Importantly, this activity must occur on the scalp; thus, the zinc carbonate, which is also a particulate material, must be codeposited with ZPT on the scalp surface to achieve the desired effect in the scalp milieu. While zinc carbonate is not an active material, its incorporation in the shampoo formula results in potentiation of the antifungal activity of ZPT.

SUMMARY

The incorporation of ZPT active material into a shampoo formula does not guarantee that the product will deliver a therapeutic benefit or that the magnitude of that benefit will be the same across different product matrices. There are many interactions with the nonactive matrix of the product that impact the delivered efficacy. Three groups of parameters are relevant to consider in developing an efficacious ZPT-based shampoo: retention, distribution, and bioavailability (Figure 59.3). Coacervate technology is used to assure that ZPT particles are retained on the scalp surface after rinsing. The nature of the coacervate must be optimized to (1) impede agglomeration of deposited particles, which hinders efficient spatial distribution of ZPT particles on the scalp surface and in the infundibula, and (2) ensure good cosmetic acceptance of the product to encourage long-term compliance. Once deposited, the formula can play a role in maintaining the molecularly dissolved ZPT in a bioactive form by codepositing a material to inhibit the natural dissociative equilibrium that can attenuate the antifungal activity of ZPT.

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60 Cosmeceuticals and Cosmetics
A Regulatory Overview

Raja K. Sivamani and Howard I. Maibach

Cosmeceuticals are a unique and rapidly growing field within dermatology and the skin care industry. The industry was estimated to have generated approximately $8.2 billion in sales in 2012 with a projected annual growth of 7.4% each year [1]. Consumers in the United States spend more on cosmeceutical products than all of Europe combined, with consumers in France and Germany spending the most within Europe [2]. Although there is no strict definition for cosmeceuticals [3], and they are not called as such everywhere, they are typically considered cosmetic products with components that may have "drug-like" benefits. Several examples of cosmeceuticals include moisturizers, serums, topical antioxidants, retinoids, peptides, and botanicals. They are believed to contain either one or a mix of ingredients that enhance skin condition and appearance without making a specific claim on skin health. Development of cosmeceutical products has distinct economic advantages as they are easier to market and require less monetary investment than a drug, which may require $800 million to upward of $1 billion [4].

REGULATORY OVERSIGHT

The term "cosmeceuticals" is derived as a blend of cosmetics and pharmaceuticals. However, the Food and Drug Administration (FDA) does not formally recognize the term "cosmeceutical" but notes that the term is used by the cosmetic industry to refer to cosmetic products that have medicinal or drug-like benefits [5]. The definitions for cosmetics, drugs, and cosmeceuticals are summarized in Table 60.1.

Regulatory oversight for the cosmeceutical industry in the United States is largely provided by the FDA. As long as no claims that would meet criteria for evaluation as a drug are met, cosmeceuticals are treated as cosmetics for regulatory purposes. The FDA does not have any legal authority in the approval of cosmetic products before they are marketed. However, they are able to take legal action against a cosmetic if it is deemed to be misbranded or adulterated. Therefore, even though cosmeceuticals may contain drug-like benefits, there is no regulatory approval process as long as the cosmeceutical does not make a claim that would qualify it as a drug.

Outside of the United States, the regulation of cosmeceuticals is not well defined. In Europe, regulatory authorities consider many cosmeceuticals as cosmetics [2]; in Japan, they are treated as quasi-drugs [2]; in India, multiple regulatory bodies may be involved with an absence of guidelines on product claims for cosmetics while the term "cosmeceutical" is not included in official legal definitions [2].

ROLE OF BOTANICALS

With the expanding demand for the use of alternative medical therapies, botanicals are playing a larger role in cosmeceuticals [7]. Studies have evaluated the ability of whole botanical extracts and specific phytochemicals to modulate cellular functions [8–10]. In this sense, botanicals may have beneficial mechanisms of action beyond serving a cosmetic purpose. However, official claims need to remain guarded to be marketed as cosmeceuticals rather than a drug.

Among botanicals, some products have blurred the boundaries of cosmeceuticals and drugs as two botanically derived dermatological preparations have received FDA approval as prescription drugs. One is an extract of *Camellia sinensis* (green tea) composed of sinecatechins that is used for the topical treatment of external genital and perianal warts [11]. The second is a purified proanthocyanidin extracted from the South American tree *Croton lechleri* for the treatment of diarrhea associated with anti-HIV drugs [12].

With growing demand for natural and botanical alternatives, there has been greater demand for "organic" cosmeceuticals. In the United States, the standard for "organic" is not set by the FDA and rather it is set by the United States Department of Agriculture (USDA). The FDA plays no role in regulating, reviewing, or enforcing these standards. Several regulatory bodies provide oversight on the "organic" distinction as outlined in Tables 60.2 through 60.5.

CONCLUSION

Although cosmeceuticals are widely used and marketed, their definition remains in flux [3], especially with regulatory oversight still in evolution. As such, there is a larger burden on the skin care provider and consumer to understand and critically evaluate the evidence [13]. Furthermore, skin care providers should understand the organic regulatory distinctions, as this can be an area of confusion for consumers.

Cosmeceuticals offer many new potentially exciting and useful skin care products and choices to the consumer. The interest and increase in botanically derived products has significantly expanded these choices. However, this array of choices can also be confusing. As the cosmeceutical industry

TABLE 60.1
Definition of Cosmetics, Drug, and Cosmeceuticals

Term	Definition	Recognized by Food and Drug Administration
Cosmetics [6]	1) Articles intended to be rubbed, poured, sprinkled, or sprayed on, introduced into, or otherwise applied to the human body or any part thereof for cleansing, beautifying, promoting attractiveness, or altering the appearance 2) Articles intended for use as a component of any such articles	Yes, approval not required for marketing
Drug [6]	1) Articles recognized in the official United States Pharmacopoeia, official Homoeopathic Pharmacopoeia of the United States, or official National Formulary, or any supplement to any of them 2) Articles intended for use in the diagnosis, cure, mitigation, treatment, or prevention of disease in man or other animals 3) Articles (other than food) intended to affect the structure or any function of the body of man or other animals 4) Articles intended for use as a component of any article specified in clause (1), (2), or (3)	Yes, approval required for marketing
Cosmeceutical [5]	Cosmetic products that have medicinal or drug-like benefits	No, approval not required for marketing if pharmaceutical claims are not made

TABLE 60.2
Organic Labeling Standards: USDA National Organic Program

Distinction	Requirement	May Display USDA Organic Seal?
100% organic	100% organic ingredients	Yes
Organic	95% organic ingredients	Yes
Made with organic ingredients	70% organic ingredients	No
No organic distinction allowed	<70% organic ingredients	No

TABLE 60.3
Organic Labeling Standards: NSF/ANSI 305 Standard for Personal Care Products (United States)

Distinction	Requirement
Contains organic ingredients	>70% composed of organic ingredients (product label must state the exact percentage of organic content)

TABLE 60.4
Organic Labeling Standards: COSMOS (Europe)

Distinction	Requirement
COSMOS organic label	1) 95% of a product's "agro-ingredients" and 20% of the entire product must be organic. 2) For products that are less than 95% organic, the label may make reference to individual organic ingredients.
COSMOS natural label	1) No minimum level of organic ingredients required, but the label must report organic ingredients on the ingredient list. 2) May report % of organic ingredients to total product or total product minus water and minerals. 3) Must not make any organic claim of products or ingredients on the front packaging label.

TABLE 60.5
Organic Labeling Standards: IOS Cosmetics Standards Established by Certech (Canada)

Distinction	Requirement
Natural and organic	95% of ingredients are of natural origin and at least 95% of plant ingredients are certified organic and 10% of all ingredients are certified organic.
Natural	95% of ingredients are of natural origin and at least 50% of plant ingredients are certified organic and 5% of all ingredients are certified organic.

continues to expand, including those that are "natural" and "organic," there will be a greater need to monitor marketing claims and the evidence behind them. Furthermore, the safety of these products will need to be evaluated closely [14].

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61 New European Legislation Concerning Efficacy Claims of Cosmetic Products

An Overview of Different Methods of Evaluation

Peter Clarys and André O. Barel

INTRODUCTION

The importance of this chapter is considered taking into account the following facts: the new European legislation is much more concerned with the protection of the consumer from misleading claims related to efficacy [1]. One observes a tightening up of the substantiation of claims: there must be relevant data of substantiation on a file to back up any claims, and there is a proposal for establishing common criteria for all types of claims. In order to help and sustain the cosmetic industry, the "Cosmetics Europe Personal Care" Association (previously COLIPA) representing the positions of the European cosmetic industry has published two guidelines.

- Cosmetic guidelines for the evaluation of the efficacy of cosmetic products [2,3]
- Cosmetic guidelines on responsible Advertising and Marketing Communication for the evaluation of the efficacy of cosmetic products [4]

A Self Regulation Project on Responsible Advertisement and Marketing Communication Information was initiated by independent regulators such as the Advertising Standard Authorities (ASA) in the United Kingdom [5]. One of the tasks of this independent self-regulatory organization of the advertising industry is to pick up eventual misleading advertising concerning cosmetic claims as presented in magazines and other media. Recent example of exaggerating claims are complaints of ASA and a competitor concerning lash claims based on retouched pictures [6]. Also it must be noted that, recently in the United States, a law firm has filed a lawsuit action against a cosmetic firm concerning claims made about its antiaging product, which has been deemed as misleading [7].

Furthermore, the U.S. Food and Drug Administration had taken umbrage with different cosmetic companies concerning their antiaging marketing claims, which could render them as drugs under the U.S. Cosmetic Act Legislation [8].

The authors of this chapter have made a critical overview of the claims proposed by various cosmetic companies in advertisements and were astonished by the variety and sometimes by the exaggeration of the claims. A critical evaluation must be made on these claims: which are acceptable and which must be considered as unacceptable because they are clearly misleading? This chapter reflects the opinions of scientists from the academic world not linked to the cosmetic industry. Finally, an overview of the different methodological approaches of claim substantiation will be given.

LEGISLATION CONCERNING COSMETIC CLAIMS

The new European legislation is much more concerned with the protection of the consumer from misleading claims related to efficacy and other characteristics of cosmetic products by tightening up the rule concerning substantiation of claims: there must be relevant data of substantiation on a file to back up any claims, and there is a project of proposals for establishing common criteria for all types of claims [1].

In 2013, cosmetic products placed on the market of the European Economic Area are obliged to be compliant with the new European Cosmetics Regulation (EC1223/2009).

The general statement concerning safety remains strictly valid: a cosmetic product made available on the market shall be safe for human health when used under normal or reasonably foreseeable conditions of use (EU Cosmetics Directive 76/768 EC) [9].

In the new European Cosmetics Regulation, Article 20 concerns the justification of the use of cosmetic claims.

Article 20.1. A general statement that cosmetic products claims are not authorized to present in the labeling, making available on the market, and advertising cosmetic products, text, names, trademarks, pictures, and figurative or other signs, characteristics, or functions that they do not have.

Article 20.2. Considering the general character of Article 20.1, the EU gives the following trends and precisions for the action plans in the future.

The commission shall, in cooperation with Member States, establish an action plan regarding claims used and fix priorities for determining common criteria justifying the use of a claim. This implies of course "the normal and reasonably foreseeable use of the cosmetic product." After

consulting the Scientific Committee for Consumer Safety (SCCS) or other relevant authorities, the Commission shall adopt a list of common criteria for claims that may be used with respect to cosmetic products, in accordance with the EC regulation concerning unfair business-to-consumer commercial practices. By July 11, 2016, the Commission shall submit to the European Parliament and the Council a report regarding the use of claim on the basis of the common criteria adopted under the second subparagraph (Article 20.2) If the report concludes that claims used in respect of cosmetic products are not in conformity with the common criteria, the Commission shall take the appropriate measures to ensure compliance with the Member States.

Article 20.3. The cosmetic company may refer on the product packaging or in any other accompanying document to the fact "that no animal testing has been carried out" only if the manufacturer and his/her suppliers have not carried out any animal testing on the finished cosmetic product or any of the ingredients contained in the final product, or used any ingredients that have been tested on an animal by others for the purpose of developing new cosmetic products.

LEGISLATION OF THE EU CONCERNING PROOF OF EFFECTS—PRODUCT INFORMATION FILE [1]

In compliance with this EU regulation (EC1223/2009) on cosmetic products, the Cosmetics Europe Organization has published in December 2011 guidelines on the Product Information File (PIF) requirements [2,3].

Where justified by the nature of the cosmetic product, proof of the effect claimed by the cosmetic product should be noticed in the PIF. The PIF should contain support information or at least a short summary of the technical support for the claimed effects. The short summary should be cross-referenced to more detailed support information. However, the detailed support information would not be held as part of the PIF. This concerns any claim made for a cosmetic product irrespective of the communication medium or type of marketing tool used and irrespective of the target audience (consumers or professionals).

All the information contained in the PIF is intellectual property of the cosmetic company and is to be made readily accessible to the competent authorities upon request. For reasons of confidentiality and commercial property, the PIF is not to be made public.

DEVELOPMENT OF COMMON CRITERIA FOR CLAIM SUBSTANTIATION OF THE EUROPEAN COMMISSION

First, common criteria to all types of claims must be considered and adopted. In specific cases, where the common general criteria may not provide an adequate and sufficient detailed framework for the protection of the consumers from misleading claims, additional common criteria for specific types of claims should be elaborated. For example, the Commission has already adopted recommendations and guidance for the specific nature of the claims concerning efficacy measurements of sunscreen products and related claims.

The general common criteria for all types of claims are that every claim should be consistent with the general principles of advertising as being based on legality, honesty, truthfulness, fairness, and evident support, allowing informed decisions by the consumer. Claims on cosmetic products should conform to the following six common criteria:

1. Legal compliance
2. Truthfulness
3. Evident support
4. Honesty
5. Fairness
6. Allow informed decisions

Supplementary explanations relating to the six criteria as proposed by a scientific expert committee from the cosmetic industry reflecting the point of view of the Cosmetics Europe Organization and the United Kingdom Cosmetic Toiletry and Perfumery Association (CTPA) together with the ASA are described in the self-regulation guiding principles on responsible advertisement and marketing communication [4,10,11].

In these guidelines concerning responsible advertisement and marketing communication, a paragraph is concerned with retouching photographic pictures.

Retouching Photographic Pictures—Image Honesty

In the list of guiding principles on responsible advertising concerning cosmetic products, special attention must be given to the criteria of Image Honesty. Digital techniques may be used to enhance the beauty of skin images in order to promote specific product benefits. However, the use of post-production modification techniques such as styling, retouching, lash inserts, hair extensions, etc., should respect the following principles:

- The advertiser should ensure that the illustration of the performance of advertised product is not misleading.
- Digital techniques should not alter the image of models such as their body shapes or features to become unrealistic and misleading with regard to the performances achievable by the product.
- Postproduction techniques are acceptable provided they do not imply that the product has characteristics or functions that it does not have.

Cosmetics Europe gives two examples of cases that would not be considered as misleading:

1. Using obvious exaggeration or stylized beauty images that are not to be intended to be taken literally
2. Using techniques to enhance the beauty of the images that are independent from the product or the effect being advertised

Unfortunately, this very interesting self-regulatory approach to cosmetic advertising suggested by Cosmetics Europe and the CTPF/ASA does not correspond always to the reality. It is known that in some publicity, photographic pictures are undergoing a retouching treatment involving longer and false eyelashes, taking away pigmentation spots, character lines, and wrinkles, whitening teeth, etc. Advertising authorities should be coming down harder on companies that persist with extreme retouching [10].

CRITICAL OVERVIEW OF COSMETIC CLAIMS PRESENTED BY THE COSMETIC COMPANIES

The EC legislation (Cosmetic Directive) clearly points out that a cosmetic claim is any public information on the content, the nature, the properties, or the efficacy of the product. In order for the claims on a cosmetic product to meet the requirements of the Cosmetic Directive without being misleading, the following conditions should be considered:

- The benefits delivered by the product should be consistent with reasonable consumer expectations created by the claims.
- Claims should be supported by sound, relevant, and clear evidence. Such evidence can be based on generally accepted data and experimental studies.

In conclusion, sincerity should be the basic and essential part of a responsible approach to the advertising of all products. The reality is sometimes very different. The consumer is overwhelmed by a wealth of various modes of actions of the cosmetic products. The following are some examples found in different media of advertising of cosmetic products: decrease of the depth of wrinkles; restore tonus, firmness, and gloss; brighter and more uniform complexion; skin looks brighter, feels softer, and more velvety; firming lift; wrinkle correction; pores of the skin are less visible; erase and filling wrinkles, lifting away the look of wrinkles; etc. These claims appear to be appropriate and reasonable.

Furthermore, some claims are very vague and do not correspond to precise properties of the skin such as the following: reduce the look of fatigue of the skin, relax facial features, the skin looks fresher, revitalize, rejuvenation, youth serum, etc.

Some claims do not correspond to the scientific reality and are misleading, although the cosmetic companies are convinced to have supporting tests for these claims. A few examples are as follows: nourishing cream for the skin, reactivation/boosting of essential genes, doubling of production of collagen after one week treatment, repairs 8 years of cumulated skin damage after 4 weeks treatment, etc. Typical examples of misleading claims are observed with anticellulite creams and gels: decrease of symptoms of cellulite is obtained after 1 week treatment.

DESCRIPTION OF METHODOLOGICAL APPROACHES OF CLAIM SUBSTANTIATION

Guidelines for the evaluation of the efficacy of cosmetic products and the substantiation of these claims have been presented to the cosmetic industry by Cosmetics Europe [2,3] and by the joint initiative in the United Kingdom CTPA and ASA [5,10]. In function of the intended use of the cosmetic product, it is possible to use and combine several approaches:

1. In vivo sensorial testing on human volunteers. Visual, tactile, and sometimes olfactive evaluation of the effects of a cosmetic product by consumers themselves or experts.
2. In vivo instrumental approach on human volunteers; noninvasive bioengineering techniques measuring specific criteria or properties of the skin.
3. Ex–in vivo instrumental testing on human materials. This testing does not reproduce normal conditions of the use of products but allows objective analyses of specific activities. For example, testing on hair tresses, skin tape strips, etc.
4. In vitro testing. The cosmetic properties of a product are examined in the laboratory on artificial media (synthetic membranes, artificial skin, culture of keratinocytes, melanocytes, adipocytes, fibroblasts, etc.).

Details of these experimental testing protocols are not accessible to the public since they remain confidential and are intellectual property.

IN VIVO VISUAL AND TACTILE TESTING ON HUMAN VOLUNTEERS

These tests are based on the appreciation of the performances of a cosmetic product through visual, tactile, and eventually olfactive evaluations by either the subjects themselves or experts.

Autoevaluations during In-Use Tests by Consumers

An in-use test evaluates the consumer's perceptions of the product efficacy and cosmetic properties based on parameters that they can observe and/or feel. Generally, these in-use tests must be carried out on a sufficient number of subjects for statistical validation. There are two types of in-use testing methodologies.

In the blind use testing procedure, the cosmetic company does not provide any information concerning specific properties of the product to be tested, which could influence the consumer's judgment and alter their perception of the product alone.

In the concept use testing, the products are tested by consumers, but important elements of information concerning the potential properties of the product to be tested are communicated to the subjects. This information helps to check whether the effects of the product as perceived by the

consumers (judgments and perception) correspond to the expectations of the cosmetic company. In this approach, clear information must be given to the subjects concerning which properties of the product must be examined.

In Vivo Visual and Tactile Evaluation on Human Subjects Carried Out by Experts

The "sensorial evaluations" using visual and tactile observations of the skin of healthy volunteers enable to investigate the proposed claims of a cosmetic product if focused on well-defined criteria. They must be conducted with the help of a panel of trained experts, according to a well-described protocol in controlled circumstances and environment. The presence of a physician is not obligatory; qualified and trained professionals (paramedical practitioners, aestheticians, or other professional in the cosmetic field) with experience in cosmetic testing are capable of carrying out these evaluations.

The chosen parameters are evaluated by clinical observation using a preestablished scale of scoring. The quantified data are compared with the initial results (before/after comparisons), or with an untreated control skin area (contralateral symmetry on the face, arms, and most body areas), or a placebo or a reference product.

In Vivo Instrumental Testing on Human Volunteers

In these testing procedures, certain properties of the skin are measured using noninvasive bioengineering techniques according to a defined protocol, following the application of cosmetic products [12–17]. The instrumental measurement is performed by a skilled technician with training in the use of this technique. The measurements are made on the subjects in controlled laboratory conditions such as a climatic experimental room where temperature and humidity are kept constant [18]. In a similar way, the quantified data are compared with the initial results (before/after comparisons), or with an untreated control skin area (contralateral symmetry on the face, arms, and most body areas), or a placebo, or a reference product. Some bioengineering instrumental testing methods can also be used on ex in vivo materials.

In this chapter, a special paragraph will be devoted to the comparison of these instrumental evaluations and the sensorial evaluations.

In Vitro Testing Methods

The effects of a cosmetic product, either a specific ingredient or the finished product, are observed in the laboratory on artificial media: synthetic membranes for diffusion studies, artificial skin, culture of cells, etc.

The in vitro results can be used in the instance where they have been correlated with an in vivo method. However, in vitro data may be used as claims without reference to an in vivo method, but support for product efficacy should not be solely based on such data. The authors would like to mention that the use of many plant extracts with slimming and anticellulite properties are solely based on in vitro tests carried out on cultures of fibroblasts and adipocytes without any in vivo experimentation.

Furthermore, in these guidelines, some sound and reasonable general principles for all testing methods are suggested.

- Studies must be relevant and using methods that are reliable and reproducible.
- Studies should follow a well-designed and scientifically based valid methodology.
- The criteria used for evaluation of product performance should be defined with accuracy and chosen in concordance of the aims of the test.
- Depending on the aims of the study, a test can be open, single blind, or double blind.
- Studies conducted on volunteers should respect ethical rules and should be executed after formal approval of a medical ethical commission.
- The target population participating in the testing has been chosen as defined by strict inclusion/exclusion criteria.

Different intrinsic factors must be taken into account when considering the selection of the human volunteers.

- Chosen skin sites for testing: face, arms, trunk, back, thighs, etc.
- Gender: males and females or only females (many testings such as antiaging are mainly carried out on females). Women are more interested than men to participate as subjects in testing procedures.
- Age: young volunteers or middle-age female volunteers (mature skin).
- Ethnic group: Caucasian, African, or Asian population?

Here in Europe, we consider mostly Caucasian subjects with different Fitzpatrick Skin Phototype Classification (going from I to VI) [19] as subjects for sensorial testing. It is known that among Caucasians, there is large variability in phototypes; however, types II and III are mostly considered in clinical studies.

TESTIMONIALS AND RECOMMENDATIONS FROM SPECIALISTS

In addition to the different methodological approaches of claim substantiations, the cosmetic company can add extra information concerning their product that is more related to testimonials from celebrities and consumers and recommendations made by specialists in order to convince the customers. Such specialists must be selected on the basis of their qualifications, expertise, or experience in the particular field.

Self-regulatory guidelines also apply on written and spoken testimonials and recommendations:

- Must be genuine, responsible, and verifiable
- Cannot replace material substantiation of a claim
- Shall avoid any misrepresentation and misinformation with regards to the nature of the product being advertised, its properties, and the achievable results

SUBSTANTIATION OF COSMETIC CLAIMS: COMPARISON BETWEEN SENSORY TESTING AND BIOENGINEERING INSTRUMENTAL TESTING

Sensory Visual Investigation of the Skin

In the dermato-cosmetic world, evaluation of the skin looks, at first glance, rather easy since the skin is very accessible for clinical evaluation [16,17]. Visual examination reveals color, dryness, oiliness, roughness, aging, etc. Tactile evaluation by feeling reveals also dryness and oiliness, roughness, firmness, laxity, aging, etc. Human vision is a very sensitive sensory and neurophysiological process. On the other side, this neurophysiological process remains subjective, mostly qualitative, and highly observer dependent, and most importantly, there is no precise memorization of color in function of time.

Since human vision does not have a precise memory for the vision process in function of time, comparison of pre/postcosmetic treatment of the skin is only possible when comparing visually high-resolution digital macrophotographic pictures of the skin surface obtained pre/postcosmetic treatment.

The eventual improvements observed on the skin surface are visually observed and evaluated by three independent experienced evaluators in order to reduce intervariability of the evaluators. Visual evaluation of specific criteria of a claim can be carried out directly in vivo on the skin or on standardized pre/postmacrophotography pictures, using a numerical scale of scoring (a 0 to 10 scale is generally used). In a preliminary phase, the evaluators are instructed and trained to which parameters must be looked at and the scoring system using typical reference pictures as examples.

In order to illustrate the methodology of a visual investigation carried out by experts, the substantiation of the efficacy of an antiaging cosmetic claim will be described.

Usually female Caucasian subjects with symptoms of photoaging on the face, based on the Griffiths photoaging scale (0 no evidence to 8 severe evidence of photoaging) [20], between 5 and 7 are selected. High-quality macroscopic pictures of the face of the subjects (subjects treated either with the active cosmetic product or with the placebo product) are taken respectively at baseline T0 and after x weeks treatment T_X. A standardized high-resolution photographic imaging system for facial pictures is used where the posture of the face of the subject is maintained in a fixed position for reproducibility (frontal and left/right positions) [21]. Furthermore, the position of illumination and nature of the light source are maintained constant, which implies possible use of polarized visible light or UV illumination. The pairs of pictures are blinded, and three independent experienced evaluators must grade the pairs of pictures in a 0 to 10 scale [22].

The following is the visual rating of photoaging on the face of frontal and left–right pictures taken under visible polarized light: global score on a 0 to 10 scale rating for the typical symptoms of aging such as coarse and fine wrinkles, crease lines, actinic keratoses, telangiectasia, rosacea, couperose, and yellowing of the skin (either specific criteria or a global evaluation based on all criteria is considered for scoring).

The following is the visual rating of photoaging on the face of frontal and left–right pictures taken under UV light: global score on a 0 to 10 scale based on the typical symptoms of aging such as presence of pigmentation spots (lentigines, ephelides, and distribution/number of spots: 0 = no spots and 10 = extreme spots).

SUBSTANTIATION OF CLAIMS USING BIOENGINEERING MEASUREMENT

Many published, noninvasive bioengineering measuring devices as tools in the evaluation of the skin are available (in *Handbook of Non-Invasive Methods and the Skin*, most bioengineering methods have been described [12,15]). The advantage of bioengineering and imaging techniques is that many aspects and properties of the living skin are now measurable and quantifiable based on objective, reliable, and reproducible techniques. Many of these instruments or techniques are nowadays commercially available and adapted for routine measurements. In this paragraph, a critical review of the advantages of testing with a bioengineering instrument and also their limitations in applications will be presented.

Advantages of Using Bioengineering Instruments

The noninvasive methods are by nature objective, quantitative, and generally investigator independent, can be calibrated and validated, and are developed to work on human volunteers. If bioengineering measurements are properly performed, under well-controlled conditions, then they can lead to a standardization of the obtained results and allow interlaboratory and interobserver comparisons [13,16,17].

Standardization, Calibration, and Validation of Different Methods

Each bioengineering instrument delivers raw data, which only become valid through a process of validation involving calibration and comparison with other methods or with a reference method [16,17]. It is strongly advised that a laboratory should use the instruments according to precisely described standard operating procedures (SOPs), which cover the calibration, validation, maintenance, performance, and condition

of the equipment used with all the details of the operation procedure [18].

Some of the measurements on the skin are strongly influenced by variations in the environmental conditions (skin hydration, transepidermal water loss [TEWL]). Therefore, the temperature and the relative humidity in the experimental measuring room should be standardized and kept constant in a climatized experimental room [13,18]. The elaboration of valid guidelines by independent and unbiased expert groups and the publication of relevant and reproducible test results according to these guidelines have been realized by the European Group of Experts for the Measurement of Efficacy of Cosmetic Products (EEMCO) [23–31].

The major limitation of bioengineering measurements is that these experimental methods deliver only a single limited property of the human skin. As a consequence, each instrument is only able to detect and measure *one single parameter* of a complex skin structure, which is not sufficient for providing an overall clinical picture [13]. As a consequence, for some investigations, it is necessary to combine more than one method.

Overview of Cosmetic Claims and Substantiation by Bioengineering Methods

When looking at the advertisements and marketing claims presented by the cosmetic industry, some performances promised are extremely imaginative but are very difficult to substantiate by bioengineering methods. A few examples taken from the publicity in the cosmetic world are as follows: reducing the look of fatigue of the skin, relaxing facial features, skin looks fresher, reenergizing the skin, the skin is invigorated, etc. How are we going to measure these claims? However, many characteristics and properties of the skin surface can be evaluated and quantified by noninvasive bioengineering methods. The following is an overview of substantiation by bioengineering methods.

Measurements of smoothness, roughness, and wrinkles of the skin surface. Different methods are available and carried out either directly on the skin or through very accurate silicone replicas [32], image analysis [22], profilometry using laser light [33], fringe projection method, PRIMOS, GFH [34], determination of microrelief and macrorelief Visioscan VC 98, Visioline VS 650, and Skin Visiometer SV 600, Courage-Khazaka [35].

Mechanical measurements on the skin. The mechanical properties of the skin (stiffness and various viscoelastic parameters) can be evaluated with different commercial devices based either on the lateral torsion method [36]; Torque Meter, Dia-Stron [37] or the vertical suction method [38]; DermaLab Skin Combo testing, Cortex [39]; BTC-20000, Surgical Research Laboratory [40]; and Cutometer MPA 580, Courage-Khazaka [41,42].

Firmness of the skin, isotropy/anisotropy of the mechanical properties of the skin. Reviscometer RVM 600, Courage-Khazaka [43].

Greasiness/oiliness of the skin surface. The determination of the quantity of sebum and the presence and topography of active sebaceous glands can be carried out with the following techniques: Sebumeter SM 815, Courage-Khazaka [44]; Sebum module Dermalab Cortex [45]; Sebum Scale, Delfin [46]; and porous absorption tapes Sebutapes, Cuderm [47], and Sebufix F 16, Courage-Khazaka [48].

Determination of superficial venous blood microcirculation and irritation. Laser Doppler flow monitor VMS-LDF and LD12-HR Imaging system, Moor [49], Periflux System 500 and Pericam PIM Imaging system, Perimed [50].

Skin complexion, skin color, erythema, and melanin. First, the specific Erythema-Redness and Melanin content instruments such as Erythema and Melanin Meter, Diastron [51]; Mexameter MX 18, Courage-Khazaka [52]; ColorMeter DSM II, Cortex [53]; and the more general determination of skin color using Tristumulus reflectance colorimeters according to the CIElab L*a*b* color system [54]: Colorimeter CL 400, Courage-Khazaka [55], and Chromameter CR 400, Minolta [56].

Evaluation and quantification of pigmentation spots, ephelides, chloasma, and hyperkeratosis spots can be carried out with a video camera under UVA illumination: VisoScan VC 98, Courage-Khazaka [35].

A standardized high-resolution photographic imaging system for facial and body pictures. The posture of the face of the subject is maintained in a fixed position for reproducibility (frontal and left/right positions). Furthermore, the position of illumination and nature of the light source are maintained constant. Possibilities of standard flat lighting, polarized light, or UV illumination; Visia CR, Canfield [21], and VisioFace RD system, Courage-Khazaka [57].

Barrier function of the skin. The integrity of the superficial structure of the skin (horny layer and most upper part of the epidermis) can be assessed by measuring the TEWL. Also, the efficacy of hydrating properties of W/O emulsions can be evaluated by this technique. Different instruments are available: Tewameter TM 300, Courage-Khazaka [58]; DermaLab TEWL probe, Cortex [59]; Vapometer, Delfin [60]; and Aquaflux, Biox [61].

Hydration of the upper parts of the epidermis and horny layer. Many simple instruments are based on the electrical properties of the horny layer/epidermis, which are related to its water content (impedance and capacitance methods) [62]: Corneometer CM 825, Courage-Khazaka [63]; DermaLab Cortex [64]; DPM 9003, Nova Technology Corporation [65]; MoistureMeter SC-2, Delfin [66]; and Skicon 200EX, I.B.S. [67]. More sophisticated instruments were recently used for determining the water content of the horny layer: Fourier-transformed infrared spectroscopy with an attenuated total reflection unit, ATR-FTIR [68]; and confocal Raman microscopy [69]. The last two instruments, although they give quantitative data directly related to the amount of water present in the horny layer, are less used in routine clinical research due to the high price of purchase.

Measurement of radiance and gloss. There exist instrumental Gloss measuring systems [70]. The skin surface is illuminated with light of a certain wavelength, and the reflected light is quantified, which gives information concerning the

gloss/radiance of the skin surface. Skin Glossmeter, Delfin [71]; Glossymeter GL 200, Courage-Khazaka [72].

Surface friction. The measurement of skin surface friction reflects the smoothness of the skin surface: Frictiometer FR 700, Courage-Khazaka [73]; KES–SE Friction surface tester, Kato Tech [74]; and Newcastle Friction Meter [75].

Thickness of the skin and structure of the dermis and hypodermis. These investigations are performed in many studies but more particularly in relation to antiaging and anticellulite claims. Specific ultrasound instruments for measurements on the skin operating at 20–25 MHz [76]. DermScan Cortex [77] and Ultrascan UC 22, Courage-Khazaka [78].

Sensitive skin. Despite the numerous different bioengineering instruments that are available, some dermatocosmetic properties of the skin remain difficult to quantify by instruments. An example is the concept of sensitive skin. The diagnosis of sensitive skin is defined by neurosensory hyperreactivity of the skin and is essentially based on self-perceived sensations of people who report facial skin discomfort as stinging, burning, and itching when their skin is exposed to some environmental factors (wind, sun, and pollution) or after application of topical products (hard water, soap, and cosmetics) [79]. Subjects with sensitive skin give a positive response profile, when questioned using a standardized Sensitive Skin Questionnaire. It appears from the literature that with the classical routine bioengineering devices (hydration, TEWL, skin color), it is very difficult to detect the presence of sensitive skin in subjects, and these parameters are not correlated with sensitive skin [79,80]. Only a very sophisticated device such as functional magnetic resonance imaging is capable to detect in the brain cortex, specific sites involved with sensitive skin [80].

CONCLUSIONS

It is now obvious that noninvasive techniques have proven to be valuable tools for measuring objectively and quantitatively many cosmetic effects on the human skin and are ideally suited for testing the efficacy of these cosmetic products [12–17]. The noninvasive technology is harmless to the human volunteers and causes almost no discomfort. Most bioengineering instruments are commercially available at affordable cost, and new techniques are constantly being developed, modified, and validated.

On the other side, sensorial (mostly visual) evaluations of the cosmetic effects by expert and trained assessors who have experience in the use of well-defined scoring systems can be considered also as a validated methodology. The sensorial/visual evaluation methods by experts can be applied to almost all cosmetic claims when using a specific, precise, and reproducible scoring system. Sensorial/visual evaluations carried out by experts provide valuable data on the product benefits, which are closer to consumer evaluations.

In both methodologies, these measurements are validated only if they are carried out under standardized conditions, ideally performed in blind conditions on a sufficient number of subjects and using appropriate statistical analysis. In conclusion, both methodologies are complementary and not concurrent.

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62 Validated Alternative Methods Available for Human Health Safety Assessment of Cosmetic Products and Their Ingredients in the European Union

Vera Rogiers

INTRODUCTION: LEGISLATIVE CHANGES AND CHALLENGES

During the last years, a lot of key changes took place in the European Union (EU) cosmetic legislation. In particular, of importance was the implementation of the so-called 6th Amendment, Council Directive 93/35/EEC, which drastically modified the policy behind Council Directive 76/768/EEC. The modification introduced can be summarized as follows: (i) notification of any cosmetic product marketed in the EU; (ii) production of an inventory of ingredients by the Commission with the help of the industry; (iii) changes in ingredient labeling, including the introduction of International Nomenclature of Cosmetic Ingredients (INCI) and labeling of the qualitative composition on the packaging; (iv) labeling of the product function where relevant; (v) for every cosmetic product on the EU market, the obligation of compiling a technical information file (TIF) or product information requirement (PIR), containing the safety assessment of the product and proof of the claims made for the product; and (vi) ban of animal testing of ingredients whenever validated methods are available. This ban was postponed twice (97/18/EC and 2000/41/EC), and the final expiry date was fixed on June 30, 2002. In the meantime, the 7th amendment became available (2003/15/EC), which established a prohibition to test finished cosmetic products and cosmetic ingredients on animals (testing ban), and a prohibition to market in the European Community finished cosmetic products and ingredients included in cosmetic products that were tested on animals (marketing ban). The testing ban on finished cosmetic products has already applied since September 11, 2004, whereas the testing ban on ingredients or combination of ingredients was applied step by step as soon as alternative methods became validated and adopted, but with a maximum cutoff date of 6 years after entry into force of the Directive, that is, March 11, 2009, irrespective of the availability of alternative nonanimal tests. The marketing ban was simultaneously applied, making that from March 11, 2009, all animal testing of cosmetic ingredients was prohibited and products in which tested ingredients (after that date) were present could not be sold anymore on the EU market. An exception was made for repeated dose toxicity tests, reproductive toxicity, and toxicokinetics. For these, testing was allowed but only when done outside the EU.

For these specific health effects, a deadline of 10 years after entry into force of the Directive was foreseen, that is, March 11, 2013, irrespective of the availability of alternative nonanimal tests.

An extensive report was made for the parliament by the Joint Research Center (JRC) with the help of several EU scientists to combine all scientific argumentation that a postponement of the deadline of 2013 was necessary because of lacking essential in vitro tests for risk assessment, for example, repeated dose toxicity, developmental toxicity, carcinogenicity, sensitization, and toxicokinetics (Adler et al. 2011). In order to decide whether or not a legislation proposal should be made, the Commission worked out an impact assessment in which a number of options were proposed, but finally no postponement of the deadline took place.

Until July 2013, Directive 76/768/EEC applied. This means that the safety of cosmetics and their ingredients brought on the EU market was the responsibility of the manufacturer, first importer in the EU, or marketer (Article 7, Council Directive 93/35/EEC). According to Article 2 of Council Directive 76/768/EEC, cosmetic products were not allowed to cause damage to human health when used under reasonable foreseeable conditions of use.

In addition, this had to be accomplished by relying on the safety of the ingredients by taking into consideration their toxicological profile, their chemical structure, and the level of exposure (Article 7a (d) of Council Directive 93/35/EEC). An extra challenge, of course, was that safety was to be guaranteed by using validated alternative methods (7th amendment, Article 4.a.1) with focus on replacement methods.

Recently, EU Regulation EC N°1223/2009 on cosmetic products (recast of Directive 76/768/EEC) was adopted (2009/1223/EU), and replaced from July 2013 onward,

Directive 76/768/EEC and its amendments. Basically, no substantial changes with respect to animal testing were introduced, and the testing and marketing bans remained untouched.

Previously posed questions remain the same, namely, "What toxicological tests are in general needed for the safety evaluation of cosmetics and their ingredients?" and "What validated *alternative methods* are available that are useful for human health safety evaluation of cosmetic ingredients?"

HAZARD IDENTIFICATION OF COSMETIC INGREDIENTS IN EUROPE

As can also be found in the 8th Revision of the "SCCS Notes of Guidance of the testing of cosmetic ingredients and their safety evaluation" (SCCS/1501/12), specific data, usually necessary for human safety assessment of cosmetic ingredients, include identification and physicochemical properties of the compound involved in hazard studies consisting of acute toxicity, irritation and corrosivity (skin, eye), skin sensitization, dermal absorption, repeated dose toxicity (28/90 days), mutagenicity/genotoxicity, developmental/reproductive toxicity, and less frequently carcinogenicity, chronic toxicity (>12 months), toxicokinetic studies, photoinduced toxicity, and information from human exposure. Until recently, most of these data were generated by animal experiments.

During more than 30 years, however, substantial efforts have been done to develop so-called 3R-alternatives according to the principle of Russell and Burch (1959) (refinement, reduction, replacement).

A major step forward was the establishment of the European Centre for the Validation of Alternative Methods (ECVAM) in 1992, which is now called the European Union Reference Laboratory for Alternatives to Animal Testing (EURL ECVAM), followed by the US equivalent, the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) in 1997, the Canadian Council on Animal Care (Environmental Health Science and Research Bureau within Health Canada, CCAC) in 2001, and the Japanese Center for the Validation of Alternative Methods (JaCVAM) in 2005. Also, the Korean Center for the Validation of Alternative Methods (KoCVAM) joined in 2011. They form together the so-called International Cooperation on Alternative Test Methods (ICATM), the Brazilian Center for Validation of Alternative Methods (BracVAM) was officially launched in 2012.

ECVAM has the mission to look for 3R-alternative methods that become available worldwide and to see whether they meet the validation criteria set before (reliability, relevance, reproducibility, with a well-defined purpose, complying with a prediction model). The tasks of EURL-ECVAM have been extended, seen the provisions in the updated Directive 86/609/EEC (Directive 2010/63/EU), in which a more active role of the Member States and EU Commission becomes mandatory.

For a number of the hazard identification tests, mentioned above, validated 3R-alternatives exist, but the list becomes shorter when only replacement methods are considered, as foreseen in Directive 76/768/EEC and its Amendments and as taken over in Regulation EC N°1223/2009 (the so-called RECAST).

VALIDATED 3R-ALTERNATIVES

Validated methods are those methods that are in compliance with the validation process, as set up in the EU by ECVAM (now called EURL-ECVAM) and its independent Advisory Committee ECVAM Scientific Advisory Committee (ESAC). This means that their relevance and reliability have been established for a particular purpose, taking into account that a prediction model was present from the start of the validation process (Balls and Fentem 1997; Worth and Balls 2001). In the meantime, the validation process has become more flexible by introducing a modular approach (Hartung et al. 2004).

Upon compliance of a particular 3R-alternative method with all modules and after peer review by independent experts, it may be taken up in the current EU legislation on chemicals (Regulation 440/2008/EC).

Until now, the practice was that once a 3R-alternative method had passed the validation procedure, the Scientific Committee on Consumer Safety (SCCS) and its predecessors analyzed its usefulness for the safety assessment of cosmetic ingredients. It is, for example, desirable that a sufficient number of relevant cosmetic ingredients, included in the Annexes of Directive 76/768/EEC (and Regulation EC N°1223/2009), are present among the reference substances included in the validation process of the replacement alternative method under consideration. Furthermore, after March 11, 2009, only replacement and validated alternative methods could be used in Europe; otherwise, the marketing ban was applied (exceptions were repeated dose toxicity, developmental toxicity, and toxicokinetics). As the actual cosmetic Regulation was implemented from March 11, 2013 onward, these exceptions do not exist anymore.

Acute Toxicity

The term "acute toxicity" is used to describe the adverse effects on health, which may result from a single exposure to a substance via the oral, dermal, or inhalation route (ECB 2003).

The in vivo acute oral toxicity test was originally developed to determine the LD_{50} value of the compound under investigation. In the current chemical legislation Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH), this LD_{50} value triggers the classification of the compound (2008/1272/EC).

The original test method (EC B.1; OECD 401), involving between three and five dosage groups, each of these comprising five to 10 animals, has been deleted (2001/59/EC) and replaced by the following alternative methods:

1. The *fixed dose method* (EC B.1 bis; OECD 420) abandons lethality as an end point and is designed not to cause death, marked pain, or distress to the

animals. By taking evident toxicity as an end point, it is a useful refinement alternative method to EC B.1/OECD 401.

2. The *acute toxic class method* (EC B.1 tris; OECD 423) does not aim to calculate a precise LD_{50} value but allows the determination of a range of exposure dosages where lethality is expected. The test follows a complex stepwise dosage scheme and may consequently take longer than the original EC B.1/OECD 401 and the alternative EC B.1 bis/OECD 420 method. Nevertheless, it offers, as a main and important advantage, a significant reduction in the number of animals tested.
3. The *up-and-down procedure* (OECD 425) allows an estimation of the LD_{50} value and confidence intervals, and the observation of signs of toxicity. The guideline significantly reduces the number of animals used in comparison to Guideline EC B.1/OECD 401.

The above alternatives are combined refinement and reduction methods, but not replacement methods. The same is true for OECD 436 and OECD 433. The former describes the acute toxic class method by the inhalation route and has been recently officially accepted (OECD 436; EC B.52) instead of OECD 403. The latter (OECD 433) is a draft guideline of the fixed concentration procedure also by inhalation. For acute toxicity testing through the dermal route, however, no validated alternatives are yet available, but a draft OECD 434 is available for the fixed dose procedure.

Usually acute toxicity data of cosmetic ingredients are already available as a result of compliance with the provisions of the 7th Amendment to Directive 67/548/EEC on the notification, classification, and labeling of dangerous substances (92/32/EEC) and/or through REACH requirements.

In the EU Research Programme, FrameWork Programme 6 (FP6), the integrated project Acute-Tox (www.acutetox.org) had as objective the development of a replacement alternative for oral acute toxicity testing. The results are available. They are, however, not concerned with acute dermal and inhalation toxicity, which are also important for cosmetic substances.

Corrosivity and Irritation of the Skin

Skin corrosion or dermal corrosion tests assess the potential of a substance to cause irreversible damage to the skin, namely, visible necrosis through the epidermis and into the dermis, following the application of a test substance for the duration period of 3 min up to 4 h. Corrosive reactions are typified by ulcers, bleeding, scabs, and, at the end of observation at 14 days, by discoloration due to blanching of the skin, complete areas of alopecia, and scars (EC B.4; OECD 404).

Corrosivity is not a feature one expects to occur with cosmetics but occasionally could occur after a manufacturing mistake or misuse by the consumer. On the other hand, a cosmetic ingredient that has the intrinsic property to be corrosive is not necessarily excluded for use in cosmetics.

It very much depends on its final concentration in the cosmetic product, the presence of "neutralizing" substances, the excipient used, the exposure route, the conditions of use, etc. According to the Classification Labelling and Packaging (CLP) regulation (2008/1272/EC) of substances and mixtures from 2011 onward, skin corrosion is called category 1 and consists of three subcategories 1A, 1B, and 1C. It is used for the classification of chemical substances and triggers (for chemicals) a number of label elements.

For skin corrosion testing, actually five validated in vitro replacement alternatives exist and are taken up in Regulation No 440/2008 (2008/440/EU). This also implies that the original in vivo corrosion test is forbidden in the EU. The alternatives available now are

- The "In vitro Skin Corrosion: Rat Skin Transcutaneous Electrical Resistance test" the TER test uses excised rat skin as a test system and its electrical resistance as an end point (EC B.40; OECD 430).
- EpiSkin, EpiDerm, SkinEthic, and EST-1000 (Epidermal skin test 1000) are four commercialized human skin model tests consisting of reconstructed human epidermal equivalent using cell viability as an end point (EC B.40bis; OECD 431).

The five tests mentioned are replacement tests, and although they are mainly used outside the cosmetic field, they can be useful in certain cases, for example, acids or bases to adjust the pH of a cosmetic formulation. A limitation of these systems with regard to the classification of volatile compounds and/or substances displaying propensity to polymerization upon contact with air was discovered recently (ESAC 2009a).

Skin irritation or dermal irritation is defined as reversible damage of the skin following the application of a test substance for up to 4 h. Originally, the standard skin irritation test consisted of an in vivo test method using three to six rabbits. Over the years, the test method has been subject to refinement and reduction measures, bringing the number of animals to a maximum of three and involving a number of steps to be taken before any in vivo study can be envisaged (EC B.4; OECD 431). These consist of

- The evaluation of existing human and animal data
- The analysis of structure activity relationships
- A study of physiochemical properties and chemical reactivity (when $pH \leq 2.0$ or $pH \geq 11.5$, corrosivity is assumed)
- Looking at available dermal toxicity data
- Taking into account the results from in vitro and ex vivo tests

A number of in vitro skin irritation tests have been officially validated (EC B.46). The EpiSkin model passed ESAC

as a validated alternative method (ESAC 2007). It is proposed as a stand-alone test that could replace the in vivo skin irritation test for the purpose (before 2011)* of distinguishing skin irritating and nonirritating substances. The end point used is cell-mediated reduction of MTT [3-(4,5)-dimethyl-2-thiazolyl-2,5-dimethyl-2H-tetrazolium bromide].

To obtain better sensitivity while maintaining similar specificity, a second end point is suggested: interleukin-1α (IL-1α) production. The test is found useful by the SCCS for cosmetic ingredients.

However, concern exists with respect to reducing substances and hair dyes and colorants since these can interfere with the formazan color evaluation (Lelièvre et al. 2007; SCCS/1392/10). When these substances need to be tested at different end point, not involving optical density quantification, should be used (SCCS/1392/10). The test has been taken up in Part B of Commission Regulation N°440/2008 (EC B.46).

The performance of the validated EpiSkin test method was used for specifying ECVAM skin irritation performance standards (May 2007).

The modified Epiderm (SIT) and the SkinEthic Reconstructed Human Epidermis (RHE) test methods were subsequently validated on the basis of these performance standards using 20 defined Reference Chemicals (ESAC 2008).

In December 2008, the EU adopted the UN Globally Harmonized System (UN GHS) for Classification, Labelling and Packaging (CLP) of Substances and Mixtures (Regulation EC N°1272/2008/EU). In agreement with the existing European system, the new EU CLP skin irritation classification system uses a single irritant category (instead of two in the UN GHS) and continues to use a total of two classification categories to distinguish irritant from nonirritant substances. However, the cutoff score shifted to an in vivo score ≥ 2.3 from a value of 2.0.

The performance (specificity and sensitivity) of all three tests has been reevaluated under the new EU CLP and was found satisfactory (ESAC 2009b). Thus, the in vitro test methods, based on reconstructed human epidermis—the EpiSkin, the modified EpiDerm, and the SkinEthic RhE—are now included in OECD 439 and were endorsed by ESAC. It is clearly mentioned that the in vitro tests can be used as a stand-alone replacement test within a testing strategy, in a weight of evidence (WoE) approach.

In the light of the imposed testing ban on cosmetic ingredients, Cosmetics Europe proposed a decision-tree approach for the integration of alternative approaches into tiered testing strategies for hazard and safety assessment of cosmetic ingredients and their use in products.

Two separate decision trees are put forward (Macfarlane et al. 2009):

1. A decision tree for hazard identification of the neat test substance, where physicochemical properties, read-across data, quantitative structure–activity relationship (QSAR) results, and in vitro skin corrosion data may lead to a classification as corrosive. If all these assays fail to indicate the substance as a skin corrosive, a validated in vitro skin irritation assay may either trigger a classification of irritant or nonirritant.
2. A decision tree for risk assessment of the neat ingredient in the final formulation(s), where the measured formulation's skin irritancy in an in vitro skin irritation test is to be compared against the measured irritancy of a benchmark control. The last step in the decision tree is called a confirmatory formulation test with human volunteers under in-use conditions.

The SCCS emphasized that in the above tiered approach, a case-by-case study of every data set remains necessary as

- The decision tree for hazard identification lacks a critical view on the applicability domain of the in vitro assays (e.g., exclusion of colorants and reducing substances, consideration of other end points, etc.).
- The decision tree for risk assessment includes a benchmark approach, which finally results in human safety testing. According to SCCNFP/0245/99, only compatibility testing in human volunteers is acceptable from an ethical point of view. It is the opinion of the SCCS that the WoE needs to be carefully considered before such human testing is applied.

Eye Irritation

Eye (mucous membrane) irritation tests have been developed to assess the production of changes in the eye following the application of a test substance to the anterior surface of the eye, which are fully reversible within 21 days of application. Eye corrosion is tissue damage in the eye, or serious deterioration of vision, following application of a test substance to the anterior surface of the eye, which is not fully reversible within 21 days of application.

There are presently no fully validated alternative methods replacing the classical Draize in vivo eye irritation test, which is an in vivo test method now involving the use of only one to three rabbits. Over the years, the test method has been subject to refinement and reduction measures, bringing the number of animals down from maximum six to maximum three, and involving a number of steps to be taken before the in vivo study can even be envisaged.

These steps consist of the following:

- The evaluation of existing human and animal data
- The analysis of structure–activity relationships
- A study of physicochemical properties and chemical reactivity (e.g., substances with a pH ≤ 2.0 or

* From 2011 onward, with Regulation No. 440/2008, skin corrosion is called category 1 and has three subcategories 1A, 1B, and 1C; skin irritation is considered as category 2. It is for chemical substances important for classification and triggers a number of label elements (GHS pictograms, signal words, hazard statements, and precautionary statements).

≥11.5 will be considered as corrosive without in vivo testing)
- Consideration of other existing information
- Taking into account the results from in vitro and ex vivo tests
- The assessment of in vivo dermal irritancy or corrosivity of the substance (EC B.5; OECD 405)

Screening methods for hazard identification (not risk assessment) to eliminate severe eye irritants are the bovine cornea opacity permeability (BCOP) and isolated chicken eye (ICE) tests. Both tests use tissues from slaughterhouses.

The tests replace the use of experimental animals to identify severe irritants, but animal testing is still required for mild and nonirritants as is often the case for cosmetic ingredients (http://ecvam.jrc.it/index.htm). This view is supported by ICCVAM. Both the BCOP and the ICE were translated into OECD (OECD 437; OECD 438) and EU guidelines (EC B.47; EC B.48).

Both tests plus two other screening tests, Isolated Rabbit Eye (IRE) and Hen's Egg Test–Chorioallantoic Membrane (HET-CAM), were taken up in the European Chemical Bureau (ECB) Manual of Decisions for Implementation of the 6th and 7th Amendments to Directive 67/548/EEC but provide only supportive evidence for cosmetic ingredient safety assessment. They are, in particular, useful within REACH (Regulation EC N°1907/2006) for labeling and transport purposes. Several tests are under validation, including human reconstructed tissue models, but these are not yet ready.

Finally, a number of cytotoxicity/cell function-based assays for water-soluble substances (the cytosensor microphysiometer test method; the fluorescein leakage test; and the neutral red release, fluorescein leakage, and red blood cell hemolysis test) underwent retrospective validation and peer review by ESAC (2009b, 2009c). These tests, however, are only screening assays and are not suitable for determining the potency of eye irritancy. The fluorescein leakage test has been adopted by the OECD and is recommended as part of a tiered testing strategy for regulatory classification and labeling but only for limited types of chemicals (i.e., water-soluble substances and mixtures) (OECD 460). For the cytosensor microphysiometer, which has been validated by ECVAM in 2009, a draft OECD guideline is in progress. This methodology is, in particular, used in the United States. Another assay based on cytotoxicity and currently under validation is the short time exposure (STE) test, which uses a rabbit corneal cell line to predict eye irritation. But again, these methods need further critical evaluation before they could be considered full replacement methods for eye irritation.

In the light of the imposed testing ban on cosmetic ingredients, Cosmetics Europe proposed a decision-tree approach for the integration of alternatives into tiered testing strategies for hazard and safety assessment of cosmetic ingredients and their use in products. It was acknowledged that, in contrast to the in vitro skin irritation tests, neither a single in vitro assay nor a testing battery has been validated as a full replacement for the rabbit Draize eye test.

Nevertheless, two separate decision trees for eye irritation were put forward (McNamee et al. 2009):

1. A decision tree for hazard identification of the neat cosmetic ingredient, where physicochemical properties, read across data, QSAR results, and in vitro eye irritation data may lead to a classification of irritant or nonirritant. It is noted that the existing in vitro models may fail to identify nonirritants and weak-to-moderate eye irritants.
2. A decision tree for risk assessment of the neat ingredient in its final formulation(s), where the measured formulation's eye irritancy in one or more in vitro eye irritation test(s) is to be compared against the measured irritancy of a benchmark control. The last step in the decision tree is called a confirmatory formulation test with human volunteers under in-use conditions.

The SCCS emphasized the fact that in the above tiered approach, human safety testing for eye irritation is the final step in the risk assessment decision tree. The committee considered that, without the existence of a validated stand-alone in vitro test/testing battery, the tiered approach is too premature to be applied. Human eye irritation testing may have serious health consequences for the volunteers involved.

Scott et al. (2010) published the outcome of an ECVAM expert meeting (held in 2005), with the aim of identifying testing strategies for eye irritation. A hazard identification testing scheme was proposed using a bottom-up (starting with test methods able to accurately identify nonirritants) or top-down (starting with test methods able to accurately identify severe irritants) progression of in vitro tests. As such, this approach intends to identify nonirritants and severe irritants, leaving all others to the (mild/moderate) irritant categories.

As identification of nonirritancy for the eye through in vitro methodology is today not yet possible, the practical value of the proposal is limited.

Skin Sensitization

A skin sensitizer is an agent that is able to cause an allergic response in susceptible individuals. The consequence of this is that following subsequent exposure via the skin, the characteristic adverse health effects of allergic contact dermatitis may be provoked (ECB 2003). As yet, there is not a fully validated and complete in vitro test methodology accepted for skin sensitization.

There are three common in vivo laboratory animal test methods to evaluate the potential of a substance to cause skin sensitization:

1. The Local lymph node assay (LLNA) (EC B.42; OECD 429) uses an inbred strain of mice and is based on the extent of stimulation of proliferation of lymphocytes in regional lymph nodes draining the

site of application of the test substance. It is an objective method giving the result as a stimulation index (SI), which is the ratio of stimulation caused by the test substance in animals versus that in vehicle-treated control animals. The test substance is applied openly to the dorsum of the ear in a suitable vehicle, and the use of Freund's complete adjuvant as an immune enhancer causing local skin inflammation is avoided.

The LLNA is an alternative method on mice that refines the methodology in comparison with the traditional guinea pig-based models as described below.

2. The Magnusson–Kligman guinea pig maximization test (GPMT) (EC B.6; OECD 406) is an adjuvant-type test, which means that the allergic response is potentiated by intradermal injection of the test substance with and without Freund's complete adjuvant. The GPMT is considered equal in sensitivity compared to the LLNA. The test result is based on the challenge response to a nonirritant patch test with the test substance.

   Thus, the test mimics the "real-life" development of allergic contact dermatitis. The method allows repeated challenges, cross reactivity, and vehicle effect studies.
3. The Buehler test (EC B.6; OECD 406) is a nonadjuvant technique that involves topical application only. The method is less sensitive compared to the GPMT. Scientific justification should be given in case the Buehler test is used.

A reduced LLNA (rLLNA) has been approved by ESAC. A retrospective analysis of published data obtained with the LLNA (Kimber et al. 2006) has taken place. It was concluded that within a tiered testing strategy in the context of REACH, a reduced version of the LLNA, using only a negative control group and the equivalent of the high-dose group from the full LLNA, can be used as a screening test to distinguish between sensitizers and nonsensitizers.

When compared with the full LLNA, the rLLNA may produce a few false negatives (3/169 in the reference document, reducing to 2/169 when negative results obtained with concentrations of <10% are considered invalid). The rLLNA does not allow the determination of the potency of a sensitizing chemical as only one dose is tested. The OECD mentions the possibility of performing a rLLNA, but with certain restrictions and certainly not when dose-response information is required (OECD 429).

Simultaneously, work at the OECD level took place to accept the LLNA using a nonradioactive methodology, for example,

- Daicel-ATP, which is a modified LLNA method using adenosine triphosphate (ATP) as an end point. The mice are exposed four times instead of three times, and the ATP content is used as a measure of the proliferation of the lymph node cells (OECD 442A).
- Cell proliferation enzyme-linked immunosorbent assay (ELISA), BrdU, which is a second-generation ELISA with calorimetric or chemiluminescent detection, which quantifies the DNA synthesis within the lymph node cells; BrdU being 5-bromo-2-deoxyuridine labeling (NICEATM-ICCVAM Web site, test method evaluation report LLNA: BrdU-ELISA) (OECD 442B).

As far as replacement in vitro tests for skin sensitization are concerned, no validated battery of assays is available yet. Advances in the field are based upon the current level of knowledge on the mechanism of skin sensitization/allergic contact dermatitis, more specifically on the five key mechanistic pathways of skin sensitization. These consist of (i) haptenation (covalent binding of chemical sensitizer to skin protein), (ii) epidermal inflammation (release of proinflammatory signals by epidermal keratinocytes), (iii) dendritic cell activation, (iv) dendritic cell migration (movement of hapten–peptide complex bearing dendritic cells from skin to draining lymph node), and (v) T-cell proliferation. In vitro tests are being developed that are representative for these steps.

It is an example of the so-called "adverse outcome pathway" (AOP) approach, which is mechanistically based and considered to be the way forward in the field of alternatives to develop new in vitro tests.

A test to assess peptide reactivity, being the first step in the AOP, namely, the Direct Peptide Reactivity Assay (DPRA), has passed ESAC and has been forwarded to OECD. This method measures the ability of chemicals to react with proteins (haptenation), a determinant step in the induction of skin sensitization. It is based on the chemical reactivity of the compound under investigation, with lysine and cysteine residues (Gerberick et al. 2004).

Another method that has passed ESAC, namely, KeratinoSens, measures direct reactivity of sensitizing material to key cysteine residues of Keap1, a regulator of Nrf2. The Nrf2-Keap1-ARE regulatory pathway is considered one of the most relevant pathways for the identification of potential skin sensitizers (Natsch 2010).

Both methods can be used in a WoE approach in an in vitro test battery for the assessment of skin sensitization.

A human cell line activation test (h-CLAT), based on the enhancement of CD86 and/or CD54 expression in THP-1 cells, is reviewed at EURL-ECVAM. Myeloid U937 Skin Sensitization Test (MUSST) is based on the same principle and uses the cell line U-937 human lymphoma. An extensive review of the actual status of in vitro testing in this field can be found in a JRC report (Adler et al. 2011).

Dermal Absorption

Human exposure to cosmetic ingredients occurs mainly via the skin. In order to reach the circulation (blood and lymph vessels), cosmetic ingredients must cross a number of cell layers of the skin, where the rate-determining layer is considered to be the stratum corneum (SC). A number of factors play

a key role in this process, including the lipophilicity of the compounds, the thickness and composition of the SC (body site), the duration of exposure, the amount of topically applied product, the concentration of target compounds, occlusion, etc. (for review, see Schaefer and Redelmeier 1996; ECETOC 1993; Howes et al. 1996). In order to have a complete picture of the toxicokinetics involved, the absorption, distribution, metabolism, and excretion (ADME) of the ingredients need to be known (see also "Toxicokinetic Studies").

In vitro dermal absorption is described in OECD Guideline 428. The Scientific Committee on Cosmetics and Non Food Products (SCCNFP) adopted a set of basic criteria (SCCNFP/0167/99), which have been updated three times (SCCNFP/0750/03; SCCP/0970/06; SCCS/1358/10). OECD 428 addresses dermal absorption from a much broader point of view than the more detailed and/or stringent test requirements for cosmetics. Therefore, the SCCS considers it essential that, for cosmetic ingredients, not only OECD Guideline 428 but also the different additions to the basic criteria are applied. An overview of the basic criteria is present in an updated version in the Eighth Revision of the SCCS's Notes of Guidance. The in vitro dermal absorption methodology is a *replacement strategy.*

Absorption of a substance through the inhalation and oral routes is also of importance for cosmetic ingredients (e.g., in sprays, aerosols, lipsticks, and toothpaste). For both, no validated in vitro alternatives are yet available.

REPEATED DOSE TOXICITY

Repeated dose toxicity comprises the adverse general (excluding reproductive, genotoxic and carcinogenic effects) toxicological effects occurring as a result of repeated daily dosing with, or exposure to, a substance for a specific part of the expected lifespan of the test species (ECB 2003).

The following in vivo repeated dose toxicity tests are available:

1.	
Repeated dose (28 days) toxicity (oral)	(EC B.7; OECD 407)
Repeated dose (28 days) toxicity (dermal)	(EC B.9; OECD 410)
Repeated dose (28 days) toxicity (inhalation)	(EC B.8; OECD 412)
2.	
Subchronic oral toxicity test: repeated dose 90-day oral toxicity study in rodents	(EC B.26; OECD 408)
Subchronic oral toxicity test: repeated dose 90-day oral toxicity study in nonrodents	(EC B.27; OECD 409)
Subchronic dermal toxicity study: repeated dose 90-day dermal toxicity study using rodent species	(EC B.28; OECD 411)
Subchronic inhalation toxicity study: repeated dose 90-day inhalation toxicity study using rodent species	(EC B.29; OECD 413)
3.	
Chronic toxicity test	(EC B.30; OECD 452)

The 28- and 90-day oral toxicity tests in rodents are the most commonly used repeated dose toxicity tests and often give a clear indication on target organs and type of systemic toxicity. From these studies, often the no observed adverse effect level (NOAEL) is retrieved, which is a key factor in quantitative risk assessment.

The inhalation route is only rarely used in repeated dose toxicity testing due to the complex study design accompanying this kind of toxicity trials as well as to the lack of relevance of this route of repeated exposure for the majority of cosmetic products.

The objective of chronic toxicity studies is to determine the effects of a test substance in a mammalian species following repeated exposure during a period covering the whole lifespan of the animals. In these tests, effects that require a long latency period or that are cumulative become manifest.

At present, no validated or generally accepted alternative methods to replace in vivo repeated dose toxicity testing on experimental animals have been proposed. The SCCS is of the opinion that evaluation of the systemic risk via repeated dose toxicity testing is a key element in evaluating the safety of new and existing cosmetic ingredients. If these data are lacking in a new cosmetic ingredient submission to the SCCS, it is considered not feasible to perform risk assessment of the compound under consideration.

An extensive review of the status in vitro testing possibilities in this field can be found in a JRC report (Adler et al. 2011).

Mutagenicity/Genotoxicity

Mutagenicity refers to the induction of permanent transmissible changes in the amount or structure of the genetic material of cells or organisms. These changes may involve a single gene or gene segment, a block of genes, or chromosomes. The term clastogenicity is used for agents giving rise to structural chromosome aberrations. A clastogen causes breaks in chromosomes that result in the loss or rearrangements of chromosome segments. Aneugenicity (aneuploidy induction) refers to the effects of agents that give rise to a change (gain or loss) in chromosome number in cells, resulting in cells that have not an exact multiple of the haploid number (2006/1907/EC).

Genotoxicity is a broader term and refers to processes that alter the structure, information content, or segregation of DNA that are not necessarily associated with mutagenicity. Thus, tests for genotoxicity include tests that provide an indication of induced damage to DNA (but not direct evidence of mutation) via effects such as unscheduled DNA synthesis (UDS), sister chromatid exchange (SCE), DNA strandbreaks, DNA adduct formation or mitotic recombination, as well as tests for mutagenicity (2006/1907/EC; EChA 2008).

The evaluation of the potential for mutagenicity of a cosmetic substance should include tests to provide information on the following genetic end points, namely, (1) mutagenicity at a gene level, (2) chromosome breakage and/or rearrangements (clastogenicity), and (3) numerical chromosome aberrations (aneugenicity). This recommendation represents the actual consensus of international groups of scientific experts (Muller et al. 2003; Dearfield et al. 2011; 2006/1907/EC;

EFSA 2011; Kacew and Lee 2013). Before undertaking any testing, a thorough review of all available (literature) data on the substance under study, including its physico-chemistry, toxicokinetic and toxicological profile, as well as data on analogous substances, is necessary.

In principle, for the base level testing of cosmetic substances, *three assays* are recommended, represented by the following test systems (SCCNFP/0755/03):

1. Tests for gene mutation	
i. Bacterial reverse mutation test	(EC B.13/14; OECD 471)
ii. In vitro mammalian cell gene mutation test	(EC B.17; OECD 476)
2. Tests for clastogenicity and aneugenicity	
i. In vitro micronucleus test	(EC B.49; OECD 487)
ii. In vitro mammalian chromosome aberration test	(EC B.10; OECD 473)

A caveat to the use of the existing in vitro tests is the relatively high rate of unexpected negative (negative for carcinogens) and, particularly, unexpected positive (positive for noncarcinogens) results. An evaluation by Kirkland et al. (2005) for combinations of two or three assays demonstrated that with an increase in the number of tests, the number of unexpected positives increases whereas the number of unexpected negatives decreases.

More recently, Kirkland et al. (2011) showed that the sensitivities of the 2- and 3-test batteries seem quite comparable when an existing database of rodent carcinogens and a new database of in vivo genotoxins, together over 950 compounds, are considered. Using data from the gene mutation test in bacteria and the in vitro micronucleus test appears to allow the detection of all relevant in vivo carcinogens and in vivo genotoxins for which data exist in these databases. The combination of these two assays would cover the three end points, as the in vitro micronucleus assay detects both structural and numerical chromosome aberrations. The European Food Safety Authority (EFSA) has already published an opinion in which the use of two tests (OECD 471 and OECD 487) is recommended as a first step in genotoxicity testing for food and feed safety assessment (EFSA 2011). The guidance of the UK Committee on Mutagenicity also recommends the two tests (Ames and micronucleus test) for stage 1 in vitro testing (COM 2012).

For nanomaterials or substances with specific structural alerts, it is necessary that specific protocol modifications/additional tests are carried out for optimal detection of genotoxicity. For nanoparticles, the bacterial reverse mutation test is not a reliable test. *Salmonella* and *Escherichia* bacteria lack the mechanisms (e.g., endocytosis) to incorporate particles. For nanoparticles, a gene mutation test in mammalian cells (*hprt* test, mouse lymphoma assay) is an accepted alternative for the bacterial test.

Although most tests will give clearly positive or clearly negative results, in some cases, the outcome has to be considered inconclusive or equivocal. Equivocal refers to a situation where some but not all the requirements for a clear positive or clear negative result have been met. A substance giving an equivocal test result should be reinvestigated, using the same test method, but varying the conditions (including sampling more cells) to obtain conclusive results. Inconclusiveness refers to a situation where no clear result was achieved due to limitation of the test or procedure. In this case, repeating the test under the correct conditions should produce a clear result.

Cells should be exposed to the test substance both in the presence and absence of an appropriate metabolic activation system. The most commonly used system is a cofactor-supplemented S9-fraction prepared from the livers of rodents (usually rat) treated with enzyme-inducing agents such as Aroclor 1254 or combination of phenobarbital and β-naphthoflavone. The choice and concentration of a metabolic activation system may depend upon the class of chemical being tested. In some cases, it may be appropriate to utilize more than one concentration of S9-mix. For azo dyes and diazo compounds, using a reductive metabolic activation system may be more appropriate (Matsushima et al. 1980; Prival et al. 1984).

In order to demonstrate that the result obtained is due to treatment with the substance, it is essential to demonstrate exposure of the bacteria or cells. A way to demonstrate exposure is through cytotoxicity. In the Ames test, a reduction in the number of spontaneous revertant colonies and/or clearing of the bacterial background lawn is sufficient to indicate cytotoxicity and thus exposure of the substance. The other tests, measuring the induction of micronuclei or gene mutations (nanoparticles) in mammalian cells, require that the cells divide through at least (and sometimes at most) one round of replication to convert DNA damage into the genetic end point scored by the test. Therefore, cytotoxicity measures based on cell proliferation are preferred and, consequently, have been incorporated into the revised OECD Test Guidelines.

In the in vitro micronucleus test, Fowler et al. (2012) have shown that the use of relative population doubling (RPD) or relative increase in cell counts (RICC) helps to increase the specificity of the in vitro micronucleus test. If cytochalasin B is used to obtain binuclear cells, determination of the reduction in the number of binuclear cells is a justified alternative way to measure cytotoxicity. In gene mutation tests (nanoparticles), relative total growth or relative survival (relative cloning efficiency) is the preferred measure of cytotoxicity.

In cases where negative results are seen in the conducted tests, a mutagenic potential is excluded. Likewise, in cases where a positive result is seen in one of the tests, the compound has to be considered as a (in vitro/intrinsic) mutagen. Under the testing/marketing ban of the Seventh Amendment of the Cosmetics Directive (2003/15/EC) on cosmetic ingredients (also taken up in Regulation 1223/2009), further in vivo testing to confirm or, predominantly, to overrule the positive in vitro findings is no longer possible. Unfortunately, at present, no validated methods are available that allow the follow-up of positive results from standard in vitro assays. A number of promising alternative methods are under development. In the future, these tests could add to a WoE approach. Examples are

- The micronucleus test in reconstructed human skin
- The comet assay in reconstructed human skin

Also, worldwide research is ongoing to overcome false positive in vitro results by incorporating in vitro toxicogenomics. The idea is that by global gene expression profiling via genome-wide transcriptomics (microarray) technology, gene patterns covering diverse mechanisms of compound-induced genotoxicity can be extracted. These gene patterns/biomarkers can be further used as a follow-up of positive findings of the standard in vitro mutagenicity/genotoxicity testing battery (Goodsaid et al. 2010; Doktorova et al. 2012; Magkoufopoulou et al. 2012).

Carcinogenicity

Substances are defined as carcinogenic if they induce tumors (benign or malignant) or increase their incidence or malignancy or shorten the time of tumor occurrence when they are inhaled, ingested, dermally applied, or injected (ECB 2003). Carcinogens can be classified into genotoxic and nongenotoxic compounds. The former induce cancer through interaction with DNA and induce mutations. The latter induce tumors through mechanisms other than DNA damage, for example, hormonal effects.

The most commonly performed in vivo carcinogenicity tests are

1. Carcinogenicity test	(EC B.32; OECD 451)
2. Combined chronic toxicity/carcinogenicity test	(EC B.33; OECD 453)

Before the testing/marketing ban of the Seventh Amendment of the Cosmetics Directive (2003/15/EC) on cosmetic ingredients, the *most commonly performed carcinogenicity tests* were the carcinogenicity test (EC B.32; OECD 451) or the combined chronic toxicity/carcinogenicity test (EC B.33; OECD 453). Under the testing and marketing ban, in vivo testing to investigate the carcinogenic potential of substances is no longer possible. Unfortunately, at present, no validated methods to study carcinogenicity are available.

The in vitro *cell transformation assay* (*CTA*) is at a late stage of development. An ESAC workgroup reviewed the prevalidation data available and came to the conclusion that the CTAs using Syrian hamster embryo (SHE) cell tests are promising but need to come to a common protocol. For the CTA using BALB/c 3T3, more work is needed (ESAC 2011). Recently, all information on the CTA has been taken up in a review booklet (Josephy et al. 2012). Also, a detailed review paper (OECD 2007) has been published, and an OECD test guideline is under development (OECD 495).

The advantage of the CTA is that it is assumed to detect both genotoxic and nongenotoxic carcinogens. Indeed, exposure of cultured cells to both types of carcinogenic substances in a CTA test can lead to cell transformation involving changes in cell behavior/phenotype. Transformed cells can lead to tumor formation in vivo when injected in a suitable host, underlining their biological relevance.

Discussions are ongoing with respect to the use of a CTA (i) as a stand-alone test for nongenotoxic carcinogens; (ii) to de-risk a positive in vitro genotoxicity test in a WoE approach (as additional information); and (iii) in combination with a positive in vitro genotoxicity test to identify a genotoxic carcinogen.

Without the 2-year bioassay, it is very difficult if not impossible to conclude on the carcinogenicity of substances. As far as genotoxic compounds are concerned, in vitro mutagenicity tests are quite well developed. Due to the relation between mutations and cancer, these genotoxicity tests can be seen as a prescreen for cancer. A positive result in one of the genotoxicity tests may be indicative enough to consider a compound as putatively carcinogenic. In combination with the CTA, this indication may even be stronger. However, as carcinogenicity is a multihit/multistep process, it cannot (for the time being) be mimicked by in vitro tests. Today, any reliable, justified conclusion on the carcinogenicity of a substance needs in vivo tests.

The situation is different for the nongenotoxic carcinogens. Before the animal testing and marketing ban, nongenotoxic carcinogens were detected by the (sub-)chronic repeated dose studies, including the carcinogenicity test. Alternatives for these in vivo tests to detect nongenotoxic carcinogens, however, are not available with the exception of the CTA. Therefore, currently, in vivo rodent studies are essential to detect nongenotoxic substances.

An extensive review of the actual status of in vitro carcinogenicity testing can be found in a JRC report (Adler et al. 2011).

Reproductive Toxicity

The term "reproductive toxicity" is used to describe the adverse effects induced (by a substance) on any aspect of mammalian reproduction. It covers all phases of the reproductive cycle, including impairment of male or female reproductive function or capacity and the induction of nonheritable adverse effects in the progeny such as death, growth retardation, and structural and functional effects (ECB 2003).

The most commonly performed in vivo reproduction toxicity studies are

1. Two-generation reproduction toxicity test	(EC B.35; OECD 416)
2. Teratogenicity test: rodent and nonrodent	(EC B.31; OECD 414)

In particular, the latter often provides a NOAEL that is used in the quantitative risk assessment process of cosmetic ingredients.

At the OECD level, there also exists a combined "Reproduction/Developmental Toxicity Screening Test" (OECD 421) and a "Combined Repeated Dose Toxicity Study with the Reproduction/Developmental Toxicity Screening Test" (OECD 422), which have not yet been taken up in Regulation EC N°440/2008 (2008/440/EC).

Recently, the Extended One-Generation Reproductive Toxicity Study has been taken up by the OECD (OECD 443).

Validated alternative methods or strategies, covering the large field of reproductive toxicity, do not yet exist. Three alternative methods restricted to embryotoxicity

(representing a limited part of the reproductive cycle) have been approved by ESAC (2001). They consist of

1. The whole embryo culture (WEC) test
2. The micromass (MM) test
3. The embryonic stem cell test (EST)

The WEC test still requires animals since pregnant animals are needed as a source of embryos. These three tests have not been taken up in regulatory testing. The applicability domain of these three alternative tests is under discussion (Marx-Stoelting et al. 2009); they cannot be used for quantitative risk assessment. Therefore, for the time being, in vivo rodent studies remain necessary. The EST can be considered as a screening test. The end point of reproduction toxicity, however, is not covered by the in vitro systems available.

An extensive review of the actual situation with respect to in vitro testing in this field can be found in a JRC report (Adler et al. 2011; JRC Scientific and Policy Report 2013).

Toxicokinetic Studies

As mentioned under "Dermal Absorption," the term "toxicokinetic studies" is used to describe the time-dependent fate of a substance within the body. This includes absorption, distribution, biotransformation, and/or excretion (ADME). The term "toxicodynamics" means the process of interaction of chemical substances with target sites and the subsequent reactions leading to adverse effects (ECB 2003).

No validated alternative methods that cover completely the field of ADME exist. Some in vitro models are suitable to study the absorption of substances from the gastrointestinal tract (e.g., caco-2 cell cultures) or the biotransformation of substances (e.g., isolated hepatocytes and their cultures), but none of the many existing models have been validated yet (Eskens and Zuang 2005; JRC Scientific and Policy Report 2013).

Although toxicokinetic data for cosmetic ingredients are only requested in certain circumstances, their relevance is high for extrapolating both in vivo and in vitro animal data to the human situation.

An in-depth review of the actual status of toxicokinetics in risk assessment of cosmetics and their ingredients is presented in a JRC report (Adler et al. 2011; JRC Scientific and Policy Report 2013).

Validation is currently performed on the human HepaRG cell line and cryopreserved human and rat hepatocytes to assess their metabolic potential (cytochrome P_{450} activity), but the final results are not yet available.

An extensive review of the actual status of this field can be found in a JRC report (Adler et al. 2011).

Photoinduced Toxicity

The "3T3 Neutral Red Uptake Phototoxicity Test (3T3 NRU PT)" is a validated in vitro replacement method based on a comparison of the cytotoxicity of a chemical when tested in the presence and in the absence of exposure to a noncytotoxic dose of UV/visible light.

The method has been formally validated and taken up in Regulation EC N°440/2008 (EC B.41; OECD 432), making its use mandatory for testing for phototoxic potential.

The reliability and relevance of the in vitro 3T3 NRU phototoxicity test was evaluated for a number of substances with a chemically different structure (Spielmann et al. 1998), including UV filters used as cosmetic ingredients. The test was shown to be predictive of acute phototoxicity effects in animals and humans in vivo.

However, the test is not designed to predict other adverse effects that may arise from combined actions of a chemical and light, for example, it does not address photoclastogenicity/photomutagenicity, photoallergy, or photocarcinogenicity.

Presently, no validated in vitro methods for detection of photosensitization are available. Nevertheless, it is expected that chemicals showing photoallergic properties are likely to give positive reactions in the 3T3 NRU PT test (EC B.41).

Recently, a postvalidation exercise of the 3T3 NRU PT took place since, in particular, for pharmaceutical substances, false positives were observed. Some measures (e.g., limit of 100 μg/ml as highest concentration) were taken to decrease this number (ECVAM/EFPIA Workshop Report 2010).

Human Data

Cosmetic products are developed to be applied to human skin and external mucosa and to be used by the general public. Occasionally, undesirable side effects, both local and systemic, may occur. Local reactions may be, among others, irritation, allergic contact dermatitis, contact urticaria, and sunlight (especially UV light) induced reactions. Skin and mucous membrane irritation are the most frequently observed reactions.

Although it is inconceivable that tests in human volunteers would replace animal tests, it is known that tests in animals and alternative methods are of limited predictive value with respect to the human situation.

Therefore, a skin *compatibility test* with human volunteers, confirming that there are no harmful effects when applying a cosmetic product for the first time to human skin or mucous membranes, may be needed scientifically and ethically.

The general ethical and practical aspects related to human volunteer compatibility studies on finished cosmetic products are described in SCCNFP/0068/98 and SCCNFP/0245/99.

A separate SCCNFP opinion addresses the conduct of human volunteer testing of potentially cutaneous irritant (mixtures of) cosmetic ingredients (SCCNFP/0003/98). Ethical and practical considerations are discussed with a specific focus on irritancy. Finally, an SCCNFP opinion has been issued concerning the predictive testing of potentially cutaneous sensitizing cosmetic (mixtures of) ingredients (SCCNFP/0120/99).

FURTHER CONSIDERATIONS

During the last years, progress has been made in the development and validation of alternative methods for regulatory testing of chemical substances in general, but also in the field of cosmetic ingredients (Rogiers and Pauwels 2006). These

tests are primarily used for hazard identification and often do not give information on potency. Most successes in the development of alternative methods are in local toxicity and short-term testing; they are often reduction/refinement methods.

The methodologies consuming the highest number of animals, however, are in long-term testing and systemic toxicity. In these fields, validated alternatives and, in particular, validated replacement alternatives are lacking. This causes serious problems for the safety assessment of new cosmetics and new cosmetic ingredients.

As experience has shown the timing needed for test development, prevalidation, validation, and regulatory acceptance before practical use of alternative methods (Eskes and Zuang 2005), the deadline of 2009 could not be met and the same was true for the cosmetic deadline of 2013. Serious concern has been expressed already by all parties involved including the SCCNFP (SCCNFP/0834/04), the Scientific Committee on Toxicity, Ecotoxicity and the Environment (CSTEE) (CSTEE 2004), the Scientific Committee on Consumer Products (SCCP), Scientific Committee on Health and Environmental Risks (SCHER), and Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR) together in the so-called Inter Committee Coordination Group of Scientific Committees (ICCG) (ICCG/1/06) and ECVAM (2007). A memorandum of the SCCS on "Alternative Methods in Safety Assessment of Cosmetic Ingredients in the EU" is available (SCCS/1294/10) and has been updated in the Eighth Revision. It is, however, clear that there is a huge problem for cosmetics and their ingredients in the EU that needs to be urgently resolved.

The implementation of REACH in 2007 has accelerated the efforts and initiatives by the commission and individual parties to develop new alternative methods and validate existing ones.

Some relevant examples are the creation of the European Partnership for Alternative Approaches to Animal Testing (*epaa*; http://ec.europa.eu/enterprise/epaa/conf.htm), the *epaa* Annual Conferences, the test strategy development for REACH by ECVAM, the revised Directive 86/609/EEC (2010/63/EU), and many other initiatives.

However, the question arises how REACH, encouraging the use of the 3Rs strategy and the application of "suitable" and "sufficiently well developed" alternatives (REACH Regulation EC No 1907/2006), will now affect cosmetic testing strategies. It is thought that the methods proposed for REACH often may not be suitable for quantitative risk assessment of cosmetic ingredients, and it is not yet clear whether results obtained under the REACH legislation for a particular compound can be used for the safety assessment of that compound as a cosmetic ingredient.

Statistics on data and alternatives used in Registration Dossiers under REACH became available (ECHA 2011). The report provides useful quantitative information about which type of data is used in the 2010 Registration Dossiers such as existing and new data, in vitro results, read across, or QSAR. One of the key messages provided by the European Chemicals Agency (ECHA), however, was that read-across and waiving were broadly used but often *not* well justified (ECHA 2011).

Nanomaterials as cosmetic ingredients (e.g., UV-filters nano-ZnO and TiO_2) pose an additional and new challenge for safety testing of cosmetics; for example, as for all validated 3R-alternative tests, nanoparticle materials have not been included in the reference compounds during the validation process. This field needs special attention (SCCP/1147/07). In the Recast (Regulation EC N°1223/2009), new provisions for nanomaterials have been taken up (e.g., notification is necessary 6 months before marketing and extensive safety data need to be available for risk assessment by the SCCS). An SCCS guidance document for risk assessment of nanomaterials became recently available (SCCS/1484/12).

CONCLUSION

Although much effort has been done by all stakeholders, only a limited number of validated replacement alternative methods are available today. In particular, in vitro methods that can be applied to get quantitative information with respect to systemic toxicity and long-term toxicity are lacking. It is, therefore, not clear how to perform quantitative risk assessment today when the NOAEL values of actives are not known and may not be determined in vivo. Margin of safety (MoS) calculations cannot be carried out for new cosmetic substances. When compounds are tested as being chemicals under REACH, it is also not yet legally clear whether these data can be used in the safety evaluation of cosmetics and their ingredients.

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63 Cosmetovigilance in the European Union

Marc Paye, E. Roni, P. Prasad, and M.G. Best

The concept of postmarketing surveillance and vigilance for medicinal products became of utmost importance after discovering, in the early 1960s, congenital anomalies in children whose mother had used thalidomide during the first months of pregnancy, although the product had fulfilled extensive toxicity and premarketing clinical studies [1]. In 1968, the World Health Organization decided to set up a pilot international project aiming at developing a system, applicable internationally, for detecting previously unknown or poorly understood adverse effects of medicinal products. This program was the basis of what is known today as pharmacovigilance. For nonmedicinal products, postmarketing vigilance programs were considered only later as the risk to cause serious adverse reactions was incomparably much lower.

For instance, cosmetics do not penetrate into the body to modify the physiological functions. Nevertheless, when a substance or ingredient comes into contact with the skin, it cannot be excluded that, in specific circumstances, or in some individuals, this substance/ingredient might cause a reaction. This is why before their introduction to the marketplace, all cosmetic products undergo an extensive safety assessment, and, for most of them, skin acceptability or compatibility studies are carefully conducted on volunteers to confirm their good tolerance [2]. Human studies may also be conducted to measure skin benefits and document product claims, or to collect consumer's feedback before the product is launched in the market. In all these studies, any undesirable health effect is taken into account to complement and strengthen the overall safety assessment on the product.

When cosmetic products are finally released in the marketplace, this is with a high degree of confidence in their safety, with undesirable health effects being rare and mostly minor when the products are used as intended or in normally foreseeable conditions.

However, undesirable health effect may never be totally excluded for the following reasons:

- The premarketing studies are run on panels of a limited size as compared to the market size. In case of reactions that are very rare, it is possible that it never appears in a limited size panel.
- The premarketing studies are on healthy volunteers, and the selection of the panel is subject to noninclusion criteria such as pregnant women, young children, use of specific medications, existing skin pathologies or injuries, etc. [3]. When the product is launched in the market, a never investigated population may be exposed to the product leading to an unidentified undesirable effect (UE) from the premarketing studies.
- When a new product enters the marketplace, the frequency of allergic reactions elicited by that product may reflect the chemical environment to which consumers were exposed prior to its launch. For instance, an ingredient with a low allergenicity potential at that time may, a few years later, become so popular and used in so many different products that the sensitized population explodes. Postmarketing surveillance could then help to identify a change in the allergenicity potential of that ingredient.
- When a counterfeit product arrives on the market, it may be with a less safe profile than the original product. Postmarketing surveillance is often a good tool to discover the existence of the counterfeit product due to its different safety profile than the original product, and allow the corrective actions to be taken.

HISTORIC AND REGULATORY CONTEXT IN EUROPE

The first European initiatives looking at the postmarketing health effects of cosmetics started from groups of dermatologists. In 1988, a multicenter project grouping several university hospitals in Germany was initiated and further developed including more than 30 centers. This group, known as "Information Network of Departments of Dermatology (IVDK)," mainly aimed at detecting new emerging allergens in the European market [4]. In 1996, this initiative expanded into a European network formed from members of the European Society of Contact Dermatitis (ESCD) called the "European Surveillance System on Contact Allergies (ESSCA)" [5]. These groups, however, were mainly focused on the allergic reactions to cosmetics and shared experience and data on skin patch testing.

Postmarketing surveillance and cosmetovigilance, as we understand them today in the new cosmetic regulation [6], were the subject of several pilot studies from the European Commission and from national initiatives in the early 1990s proposing harmonized forms for health care professionals (HCPs) to use to report undesirable health effects. However, the return of such forms was relatively disappointing in the

absence of a legislative framework. In 1993, the first regulatory basis was set up in the 6th Amendment [7] (Art 7a [1] (f)) of the European Cosmetic Directive 76/768/EEC [8] requiring that "Existing data on undesirable effects on human health resulting from use of the cosmetic product" shall be readily accessible to the competent authorities (CAs). The 6th Amendment marked a big step forward in the creation of cosmetovigilance systems by forcing companies to develop internal processes to handle and evaluate undesirable events, as well as to keep their database of undesirable events constantly updated. However, the regulatory framework was relatively vague, and most companies developed their internal rules and used the vigilance data for continuous reevaluation of the safety of their products. The quality of UE data was very variable between companies. Consumers and HCPs were not educated on the need to report UEs to the manufacturers. There was no exchange of vigilance information between companies, and, in such conditions, when CAs requested to have access to the vigilance data of some companies, data were not always very useful.

In February 2003, Directive 2003/15/EC on the approximation of the laws of the EU member states relating to cosmetics products was adopted as the 7th Amendment to the Cosmetics Directive [9]. The 7th Amendment required that all vigilance data "shall be made easily accessible to the public by any appropriate means, including electronic means." One year later, Cosmetic Europe (formerly COLIPA, the European Federation of the Perfume, Cosmetics, and Toiletries Industry) set up a project team "cosmetovigilance" to develop guidelines for the cosmetic companies in order to handle and make accessible cosmetovigilance data in a more harmonized way. The first COLIPA guidelines "Management of undesirable event reports" on postmarketing surveillance were issued in 2005 [10] and provided instructions to the cosmetic industry on receiving, handling, evaluating, classifying, and reporting undesirable event reports associated with the use of cosmetic products.

In the meantime, in 2004, the Council of Europe set up a Committee of Experts on cosmetic products who initiated a pilot study on cosmetovigilance in which four countries participated: Austria, France, Norway, and Denmark. The outcome of those studies was that UEs on cosmetics are not so rare but are underreported by the consumers who usually discontinue the product and move to a different one rather than complain. It was also concluded that some of these effects may be serious and that the development of a well-organized cosmetovigilance system at a European level would be extremely useful. Based on these conclusions, the Council of Europe published in 2006 a resolution in which they proposed a definition for UEs and serious UEs (SUEs), suggested that SUEs should be immediately reported to CAs, developed the basis for causality relationship assessment between the reported effect and the cosmetic product, and tried to set up the respective roles and responsibilities of the different stakeholders (consumers, HCPs, manufacturers, distributors, and authorities) [11]. They also proposed some standard reporting forms to harmonize the exchange of relevant and sufficient information. In order to better align with the resolution of the Council of Europe, Cosmetic Europe developed the second edition of their guidelines in 2008 [12].

On the November 30, 2009, the new European Cosmetic Regulation, EC 1223/2009, was published in the official journal of the European Commission [6] and aimed at replacing the current Cosmetics Directive 76/768/EEC. Significant provisions related to cosmetovigilance were included, which constitute a real launch for a European harmonized system and are applicable from July 11, 2013.

The provisions related to cosmetovigilance in the European Cosmetic Regulation EC 1223/2009 are as follows:

- Art 23—Responsible persons and distributors must report to the CAs of the European countries (including Norway and Switzerland) where they occur, all SUEs on cosmetic products.
- Art 21—Existing data on UEs and SUEs resulting from the use of the cosmetic product shall be made accessible to the public by any appropriate means.
- Art 1—All available data on the UEs and SUEs from the cosmetic product or, where relevant, other cosmetic products must be incorporated into the Cosmetic Product Safety Report (CPSR), including statistical analysis.

This chapter will describe what is meant by each of these three requirements and where to find guidance to comply with them.

Definitions

Undesirable Effect

As defined in the European Cosmetic Regulation, EC 1223/2009, a UE is "an adverse reaction for human health attributable to the normal or reasonably foreseeable use of a cosmetic product."

Undesirable Event

An undesirable event is any medical occurrence for human health before a causal relationship has been established with the use of a cosmetic product, or which has not been attributed to the use of the cosmetic product after the causality assessment.

For the purpose of this chapter, both undesirable events and effects will be abbreviated by "UE," unless there is a need to make the difference.

Valid Undesirable Event/Effect

In order to be considered valid, the minimum data elements for a case are an identified consumer or identified reporter if the reporter is different from the consumer, a well-defined cosmetic product, the nature of the undesirable event/effect, and the date of its onset.

Serious Undesirable Event/Effect

A SUE is an undesirable event/effect that results in one of the following outcomes:

- Death
- Immediate vital risk
- Hospitalization
- Congenital anomaly
- Disability
- Temporary or permanent functional incapacity

The seriousness of an undesirable event/effect is thus determined on qualitative criteria related to a potential outcome of the reaction and *not on the severity of the reaction*, which is a quantitative criterion and results in a classification of a serious or nonserious undesirable event/effect as mild, moderate, or severe.

Spontaneous Reports

They are undesirable event/effect reports that are spontaneously communicated by a consumer, HCP, or any other reporter, provided they were not solicited.

Health Care Professional

The International Committee for Harmonisation has defined HCPs in its ICH E2D Notes for guidance [13] as medically qualified persons such as physicians, pharmacists, dentists, nurses, coroners, or as otherwise specified by local regulations. An undesirable event/effect reported by or confirmed by an HCP is said to be "medically confirmed."

Cosmetovigilance

As defined in the "EC Guidances for SUE reporting" [14], cosmetovigilance consists of the collection, evaluation, and monitoring of spontaneous reports of undesirable events observed during or after normal or reasonably foreseeable use of a cosmetic product.

STEPS IN COSMETOVIGILANCE

Cosmetovigilance consists of a series of successive steps with the aim to oversee the health safety behavior of cosmetic products when released on the marketplace and, if appropriate, take the relevant preventive or corrective actions. Cosmetovigilance is a major tool contributing to postmarketing surveillance. The steps in cosmetovigilance are described in Figure 63.1.

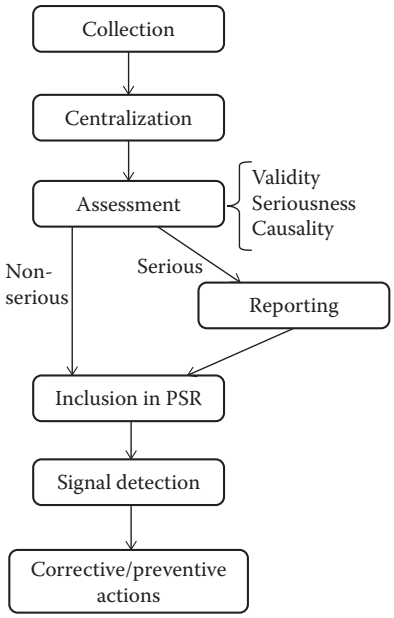

FIGURE 63.1 Steps in cosmetovigilance. UEs are collected and centralized to be available by appropriately qualified persons who assess the case for validity, seriousness, and causal relatedness with the product. If the case is nonserious, data are incorporated into the CPSR of the Product Information File. If the case is serious, it is first reported to the appropriate CA before incorporation into the CPSR. All cases are used for signal detection, which, if relevant and appropriate, may lead to the setup of preventive or corrective actions.

Data Collection: Initial and Follow-Up

A good process for undesirable event data collection is the basis of any vigilance system. Without good and sufficient data to describe a UE, it is not possible to make a good assessment of a case report. It is thus crucial to set up an efficient and clear internal system for the data collection, with all people involved in the process being trained on the minimum criteria to be collected. Usually, as much information as possible should be collected from the initial contact with the reporter as it is not always easy or even possible to establish further contacts. When the information is not sufficient for a complete assessment of the case, it is necessary to attempt to recontact the reporter to complete the information or to document the evolution/outcome of the reaction. When the consumer who experienced the UE is different from the reporter, it is usually more appropriate to directly contact the consumer to get the additional information. However, in order to respect data privacy regulations, it is necessary to obtain first the written authorization of the reporter to contact the consumer before initiating any contact with him/her. Similarly, when the consumer has consulted an HCP about the UE, a direct contact with the HCP is always very helpful to get a professional description of the reaction and to better understand its cause. A written consent from the consumer is also mandatory before any contact with the HCP to allow the latter to provide additional information.

In order to harmonize and facilitate data collection inside a company, mainly when products are marketed in many different members states, companies usually develop "data collection forms" to help in getting the maximum of information during the initial contact with the reporter. The form developed by the EU Commission to report SUE to the authorities may be partly used for such a purpose, but other simplified and better adapted forms may also be used. What is very important when using a form is to first let the reporter describe spontaneously the case with his/her words and collect the reporter's verbatim. The form should only be used

TABLE 63.1
Complaint Habits in the EU

High Level of Complaints	Moderate Level of Complaints	Low Level of Complaints
Germany	Spain	Belgium
United Kingdom	Cyprus	Bulgaria
Ireland	Latvia	Czech Republic
Austria	Lithuania	Denmark
Iceland	Luxembourg	Estonia
Finland	Poland	France
	Slovenia	Italy
		Hungary
		The Netherlands
		Portugal
		Slovakia
		Norway

Note: Level of complaint habits per country (based on a 2008 survey sponsored by the EU Commission). All types of complaints have been included in the survey.

afterward to complete the information, which would not have been provided spontaneously. It may seem easy to have a list of symptoms, ask the reporter if the consumer experienced any of them, and check a "yes or no" box during the call. However, this is a system that often leads to an overreporting of reactions associated with the event and does not provide a good picture of what really happened. This is thus not encouraged.

More and more, it is observed that consumers contact the companies to report UEs by e-mails instead of calling. This is positive in the sense that this is an easy way for the consumers to report complaints, and companies receive much more UE reports than in the past. This type of communication provides an overall much better and quicker overview of any issue that could happen with the product on the market. Nevertheless, UEs received by e-mail very often provide a minimal description of the reaction(s), mainly about the chronology of events, and are often more difficult to assess. Furthermore, only some of the consumers developing a UE with a cosmetic product complain to the company or consult with their HCP for the UE. With cosmetics, the UEs are usually minor and transitory, and many consumers just change their cosmetic product without reporting the effect. A survey from the EU Commission of the complaints reporting habits in the different member states has been conducted for complaints related to any type of product or any kind of service [15]. The outcome of that survey is to document the huge differences between the member states, with some countries having a high rate of complaints per million of inhabitants, while other countries have an extremely low level of complaints (Table 63.1).

Other Potential Sources of UE

Although this is rare, it sometimes happens that a UE on a cosmetic is reported in a newspaper or other journals. Provided the information is coming from a spontaneous, and not a solicited, source and that the level of information is sufficient to validate the case, such information cannot be ignored and has to be considered as a UE. Follow-up is, however, rarely possible. On Internet Web sites like Facebook, Twitter, or others, it may be more frequent to discover UE complaints. There is no obligation for companies to actively screen Internet Web sites for UE on their products. However, and similar to a newspaper, once a valid case is identified, it cannot be ignored. The difficulty with the Internet is to identify the reporter and be sure that the case is real. Each company should decide and develop their own process to deal with Internet-received cases in order to confirm their validity. For instance, the process could be to ask the reporter to contact the company directly to report the case independently of the Web site.

National CAs (NCAs) will receive SUEs directly from consumers and HCPs. Before sharing the information on the SUEs with the CAs of the other member states, the receiving CA will notify the case to the responsible person of the manufacturer of the cosmetic product. NCAs will thus be a potential and likely growing source of SUEs for the companies. Such cases have to be incorporated in the UE database of the company, and cooperation with the reporting NCAs is expected for sharing respective information on the case and on the product.

Table 63.2 provides a checklist of information that should be attempted to be collected during the initial or follow-up contacts.

Data Centralization

After collecting information on a UE, the second step is to record and centralize the information in a database. In cosmetovigilance, there is no obligation or even recommendation to use a specific database for UEs. A company who may receive very few UEs per year could, for instance, keep all the information in a paper file, without using a database. However, for products being largely distributed in many different countries, it is highly recommended to keep relevant information on UEs in a database, which could be from a simple Excel file to a very sophisticated vigilance database, depending on each company's specific situation. Whatever the internal "database" is used, what is necessary is that the system is well controlled and reliable. A process should be in place to ensure that the information could not be lost or altered with time.

The main reasons and interests for using a database are as follows:

- A single expert person for the evaluation may be used for the assessment of all UEs received by the company whatever the country of origin, provided all the information is translated in an easily understandable language.
- The information related to all UEs is kept in the same and harmonized format facilitating the

TABLE 63.2
Checklist for Data Collection

Administrative Information
Date of first awareness by a member of the company
Name of the company recipient
Identification and contact information of the reporter
Is the reporter an HCP?
Identification of the consumer (any information that proves the existence of a consumer such as initials, sex, age group, relationship with the reporter)
Product
Brand and variant identification as precise as possible
Batch number
Date of use and duration of use
Dosage and frequency of use
Continued, stopped, or interrupted after the reaction occurred
Concomitant Products
Other cosmetic products used concomitantly
Other products used concomitantly with the cosmetic products
Any change in the habits before the reaction occurred
Reaction
Detailed description as provided by the reporter including list of reported symptoms, precise location of the events, severity level of the events, consequence of the events for the consumer, etc.
Date of occurrence
Time sequence since the last use of the product as precise as possible
Outcome after stopping the product
Potential Treatment Provided for the Reaction
Detailed description of the treatment
Outcome of the reaction after the treatment
Medical Confirmation of the Reaction
Was the reaction or the causal relatedness with the product confirmed by a health care professional?
Contact information of the HCP
Authorization from the consumer or reporter to contact the HCP to get more details on the reaction
Medical dossier or documents about the reaction including medical certificate in case of functional incapacity or disability
Results of any additional investigations (e.g., patch testing) about the reaction
Medical History of the Consumer
History of allergies
Other products to which the consumer developed similar reactions in the past
Other relevant pathologies
Medications

retrieval of specific information and the identification of missing information.

- UEs should be coded under medical terms coming from either internal listing of terms or a medical dictionary like the MedDRA dictionary [16], which is used for pharmacovigilance. Having the coding done by a single and central person ensures a greater consistency.
- An increase in the frequency of a specific UE (e.g., increase in allergic reactions) may be a signal requiring the company to take preventive or corrective actions. Some databases allow running periodic or on-demand reports in order to follow the frequency of occurrence of specific UEs.

Data Validation

Only valid UEs qualify for reporting to CAs, for inclusion in the safety dossier of the product information file, and for being accessible to the public. All reports should thus be validated before any further use to be sure that the minimum criteria are available. The minimum criteria necessary to validate a report are

- An identifiable reporter characterized by its qualification, name, initials, address, or contact details (e.g., e-mail address). If the reporter is not the consumer, an identifiable consumer is also required. For data privacy reasons, the consumer may be identified,

for instance, by the name initials, the gender, and the age or age group depending on local and European data privacy regulations.
- An identified product. The product should be identified as precisely as possible, with the brand name, the variant name, and, whenever feasible, the batch or lot number.
- A well-defined reaction with detailed symptoms and, whenever feasible, their location. For instance, a report saying that a consumer has had a reaction to a well-defined product would not qualify as a valid report if the kind of reaction is not better defined.
- A date of occurrence of the reaction. The date should be as precise as possible and must contain, at the minimum, the year of occurrence. For instance, a physician reporting that during his/her career, he/she has seen at least 10 consumers developing an allergic reaction to a given product would not qualify as a valid report if the physician is not able to provide the year of occurrence and some characterization of the consumers.

The lack of any of these four elements means that the case is considered incomplete and does not qualify as a valid report. In such a case, it is expected that the company exercise due diligence in following up the case to try to collect the missing data elements.

Data Assessment*

Once a report has been validated, it must be assessed for

- *Seriousness.* It is key in any vigilance system to determine immediately if a case is serious or nonserious, as all serious cases will have to be reported to health authorities within a short time frame. The essential element in assessing the seriousness of a UE report is to understand the difference between a serious reaction and a severe reaction, which are two different evaluations.

  The severity of a reaction is based on a quantitative assessment leading to a classification of the reaction as mild, moderate, or severe. Such a classification does not link to a reporting obligation of the case.

  On the other hand, the seriousness assessment is based on qualitative criteria linked to the outcome of the reaction and will be critical in the decision on the use of the data. A UE is considered serious if it falls into any of the following outcomes:
  - The death of the consumer.
  - An immediate vital risk for the consumer. It is often important to get the opinion of the HCP when the consumer has been treated for the reaction or of the potential outcome of the reaction if the consumer would not have been treated. It should be pointed out that a reaction that has not led to a vital risk at the time of the reaction but that might lead to a vital risk if it reoccurs again does not qualify as a serious reaction.
  - A congenital anomaly of the baby related to the use of a cosmetic product by the mother during pregnancy or the mother or father at the time of conception.
  - A disability that can be defined as the impairment or the loss of a body function or structure and is usually quantified by a percentage of disability.
  - A transitory or permanent functional incapacity. The functional incapacity is a parameter that can refer to a physiological function (e.g., vision, smell, taste, hearing, touch, mobility, etc.) or to a social function (incapacity to work, to go to school, to drive, to practice a sport, etc.). With social incapacity being very subjective and subject-dependent, companies will have to exert great care and define precise and relevant rules in order to validate the criteria.
- *Causal relatedness.* All valid reports of UEs should be assessed for the causal relatedness with the use of the cosmetic product. Indeed, it is not so infrequent in some countries that consumers complain about a product with the hope of being reimbursed, or getting a replacement product, or even simply for joking (lots of children complaints in some countries). Furthermore, it is very rare that a consumer only uses one single cosmetic product, and when a reaction is reported, it is not always obvious to determine which of the numerous products used may be attributed to the reaction.

  A "causality assessment method" for cosmetics [14,17,18] has been developed and is expected to be used as the single assessment method by all partners in cosmetovigilance in order to facilitate communication.

  The causality assessment method is based on the following criteria:
  - *The chronology of events.* The time sequence of the appearance of the UE(s) since the use of the cosmetic product is estimated to be compatible, partly compatible, or incompatible for the developed symptoms and the type of product. The disappearance of the symptoms after stopping the use of the product is also taken into account for such an assessment. When insufficient information is available to clearly characterize the chronology of events, it is considered that the chronology is unavailable, which is, by convention, equivalent to "partly compatible."
  - *The symptomatology or semiology.* The symptoms reported by the consumer are evaluated

* At the time of printing this chapter, the European Commission is in the process of updating their guidance and providing up-to-date interpretation of the seriousness criteria; the readers are thus referred to check updated reference 14 for the most up-to-date interpretation and examples of seriousness criteria.

independently of any chronology consideration. The symptoms may be considered as evocative or nonevocative for the use of the cosmetic product. A symptom that is evocative is one that could be reasonably expected from the use of that type of product. Such an assessment is thus largely based on the experience of the evaluator. However, it is also important to consider as evocative unusual reactions for that type of product but that occurred at the site of application of the product, and which might not be excluded, even if not really expected from the current knowledge. Not only local effects may be evocative but also symptoms resulting from a systemic reaction.

- *The reexposure to the product*. Most of the time, when a consumer reacts to a cosmetic product, he/she withdraws the product and moves to another similar product. Nevertheless, sometimes the consumer wishes to retry the same product that caused the initial reaction, either because the consumer likes that product very much or because he/she does not believe that the reaction was due to the product.

  When a consumer stops using the product after having experienced a UE, the reaction totally resolved and then the consumer starts reusing the product under the same conditions as the previous time, it is considered to be a rechallenge. Three situations can then happen:

  1. The consumer redevelops the same reaction as the first time: this is a positive rechallenge.
  2. The consumer does not react anymore to the product: this is a negative rechallenge.
  3. The consumer develops a reaction that is different from the first reaction: this is a nonconclusive rechallenge.

  When there is no rechallenge, the reexposure criterion is considered as equivalent to a "nonconclusive rechallenge."
- *The medical investigation*. Medical investigation is considered to be any medical dossier that completes information about the causal relatedness between the reaction and the use of the product. For instance, allergic patch tests results that prove or deny an allergic reaction to one of the ingredients of the incriminated product, blood analysis results, written medical dossier with a clear assessment by the HCP, etc.

  The medical investigation may result in confirming the causal relatedness with the use of the product, in denying that relationship, or in being nonconclusive in one way or the other. An absence of medical investigation is, by convention, considered equivalent to "nonconclusive medical investigation."

After having evaluated the chronology of events (compatible, incompatible, partly compatible), the symptomatology (evocative, nonevocative), the availability of reexposure data (positive, negative, nonconclusive, or unavailable), and the existence of medical investigation data (positive, negative, nonconclusive, or unavailable), the evaluator will conclude on the causal relatedness of the reaction with the cosmetic product by using a decision tree or a decision table described in the SUE reporting guidelines of the European Commission [14]. The conclusion will lead to one of the five following causality levels:

1. The causal relatedness is *excluded* if the chronology of events is clearly incompatible (e.g., the events occurred before using the product or so late after using the product that it cannot be attributed to the product).
2. The causality relatedness is *unlikely* attributable to the cosmetic product.
3. The causality relatedness is not *clearly attributable* to the cosmetic product.
4. The causality relatedness is *likely* attributable to the cosmetic product.
5. The causality relatedness is *very likely* attributable to the cosmetic product.

When another concomitant product has been used and is suspected to be likely or very likely the cause of the UE, the causal relatedness with the cosmetic product may be downgraded. A case-by-case responsible judgment should then be exercised.

- *Medical confirmation*. Although the medical confirmation or not of a UE report will not affect the decision to report it to the CAs, to include it in the safety dossier of the product information file, or to make it accessible to the public, it is generally recommended to differentiate the cases that have been medically confirmed from the others.

  To be medically confirmed, a case report must be
  - Reported by an HCP
  - Confirmed by an HCP during the follow-up
  - Confirmed by a medical dossier or document provided to the company

Data Reporting: SUEs

Article 25 of the Cosmetic Regulation requires that all SUEs occurring in one of the member states of the European Union shall be reported to the NCA of the member state where it occurred ("receiving authority") in a maximum of 20 calendar days. If after 20 days, there is still insufficient information for a proper assessment, but the criteria to validate the case and classify it as serious are available, the case shall be reported anyway while the company attempts to obtain additional information.

When a report is classified as nonserious based on the initial information received by the company and becomes serious based on the information collected during the follow-up of the case, the date when the follow-up information has been received is used as day 0 to calculate the 20 calendar days reporting time frame.

When a report has been sent as serious to the CAs and the follow-up information denies the causal relatedness of the reaction with the cosmetic product (e.g., an allergy patch test clearly attributes the reaction to another concomitantly used product), a follow-up report may be submitted to the authority to declassify the case, with clear documentation of the reason why.

To report an SUE to the CA, the responsible person completes and submits a reporting form A (accessible on the European Commission Web site [14]) with an assessment of the causal relatedness between the cosmetic product and the reaction. The submission address should be available from the Web site of each CA or, as a list, on the Web site of the European Commission (http://ec.europa.eu/consumers/sectors/cosmetics/documents/guidelines/sue_en.htm). After receiving form A, the receiving authority will review all data, complete form B (accessible on the European Commission Web site), and include their own assessment of the case in terms of seriousness and causal relatedness before sending back forms A and B to the responsible person (recommended). In case of disagreement, the responsible person shall explain on form A why there is a divergence of view in the assessment. Both forms A and B will then be distributed by the receiving CA to the NCA of all other member states of the European Union.

If one of the member states wishes to express specific comments or question, it will send the comments/questions to the receiving member states, which will recontact the responsible person.

A SUE may be reported directly to a distributor who would become responsible for reporting the SUE to the NCA of the member state where the SUE occurred. However, distributors shall communicate first with the responsible person to inform that one about the SUE and to collect missing information to complete form A. The responsible person becoming aware of the SUE would then also be responsible for reporting the case to the NCA. It is expected that clear roles and responsibilities between the distributors and the manufacturers are in place in a contractual agreement to ensure good communication between them on SUEs and ensure that both the distributor and the responsible person are compliant with their reporting obligation.

Consumers and HCPs also have the opportunity to report SUEs directly to the CA. In such a case, the authority will complete form C and work with the responsible person to collect all the relevant information on the product before distributing the form to the other member states.

Detailed description of the communication flow, copies of the forms, and instructions to complete the different forms are available on the Web site of the EU Commission [14].

Data Inclusion in CPSR

Annex I of Cosmetics Regulation requires that "All available data on the undesirable effects and serious undesirable effects to the cosmetic product or, where relevant, other cosmetic products" are included in the CPSR and that "This includes statistical data."

The expectation of the European Commission is that "All available data" are included in the dossier, which means data on all UEs collected on the cosmetic product whether the effect is serious or nonserious and whether the UEs are very likely, likely, not clearly, or unlikely attributable to the use of the product. Only cases where the causality relationship has been excluded will not be included in the CPSR. For nonserious UEs, the information may be limited to the kind of event that has been reported (e.g., skin irritation, allergic reaction, breathing difficulty, etc.) for the given product. In the case of SUEs, all SUEs on the product, worldwide, shall be included in the dossier including, for those having been reported to a CA, the full SUE notification dossier. Although a corrective action is not expected for each SUE if no special risk for the general population is identified, if a corrective or preventive action has been implemented, it shall be described and explained in the CPSR.

The objective of including cosmetovigilance data in the CPSR is to provide all relevant information to the safety assessor to permit a constant reevaluation of the safety of the cosmetic product. It is thus recommended to present all data on the UEs in a way that helps to discriminate between the least and the most relevant case reports and, for instance, clearly separate the cases that have been medically confirmed from those that have not, and the cases that are very likely or likely related to the use of the product from those that are not clearly or unlikely attributable to the use of the product.

"Where relevant" data on "other cosmetic products" should also be included in the CPSR. This provision of the Cosmetic Regulation allows the manufacturer to include in the CPSR data on different product(s) than the product for which the dossier is built. As the CPSR's only objective is to provide a tool for the safety assessor to estimate the safety of the product, it will be the responsibility of the responsible person to decide which data on "other" products may be relevant for assessing the safety of the product. Some examples are given below that have no other purpose than to help understand what could be included under this provision:

- When a new product is launched in the market, the cosmetovigilance data will be empty, unless some "premarket" consumer experience is available. In such circumstances, cosmetovigilance data on "similar" products may be the only data that could be provided to the safety assessor. UEs collected on other products with small formula differences (e.g., other variants with different perfumes) or UEs collected on all products from the same category (e.g., on all shower gels from the manufacturer) could provide relevant premarket information for conducting the safety assessment.

- When there is a concern related to a specific ingredient in a product that could be the cause of some allergic reactions (or other kind of reactions), it could be appropriate to include in the CPSR vigilance data on all products containing the same ingredient in order to evaluate if further allergic reactions have been observed on that ingredient.

The Cosmetic Regulation also states that "This [the CPSR] includes statistical data." However, the kind of statistical data expected is not explained. The purpose of statistical data should be to facilitate the comparison of data with those of other products, or from period to period in order to detect any signal that the safety of the product is not as good as the safety of similar products, or that the safety of the cosmetic product is changing over time. By consequence, at the minimum, the data on the UEs shall be expressed relatively to the level of exposure by consumers (e.g., the number of specific UEs per millions of units of the product placed on the market) or relatively to a period of sales when the exposure of consumers is stable over time (e.g., the number of specific UEs per year of sale).

Data Transparency

Art 21 of the Cosmetic Regulation stipulates that "existing data on undesirable effects and serious undesirable effects resulting from the use of the cosmetic product are made accessible to the public by any appropriate means."

Under the Cosmetic Regulation, an undesirable *event* that is not attributable to the use of a cosmetic product under normal or reasonably foreseeable conditions of use does not qualify to be defined as an undesirable *effect*. The information to be provided to the public thus concerns all UEs and SUEs that have been assessed as likely or very likely related to the use of the product. The information provided has to be easily understood by the consumers, avoiding, for instance, too specific medical terms and providing the data in a clear and simple manner such as the number of specific types of reaction per year or millions of units placed on the market.

The data must be made easily accessible to the public by any appropriate means. There is no obligation for a responsible person or a company to publish the data on the cosmetic products. The information shall be made available to the public upon request only. That means that consumers, or the public, must be able to easily find where to request such data. The contact address may be provided by "any appropriate means," which can be a contact phone number, e-mail, postal address, or others placed on a company Web site, on the product label, or others.

Data Internal Use

UE collection, validation, recording, and assessment will not have any other value than complying with some regulatory requirements if they are not accompanied by a good and appropriate system for signal detection.

Signal detection is the main purpose of a market surveillance system in order to detect if the safety of the product anticipated from the premarket studies and assessments is confirmed on the marketplace and if the product remains safe over time. Signal detection involves the consideration of a large series of parameters such as specific unexpected SUEs or an increase in the frequency of any type of UEs, but also any other type of information that the company would judge relevant for the overall assessment of a change in the safety of the product.

In case of the detection of a signal on a cosmetic product, the signal has to be evaluated and validated according to internal rules (e.g., by additional patch testing, by searching confirmatory information from other products or from literature) and the risk related to the change in the safety profile of the product quantified [19,20]. It is important to mention that the new reporting requirement of SUEs to CAs according to the Cosmetic Directive does not replace the obligations of the responsible person to comply with the requirements of other safety regulations, should any notification of a serious risk for the general population become necessary.

Corrective and Preventive Actions

Implementing corrective and preventive action (CAPA) is not directly linked to the reporting of SUEs. Most of the reported SUEs will never lead to a CAPA. On the other hand, a CAPA may be initiated without having received any SUE on the cosmetic product. The decision to initiate a CAPA should be based on the existence of an increased risk for the population that could be minimized by taking the appropriate action.

CAPA may have multiple components (Table 63.3) and should always be proportionate to the risk level for the population. They are usually decided by the responsible person or, more precisely, by a Company Risk Minimization Committee on behalf of the responsible person, but, in some instances, the NCAs may also mandate CAPA to the responsible person in case of a different risk assessment of the situation (e.g., in case of additional information on similar products from other

TABLE 63.3
Examples of CAPA

- Applying a warning on the label
- Limiting the use of the product to some populations
- Slight reformulation
  - Reducing the concentration of an ingredient
  - Replacing an ingredient
  - Changing the specification requirements of an ingredient (e.g., purity)
- Complete reformulation
- Changing the delivery system to decrease the dosage
- Stopping the distribution of the product until additional safety studies have confirmed the safety of the product
- Recalling the product from the market
- Others

companies that the responsible person would not be aware of).

QUALITY REQUIREMENTS OF A GOOD VIGILANCE SYSTEM

The responsible person should have a quality management system in place to support the activities in place to meet with the cosmetovigilance requirements and ensure that

- The process for UE handling is consistent between the different affiliates, aligned with the global established rules for the whole company and reproducible with time.
- The roles and responsibilities between the different partners inside the company or external are clear to all.
- All employees involved in cosmetovigilance activities are well aware of their respective tasks and are compliant with.
- All data related to UEs are retained as appropriate and for a duration that fulfills European, national, and company's regulations and requirements, including data privacy protection provisions.

Written Procedures

An essential element of any vigilance system is that there are clear written procedures in place to ensure that the system functions properly, that the roles and responsibilities and required tasks are clear to all parties involved, and that there is provision for proper control and, when needed, change of the system. Written procedures may take the form of SOPs, policy documents, working practices, guidelines, work instructions, contractual agreements, etc.

Written instructions usually cover, at least, the following steps:

- The collection of UEs and the process to follow-up with the reporter when information is missing
- The flow of information from the collection of data on a UE to entry in the database or to communication to the evaluator, including the time frame
- The process and the rules for the assessment of the UEs, including back-up procedure when the main assessor is not available in order to meet the 20 days reporting timing for SUEs
- The process and the roles and responsibilities for communicating SUEs to the CAs
- The process to incorporate all UE data into the Cosmetic Products Safety Report of the Product Information File, including the format of the data and the update rules
- The process followed to identify any new signal and actions taken once a signal is detected
- The respective roles and responsibilities, and communication requirements between the responsible person and the distributors in order to fulfill their obligation to report SUEs to the CAs

Training

Staff involved in cosmetovigilance activities should be appropriately trained for performing their task and ensure compliance with the requirements of the European Regulation and of internal procedures.

In terms of other employees of the company, they should also be well aware that they have a duty to communicate to the appropriate person of their entity any information about a UE on a company cosmetic product that was spontaneously reported to them.

Quality Assurance

In order to ensure data accuracy and overall compliance with the vigilance requirement, quality assurance procedures are usually in place. Such procedures may differ from company to company and may depend on the organization of each company, on the number of UEs they collect on their product, on the qualification of the personnel involved in cosmetovigilance, etc. Quality assurance steps may include internal audits, recording of the phone contacts with consumers, quality check of the data transcription in the database, etc.

COMPARISON TO OTHER VIGILANCE SYSTEMS: PHARMACOVIGILANCE AND MATERIOVIGILANCE

The largest companies often manufacture products that belong to different regulatory statuses such as cosmetics, medical devices, drugs, and others. It is essential for such companies to integrate the requirements of the respective vigilance regulations in their system in order to ensure the practicality of their system and the compliance with the legal obligations.

Although in the overall principle, vigilance for all types of products involves the same steps (collection of information on health issues, recording and centralization of the information, assessment, reporting of serious health issues, signal detection and implementation of CAPA whenever relevant), some important difference exists, which are summarized in Table 63.4.

CONCLUSION

Cosmetovigilance has been used by companies for many years in order to follow up on and confirm the safety of their products once they are placed on the market. However, the European Cosmetic Directive provides a new framework to harmonize the process between companies and with member states. It

TABLE 63.4
Comparison between Cosmetovigilance, Pharmacovigilance, and Materiovigilance in Europe

	Cosmetovigilance	Pharmacovigilance	Materiovigilance
References	Cosmetic Regulation (EC) 1223/2009 [12] SUE reporting guidelines [14]	EU Pharmacovigilance Regulation EC 1235/2010 [21] EU Pharmacovigilance Directive 2010/84/EU [22]	Meddev 2.12-1 rev 7 [23]
Product Type	Cosmetics	Medicinal products	Medical devices
Name of the (Potential) Health Concern	Undesirable event/effect	Adverse event/effect	Incident
Temporal Assessment of Seriousness	Based on the events that really occurred	Based on the events that really occurred or that are deemed serious by the reporter	Based on the events that really occurred or that might have occurred or that might occur if the same situation reappears
Seriousness Assessment Based on	Health effects	Health effects	Health effects or product quality defects that might cause a health effect
Seriousness Criteria	UE that results in – Temporary or permanent functional incapacity – Disability – Hospitalization – Congenital anomalies – Immediate vital risk – Death	Adverse event that results in – Persistent or significant incapacity – Persistent or significant incapacity – Inpatient hospitalization or prolongation of existing hospitalization – Congenital anomaly/birth defect – Life threatening situation – Death	Defined as a serious deterioration of the state of health with the medical devices being suspected as the cause Serious deterioration of the state of health is (a) Life-threatening illness (b) Permanent impairment of a body function or permanent damage to a body structure (c) A condition necessitating medical or surgical intervention to prevent (a) or (b) (d) A condition that requires hospitalization or significant prolongation of existing hospitalization (e) Any indirect harm as a consequence of an incorrect diagnostic or IVD test results when used within manufacturer's instructions for use (f) Fetal distress, fetal death, or any congenital abnormality or birth defects Any malfunction or deterioration in the characteristics and/or performance of a device, as well as any inadequacy in the labeling or the instructions for use that, directly or indirectly, might lead to or might have led to the death of a patient, or user, or other persons or to a serious deterioration in their state of health
Reporting Timeframe for Serious Cases	Within 20 calendar days from the date of receipt of the reports	Within 15 calendar days from the date of receipt of the reports	Serious health threat: immediately and not later than 2 days Death or serious deterioration of the state of health: immediately and not later than after 10 days
Use of Nonserious Cases	In the cosmetic product safety report available to authorities upon request	In periodic safety update report submitted periodically to authorities, or in risk–benefit assessment report kept internally. Reporting of individual cases to authorities in 90 days (not yet implemented by all EU member states)	In risk management plan available to authorities upon requests

also clearly sets up a collaborative and transparent framework between all stakeholders whether they are the manufacturers, consumers, HCPs, the distributors, or the NCAs.

Such a high level of collaboration is expected to improve the knowledge of the impact of cosmetic products or ingredients on the safety of consumers and to detect earlier than ever any unanticipated negative health-related effect to immediately take the appropriate corrective or preventive actions.

Disclaimer: Although some parts of this chapter may have been inspired from discussions held at the Cosmetic Europe Task Force on Cosmetovigilance, its overall content has been written from a personal perspective of the authors.

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64 Trends in Cosmetic Regulations in the United States

F. Anthony Simion

INTRODUCTION

The regulatory environment for cosmetics in the United States continues to evolve rapidly. This is not due to changes in the US regulatory framework for cosmetics; rather it is the effect of globalization. Globalization impacts cosmetic regulation in two somewhat related ways. First, as manufacturers of both cosmetic raw materials and finished products try to sell their goods in multiple markets, they must abide by the regulations in each market. The United States is just one of the major markets in which companies sell their products. Other large, key markets include the European Union (EU), Japan, and China. The framework developed by the EU has become the model that many countries and regions such as Mexico, Israel, and the Association of South East Asia Nations (ASEAN) have gravitated to. As a result, if an ingredient or product is to be compliant on a global, rather than on a national or even regional basis, it must take account of EU requirements. Recently, China has started to have an impact on companies' overall view of the regulatory landscape, although it has not as yet had a tangible effect on ingredients and products in the United States.

The second impact of globalization is rapid communications. News stories about issues and problems are rapidly transmitted between countries and regions. Unfortunately, the Internet does not assess the accuracy or validity of the information. Furthermore, interested parties such as nongovernmental organizations (NGOs) and activists in different countries rapidly communicate with and learn from each other and determine what works and what does not. The NGOs have been very effective at molding public opinion, especially in Europe, and building coalitions that can change the regulatory environment at the legislative level.

The US cosmetics industry through its trade association, the Personal Care Products Council (PCPC), is working to meet these challenges. The PCPC is working in parallel with trade associations in other regions, such as Cosmetics Europe (formerly Colipa) in the EU, to provide a balancing opinion as well as to influence the legislative and regulatory processes. This will become an even more important initiative in the future, if the US cosmetic industry is to remain innovative and, to a large degree, self-regulating.

FEDERAL COSMETIC LAWS AND REGULATIONS

The underlying regulatory framework for cosmetic products at the federal level in the United States has remained unchanged for 75 years. The Federal Food, Drug and Cosmetics Act of 1938[1] defines a cosmetic as

> …articles intended to be rubbed, poured, sprinkled, or sprayed on, introduced into, or otherwise, applied to the human body or any part thereof for cleansing, beautifying, promoting attractiveness or altering the appearance.

It is the intent of the product, as defined as the claims made for its benefits, which determines whether the product is a drug or cosmetic. One result has been the ability of marketers to sail closer and closer to this divide, making claims that suggest true physiological benefits that would make them a drug, only to cleverly phrase the claim so it relates to appearance, making it a cosmetic. Frequently, these products are incorrectly termed "cosmeceuticals," which is a marketing term and not a legal category. As one company makes a questionable claim, others rush to meet it and push the claim even closer to drug status. Recently, the US Food and Drug Administration (FDA), believing that some advertising had crossed into drug claims, has sent "Warning" or "Regulatory" letters to companies. This also happened in the mid-1960s with the "Wrinkle Remover" cases and again in the late 1980s when the focus was on claims of antiaging and cell rejuvenation. With each such episode, the cosmetic industry recalibrates itself, learns where the new boundaries are, and pursues other directions. The current interest in cosmeceuticals must be evaluated against the regulatory framework of only having drugs and cosmetics in the United States. As there is no cosmeceutical category in the United States, these products must fall into one of the two established statutory categories.

Another effect of the U.S. definitions of drugs and cosmetics is that a single formula can be either a drug or cosmetic, depending on the claims made. One good example is a soap containing triclocarban. If it is sold as a deodorant soap, it is cosmetic as deodorancy improves attractiveness. In contrast,

the same formula being sold as antimicrobial soap, making disease-prevention claims, is a drug.[2] Another example is a clear antiperspirant gel made by Gillette in 1995, which was originally marketed as a deodorant. It made the claim of higher deodorancy than other deodorant products. Gillette successfully defended this claim with the industry self-regulatory "watchdog," the National Advertising Division (NAD) of the Better Business Bureau, based on the antiperspirant technology being more effective at reducing body odor than that used in deodorants, which do not contain aluminum salt.[3] The one exception to that general rule is where the included therapeutic ingredient, such as penicillin, is so well known, that its very presence in a product would imply therapeutic, that is, drug benefits.[2]

There is no premarketing review or approval required before selling a new cosmetic in the United States. Thus, the US Food and Drug Administration (FDA) only regulates cosmetic products once they have reached the marketplace. This approach was reconfirmed several times over in the last few years, especially in respect to nanotechnology. For cosmetics that contain "nanomaterials," the FDA believes that the current approaches to and methods for evaluating product safety are sufficient, as long as companies take full account of the differences in properties and behaviors of ingredients caused by their smaller size. Last year, in their Guidance for Nanotechnology in cosmetics, the FDA reconfirmed this approach.[4–6] The FDA does have the ability to exclude ingredients that they believe to be harmful. In the past, they have declared that the presence of halogenated salicylanalides such as tribromsalan (TBS, 3,4′,5-tribromosalicylanilide) or hexachlorophene in a cosmetic would result in an adulterated product. In 2005, the FDA issued a guidance to the effect that cosmetic products on sun-exposed areas, containing alpha hydroxyl acids (AHA), should carry the Sun Burn Alert, unless the manufacturer had data that showed that the increased AHA-induced UV damage to the skin would not occur.[7]

There are many consumer products that carry both drug and cosmetic claims and are therefore governed by both sets of regulations. In practice, as the drug regulations are more stringent for many aspects including good manufacturing practice (GMP) and serious and unexpected (adverse) event reporting, these combination products are effectively regulated as drugs. Most of these combinations are over-the-counter (OTC) drugs, and as long as the monograph requirements are met, preapproval to enter the marketplace is not required. OTC regulations continue to slowly evolve. Some of the monographs that impact personal care products have been finalized such as antiacne and antidandruff. Others are still at the tentative final stage such as antimicrobial. The monograph for sunscreen products is still a work in progress. In December 2012, the final monograph of testing and labeling of sunscreen products came into effect.[8] However, it is unclear when the rules covering sunscreen actives will be finalized.

Like the personal care industry, the FDA has recognized the increasing concern of some of the general public over the safety of cosmetics. In 2004, the Environmental Working Group (EWG), an activist group, petitioned the FDA to take a more active regulatory role including the review and approval of cosmetics before they are marketed. The following year, the FDA rejected the petition, believing that it already had sufficient authority to regulate cosmetics and ensure the safety of cosmetics.[9] This is a similar stance to the position that the Agency took in respect to nanotechnology.

Since then, all—the FDA, industry, and the NGOs—have moved forward. The FDA has reintroduced the voluntary registration of manufacturing premises and ingredient statements, which existed from the 1970s to the mid-1990s. The voluntary annual reporting of cosmetic adverse events, which was discontinued at the same time, has not been reintroduced by the FDA. However, the reporting of serious and unexpected (adverse) events for OTC drugs was made mandatory. The NGOs have focused much of their efforts on legislation at the federal and states level. They have supported new bills to enact new federal legislation to regulate cosmetics, such as the Safe Cosmetics bill (HR 2359) introduced in 2011 by Representatives Schakowsky, Markey, and Baldwin. At the state level, the NGOs have worked to influence green chemistry regulations in California and laws in Washington state that regulate products primarily for children.

Over the last 40 years, the US cosmetics industry has successfully remained mainly self-regulatory by continuing to develop comprehensive safety programs that meet the government's and the public's expectations, while maintaining an excellent safety record in the marketplace. To try to maintain the public's confidence, in 2006, the PCPC proactively introduced a program, the Consumer Commitment Code, to make the safety assurance process for cosmetics more complete and transparent. It is described in more detail in Section V (US Cosmetic Industry's response) and is in part based on the work of the Cosmetic Ingredient Review (CIR). The CIR is a board of independent dermatologists and toxicologists that review the safety of cosmetic ingredients. The FDA has a presence and participates in the CIR process on a nonvoting basis. Additionally the US cosmetics industry has supported alternative legislative initiatives to update cosmetic law in the US Congress, based on giving the CIR's reviews regulatory weight.

The FDA is not the only federal agency that has jurisdiction over cosmetics and the personal care industry. The Consumer Product Safety Commission regulates some aspects of product labeling under the Fair Packaging and Labeling Act (FPLA). The Bureau of Alcohol, Tobacco, and Firearms (ATF) also regulates the packaging and labeling of those cosmetic products that contain ethanol.[10] The Federal Trade Commission (FTC) has jurisdiction over the fairness of advertising, especially as it relates to false or misleading claims. In the last few years, the FTC has become more active in ensuring compliance with advertising rules for both cosmetics and dietary supplements, especially in the areas of weight loss and hair growth, where there has been a long history on questionable claims.

STATE LAWS AND REGULATION OF COSMETICS IN THE UNITED STATES

For many years, individual states in the United States have had laws and regulations that impact cosmetics. Primary examples of this are from California where the Volatile Organic Compound (VOC) regulations have changed the composition of many cosmetic products such as hairsprays. The California Proposition 65 limits the level of potential carcinogens and reproductive toxicants that a person can be exposed to per day; otherwise warning labeling is required. Such regulations can impact personal care products across the United States. It is difficult to control distribution of a product once it leaves the manufacturer's warehouse and reaches mass merchandisers. Therefore, many manufacturers follow the requirements of California across the entire country.

In 2005, California passed the Safe Cosmetics Act (2005), which requires manufacturers to disclose to the state the intentional addition of chemicals suspected to cause cancer, birth defects, or other reproductive harm. California then posts this information on a public Web site. The amount of information on this Web site is huge, and perhaps overwhelming. This may be one of the key reasons why this Act has not had a great impact.

In 2008, California passed Green Chemistry legislation (Green Chemistry: Chemicals of Concern and Alternative Analysis Process [AB 1879] and Green Chemistry: Online Toxics Clearinghouse [SB509]) collectively known as the Green Chemistry Initiative. The aim is to reduce the potentially harmful effect of products (not just cosmetics) on human health and the environment. The regulations that are required to implement this initiative have recently been published (October 2013). Thus, the law's impact is not yet clear, is potentially great, and may affect the whole of the United States due to the national distribution of products. Other states have discussed new green chemistry laws, and some have been enacted into law. Many of these focus on products that are designed for use on children. Laws in Washington state and Minnesota are examples.

Obviously, the personal care industry prefers to have a single set of laws and regulations to follow, rather than separate requirements in each state. Furthermore, since many of the laws related to product safety utilize information and analysis published by panels or committees of experts, industry is trying to ensure that a few lists of the highest scientific quality are used as the basis of the regulations.

IMPACT OF OTHER COUNTRIES' AND REGIONS' LAWS AND REGULATIONS ON US COSMETICS INDUSTRY

Although they do not have the force of law in the United States, EU and Canadian cosmetic regulations are having a great impact on the development of raw materials and cosmetic products in this country. This is because cosmetics manufacturers want to be able to develop global formulas or at least ones that can be sold in the major markets of North America and the EU. To do this, the products must be compliant with both sets of cosmetic regulations, and in this way, the EU impacts cosmetics produced in the United States. Furthermore, Canada and Mexico are taking account of EU regulations as they develop their own. In this way, a Pan-North American personal care product will be impacted by the EU, even if the product is not intended for distribution in Europe. Indeed, ASEAN has modeled their new cosmetic regulations on the EU's.

In the last few years, China's economy has grown rapidly and now is the second largest for an individual country; the country's demand for cosmetics has grown in parallel. As cosmetic regulations in China are developed, these are likely to impact the cosmetics business around the world.

For ingredient safety, there is already a degree of global harmonization. The science of toxicology is the same around the world. Thus, the criteria in evaluating safety that are applied by the advisory bodies in the United States (CIR) and in the EU (the Scientific Committee on Cosmetic Safety [SCCS]) tend to be similar. This contrasts with labeling regulations where there is far less commonality among different regions, based on language requirements, as well as national sensibilities and expectations around product performance claims. The exception to this is the Ingredient Declaration, where the International Nomenclature of Cosmetic Ingredients (INCI) nomenclature is becoming the global standard.

In the future, the regulation burden on cosmetic ingredients will increase in the EU with the introduction of the Registration Evaluation and Authorization of Chemicals (REACH) legislation and the animal testing ban. The latter now applies to both single and multiple dose method. It is not clear whether the effect of REACH will be as great in the United States as it will be in Europe. Many raw materials will have to be REACH compliant: they will have to meet EU regulations to be sold in Europe, and precluding the EU market will significantly reduce the ingredient manufacturers' return on their investment. How the animal testing ban, which came into full effect for Europe in March 2013, will impact the development of new raw materials for cosmetics remains unclear, as several countries such as China and Japan (for quasi-drugs) do require testing of raw materials for products that are regulated in the EU as cosmetics. Even with fragrances, EU regulations can impact formulas sold in the United States. The EU requires labeling if the presence of fragrance ingredients that are putative allergens exceeds specific thresholds. Many cosmetic manufacturers want to avoid such labeling for, at the minimum, it can significantly lengthen the ingredient statement. If a cosmetic manufacturer is going to have a global fragrance for the global product, again, EU regulations will have an impact. However, for other areas of package labeling, there is less global harmonization of practice or regulation. For instance, the local language(s) are used to communicate with consumers where the products are sold. The claims made for the products reflect local consumers' interests and sensibilities. Currently, the EU is trying to develop regulations and guidelines for claims, and their substantiation, as part of the new EU cosmetic regulations (Recast).

In the past, the attempts to harmonize cosmetic regulations at the governmental level have been unsuccessful. However, industry pressures such as global economy of scales have led to a de facto harmonization of ingredients in products. Currently, the International Committee on Cosmetic Regulations (ICCR), which include industry as well as governmental agencies from the EU, Japan, Canada, and the United States, are working to harmonize approaches on many issues, such as traces, nanotechnology, and in vitro (especially in silico) alternatives to animal testing, so that single maximum levels of traces, definitions, and testing methods can be used across different jurisdictions and regulatory frameworks. Issues discussed include maximum level of traces, safety testing of cosmetics, and nanotechnology.[11] At the July 2012 meeting, there were presentations from NGOs, and additional countries had observer status.

US COSMETIC INDUSTRY'S RESPONSE TO THE CHANGING REGULATORY ENVIRONMENT

In the last decade, the NGOs in the United States have had a significant impact in the court of public opinion and therefore with state legislators. Examples of these are the pressure that was exerted in California that resulted in passage of the Safe Cosmetic Act (2005) and Green Chemistry Initiative in 2008 (AB 1789 and SB 509). Seeing this trend and recognizing that what has already happened in the EU could portend what happens in the United States, the US cosmetic industry is becoming more proactive in meeting this challenge. This has happened in two ways. First, industry has realized that it needs to go beyond its historical lobbying efforts at the federal and state levels and become more active in preventing the passage of unreasonable and burdensome laws as well as supporting federal legislation that is both based on good science and effective.

Second, it has realized that it needs to increase its efforts to provide accurate information to the public and the press, and use this as the starting point in influencing public opinion. The result is several initiatives from the PCPC (the Council), which include

- The Consumer Commitment Code: industry is working to make the safety assessment process more transparent to the press and public in general. The code emphasizes that cosmetic manufacturers have a solid scientific basis for the safety of both the ingredients that they use and the final product.
  - For ingredients, it is recommended that companies use the assessments of expert authoritative bodies such as the CIR, FDA, SCCS, or NICNAS (Australia's National Industrial Chemicals Notification and Assessment Scheme) as the basis of the safety assurance process. Indeed ingredients that the CIR finds to be unsafe should be excluded from products. Ingredients with a CIR "insufficient" data finding should only be used if the company has enough scientifically valid data to support its safe use.
  - Serious and unexpected adverse events (reactions) to cosmetics should be reported to the FDA in a timely manner.
  - The US FDA can make written requests to review the data that forms the basis of a product's safety assessment.
- The Council has set up a Web site to give accurate information to the press and public in a way that is easier for them to understand. Its address is www.cosmeticsinfo.org.

These efforts are a start in the industry's goal to remain self-regulating. To do this successfully will require the industry to effectively manage attacks from the activist NGOs. They have been active and effective in the EU and their counterparts in the United States have been following their lead. The US industry must watch what is happening in the EU, because with the rapid communications across the Atlantic, those regulations will be proposed in the United States very quickly.

CONCLUSIONS

Although the legal framework for cosmetic regulation has not significantly changed in the United States for seventy-five years, globalization has made the US regulatory environment more complex and hostile in practice. By globalizing raw materials and cosmetic formulas, the US cosmetic industry is impacted by regulatory regimes from the different regions in which the ingredients and products are sold. This is especially true for the framework developed in the EU. Indeed, many countries and regions are following the EU's model, or at minimum, incorporating some of the EU's approaches in their regulatory framework. Therefore, it is important for US companies and industry to work closely with and support their EU counterparts, as the Europeans try to influence their legislative and regulatory processes.

The second effect of globalization is the increased speed of communications around the world. Ideas and approaches for cosmetic issues can be rapidly passed between NGOs and consumers in different countries or continents. While activist groups have been more politically influential in Europe than they are in the United States, those in the United States have become more vocal and successful in influencing public opinion and legislators, especially around five years ago. In California, this resulted in the passage of the Safe Cosmetics Act (2005) and the Safer Consumer Products Act (2008), that is, the Green Chemistry law. The US personal care industry has not been willing to cede the "court of public opinion" to the activists. To this end, the PCPC (industry association) has several initiatives that include

- Development of a Web site (www.cosmeticsinfo.org) to provide accurate information to the public and especially to the press.

- Working to make the product safety assurance process more transparent through the introduction of the Consumer Commitment Code.
- Finally, the PCPC has supported legislation to update US Federal cosmetic law in a practical and science-based manner, as well as working at both the state and federal levels to prevent passage of laws that are unreasonable and unworkable.

The FDA is planning to maintain its current approach: in response to pressure from the activist NGOs, the Agency has stated that the current regulatory structure is comprehensive and adaptive to evaluate scientific advances such as nanotechnology.

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