Section I

Basic Concepts

Part 1: Skin Physiology Pertinent to Cosmetic Dermatology

Chapter 1: Epidermal barrier

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BASIC CONCEPTS

- The outer surface of the skin, the epidermis, along with its outermost layer, the stratum corneum, forms the epidermal barrier.
- The stratum corneum is a structurally heterogeneous tissue composed of non-nucleated, flat, protein-enriched corneocytes and lipid-enriched intercellular domains.
- The roles of the skin barrier include preventing microbes from entering the skin, protecting from environmental toxins, maintaining skin hydration, and diffusing oxidative stress.
- Delivery technologies such as lipid systems, nanoparticles, microcapsules, polymers, and films can improve the barrier properties of the skin.

Introduction

Skin is the interface between the body and the environment. There are three major compartments of the skin: the epidermis, the dermis, and the hypodermis. Epidermis is the outermost structure and it is a multilayered, epithelial tissue divided into several layers. The outermost structure of the epidermis is the stratum corneum (SC) which forms the epidermal permeability barrier that prevents the loss of water and electrolytes. Other protective or barrier roles for the epidermis include: immune defense, UV protection, and protection from oxidative damage. Changes in the epidermal barrier caused by environmental factors, age, or other conditions can alter the appearance as well as the functions of the skin. Understanding the structure and function of the SC and the epidermal barrier is vital because it is the key to healthy skin and its associated social ramifications.

Structural components of the epidermal barrier

The outer surface of the skin, the epidermis, mostly consists of epidermal cells, known as keratinocytes, which are arranged in several stratified layers – the basal cell layer, the spinous cell layer and the granular cell layer – whose differentiation eventually produces the SC. Unlike other layers, the SC is made of anucleated cells called corneocytes which are derived from keratinocytes. The SC forms the major protective barrier of the skin, the epidermal permeability barrier. Figure 1.1 shows the different layers of the epidermis and the components that form the epidermal barrier. The SC is a structurally heterogeneous tissue composed of non-nucleated, flat, protein-enriched corneocytes and lipid-enriched intercellular domains [1]. The lipids for barrier function are synthesized in the keratinocytes of the nucleated epidermal layers, stored in the lamellar bodies, and extruded into the intercellular spaces during the transition from the stratum granulosum to the SC forming a system of continuous membrane bilayers [1,2]. In addition to the lipids, other components such as melanins, proteins of the SC and epidermis, free amino acids and other small molecules also have important roles in the protective barrier of the skin. A list of the different structural as well as functional components of the SC is shown in Table 1.1.

Corneocytes

Corneocytes are formed by the terminal differentiation of the keratinocytes from the granular layer of the epidermis. The epidermis is comprised of 70% water, as are most tissues, yet the SC is comprised of only 15% water. Alongside this change in water content the keratinocyte nuclei and virtually all the subcellular organelles begin to disappear in the granular cell layer leaving a proteineous core containing keratins, other structural proteins, free amino acids and amino acid

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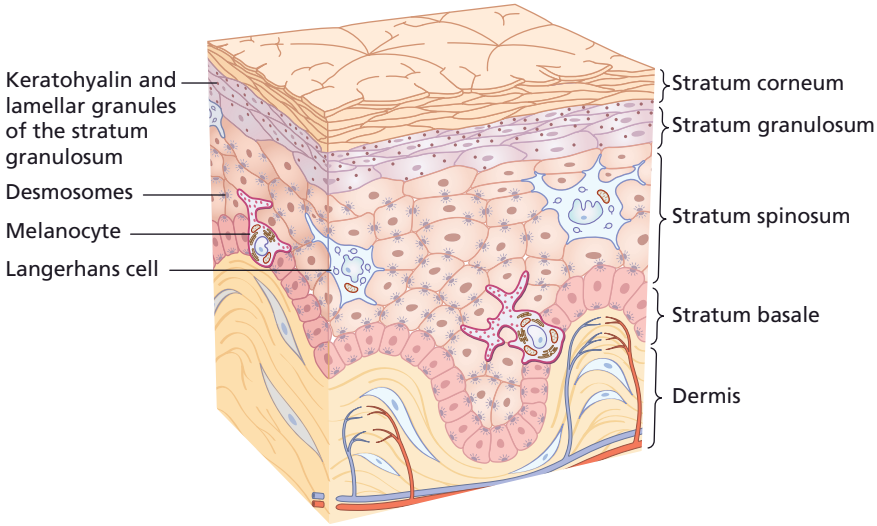

Figure 1.1 Diagram of the epidermis indicating the different layers of the epidermis and other structural components of the epidermal barrier.

Table 1.1 Structural and functional components of the stratum corneum.

Components	Function	Location
SC	Protection	Topmost layer of epidermis
CE	Resiliency of SC	Outer surface of the SC
Cornified envelope precursor proteins	Structural proteins that are cross-linked to form CE	Outer surface of SC
LG	Permeability barrier of skin	Granular cells of epidermis
SC interfacial lipids	Permeability barrier of skin	Lipid bilayers between SC
Lipid–protein cross-links	Scaffold for corneocytes	Between SC and lipid bi-layers
Desmosomes and corneodesmosomes	Intercellular adhesion and provide shear resistance	Between keratinocytes and corneocytes
Keratohyalin granules	Formation of keratin "bundles" and NMF precursor proteins	Stratum granulosum
NMF	Water holding capacity of SC	Within SC
pH and calcium gradients	Provides differentiation signals and LG secretion signals	All through epidermis
Specialized enzymes (lipases, glycosidases, proteases)	Processing and maturation of SC lipids, desquamation	Within LG and all through epidermis
Melanin granules and "dust"	UV protection of skin	Produced by melanocytes of basal layer, melanin "dust" in SC

CE, cornified envelope; LG, lamellar granules; NMF, natural moisturizing factor; SC, stratum corneum.

derivatives, and melanin particles which persist throughout the SC. From an oval or polyhedral shape of the viable cells in the spinous layers, the keratinocyte starts to flatten off in the granular cell layer and then assumes a spindle shape and finally becomes a flat corneocyte. The corneocyte itself develops a tough, chemically resistant protein band at the periphery of the cell, called the cornified cell envelope, formed from cross-linked cytoskeletal proteins [3].

Proteins of the cornified envelope

The cornified envelope (CE) contains highly cross-linked proteins formed from special precursor proteins synthesized in the granular cell layer, particularly involucrin, loricrin, and cornifin. In addition to these major protein components, several other minor unique proteins are also cross-linked to the cornified envelope. These include proteins with specific functions such as calcium binding proteins, antimicrobial and immune functional proteins, proteins that provide structural integrity to SC by binding to lipids and desmosomes, and protease inhibitors. The cross-linking is promoted by the enzyme transglutaminase which is detectable histochemically in the granular cell layer and lower segments of the stratum corneum. The γ-glutamyl link that results from transglutaminase activity is extremely chemically resistant and this provides the cohesivity and resiliency to the SC.

Lamellar granules and inter-corneocyte lipids

Lamellar granules or bodies (LG or LB) are specialized lipid-carrying vesicles formed in suprabasal keratinocytes, destined for delivery of the lipids in the interface between the corneocytes. These lipids form the essential component of the epidermal permeability barrier and provide the "mortar" into which the corneocyte "bricks" are laid for the permeability barrier formation. When the granular keratinocytes mature to the SC, specific enzymes within the LB process the lipids, releasing the non-polar epidermal permeability barrier lipids, namely, cholesterol, free fatty acids and ceramides, from their polar precursors – phospholipids, glucosyl ceramides, and cholesteryl sulfate, respectively. These enzymes include: lipases, phospholipases, sphingomyelinases, glucosyl ceramidases, and sterol sulfatases [4,5]. The lipids fuse together in the SC to form a continuous bi-layer. It is these lipids, along with the corneocytes, that constitute the bulk of the water barrier property of the SC [6,7].

Lipid–protein cross-links at the cornified envelope

LG are enriched in a specific lipid unique to the keratinizing epithelia such as the human epidermis. This lipid (a ceramide) has a very long chain omega-hydroxy fatty acid moiety with linoleic acid linked to the omega hydroxyl group in ester form. This lipid is processed within the SC to release the omega hydroxyl ceramide that becomes cross-linked to the amino groups of the cornified envelope proteins. The molecular structure of these components suggests that the glutamine and serine residues of CE envelope proteins such as loricrin and involucrin are covalently linked to the omega hydroxyl ceramides [8]. In addition, other free fatty acids (FFA) and ceramides (Cer), may also form protein cross-links on the extracellular side of the CE, providing the scaffold for the corneocytes to the lipid membrane of the SC.

Desmosomes and corneodesmosomes

Desmosomes are specialized cell structures that provide cell–cell adhesion (Figure 1.1). They help to resist shearing forces and are present in simple and stratified squamous epithelia as in human epidermis. Desmosomes are molecular complexes of cell adhesion proteins and linking proteins that attach the cell surface adhesion proteins to intracellular keratin cytoskeletal filaments proteins. Some of the specialized proteins present in desmosomes are cadherins, calcium binding proteins, desmogleins, and desmocollins. Cross-linking of other additional proteins such as envoplakins and periplakins further stabilizes desmosomes. Corneodesmosomes are remnants of the desmosomal structures that provide the attachment sites between corneocytes and cohesiveness for the corneocytes in the SC. Corneodesmosomes have to be degraded by specialized proteases and glycosidases, mainly serine proteases, for the skin to shed in a process called desquamation [9].

Keratohyalin granules

Keratohyalin granules are irregularly shaped granules present in the granular cells of the epidermis, thus providing these cells their granular appearance (Figure 1.1). These organelles contains abundant amount of keratins "bundled" together by a variety of other proteins, most important of which is filaggrin (filament aggregating protein). An important role of this protein, in addition to bundling of the major structural protein, keratin of the epidermis, is to provide the natural moisturizing factor (NMF) for the SC. Filaggrin contains all the amino acids that are present in the NMF. Filaggrin, under appropriate conditions, is dephosphorylated and proteolytically digested during the process when granular cells mature into corneocytes. The amino acids from filaggrin are further converted to the NMF components by enzymatic processing and are retained inside the corneocytes as components of NMF [4,9].

Functions of epidermal barrier

Water evaporation barrier (epidermal permeability barrier)

Perhaps the most studied and the most important function of the SC is the formation of the epidermal permeability barrier [1,4,10]. The SC limits the transcutaneous movement

of water and electrolytes, a function that is essential for terrestrial survival. Lipids, particularly ceramides, cholesterol, and FFA, together form lamellar membranes in the extracellular spaces of the SC which limit the loss of water and electrolytes. Corneocytes are embedded in this lipid-enriched matrix, and the cornified envelope, which surrounds corneocytes, provides a scaffold necessary for the organization of the lamellar membranes. Extensive research, mainly by Peter Elias' group has elucidated the structure, properties, and the regulation of the skin barrier by integrated mechanisms [5,7,11]. Barrier disruption triggers a cascade of biochemical processes leading to rapid repair of the epidermal barrier. These steps include increased keratinocyte proliferation and differentiation, increased production of corneocytes, and production, processing, and secretion of barrier lipids, ultimately leading to the repair of the epidermal permeability barrier. These events are described in more detail in the barrier homeostasis section below. A list of the different functions of human epidermis is shown in Table 1.2.

Mechanical barrier

Cornified envelope provides mechanical strength and rigidity to the epidermis, thereby protecting the host from injury. Specialized protein precursors and their modified amino acid cross-links provide the mechanical strength to the SC. One such protein, trichohyalin, is a multifunctional cross-bridging protein that forms intra- and inter-protein cross-links between cell envelope structure and cytoplasmic keratin filament network [12]. Special enzymes called transglutaminases, some present exclusively in the epidermis (transglutaminase 3), catalyze this cross-linking reaction. In addition, adjacent corneocytes are linked by corneodesmosomes, and many of the lipids of the SC barrier are also chemically cross-linked to the cornified envelope. All these chemical links provide the mechanical strength and rigidity to the SC.

Antimicrobial barrier and immune protection

The epidermal barrier acts as a physical barrier to pathogenic organisms that attempt to penetrate the skin from the outside environment. Secretions such as sebum and sweat and their acid pH provide antimicrobial properties to skin. Innate immune function of keratinocytes and other immune cells of the epidermis such as Langerhans cells and phagocytes provide additional immune protection in skin. Epidermis also generates a spectrum of antimicrobial lipids, peptides, nucleic acids, proteases, and chemical signals that together forms the antimicrobial barrier (Table 1.3). The antimicrobial peptides are comprised of highly conserved, small, cysteine rich, cationic proteins that are expressed in large amounts in skin. Desquamation, which causes the outward movement of corneocytes and their sloughing off at the surface, also serves as a built-in mechanism inhibiting pathogens from colonizing the skin.

NMF and skin hydration and moisturization

NMF is a collection of water-soluble compounds that are found in the stratum corneum (Table 1.4). These compounds compose approximately 20–30% of the dry weight of the

Table 1.2 Barrier functions of the epidermis.

Function	Localization/components involved
Water and electrolyte permeability barrier	SC/corneocyte proteins and extracellular lipids
Mechanical barrier	SC/corneocytes, cornified envelope
Microbial barrier/immune function	SC/lipid components/viable epidermis
Hydration/moisturization	SC/NMF
Protection from environmental toxins/drugs	SC/corneocytes, cornified envelope
Desquamation	SC, epidermis/proteases and glycosidases
UV barrier	SC/melanins of SC/epidermis
Oxidative stress barrier	SC, epidermis/antioxidants

NMF, natural moisturizing factor; SC, stratum corneum.

Table 1.3 Antimicrobial components of epidermis and stratum corneum.

Component	Class of compound	Localization
Free fatty acids	Lipid	Stratum corneum
Glucosyl ceramides	Lipid	Stratum corneum
Ceramides	Lipid	Stratum corneum
Sphingosine	Lipid	Stratum corneum
Defensins	Peptides	Epidermis
Cathelicidin	Peptides	Epidermis
Psoriasin	Protein	Epidermis
RNAse 7	Nucleic acid	Epidermis
Low pH	Protons	Stratum corneum
"Toll-like" receptors	Protein signaling molecules	Epidermis
Proteases	Proteins	Stratum corneum and epidermis

Table 1.4 Approximate composition of skin natural moisturizing factor.

Components	Percentage levels
Amino acids and their salts (over a dozen)	30–40
Pyrrolidine carboxylic acid sodium salt, urocanic acid, ornithine, citruline (derived from filaggrin hydrolysis products)	7–12
Urea	5–7
Glycerol	4–5
Glucosamine, creatinine, ammonia, uric acid	1–2
Cations (sodium, calcium, potassium)	10–11
Anions (phosphates, chlorides)	6–7
Lactate	10–12
Citrate, formate	0.5–1.0

corneocyte. Many of the components of NMF are derived from the hydrolysis of filaggrin, a histidine- and glutamine-rich basic protein of the keratohyalin granule. The SC hydration level controls the protease that hydrolyzes filaggrin and histidase that converts histidine to urocanic acid. As NMF is water soluble and can easily be washed away from the SC, the lipid layer surrounding the corneocyte helps seal the corneocyte to prevent loss of NMF.

In addition to preventing water loss from the organism, the SC also acts to provide hydration and moisturization to skin. NMF components absorb and hold water allowing the outermost layers of the SC to stay hydrated despite exposure to the harsh external environment. Glycerol, a major component of the NMF, is an important humectant present in skin which contributes skin hydration. Glycerol is produced locally within the SC by the hydrolysis of triglycerides by lipases, but also taken up into the epidermis from the circulation by specific receptors present in the epidermis called aquaporins [13]. Other humectants in the NMF include urea, sodium and potassium lactates, and pyrrolidine carboxylic acid (PCA) [9].

Protection from environmental toxins and topical drug penetration

The SC also has the important task of preventing toxic substances and topically applied drugs from penetrating the skin. The SC acts as a protective wrap because of the highly resilient and cross-linked protein coat of the corneocytes and the lipid-enriched intercellular domains. Pharmacologists and topical or "transdermal" drug developers are interested in increasing SC permeation of drugs into the skin. The multiple route(s) of penetration of drugs into the skin can be via hair follicles, interfollicular sites, or by penetration through corneocytes and lipid bilayer membranes of the SC [10]. The molecular weight, solubility, and molecular configuration of the toxins and drugs greatly influence the rate of penetration. Different chemical compounds adopt different pathways for skin penetration.

Desquamation and the role of proteolytic enzymes

The process by which individual corneocytes are sloughed off from the top of the SC is called desquamation. Normal desquamation is required to maintain the homeostasis of the epidermis. Corneocyte to corneocyte cohesion is controlled by the intercellular lipids as well as the corneodesmosomes that bind the corneocytes together. The presence of specialized proteolytic enzymes and glycosidases in the SC help in cleavage of desmosomal bonds resulting in release of corneocytes [9]. In addition, the SC also contains protease inhibitors that keep these proteases in check and the balance of protease–protease inhibitors have a regulatory role in the control of the desquamatory process. The desquamatory process is also highly regulated by the epidermal barrier function.

The SC contains three families of proteases (serine, cysteine, and aspartate proteases), including the epidermal-specific serine proteases (SP), kallikrein-5 (SC tryptic enzyme [SCTE]), and kallikrein-7 (SC chymotryptic enzyme), as well as at least two cysteine proteases, including the SC thiol protease (SCTP), and at least one aspartate protease, cathepsin D. All these proteases have specific roles in the desquamatory process at different layers of the epidermis.

Melanin and the UV barrier

Although melanin is not typically considered a functional component of the epidermal barrier, its role in the protection of the skin from UV radiation is indisputable. Melanins are formed in specialized dendritic cells called melanocytes in the basal layers of the epidermis. The melanin produced is transferred into keratinocytes in the basal and spinous layers. There are two types of melanins, depending on the composition and the color. The darker eumelanin is most protective to UV than the lighter, high sulfur-containing pheomelanin. The keratinocytes carry the melanins through the granular layer and into the SC layer of the epidermis. The melanin "dust" present in the SC is structurally different from the organized melanin granules found in the viable deeper layers of the epidermis. The content and composition of melanins also change in SC depending on sun exposure and skin type of the individual.

Solar UV radiation is very damaging to proteins, lipids, and nucleic acids and causes oxidative damage to these macromolecules. The SC absorbs some UV energy but it is the melanin particles inside the corneocytes that provide the most protection. Darker skin (higher eumelanin content) is significantly more resistant to the damaging effects of UV on DNA than lighter skin. In addition, UV-induced apoptosis

(cell death that results in removal of damaged cells) is significantly greater in darker skin. This combination of decreased DNA damage and more efficient removal of UV-damaged cells plays a critical part in the decreased photocarcinogenesis seen in individuals with darker skin [14]. In addition to melanin, trans-urocanic acid (tUCA), a product of histidine deamination produced in the SC, also acts as an endogenous sunscreen and protects skin from UV damage.

Oxidative stress barrier

The SC has been recognized as the main cutaneous oxidation target of UV and other atmospheric oxidants such as pollutants and cigarette smoke. UVA radiation, in addition to damaging the DNA of fibroblasts, also indirectly causes oxidative stress damage of epidermal keratinocytes. The oxidation of lipids and carbonylation of proteins of the SC lead to disruption of epidermal barrier and poor skin condition [15]. In addition to its effects on SC, UV also initiates and activates a complex cascade of biochemical reactions within the epidermis, causing depletion of cellular antioxidants and antioxidant enzymes such as superoxide dismutase (SOD) and catalase. Acute and chronic exposure to UV has been associated with depletion of SOD and catalase in the skin of hairless mice [16]. This lack of antioxidant protection further causes DNA damage, formation of thymine dimers, activation of proinflammatory cytokines and neuroendocrine mediators, leading to inflammation and free radical generation [17]. Skin naturally uses antioxidants to protect itself from photodamage. UV depletes antioxidants from outer SC. A gradient in the antioxidant levels (alfa-tocopherol, vitamin C, glutathione, and urate) with the lowest concentrations in the outer layers and a steep increase in the deeper layers of the SC protects it from oxidative stress [18]. Depletion of antioxidant protection leads to UV-induced barrier abnormalities. Topical application of antioxidants would support these physiologic mechanisms and restore a healthy skin barrier [19,20].

Regulation of barrier homeostasis

The epidermal barrier is constantly challenged by environmental and physiologic factors. Because a fully functional epidermal barrier is required for terrestrial life to exist, barrier homeostasis is tightly regulated by a variety of mechanisms.

Desquamation

Integral components of the barrier, corneocytes, and the intercellular lipid bilayers are constantly synthesized and secreted by the keratinocytes during the process of terminal differentiation. The continuous renewal process is balanced by desquamation which removes individual corneocytes in a controlled manner by degradation of desmosomal constituent proteins by the SC proteases. The protease activities are under the control of protease inhibitors which are co-localized with the proteases within the SC. In addition, the activation cascade of the SC proteases is also controlled by the barrier requirement. Lipids and lipid precursors such as cholesterol sulfate also regulate desquamation by controlling the activities of the SC proteases [21].

Corneocyte maturation

Terminal differentiation of keratinocytes to mature corneocytes is controlled by calcium, hormonal factors, and by desquamation. High calcium levels in the outer nucleated layers of epidermis stimulate specific protein synthesis and activate the enzymes that induce the formation of corneocytes. A variety of hormones and cytokines control keratinocyte terminal differentiation, thereby regulating barrier formation. Many of the regulators of these hormones are lipids or lipid intermediates which are synthesized by the epidermal keratinocytes for the barrier function, thereby exerting control of barrier homeostasis by affecting the corneocyte maturation. For example, the activators and/or ligands for the nuclear hormone receptors (e.g. peroxisome proliferation activator receptor [PPAR] and vitamin D receptor) that influence keratinocyte terminal differentiation are endogenous lipids synthesized by keratinocytes.

Lipid synthesis

Epidermal lipids, the integral components of the permeability barrier, are synthesized and secreted by the keratinocytes in the stratum granulosum after processing and packaging into the LB. Epidermis is a very active site of lipid synthesis under basal conditions and especially under conditions when the barrier is disrupted. Epidermis synthesizes ceramides, cholesterol, and FFA (a major component of phospholipids and ceramides). These three lipid classes are required in equimolar distribution for proper barrier function. The synthesis, processing, and secretion of these lipid classes are under strict control by the permeability barrier requirements. For example, under conditions of barrier disruption, rapid and immediate secretion by already packaged LB occurs as well as transcriptional and translational increases in key enzymes required for new synthesis of these lipids to take place. In addition, many of the hormonal regulators of corneocyte maturation are lipids or lipid intermediates synthesized by the epidermis. SC lipid synthesis and lipid content are also altered with various skin conditions such as inflammation and winter xerosis [22,23].

Environmental and physiologic factors

Barrier homeostasis is under control of environmental factors such as humidity variations. High humidity (increased

SC hydration) downregulates barrier competence (as assessed by barrier recovery after disruption) whereas low humidity enhances barrier homeostasis. Physiologic factors can also have influence on barrier function. High stress (chronic as well as acute) increases corticosteroid levels and causes disruption of barrier homeostasis. Conditions that cause skin inflammation can stimulate the secretion of inflammatory cytokines such as interleukins, induce epidermal hyperplasia, cause impaired differentiation, and disrupt epidermal barrier functions.

Hormones

Barrier homeostasis and SC integrity, lipid synthesis is all under the control of different hormones, cytokines, and calcium. Nuclear hormone receptors for both well-known ligands, such as thyroid hormones, retinoic acid, and vitamin D, and "liporeceptors" whose ligands are endogenous lipids control barrier homeostasis. These liporeceptors include peroxisome proliferator activator receptor (PPAR alfa, beta, and gamma) and liver X receptor (LXR). The activators for these receptors are endogenous lipids and lipid intermediates or metabolites such as certain FFA, leukotrienes, prostanoids, and oxygenated sterols. These hormones, mediated by their receptors, control barrier at the level of epidermal cell maturation (corneocyte formation), transcriptional regulation of terminal differentiation proteins and enzymes required for lipid processing, lipid transport, and secretion into LB [5].

pH and calcium

Outermost SC pH is maintained in the acidic range, typically in the range 4.5–5.0, by a variety of different mechanisms. This acidity is maintained by formation of FFA from phospholipids; sodium proton exchangers in the SC and by the conversion of histidine of the NMF to urocanic acid by histidase enzyme in the SC. In addition, lactic acid, a major component of the NMF, has a major role in maintaining the acid pH of the SC. Maintenance of an acidic pH in the SC is important for the integrity and cohesion of the SC as well as the maintenance of the normal skin microflora. The growth of normal skin microflora is supported by acidic pH while a more neutral pH supports pathogenic microbes' invasion of the skin.

This acidic pH is optimal for processing of precursor lipids to mature barrier forming lipids and for initiating the desquamatory process. The desquamatory proteases present in the outer SC such as the thiol proteases and cathepsins are more active in the acidic pH, whereas the SCCE and SCTE present in the lower SC are more active at neutral pH. When the pH gradient is disrupted, desquamation is decreased resulting in dry scaly skin and disrupted barrier function.

In the normal epidermis, there is a characteristic intraepidermal calcium gradient, with peak concentrations of calcium in the granular layer and decreasing all the way up to the SC [24]. The calcium gradient regulates barrier properties by controlling the maturation of the corneocytes, regulating the enzymes that process lipids and by modulating the desquamatory process. Calcium stimulates a variety of processes including the formation and secretion of LB, differentiation of keratinocytes, formation of cornified envelope precursor proteins, and cross-linking of these proteins by the calcium inducible enzyme transglutaminase. Specifically, high levels of calcium stimulate the expression of proteins required for keratinocyte differentiation, including key structural proteins of the cornified envelope, such as loricrin, involucrin, and the enzyme, transglutaminase 1, which catalyzes the cross-linking of these proteins into a rigid structure.

Coordinated regulation of multiple barrier functions

Co-localization of many of the barrier functions allows regulation of the functions of the epidermal barrier to be coordinated. For example, epidermal permeability barrier, antimicrobial barrier, mechanical protective barrier, and UV barrier are all co-localized in the SC. A disruption of one function can lead to multiple barrier disruptions, and therefore multiple barrier functions are coordinately regulated [5]. Disruption of the permeability barrier leads to activation of the cytokine cascade (increased levels of primary cytokines, interleukin-1, and tumor necrosis factor-alfa) which in turn activates the synthesis of antimicrobial peptides of the SC. Additionally, the cytokines and growth factors released during barrier disruption lead to corneocyte maturation, thereby strengthening the mechanical and protective barrier of the skin. Hydration of the skin itself controls barrier function by regulating the activities of the desquamatory proteases (high humidity decreases barrier function and stimulates desquamation). In addition, humidity levels control filaggrin hydrolysis which releases the free amino acids that form the NMF (histidine, glutamine arginine, and their by-products) and trans-urocanic acid (deamination of histidine) which serves as a UV barrier.

Methods for studying barrier structure and function

Physical methods

SC integrity and desquamation can be measured using tape stripping methods. Under dry skin conditions, when the barrier is compromised, corneocytes do not separate singly but as "clumps." This can be quantified by using special tapes and visualizing the corneocytes removed by light microscopy. Another harsher tape-stripping method involves stripping of the SC using cyanoacrylate glue. These physical methods provide a clue to the binding forces that hold the

corneocyte together. The efficacy of treatment with skin moisturizers or emollients that improve skin hydration and reduce scaling can be quantitated using these methods.

Instrumental methods

The flux of water vapor through the skin (transepidermal water loss [TEWL]) can be determined using an evaporimeter [25]. This instrument contains two water sensors mounted vertically in a chamber one above the other. When placed on the skin in a stable ambient environment the difference in water vapor values between the two sensors is a measure of the flow of water coming from the skin (TEWL). There are several commercially available evaporimeters (e.g. Tewameter® [Courage & Khazaka, Köln, Germany]), which are widely used in clinical practice as well as in investigative skin biology. Recovery of the epidermal barrier (TEWL) after disruption using physical methods (e.g. tape strips) or chemical methods (organic solvent washing) provides valuable information on epidermal barrier properties [26].

Skin hydration can be measured using the Corneometer® (Courage & Khazaka, Köln, Germany). The measurement is based on capacitance of a dielectric medium. Any change in the dielectric constant caused by skin surface hydration variation alters the capacitance of a precision measuring capacitor. The measurement can detect even the slightest changes in hydration level. Another important recent development in skin capacitance methodology is the SkinChip® (L'Oreal, Paris, France). Skin capacitance imaging of skin surface can be obtained using the SkinChip. This method provides information on skin microrelief, level of SC hydration, and sweat gland activity. SkinChip technology can be used to quantify regional variation in skin, skin changes with age, effects of hydrating formulations, surfactant effects on corneocytes, acne, and skin pore characteristics [27].

Several other recently developed methods for measuring epidermal thickness such as confocal microscopy, dermatoechography, and dermatoscopy can provide valuable information on skin morphology and barrier abnormalities [28]. Other more sophisticated (although not easily portable) instrumentation techniques such as ultrasound, optical coherence tomography, and magnetic resonance imaging (MRI) can provide useful information on internal structures of SC and/or epidermis and its improvements with treatment. MRI has been successfully used to evaluate skin hydration and water behavior in aging skin [29].

Biologic methods

Ultrastructural details of SC and the intercellular spaces of the SC can be visualized using transmission electron microscopy of thin vertical sections and freeze–fracture replicas, field emission scanning electron microscopy, and immunofluorescence confocal laser scanning microscopy [30]. The ultrastructural details of the lipid bi-layers within the SC can be visualized by electron microscopy after fixation using ruthenium tetroxide. The existence of corneodesmosomes in the SC, and their importance in desquamation, can be measured by scanning electron microscopy of skin surface replicas.

The constituent cells of the SC, the corneocytes, can be visualized and quantitated by scraping the skin surface or by use of a detergent solution. The suspension so obtained can be analyzed by microscopy, biochemical or immunologic techniques.

Punch or shaved biopsy techniques can be combined with immunohistochemistry using specific SC and/or epidermis specific antibodies to quantify the SC quality. Specific antibodies for keratinocyte differentiation specific proteins, desmosomal proteins, or specific proteases can provide information on skin barrier properties.

Relevance of skin barrier to cosmetic product development

Topical products that influence barrier functions

The human skin is constantly exposed to a hostile environment: changes in relative humidity, extremes of temperature, environmental toxins, and daily topically applied products. Daily exposure to soaps and other household chemicals can compromise skin barrier properties and cause unhealthy skin conditions. Prolonged exposure to surfactants removes the epidermal barrier lipids and enhances desquamation leading to impaired barrier properties [4,10]. Allergic reactions to topical products can result in allergic or irritant contact dermatitis, resulting in itchy and scaly skin and skin redness leading to barrier perturbations.

Cosmetics that restore skin barrier properties

Water is the most important plasticizer of SC. Cracking and fissuring of skin develops as SC hydration declines below a critical threshold. Skin moisturization is a property of the outer SC (also known as stratum disjunctum) as corneocytes of the lower SC (stratum compactum) are hydrated by the body fluids. "Moisturizers" are substances that when applied to skin add water and/or retains water in the SC. The NMF components present in the outer SC act as humectants, absorb moisture from the atmosphere, and are sensitive to humidity of the atmosphere. The amino acids and their metabolites, along with other inorganic and organic osmolytes such as urea, lactic acid, taurine, and glycerol act as humectants within the outer SC. Secretions from sebaceous glands on the surface of the skin also act as emollients and contribute to skin hydration. A lack of any of these components can contribute to dry scaly skin. Topical application of all of the above components can act as humectants, and can relieve dry skin condition and improve skin moisturization

and barrier properties. Film-forming polysaccharide materials such as hyaluronic acid binds and retains water and helps to keep skin supple and soft.

In addition to humectants, emollients such as petroleum jelly, hydrocarbon oils and waxes, mineral and silicone oils, and paraffin wax provide an occlusive barrier to the skin, preventing excessive moisture loss from the skin surface.

Topically applied barrier compatible lipids also contribute to skin moisturization and improved skin conditions. Chronologically aged skin exhibits delayed recovery rates after defined barrier insults, with decreased epidermal lipid synthesis. Application of a mixture of cholesterol, ceramides, and essential/non-essential FFAs in an equimolar ratio was shown to lead to normal barrier recovery, and a 3:1:1:1 ratio of these four ingredients demonstrated accelerated barrier recovery [31].

Topical application of antioxidants and anti-inflammatory agents also protects skin from UV-induced skin damage by providing protection from oxidative damage to skin proteins and lipids [19,20].

Skin irritation from cosmetics

Thousands of ingredients are used by the cosmetic industry. These include pure compounds, mixtures, plant extracts, oils and waxes, surfactants, detergents, preservatives, and polymers. Although all the ingredients used by the cosmetic industry are tested for safety, some consumers may still experience reactions to some of them. Most common reactions are irritant contact reactions while allergic contact reactions are less common. Irritant reactions tend to be more rapid and cause mild discomfort and redness and scaling of skin. Allergic reactions can be delayed, more persistent, and sometimes severe. Ingredients previously considered safe can be irritating in a different formulation because of increased penetration into skin. More than 50% of the general population perceives their skin as sensitive. It is believed that the perception of sensitive skin is, at least in part, related to skin barrier function. People with impaired barrier function may experience higher irritation to a particular ingredient because of its increased penetration into deeper layers of the skin.

Conclusions and future trends

Major advances have been made in the last several decades in understanding the complexity and functions of the SC. Extensive research by several groups has elucidated the metabolically active role of SC and characterized the major components within it and their importance in providing protection from the external environment. New insights into the molecular control mechanisms of desquamation, lipid processing, barrier function, and antimicrobial protection have been elucidated in the last decade.

Knowledge of other less well-known epithelial organelles such as intercellular junctions, tight junctions, and gap junctions and their role in barrier function in the skin is being elucidated. Intermolecular links that connect intercellular lipids with the corneocytes of the SC and their crucial role for maintaining barrier function is an area being actively researched.

New knowledge of the corneocyte envelope structure and the physical state of the intercellular lipid crystallinity and their interrelationship would lead to development of new lipid actives for improving SC moisturization and for treatment of skin barrier disorders. Further research in the cellular signaling events that control the communication between SC and the viable epidermis will shed more light on barrier homeostasis mechanisms.

Novel delivery systems have an increasingly important role in the development of effective skin care products. Delivery technologies such as lipid systems, nanoparticles, microcapsules, polymers, and films are being pursued not only as vehicles for delivering cosmetic actives through skin, but also for improving barrier properties of the skin.

Undoubtedly, skin care and cosmetic companies will exploit this new knowledge in developing novel and more efficacious products for strengthening the epidermal barrier and to improve and enhance the functional and aesthetic properties of the human skin.

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Chapter 2: Photoaging

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BASIC CONCEPTS

- UV radiation damages human skin connective tissue through several interdependent, but distinct, processes.
- The normal dermal matrix is maintained through signaling transduction pathways, transcription factors, cell surface receptors, and enzymatic reactions.
- UV radiation produces reactive oxygen species which inhibit procollagen production, degrade collagen, and damage fibroblasts.

Introduction

Skin, the largest human organ, is chronically exposed to UV radiation from the sun. Thinning of the ozone layer, which increases UV transmittance to the Earth, has heightened awareness of the potential injurious skin effects of exposure to UV radiation: photoaging, sunburn, immunosuppression, and carcinogenesis. Photoaging, the most common form of skin damage caused by UV exposure, produces damage to connective tissue, melanocytes, and the microvasculature [1]. Recent advances in understanding photoaging in human skin have identified the physical manifestations, histologic characteristics, and molecular mechanisms of UV exposure.

Definition

Photoaging is the leading form of skin damage caused by sun exposure, occurring more frequently than skin cancer. Chronic UV exposure results in premature skin aging, termed cutaneous photoaging, which is marked by fine and coarse wrinkling of the skin, dyspigmentation, sallow color, textural changes, loss of elasticity, and premalignant actinic keratoses. Most of these clinical signs are caused by dermal alterations. Pigmentary disorders such as seborrheic keratoses, lentigines, and diffuse hyperpigmentation are characteristic of epidermal changes [2].

These physical characteristics are confirmed histologically by epidermal thinning and disorganization of the dermal connective tissue (see p. 14). Loss of connective tissue interstitial collagen fibrils and accumulation of disorganized connective tissue elastin leads to solar elastosis, a condition characteristic of photoaged skin [3]. Similar alterations in the cellular component and the extracellular matrix of the connective tissue of photoaged skin may affect superficial capillaries, causing surface telangiectasias [4].

Physiology

Photoaged versus chronically aged skin

Skin, like all other organs, ages over time. Aging can be defined as intrinsic and extrinsic. Intrinsic aging is a hallmark of human chronologic aging and occurs in both sun-exposed and non-sun-exposed skin. Extrinsic aging, on the contrary, is affected by exposure to environmental factors such as UV radiation. While sun-protected chronically aged skin and photoaged chronically aged skin share common characteristics, many of the physical characteristics of skin that decline with age show an accelerated decline with photoaging [5]. Compared with photodamaged skin, sun-protected skin is characterized by dryness, fine wrinkles, skin atrophy, homogeneous pigmentation, and seborrheic keratoses [6]. Extrinsically aged skin, on the contrary, is characterized by roughness, dryness, both fine and coarse wrinkles, atrophy, uneven pigmentation, and superficial vascular abnormalities (e.g. telangiectasias) [6]. It is important to note that these attributes are not absolute and can vary according to Fitzpatrick skin type classification and history of sun exposure.

While the pathophysiology of photoaged and photo-protected skin differ, the histologic features of these two entities are distinct. In photo-protected skin, a thin epidermis is present with an intact stratum corneum, the dermoepidermal junction and the dermis are flattened, and dermal fibroblasts produce less collagen. In photoaged skin,

Cosmetic Dermatology: Products and Procedures. Edited by Z.D. Draelos.

the thickness of the epidermis can either increase or decrease, corresponding to areas of keratinocyte atypia. The dermoepidermal junction is atrophied in appearance and the basal membrane thickness is increased, reflecting basal keratinocyte damage.

Changes in the dermis of photoaged skin can vary based on the amount of acquired UV damage. Solar elastosis is the most prominent histologic feature of photoaged skin. The quantity of elastin in the dermis decreases in chronically aged skin, but in UV-exposed skin, elastin increases in proportion to the amount of UV exposure [7,8]. Accumulated elastic fibers occupy areas in the dermal compartment previously inhabited by collagen fibers [9]. This altered elastin deposition is manifest clinically as wrinkles and yellow discoloration of the skin.

Another feature of photoaged skin is collagen fibril disorganization. Mature collagen fibers, which constitute the bulk of the skin's connective tissue, are degenerated and replaced by collagen with a basophilic appearance, termed basophilic degeneration. Additional photoaged skin characteristics include an increase in the deposition of glycosaminoglycans and dermal extracellular matrix proteins [10,11]. In fact, the overall cell population in photodamaged skin increases, leading to hyperplastic fibroblast proliferation and infiltration of inflammatory substrates that cause chronic inflammation (heliodermatitis) [12]. Changes in the microvasculature also occur, as is clinically manifested in surface telangiectasias and other vascular abnormalities.

Photobiology

In order to fully understand the molecular mechanisms responsible for photoaging in human skin, an awareness of the UV spectrum is crucial. The UV spectrum is divided into three main components: UVC (270–290 nm), UVB (290–320 nm), and UVA (320–400 nm). While UVC radiation is filtered by ozone and atmospheric moisture, and consequently never reaches the Earth, UVA and UVB rays do reach the terrestrial surface. Although the ratio of UVA to UVB rays is 20:1 [13] and UVB is greatest during the summer months, both forms of radiation have acute and chronic effects on human skin.

Photoaging is the superposition of UVA and UVB radiation on intrinsic aging. In order to exert biologic effects on human skin, both categories of UV rays must be absorbed by chromophores in the skin. Depending on the wavelength absorbed, UV light interacts with different skin cells at different depths (Figure 2.1). More specifically, energy from UVB rays is mostly absorbed by the epidermis and affects epidermal cells such as the keratinocytes, whereas energy from UVA rays affects both epidermal keratinocytes and the deeper dermal fibroblasts. The absorbed energy is converted into varying chemical reactions that cause histologic and clinical changes in the skin. UVA absorption by chromophores mostly acts indirectly by transferring energy to oxygen to generate reactive oxygen species (ROS), which subsequently causes several effects such as transcription factor activation, lipid peroxidation, and DNA-strand breaks. On the contrary, UVB has a more direct effect on the absorb-

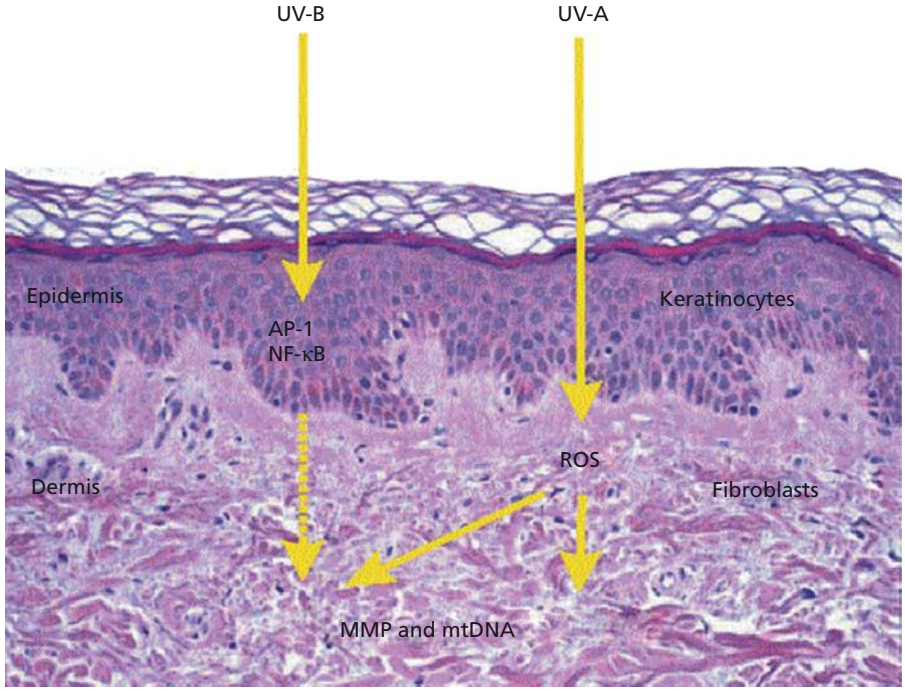


Figure 2.1 Ultraviolet light interacts with different skin cells at different depths. More specifically, energy from UVB rays is mostly absorbed by the epidermis and affects epidermal cells such as the keratinocytes. Energy from UVA rays affects both epidermal keratinocytes and the deeper dermal fibroblasts. AP-1, activator protein 1; NF-κB, nuclear factor κB; MMP, matrix metalloproteinase; mtDNA, mitochondrial DNA; ROS, reactive oxygen species. (Reproduced by permission of: Blackwell Publishing. This figure was published in: Berneburg M, Plettenberg H, Krutmann J. (2000) Photoaging in human skin. *Photodermatol Photoimmunol Photomed* **16**, figure 1, p. 240.)

ing chromophores and causes cross-linking of adjacent DNA pyrimidines and other DNA-related damage [14]. Approximately 50% of UV-induced photodamage is from the formation of free radicals, while mechanisms such as direct cellular injury account for the remainder of UV effects [15].

Cutaneous microvasculature

Intrinsically aged skin and photodamaged skin share similar cutaneous vasculature characteristics, such as decreased cutaneous temperature, pallor, decreased cutaneous vessel size, reduced erythema, reduced cutaneous nutritional supply, and reduced cutaneous vascular responsiveness [16–18]. However, there are also significant differences in the microvasculature of chronologic sun-protected versus photoaged skin. Studies have reported that the blood vessels in photoaged skin are obliterated and the overall horizontal architecture of the vascular plexuses is disrupted [19]. In contrast to photodamaged skin, intrinsically aged skin does not display a greatly disturbed pattern of horizontal vasculature. Additionally, while cutaneous vessel size has been reported to decrease with age in both scenarios, only photoaged skin exhibits a large reduction in the number of dermal vessels. This reduction is especially highlighted in the upper dermal connective tissue, where it is hypothesized that chronic UV-induced degradation of elastic and collagen fibers is no longer able to provide the physical support required for normal cutaneous vessel maintenance [16].

Furthermore, preliminary studies have reported that the effects of exposure to acute UV radiation differ from chronic exposure. Recent studies have implied that a single exposure to UVB radiation induces skin angiogenesis in human skin *in vivo* [20,21]. The epidermis-derived vascular endothelial growth factor (VEGF) is an angiogenic factor that is significantly upregulated with UV exposure in keratinocytes *in vitro* and in human skin *in vivo*. Chung and Eun [16] have demonstrated that, compared to low VEGF expression in non-UV-irradiated control skin, epidermal VEGF expression increased significantly on days 2 and 3 post-UV-irradiation, consequently inducing cutaneous angiogenesis. Therefore, acute UV exposure has been shown to induce angiogenesis. However, chronic UV-exposed photodamaged skin exhibits a significant reduction in the number of cutaneous blood vessels. The reasons for this discrepancy between the effects of acute and chronic UV exposure on angiogenesis *in vivo* are still under investigation.

Molecular mechanisms of photoaging

During the last few years substantial progress has been made in exposing the molecular mechanisms accountable for photoaging in human skin. One major theoretical advance that has been elucidated by this work is that UV irradiation damages human skin by at least two interdependent mechanisms:

1 Photochemical generation of ROS; and

2 Activation of cutaneous signal transduction pathways.

These molecular processes and their underlying components are described in detail below. Before these processes are highlighted, however, the structure and function of collagen must be understood.

Collagen

Type I collagen accounts for greater than 90% of the protein in the human skin, with type III collagen accounting for a smaller fraction (10%). The unique physical characteristics of collagen fibers are essential for providing strength, structural integrity, and resilience to the skin. Dermal fibroblasts synthesize individual collagen polypeptide chains as precursor molecules called procollagen. These procollagen building blocks are assembled into larger collagen fibers through enzymatic cross-linking and form the three-dimensional dermal network mainly made of collagen types I and III. This intermolecular covalant cross-linking step is essential for maintenance and structural integrity of large collagen fibers, especially type I collagen.

Natural breakdown of type I collagen is a slow process and occurs through enzymatic degradation [22]. Dermal collagen has a half-life of greater than 1 year [22], and this slow rate of type I collagen turnover allows for disorganization and fragmentation of collagen which impair its functions. In fact, fragmentation and dispersion of collagen fibers is a feature of photodamaged skin that is clinically manifest in the changes associated with photodamaged human skin.

The regulation of collagen production is an important mechanism to understand before discussing how this process is impaired. In general, collagen gene expression is regulated by the cytokine, transforming growth factor β (TGF-β), and the transcription factor, activator protein (AP-1), in human skin fibroblasts. When TGF-βs bind to their cell surface receptors (TβRI and TβRII), transcription factors Smad2 and Smad3 are activated, combine with Smad4, and enter the nucleus, where they regulate type I procollagen production. AP-1 has an opposing effect and inhibits collagen gene transcription by either direct suppression of gene transcription or obstructing the Smad complex from binding to the TGF-β target gene (Figure 2.2) [23]. Therefore, in the absence of any inhibiting factors, the TGF-β/Smad signaling pathway results in a net increase in procollagen production.

How does UV irradiation stimulate photoaging?

UV irradiation stimulates photoaging through several molecular mechanisms, discussed in detail below.

Reactive oxygen species

Approximately 50% of UV-induced photodamage is from the formation of free radicals, while mechanisms such as

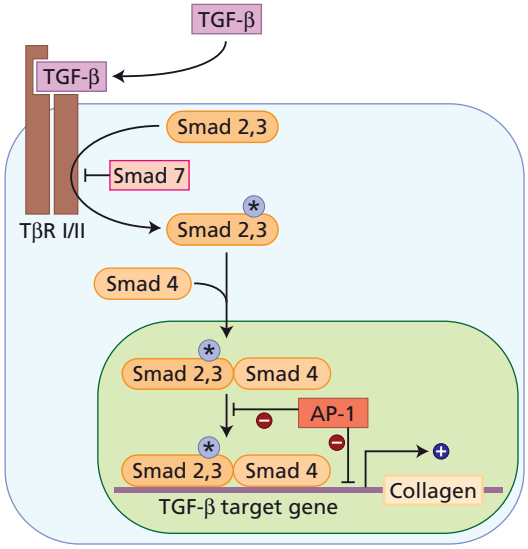


Figure 2.2 The regulation of procollagen production: the TGF-β/Smad signaling pathway. AP-1, activator protein 1; TβR, TGF-β receptor; TGF-β, transforming growth factor β. (Reproduced by permission of: Elsevier Ltd. This figure was published in: Kang S, Fisher G, Voorhees JJ. (2001) Photoaging pathogenesis, prevention, and treatment. *Clin Geriatric Med* **17**(4), figure 1, p. 645.)

direct cellular injury account for the remainder of UV effects [15]. Proposed in 1954, the free radical theory of aging suggests that aging is a result of reactions caused by excessive amounts of free radicals, which contain one or more unpaired electrons [24]. Generation of ROS occurs during normal chronologic aging and when human skin is exposed to UV light in photoaging [25]. ROS mediate deleterious post-translational effects on aging skin through direct chemical modifications to mitochondrial DNA (mtDNA), cell lipids, deoxyribonucleic acids (DNA), and dermal matrix proteins, including collagens. In fact, a 4977 base-pair deletion of mtDNA was recently found in dermal human fibroblast cells. This deletion is induced by UVA via ROS and is a marker of UVA photodamage [26].

UV radiation inhibits procollagen production: TGF-β/Smad signaling pathway

UV light inhibits procollagen production through two signaling pathways: downregulation of TβRII and inhibition of target gene transcription by AP-1. UV radiation has been reported to disrupt the skin collagen matrix through the TGF-β/Smad pathway [1]. More specifically, UV radiation downregulates the TGF-β type II receptor (TβRII) and results in a 90% reduction of TGF-β cell surface binding, consequently reducing downstream activation of the Smad 2, 3, 4 complex and type I procollagen transcription.

Additionally, UV radiation activates AP-1, which binds factors that are part of the procollagen type I transcriptional complex. This, in turn, reduces TGF-β target gene expression, such as expression of type I procollagen [27].

UV-induced matrix metalloproteinases stimulate collagen degradation

It has been demonstrated that UV irradiation affects the post-translational modification of dermal matrix proteins (through ROS) and also downregulates the transcription of these same proteins (through the TGF-β/Smad signaling pathway). UVA and UVB light also induces a wide variety of matrix metalloproteinases (MMPs) [28]. As their name suggests, MMPs degrade dermal matrix proteins, specifically collagens, through enzymatic activity. UV-induced MMP-1 initiates cleavage of type I and III dermal collagen, followed by further degradation by MMP-3 and MMP-9.

Recall that type I collagen fibrils are stabilized by covalent cross-links. When undergoing degradation by MMPs, collagen molecules can remain cross-linked within the dermal collagen matrix, thereby impairing the structural integrity of the dermis. In the absence of perfect repair mechanisms, MMP-mediated collagen damage can accrue with each UV exposure. This type of collective damage to the dermal matrix collagen is hypothesized to have a direct effect on the physical characteristics of photodamaged skin [14].

In addition to UV induction of MMPs, transcription factors may cause MMP activation. It has been reported that within hours of UV exposure, the transcription factors AP-1 and NF-κB are activated which, in turn, stimulate transcription of MMPs (Figure 2.3) [29].

Fibroblasts regulate their own collagen synthesis

Fibroblasts have evolved to regulate their output of extracellular matrix proteins (including collagen) based on internal mechanical tension [30]. Type I collagen fibrils in the dermis serve as mechanical stabilizers and attachment sites for fibroblasts in sun-protected skin. Surface integrins on the fibroblasts attach to collagen and internal actin–myosin microfilaments provide mechanical resistance by pulling on the intact collagen. In response to this created tension, intracellular scaffolding composed of intermediate filaments and microtubules pushes outward to causing fibroblasts to stretch. This stretch is an essential cue for normal collagen and MMP production by fibroblasts (Figure 2.4) [30].

This mechanical tension model is different in photoaged human skin. Fibroblast–integrin attachments are lost, which prevents collagen fragments from binding to fibroblasts. Collagen–fibroblast binding is crucial for maintenance of normal mechanical stability. When mechanical tension is reduced, as in photoaged skin, fibroblasts collapse, which causes decreased procollagen production and increased collagenase (COLase) production [30]. Collagen is continually lost as this cycle repeats itself.

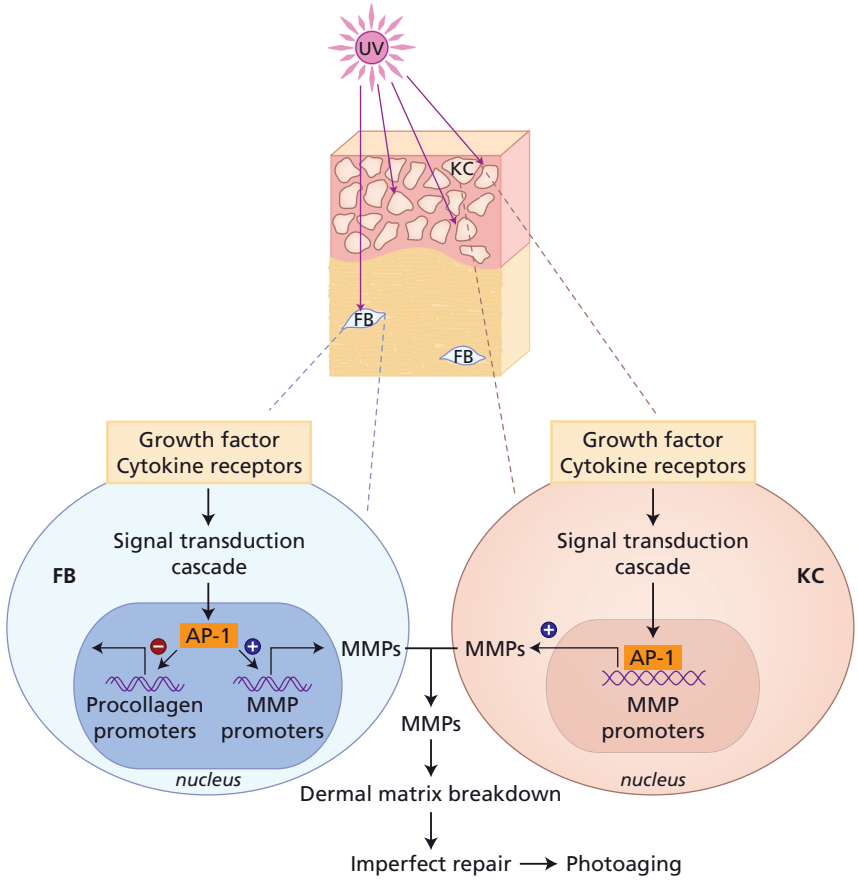
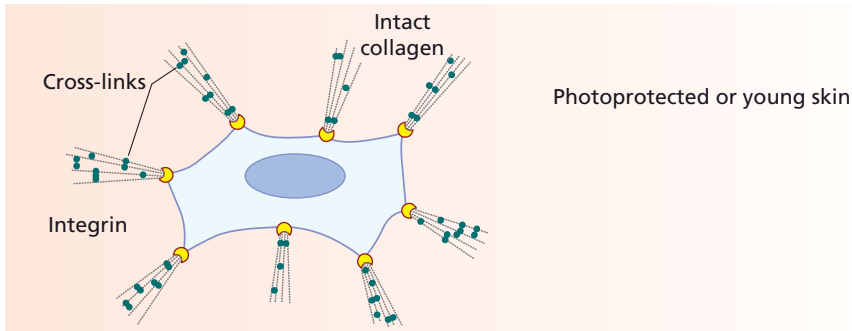
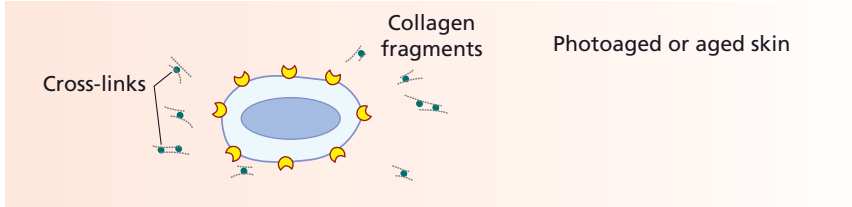


Figure 2.3 Model depicting the effects of UV irradiation on epidermal keratinocytes (KC) and dermal fibroblasts (FB). AP-1, activator protein 1; MMP, matrix metalloproteinase. (Reproduced by permission of: Fisher GJ, *et al.* Mechanisms of photoaging and chronological skin aging. *Arch Dermatol* 2002; **138**: figure 1 p. 1463. Copyright 2002, American Medical Association. All Rights reserved.)



(a) Stretched fibroblast



(b) Collapsed fibroblast

Figure 2.4 Fibroblasts have evolved to regulate their output of collagen based on internal mechanical tension. Model depicting the effects of mechanical tension on procollagen production in (a) sun-protected human skin versus (b) photodamaged human skin. (Reproduced by permission of: Fisher GJ, Varani J, Voorhees, JJ. Looking older. *Arch Dermatol* 2008; **144**(5), figure 2, p. 669. Copyright 2008, American Medical Association. All rights reserved.)

Ethnic skin: photoaging

All races are susceptible to photoaging. However, people with Fitzpatrick skin phototypes IV–VI are less susceptible to the deleterious effects of UV irradiation than people with a lower Fitzpatrick skin type classification. This phenomenon is most likely a result of the protective role of melanin [31]. Studies reporting ethnic skin photoaging are few and far between. However, for the purposes of this discussion, characteristics of photoaging in different ethnic skin categories are briefly highlighted.

In one of the first studies comparing UV absorption amongst different skin types, Kaidbey *et al.* [32] compared the photoprotective properties of African-American skin with Caucasian skin exposed to UVB irradiation. It was known that only 10% of the total UVB rays penetrated the dermis. However, the mean UVB transmission into the dermis by African-American dermis (5.7%) was found to be significantly less than for Caucasian dermis (29.4%). Similar experiments were performed with UVA irradiation. Although only 50% of the total UVA exposure penetrates into the papillary dermis, UVA transmission into African-American dermis was 17.5% compared to 55% for white epidermis [32]. The physiologic reason behind this difference in black and white skin lies at the site of UV filtration. The malpighian layer (basal cell layer) of African-American skin is the main site of UV filtration, while the stratum corneum absorbs most UV rays in white skin. The malpighian layer of African-American skin removes twice as much UVB radiation as the overlying statum corneum, thus mitigating the deleterious effects of UV rays in the underlying dermis [33].

In African-Americans, photoaging may not be clinically apparent until the fifth or sixth decade of life and is more common in individuals with a lighter complexion [34]. The features of photoaging in this ethnic skin group manifest as signs of laxity in the malar fat pads sagging toward the nasolabial folds [35]. In patients of Hispanic and European descent, photoaging occurs in the same frequency as Caucasians and clinical signs are primarily wrinkling rather than pigmentary alterations. The skin of East and South-East Asian patients, on the contrary, mainly exhibits pigmentary alterations (seborrheic keratoses, hyperpigmentation, actinic lentigines, sun-induced melasma) and minimal wrinkling as a result of photoaging [36,37]. Finally, very few studies have reported on the signs of photoaging in South Asian (Pakistanis, Indians) skin. UV-induced hyperpigmentation, dermatosis papulosa nigra, and seborrheic keratosis are noted [38].

Despite all of these differences, it is important to note that the number of melanocytes per unit area of skin does not vary across ethnicities. Instead, it is the relative amount of melanin packaged into melanocytes that accounts for the physiologic differences between Caucasian skin and ethnic skin [39].

Prevention

Although the effects of the sun's rays appear daunting, there are some ways to avoid the deleterious effects of photoaging. Avoiding photoaging can often prove to be more cost-effective than trying to reverse the signs of photoaging after they have manifested.

Primary prevention

Sun protection

UV rays are especially prevalent during the hours of 10AM–4PM and sun protection should be encouraged during this time. Sun protection can be offered to patients in the form of sunscreens, sun-protective clothing, and/or sun avoidance. Sun-protective clothing includes any hats, sunglasses, or clothing that would help block the sun's rays. Photoprotective clothing is given a UV protection factor (UPF) rating, which is a measurement of the amount of irradiation that can be transmitted through a specific type of fabric. A UPF of 40–50 is recommended by most dermatologists, as it transmits less than 2.6% of UV irradiation [5].

Traditionally, sunscreens contain one or more chemical filters – those that physically block, reflect, or scatter specific photons of UV irradiation and those that absorb specific UV photons. UVA sunblocks contain the inorganic particulates titanium dioxide or zinc oxide, while UVA-absorbing sunscreens contain terephthalylidene dicamphor sulfonic acid or avobenzene. UVB-absorbing sunscreens can contain salicylates, cinnamates, *p*-aminobenzoic acid, or a combination of these [40]. The US Food and Drug Administration (FDA) recommended dose of sunscreen application is 2 mg/cm^2 [41].

The sun protection factor (SPF) is an international laboratory measure used to assess the efficacy of sunscreens. The SPF can range from 1 to over 80 and indicates the time that a person can be exposed to UVB rays before getting sunburn with sunscreen application relative to the time a person can be exposed without sunscreen. SPF levels are determined by the minimal amount of UV irradiation that can cause UVB-stimulated erythema and/or pain. The effectiveness of a particular sunscreen depends on several factors, including the initial amount applied, amount reapplied, skin type of the user, amount of sunscreen the skin has absorbed, and the activities of the user (e.g. swimming, sweating).

The sun protection factor is an inadequate determination of skin damage because it does not account for UVA rays. Although UVA rays have an important role in photoaging, their effects are not physically evident as erythema or pain, as are UVB rays. Therefore, it has been suggested that SPF may be an imperfect guide to the ability of a particular sun-

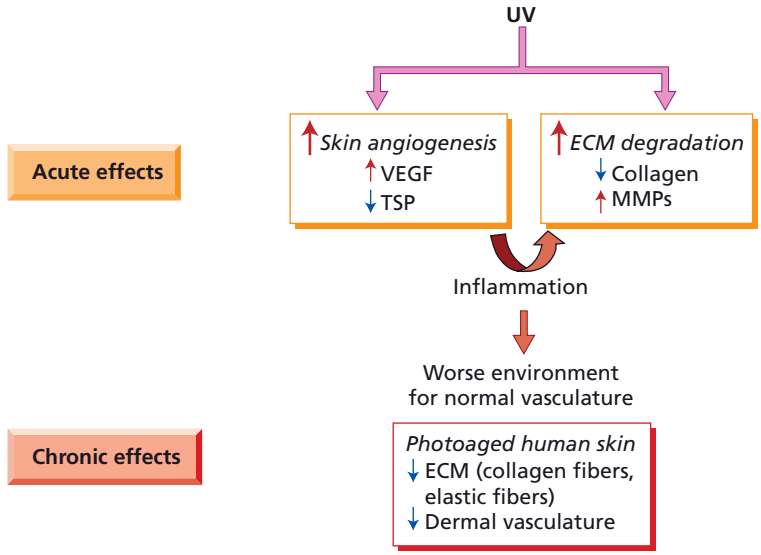


Figure 2.5 Model depicting the acute and chronic effects of UV irradiation on skin angiogenesis and extracellular matrix (ECM) degradation in human skin. MMP, matrix metalloproteinase; TSP, thrombospondin-1 (ECM protein; inhibitor of angiogenesis in epithelial tissues); VEGF, vascular endothelial growth factor. (Reproduced by permission of: Blackwell Publishing. This figure was published in: Chung JH, Eun HC. (2007) Angiogenesis in skin aging and photoaging. *J Dermatol* **34**, figure 1, p. 596.)

screen to shield against photoaging [5]. As a result, combination UVA–UVB sunscreens have been developed and are recommended to protect the human skin from both types of irradiation.

Secondary prevention

Retinoids

A large number of studies have reported that topical application of 0.025–0.1% all-*trans* retinoic acid (tRA) improves photoaging in human skin [42,43]. Results vary based on treatment duration and applied tRA dose. Although there have been a variety of clinical trials on the topic, the molecular mechanisms by which tRA acts are still waiting to be discovered. Retinoic acids have been used in an *ex post facto* manner to reverse the signs of photodamage and in a preventative fashion to avoid photoaging.

More specifically, tRA has been shown to induce type I and III procollagen gene expression in photoaged skin [44]. It has been observed that topical tRA induces TGF-β in human skin [45], which stimulates the production of type I and III procollagen.

In addition, tRA has been used in a preventive fashion to avert UV-induced angiogenesis. Kim *et al.* [20] demonstrated that topical application of retinoic acid before UV exposure inhibited UV-induced angiogenesis and increases in blood vessel density. In general, extracellular signal-related kinases (ERKs, or classic MAP kinases) positively regulate epidermally derived VEGF. VEGF stimulates angiogenesis upon UV induction. Retinoic acid inhibits ERKs, which can potentially lead to downregulation of VEGF expression, UV-induced angiogenesis, and angiogenesis-associated photoaging (Figures 2.5 and 2.6) [16].

Finally, tRA has been reported to prevent UV-stimulated MMP expression. The transcription factor, c-Jun, is a key component in forming the AP-1 complex. Recall that the AP-1 complex both inhibits types I and III procollagen and stimulates transcription of MMPs. Retinoic acid blocks the accumulation of c-Jun protein, consequently inhibiting the formation of the AP-1 complex and dermal matrix-associated degradation [46].

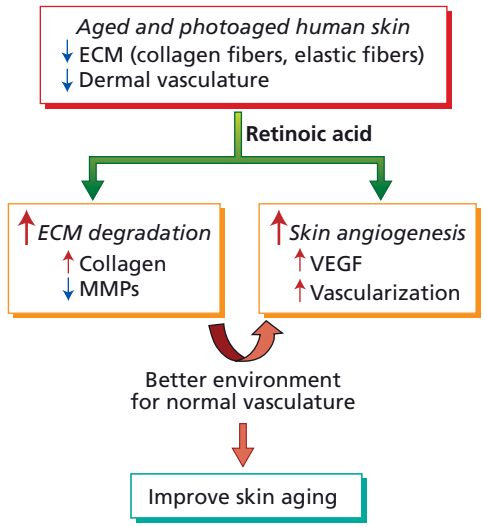


Figure 2.6 Model depicting the effect of topical retinoids on photoaged human skin. ECM, extracellular matrix; MMP, matrix metalloproteinase; VEGF, vascular endothelial growth factor. (Reproduced by permission of: Blackwell Publishing. This figure was published in: Chung J, Eun HC. (2007) Angiogenesis in skin aging and photoaging. *J Dermatol* **34**, figure 5, p. 599.)

Antioxidants

It is important to highlight briefly the role of antioxidants in the reduction of photoaging. *In vitro* studies have

discovered a large number of antioxidants that either forestall or reverse the clinical signs of photodamage caused by ROS. *In vivo* studies investigating these same antioxidants are ongoing. One such antioxidant, vitamin C, has been shown to mitigate photodamaged keratinocyte formation and erythema post-UV-irradiation [47].

Inherent defense mechanisms

Although science has developed exogenous mechanisms to prevent and reverse the clinical signs of photoaging, the human skin possesses endogenous machinery built to protect the skin from UV-induced damage. These inherent defense mechanisms include, but are not limited to, increased epidermal thickness, melanin distribution, DNA repair mechanisms and apoptosis of sunburned keratinocytes, MMP tissue inhibitors, and antioxidants [5,32,48–50].

Failure of prevention: immunosuppression

Although photoaging is the most prevalent form of skin damage, local and systemic immunosuppression, leading to skin carcinoma, can result from overexposure to the sun's rays. This immunosuppression is mediated by a combination of DNA damage, epidermal Langerhans' cell depletion, and altered cytokine expression [51,52].

Conclusions

The pathophysiology of photoaging derives from the ability of UV irradiation to exploit established molecular mechanisms which have evolved to maintain the internal milieu of human skin connective tissue. Disruption of the normal skin architecture does not occur through one pathway, but rather is the culmination of several interdependent, but distinct, processes that have gone awry. The integrity of the normal dermal matrix is maintained through signaling transduction pathways, transcription factors, cell surface receptors, and enzymatic reactions that are intertangled and communicate with one another. When UV irradiation is introduced into this homeostatic picture, deleterious effects can be implemented. Production of ROS, inhibition of procollagen production, collagen degradation, and fibroblast collapse are only a few known processes amongst the medley of mechanisms still waiting to be discovered that contribute to photoaging. Although human skin is equipped with inherent mechanisms to protect against photoaging and methods of prevention and therapeutics are widely available, these alternatives are not absolute and do not necessarily guarantee a perfect escape from the sun's UV irradiation. With each passing day, scientists continue to discover novel cutaneous molecular mechanisms affected by UV irradiation and, consequently, search for new solutions to photodamage.

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Chapter 3: Self-perceived sensitive skin

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BASIC CONCEPTS

- Sensitive skin is a term used by individuals who perceive their skin as being more intolerant or reactive than the general population.
- Sensitive skin is clinically characterized by subjective, sensorial signs: facial discomfort with stinging, burning, and itching.
- The clinical signs of sensitive skin appear in specific conditions, provoked by reactivity factors: environmental factors: wind, sun, cold weather, fast changes in temperature; topical factors: hard water, cosmetics; internal factors: life stress, menstruation, or spicy or hot foods.

Introduction

Sensitive skin is a clinical syndrome, first described in the 1960s by Thiers [1]. A protocol for clinical evaluation of sensitive skin using lactic acid sting testing was first introduced in the 1970s by Frosch and Kligman [2]. Subsequent to that, interest in the field of sensitive skin exploded based on "subjective discomfort, namely, delayed stinging from topical agents applied to the skin." In spite of the contrary opinion expressed by Maibach *et al*. [3] at the end of the 1980s, that "the plausibility of the concept of the sensitive skin evokes discussion and often amusement because of the variance of the number of opinions compared with the amount of data, at least until recently," significant progress has been made on sensitive skin research in recent years. Based on current opinion, sensitive skin is now well accepted as a clinical syndrome.

Based on consumer complaints, it is clear that sensitive skin is a term used by individuals who perceive their skin as being more intolerant or reactive than the general population. Consequently, sensitive skin could be defined as a hyperreactive skin, characterized by exaggerated sensorial reaction to environmental or topical factors, including hard water and cosmetics. Consequently, instead of "sensitive skin," it is better to call this syndrome "self-perceived sensitive skin" (SPSS).

In the last decade, some new understanding on the mechanisms of sensitive skin, involving sensitive epidermal nerves has been emphasized [4].

Cosmetic Dermatology: Products and Procedures. Edited by Z.D. Draelos.

Clinical features

Clinical signs and provocative factor

Sensitive skin is clinically characterized by subjective, sensorial signs: facial discomfort with stinging, burning, and itching. SPSS is more frequent in young women, and decreases with age.

The clinical signs of sensitive skin appear in specific conditions, provoked by reactivity factors:

- Environmental factors: wind, sun, cold weather, fast changes in temperature;
- Topical factors: hard water, cosmetics;
- Internal factors: life stress, menstruation, or spicy or hot foods.

Clinical subgroups

Although the distribution of sensitive skin occurs throughout the population, multivariate analysis shows that several subgroups could be defined [4,5], according to the severity of sensitive skin and to the provocative factors:

1 Severe sensitive skin;
2 Sensitive skin to environment;
3 Sensitive skin to topical factors.

Severe sensitive skin

Severe sensitive skin demonstrates very high facial skin reactivity to all kinds of factors: topical, environmental including atmospheric pollution and also internal factors such as stress and tiredness. Severe sensitive skin could present as "crisis phases" occurring for several days or weeks. During these phases, known as "status cosmeticus," the skin becomes intolerant to all applied products, even products that are usually very well tolerated by the consumer [6].

Sensitive skin to topical factors

Around 25% of women have sensitive skin to topical factors. In this subgroup of sensitive skin, the provocative factor is the application of a product on the skin. It is important to underline that the intolerance observed appears immediately or in the minutes following application, sometimes from the first application.

Sensitive skin to environmental factors

Around 15–20% of women have sensitive skin to environmental factors such as heat, rapid changes in temperature, or wind.

Diathesis factors

In most cases of sensitive skin, the skin hyperreactivity is constitutional. Thiers [1], who was the first to describe this syndrome, has suggested that diathesis features could exist. We also found that a familiar history of sensitive skin exists. Sensitive skin is more frequently found in subjects with fair complexion, and/or redness on the cheekbones [7,8]. Severe dry skin could be as affected as severe oily skin by skin hyperreactivity.

Acquired skin hyperreactivity could mimic the signs observed during sensitive skin syndrome. This acquired "sensitive skin," characterized by a temporary decrease of the threshold of sensorial reactivity of the skin, could be linked to topical irritants improperly applied such as retinoids or hydroxy-acids. In these cases, it is possible that skin that is usually "non-reactive" becomes "reactive" for a period of time. The presence of active facial dermatitis such as seborrheic dermatitis or rosacea could also lower the threshold of skin reactivity. However, although an outbreak of facial atopic dermatitis increases skin reactivity, it is incorrect to consider all sensitive skin as atopic skin.

Sensitive skin and immuno-allergologic pattern

An important point about sensitive skin comes from controversial opinions that exist regarding allergic status [5,7]. To explore this, skin patch test reactivity was studied in 152 female adult volunteers [9]. Eighty-eight declared themselves as having sensitive skin, and 64 as having non-sensitive skin.

A series of 44 different topical ingredients known to be potential allergens were applied to the back under Finn Chambers (Table 3.1). The patches were removed after 47 hours and the reactions read after 1 hour and 2 days. For each ingredient, the incidence of positive reactions was compared between the two populations, using the χ^2 test. Positive reactions were recorded for 19 out of the 44 tested compounds. No significant difference in the incidence of positive reactions was found between sensitive and non-sensitive skin subjects for any of the patch-tested ingredients.

Currently, sensitive skin must not be considered as a syndrome linked to an immuno-allergologic pattern.

Table 3.1 List of tested allergens on self-perceived sensitive skin and non-self-perceived sensitive skin subjects. (From [4] and [9].)

Diazolidinyl urea	Hydroquinone
Colophon	Cocamidopropylbetaine
Formaldehyde	Ethylene diamine
Balsam of Peru	Ortho-aminophenol
Benzoic acid	Glyceryl monothioglycolate
Pyrogallol	Ammonium thioglycolate
Parabens mix	Dowicil
Ammonium persulfate	Isothiazolinones
p-aminodiphenylamine	Fragance mix
Wool alcohols	

Diagnosis

Provocative tests

The diagnosis of SPSS must be based on clinical signs, which are neurosensorial (i.e. subjective). In fact, facial stinging, burning, and itching are clinical signs directly felt by the subject but not seen by the observer. It corresponds to the concept of "invisible dermatoses" [10], as is also the case for all sensorial signs encountered in dermatology (e.g. itching, pain).

Pertinent clinical questionnaires are probably the best tools to diagnose this syndrome. Provocative tests could be of help.

The lactic acid stinging test was first described by Frosch and Kligman [2,11]. A solution of 10% lactic acid is applied to a nasolabial fold and the provoked stinging feeling is quantified. Generally, the stinging is measured every minute for 5 minutes on a scale from 0 to 3. The lactic acid reaction is compared with the other nasolabial fold where a control solution (saline solution) is applied. The test discriminates between "stingers" and "non-stingers," but does not affect the discrimination between sensitive skin and non-sensitive skin subjects [12]. In our opinion, the lactic acid stinging test is of interest to assess efficacy of products, but not for diagnostic purposes.

Considering the clinical signs linked to SPSS (stinging, burning, itching), we have hypothesized that the main player is the sensitive epidermal nerve, C-fibers [13]. According to this physiologic hypothesis, we have proposed to test the skin reactivity by using capsaicin [14], an irritant compound extracted from red pepper which acts on vanilloid receptors of the nociceptive C-fibres and provokes the release of neuropeptides as substance P and calcitonin gene-related peptide (CGRP) [15,16].

Capsaicin cream (0.075%) was applied at the angle of the jaw over an area of 4 cm^2. The neurosensorial signs (stinging, burning, and itching [SBI]) were assessed at 3, 5, 10, 15 and 20 minutes according to a scale score (0, 1, 2, 3). The sum of the scores gives the global SBI score.

The results we obtained on two groups of subjects clearly showed that the sensitive skin subjects' (n = 64) reactions were significantly higher than the non-sensitive subjects (n = 88) (Figure 3.1). The capsaicin test allows one to discriminate quite well between SPSS subjects and non-SPSS subjects.

On the same population sample, we compared the scores obtained with the capsaicin test with those from the lactic acid stinging test. The results are presented in Table 3.2. With capsaicin, the scores showed a better correlation to the SPSS than those recorded with lactic acid. Furthermore, there is a real relationship between the severity of the sensitive skin and the response to the capsaicin test. The higher the severity of SPSS, the higher the capsaicin score.

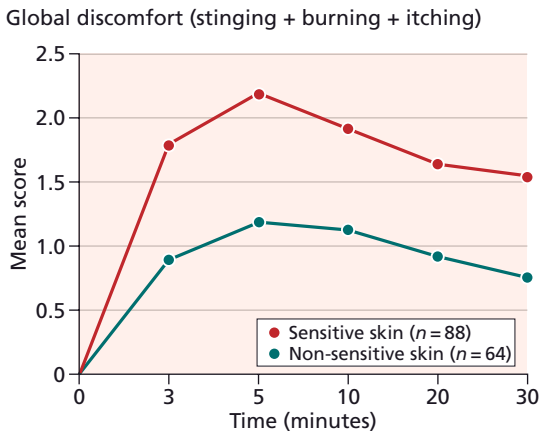

Figure 3.1 Stinging, burning, and itching (SBI) score with capsaicin test on self-perceived sensitive skin and non-self-perceived sensitive skin subjects. Scores are significantly different at each experimental time ($p < 0.01$). (From [4] and [14].)

Sensitive skin and populations

Epidemiologic data

The prevalence of SPSS is estimated at 51–56% in Europe, USA, and Japan [8,17–20].

Willis *et al.* [8] published an epidemiologic study in the UK on sensitive skin where 2058 people (up to 18 years of age) were investigated. Of those who reposonded, 51% of the women and 38% of the men declared themselves to have sensitive skin.

In the San Francisco area, the reported prevalence of SPSS in four ethnic groups (African-American, Asian, Euro-American, and Hispanic Central American) is 52% [19]. No significant difference of prevalence in each group was found: 52% of African-Americans had sensitive skin, 51% of Asians, 50% of Euro-Americans, and 54% of South Americans.

Yang *et al.* [21] studied the sensitive skin in four cities of China: Beijing and Harbin (northern cities), Chengdu and Suzhou (southern cities). Two thousand Chinese women, aged 18–75 years, were included. The global prevalence of sensitive skin was 36%. The prevalence decreases with age (47% at 21–25 years; 20.8% at 51–55 years).

Clinical features

Although the comparison of groups living in San Francisco (African-Americans, Asians, Euro-Americans, and Hispanics) gave the same prevalence of sensitive skin (52%), some differences (10) were observed for factors of skin reactivity and, to a lesser extent, its clinical symptoms. Euro-Americans were characterized by higher skin reactivity to the wind and tended to be less reactive to cosmetics. African-Americans presented less skin reactivity to most environmental factors and a lower frequency of recurring facial redness. Asians appeared to have greater skin reactivity to sudden changes in temperature, to the wind, and also to spicy foods. They tended to experience itching more frequently. In addition, the frequency of skin reactivity to alcoholic beverages was significantly lower in the African-

Table 3.2 (a) Stinging and itching scores with capsaicin test according to the different self-assessed level of self-perceived sensitive skin. (From [4].)

	Non-sensitive (n = 64)	Sensitive (n = 88)			Significance
		Weak (n = 42)	Medium (n = 39)	Strong (n = 7)	
Stinging	2.6 ± 0.6	3 ± 0.6	4.3 ± 0.6	5 ± 0.6	$p < 0.02$
Itching	0.6 ± 0.4	1.6 ± 0.4	2 ± 0.4	2.9 ± 0.4	$p < 0.02$

Table 3.2 (b) Stinging scores during lactic acid stinging test according to the different self-assessed level of self-perceived sensitive skin. (From [4].)

Non-sensitive (n = 64)	Sensitive (n = 88)			Significance
	Weak (n = 42)	Medium (n = 39)	Strong (n = 7)	
2 ± 0.3	2 ± 0.2	3.3 ± 0.3	3 ± 0.3	
		$p < 0.001$	$p < 0.001$	$p < 0.01$

American and Hispanic sensitive groups and higher in the Asian group.

In China, Yang *et al*. [21] have reported that sensitive skin was strongly reactive to environmental factors, but not to cosmetic use. A significantly higher prevalence (55.8%) of sensitive skin was found in Chengdu (Sichuan), where the food is very spicy. By studying the link between chili consumption and sensitive skin prevalence, it has been confirmed that sensitive skin was strongly linked to spicy food intake.

Physiologic mechanisms involved in self-perceived sensitive skin

Barrier function and sensitive skin

It is currently believed that sensitive skin is linked to the skin barrier alteration which could explain the increase in skin reactivity to physical or chemical factors.

In fact, transepidermal water loss (TEWL) has been reported to be increased in subjects with sensitive skin [18]. In addition, an increase in TEWL has also been reported in the "lactic acid stingers" subjects [12]. The alteration of the skin barrier function is certainly involved in the physiology of some patterns of sensitive skin, but it is not unequivocal.

Epidermal sensitive nerves and sensitive skin

In the last decade, additional evidence has been discovered implicating the key role for sensitive nerves in the physiologic mechanisms involved in sensitive skin.

The neurosensorial signs of the pattern of capsaicin reactivity of sensitive skin suggest a neurogenic origin [14]. Recent data that emphasize the role of C-fibres in the itching process must also be considered [13].

It is observed that there is a decrease with age in the epidermal sensitive nerve density on the face [22]. It should also be noticed that there is a similar decrease in the facial skin reactivity to capsaicin and in the prevalence of sensitive skin, suggesting a direct involvement of epidermal sensitive nerves in skin reactivity.

Specific brain activation on sensitive skin subjects

To investigate the possible involvement of the central nervous system (CNS) in SPSS patterns, we measured cerebral responses to cutaneous provocative tests in sensitive and in non-sensitive skin subjects using functional magnetic resonance imaging (fMRI) [23]. According to their responses to validated clinical questions about their skin reactivity, subjects were divided into two balanced groups: severe SPSS and non-SPSS subjects. Event-related fMRI was used to measure cerebral activation induced by split-face application of lactic acid and of its vehicle (control). In sensitive skin subjects, prefrontal and cingulate activity was significantly higher demonstrating a CNS involvement in sensitive skin physiologic pathways.

Conclusions

Sensitive skin is a syndrome observed all over the world. The key clinical features of sensitive skin are neurosensorial signs, mainly provoked by climatic factors, or by topical applications usually well-tolerated on skin.

The hypothesis of the neurogenic origin of sensitive skin is becoming more and more predominant.

1 Sensitive skin subjects demonstrate a significantly higher skin hyperreactivity to capsaicin which specifically stimulates the C-fibers.

2 With age, as sensitive skin is decreasing, facial sensitive epidermal nerve density is also decreasing.

3 Spicy food (rich in capsaicin) increases the prevalence of sensitive skin.

4 The results obtained with fMRI show that sensitive skin subjects demonstrate a specific pattern on cerebral activation, with a higher brain activity for sensitive skin subjects in prefrontal and cingulated areas.

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Chapter 4: Pigmentation and skin of color

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BASIC CONCEPTS

- Differences in the structure, function, and physiology of the hair and skin in individuals of skin of color are important in understanding the structural and physiologic variations that exist and influence disease presentations.
- Melanin, the major determinant of skin color, absorbs UV light and blocks free radical generation, protecting the skin from sun damage and aging.
- UV irradiation of keratinocytes induces pigmentation by the upregulation of melanogenic enzymes, DNA damage that induces melanogenesis, increased melanosome transfer to keratinocytes, and increased melanocyte dendricity.
- Racial differences in hair include the hair type, shape, and bulb.

Introduction

The demographics of the USA reflect a dynamic mixture of people of various ethnic and racial groups. Currently, one in three residents in the USA is a person of skin of color [1]. Persons of skin of color include Africans, African-Americans, Afro-Caribbeans, Asians, Latinos (Hispanics), Native Americans, Middle Easterners, and Mediterraneans. The term "black" as in black skin refers to individuals with African ancestry, including Africans, African-Americans, and Afro-Caribbeans. Subgroups exist within each ethnoracial group. The differences in the structure, function, and physiology of the hair and skin in individuals of skin of color are important in understanding the structural and physiologic variations that exist and influence disease presentations. Pigmentation is especially important in patients of skin of color because pigmentary disorder is the most common reason for a visit to a dermatologist in this group [2].

Melanocytes

Melanin, the major determinant of skin color, absorbs UV light and blocks free radical generation, protecting the skin from sun damage and aging. Melanocytes, the cells that produce melanin, synthesize melanin in special organelles, melanosomes. Melanin-filled melanosomes are transferred from one melanocyte to 30–35 adjacent keratinocytes in the basal layer [3]. The number of melanocytes also decreases with age.

Cosmetic Dermatology: Products and Procedures. Edited by Z.D. Draelos.

There is more than one type of melanin: eumelanin, a dark brown–black pigment; and pheomelanin, a yellow–reddish pigment. Eumelanin is deposited in ellipsoidal melanosomes which contain a fibrillar internal structure. Synthesis of eumelanin increases after UV exposure (tanning). Pheomelanin has a higher sulfur content than eumelanin because of the sulfur-containing amino acid cysteine. Pheomelanin is synthesized in spherical melanosomes and is associated with microvesicles [4]. Although not obvious to the naked eye, most melanin pigments of the hair, skin and, eyes are combinations of eumelanin and pheomelanin [5]. It is generally believed that genetics determine the constitutive levels of pheomelanin and eumelanin. Eumelanin is more important in determining the degree of pigmentation than pheomelanin. Eumelanin, and not pheomelanin, increases with visual pigmentation [5]. Lighter melanocytes have higher pheomelanin content than dark melanocytes. In one study [5], white persons had the least amount of eumelanin, Asian Indians had more, and African-Americans had the highest. Of note, adult melanocytes contain significantly more pheomelanin than cultured neonatal melanocytes.

Melanosomes also differ among different races. In black persons they are mostly in the basal layer, but those of white persons are mostly in the stratum corneum. This is evident in the site of UV filtration: the basal and spinous layers in blacks and the stratum corneum in white persons. Of note, the epidermis of black skin rarely shows atrophied areas [6]. In black skin, melanocytes contain more than 200 melanosomes. The melanosomes are 0.5–0.8 mm in diameter, do not have a limiting membrane, are stuck closely together, and are individually distributed throughout the epidermis. In white skin, the melanocytes contain less than 20 melanosomes. The melanosomes are 0.3–0.5 mm in

diameter, associated with a limiting membrane, and distributed in clusters with spaces between them. The melanosomes of lighter skin degrade faster than that of dark skin. As a result, there is less melanin content in the upper layers of the stratum corneum. Thus, the melanocytes in black skin are larger, more active in making melanin, and the melanosomes are packaged, distributed, and broken down differently from in white skin.

There is also a difference in melanosomes between individuals within the same race but with varying degrees of pigmentation. Despite greater melanin content in darker skins, there is no evidence of major differences in the number of melanocytes [7]. Also, dark Caucasian skin resembles the melanosome distribution observed in black skin [8]. Black persons with dark skin have large, non-aggregated melanosomes and those with lighter skin have a combination of large non-aggregated and smaller aggregated melanosomes [9]. White persons with darker skin have non-aggregated melanosomes when exposed to sunlight and white persons with lighter skin have aggregated melanosomes when not exposed to sunlight [7,8,10].

The steps of melanogenesis are as follows. The enzyme tyrosinase hydroxylates tyrosine to dihydroxyphenylalanin (DOPA) and oxidizes DOPA to dopaquinone. Dopaquinone then undergoes one of two pathways. If dopaquinone binds to cysteine, the oxidation of cysteinyldopa produces pheomelanin. In the absence of cysteine, dopaquinone spontaneously converts to dopachrome. Dopachrome is then decarboxylated or tautomerized to eventually yield eumelanin. Melanosomal P-protein is involved in the acidification of the melanosome in melanogenesis [11]. Finally, the tyrosinase activity (not simply the amount of the tyrosinase protein) and cysteine concentration determine the eumelanin–pheomelanin content [5].

Tyrosinase and tyrosinase-related proteins 1 and 2 (TRP-1 and TRP-2) are upregulated when α-melanocyte-stimulating hormone (α-MSH) or adrenocorticotropin binds to melanocortin-1 receptor (MC1R), a transmembrane receptor located on melanocytes [11–14]. The MC1R loss-of-function mutation increases sensitivity to UV-induced DNA damage. Gene expression of tyrosinase is similar between black and white persons, but other related genes are expressed differently. The MSH cell surface receptor gene for melanosomal P-protein is expressed differently between races. This gene may regulate tyrosinase, TRP-1, and TRP-2 [5].

In addition to the MC1R, protease-activated receptor 2 (PAR-2) is another important receptor that regulates epidermal cells and affect pigmentation [15]. PAR-2 is expressed on many cells and several different organs. Accordingly, the receptor is involved in several physiologic processes, including growth and development, mitogenesis, injury responses, and cutaneous pigmentation. In the skin, PAR-2 is expressed in the keratinocytes of the basal, spinous, and granular layers of the epidermis, endothelial cells, hair follicles, myeoepithelial cells of sweat glands, and dermal dendritic-like cells [16,17]. PAR-2 is a seven transmembrane domain G-protein-coupled receptor which undergoes activation via proteolytic cleavage of the NH2 terminus which acts as a tethered ligand which then activates the receptor (autoactivation).

PAR-2 activating protease (PAR-2-AP), endothelial cell-released trypsin, mast cell-released trypsin and chymase, and SLIGKV all irreversibly activate PAR-2 while serine protease inhibitors interferes with the activation of the receptor [18–20]. SLIGKV and trypsin activate PAR-2 to use a Rho-dependent signaling pathway to induce melanosomal phagocytosis by keratinocytes. The result is an increase in pigmentation to the same degree as UV radiation [17–21]. Serine proteases are regulatory proteins involved in tumor growth, inflammation, tissue repair, and apoptosis in various tissues [17]. In the skin, serine protease inhibitors prevent the keratinocytes from phagocytosing melanosomes from the presenting dendritic tip of the melanocyte. This leads to a dose-dependent depigmentation without irritation or adverse events.

PAR-2 also has a proinflammatory affect in the skin [17]. The activation of PAR-2 expressed on endothelial cells by tryptase, trypsin, or PAR-2-AP leads to an increase in proinflammatory cytokines interleukin 6 (IL-6) and IL-8 and also stimulates NF-κB, an intracellular proinflammatory regulator [18]. Mast cells interact with endothelial cells to regulate inflammatory responses, angiogenesis, and wound healing, and PAR-2 has a regulatory role in this cell–cell interaction [17,18].

UV irradiation of keratinocytes induces pigmentation in several ways: upregulation of melanogenic enzymes, DNA damage that induces melanogenesis, increased melanosome transfer to keratinocytes and increased melanocyte dendricity. UV radiation (UVR) increases the secretion of proteases by keratinocytes in a dose-dependent manner. Specifically, UVR directly increases the expression of PAR-2 *de novo*, upregulates proteases that activate PAR-2, and activates dermal mast cell degranulation [21].

Data on whether PAR-2 is expressed differently in skin of color compared to white skin are needed. One study did find differences in skin phototypes I, II, and III [21]. UVR increases the expression of PAR-2 in the skin and activated PAR-2 stimulates pigmentation. This study found that the response of PAR-2 to UVR is an important determinant of one's ability to tan. In the non-irradiated skin, PAR-2 expression was confined to the basal layer and just above the basal layer. Irradiated skin showed *de novo* PAR-2 expression in the entire epidermis or upper two-thirds of the epidermis. Skin phototype I had a delayed upregulation of PAR-2 expression compared to phototypes II and III.

Dyspigmentation

After cutaneous trauma or inflammation, melanocytes can react with normal, increased, or decreased melanin production; all of which are normal biologic responses. Increased and decreased production results in postinflammatory hyperpigmentation or hypopigmentation. Postinflammatory hyperpigmentation (PIH) is an increase in melanin production and/or an abnormal distribution of melanin resulting from inflammatory cutaneous disorders or irritation from topical medications [22,23]. Examples include acne, allergic contact dermatitis, lichen planus, bullous pemphigoid, herpes zoster, and treatment with topical retinoids. Often, the PIH resulting from acne is more distressing to darker skinned individuals than the initial acute lesion. The color of the hyperpigmentation in PIH depends on the location of the melanin. Melanin in the epidermis appears brown, while melanin in the dermis appears blue-gray. Wood's lamp examination distinguishes the location of the melanin: the epidermal component is enhanced and the dermal component becomes unapparent [24]. Postinflammatory hypopigmentation shares the same triggers as PIH but instead results from decreased melanin production with clinically apparent light areas [23]. The Wood's lamp examination does not accentuate hypopigmentation in postinflammatory hypopigmentation; it is useful for depigmented disorders such as vitiligo and piebaldism.

The pathogenesis of PIH and postinflammatory hypopigmentation are unknown. It is likely that an inflammatory process in the skin stimulates keratinocytes, melanocytes, and inflammatory cells to release cytokines and inflammatory mediators that lead to the hyperpigmentation or hypopigmentation. The cytokines and inflammatory mediators include leukotriene (LT), prostaglandins (PG), and thromboxane (TXB) [25]. Specifically for PIH, *in vitro* studies revealed that LT-C4, LT-D4, PG-E2, and TXB-2 stimulate human melanocyte enlargement and dendrocyte proliferation. LT-C4 also increases tyrosinase activity and mitogenic activity of melanocytes. Transforming growth factor-α and LT-C4 stimulate movement of melanocytes. In postinflammatory hypopigmentation, the pathogenesis likely involves inflammatory mediators inducing melanocyte cell-surface expression of intercellular adhesion molecule 1 (ICAM-1) which may lead to leukocyte–melanocyte attachments that inadvertently destroy melanocytes. These inflammatory mediators include interferon-gamma, tumor necrosis factor α (TNF-α), TNF-β, IL-6, and IL-7.

Natural sun protective factor in skin of color

It is clear that those who fall within Fitzpatrick skin phototypes IV–VI are less susceptible to photoaging; this is most likely due to of the photoprotective role of melanin [26,27]. The epidermis of black skin has a protective factor (PF) for UVB of 13.4 and that of white skin is 3.4 [28]. The mean UVB transmission by black epidermis is 5.7% compared to 29.4% for white epidermis. The PF for UVA in black epidermis is 5.7 and in white epidermis is 1.8 [28]. The mean UVA transmission by black epidermis is 17.5% and 55.5% for white epidermis. Hence, 3–4 times more UVA reaches the upper dermis of white persons than that of black persons.

The main site of UV filtration in white skin is the stratum corneum, whereas in black skin it is the basal layer [28]. The malphigian layer of black skin removes twice as much UVB radiation as the stratum corneum [29]. It is possible that even greater removal of UVA occurs in black skin basal layers [29]. While the above characteristics of natural sun protective factor were studied in black skin, they can probably be extrapolated to most persons of skin photoypes IV–VI.

Skin of color

Epidermis

The epidermal layer of skin is made up of five different layers: stratum basale, stratum spinosum, stratum granulosum, stratum lucidum, and stratum corneum. The stratum basale (also termed the basal layer) is the germinative layer of the epidermis. The time required for a cell to transition from the basal layer through the other epidermal layers to the stratum corneum is 24–40 days. The morphology and structure of the epidermis is very similar among different races, although a few differences do exist.

Stratum corneum

The stratum corneum, the most superficial layer, is the layer responsible for preventing water loss and providing mechanical protection. The cells of the stratum corneum, the corneocytes, are flat cells measuring 50 μm across and 1 μm thick. The corneocytes are arranged in layers; the number of layers varies with anatomic site and race. There are no differences between races in corneocyte surface area, which has a mean size of 900 μm [2,30]. The stratum corneum of black skin is more compact than that of white skin. While the mean thickness of the stratum corneum is the same in black and white skin, black skin contains 20 cell layers while white skin contains 16. The answer to whether or not there are racial differences in spontaneous desquamation is inconclusive [29–31]. Parameters for skin barrier function (stratum corneum hydration, sebum secretion, erythema, and laser Doppler flowmetry) are similar, even after an objective epicutaneous test with sodium lauryl sulfate [32].

Transepidermal water loss

Transepidermal water loss (TEWL) is the amount of water vapor loss from the skin, excluding sweat. TEWL increases with the temperature of the skin. Concrete evidence regarding the difference in TEWL between different races has yet to be established. Aside from TEWL, hydration is also a characteristic of skin. One of the ways to measure hydration, or water content, is conductance. Conductance, the opposite of resistance, is increased in hydrated skin because hydrated skin is more sensitive to the electrical field [33]. Skin conductance is higher in black persons and Hispanics than white persons [33]. Lipid content in black skin is higher than that of white skin [34]. However, black skin is more prone to dryness, suggesting that a difference in lipid content has a role. This includes the ratio of ceramide : cholesterol : fatty acids, the type of ceramides, and the type of sphingosine backbone. One study suggests that the degree of pigmentation influences lipid differences [35].

Pigmentation affects skin dryness. Skin dryness is greater on sun-exposed (dorsal arm) sites for lighter skin, such as Caucasian and Chinese skin, than sites that are primarily out of the sun (ventral arm) [36]. There is no difference in skin dryness between sites for darker skin, such as African-Americans and Mexicans. For adults less than 51 years of age, skin dryness does not change as a function of ethnicity (African-American, Caucasian, Chinese, and Mexican) for sun-exposed sites and sites that are not primarily sun-exposed. For those 51 years of age and older, skin dryness is higher for African-Americans and Caucasians than for Chinese and Mexicans. As a function of age, skin dryness in African-American skin increases 4% on the dorsal site and 3% on the ventral site; in Caucasian skin, it increases 11% on the dorsal site and 10% on the ventral site. All of the above findings suggest that sun exposure can dry the skin and that melanin provides protection.

Skin reactivity

Mast cells

Sueki *et al.* [37] studied the mast cells of four African-American men and four white men (mean age 29 years) by evaluating punch biopsies of the buttocks with electron microscopy, with the following results. The mast cells of black skin contained larger granules (the authors attributed this to the fusion of granules). Black skin also had 15% more parallel-linear striations and 30% less curved lamellae in mast cells. Tryptase reactivity was localized preferentially over the parallel-linear striations and partially over the dark amorphous subregions within granules of mast cells from black skin, whereas it was confined to the peripheral area of granules, including curved lamellae, in white skin. Cathepsin G reactivity was more intense over the electron-dense amorphous areas in both groups, while parallel-linear striations in black skin and curved lamellae in white skin were negative.

Patch test antigens

Contact dermatits

Irritant contact dermatitis (ICD) is the most common form of dermatitis and loosely defined as non-specific damage to the skin after exposure to an irritant. The various clinical manifestations are influenced by the concentration of chemicals, duration of exposure, temperature, humidity, and anatomic location, and other factors. Acute contact dermatitis presents with the classic findings of localized superficial erythema, edema, and chemosis. Cumulative contact dermatitis presents with similar findings, but with repeated exposure of a less potent irritant [38].

The susceptibility to ICD differs between black and white skin [39]. The structural differences in stratum corneum of black skin (e.g. compact stratum corneum, low ceramide levels) are credited with decreasing the susceptibility to irritants. Reflectance confocal microscopy (RCM) is an imaging tool that permits real-time qualitative and quantitative study of human skin; when used with a near-infrared laser beam, one can create "virtual sections" of live tissue with high resolution, almost comparable with routine histology. Measuring skin reactivity to chemical irritants with RCM and TEWL reveal that white skin had more severe clinical reactions than black skin. The pigmentation in darker skin can make the assessment of erythema difficult and interfere with identification of subclinical degrees of irritancy. Even without clinical evidence of irritation, RCM and histology reveal parakeratosis, spongiosis, perivascular inflammatory infiltrate, and microvesicle formation. Mean TEWL after exposure to irritants is greater for white skin than for black skin. This supports the concept that the stratum corneum of black skin enhances barrier function and resistance to irritants.

There are no differences between white persons and African-Americans in objective and subjective parameters of skin such as dryness, inflammation, overall irritation, burning, stinging, and itching [40]. Acute contact dermatitis with exudation, vesiculation, or frank bullae formation is a more common reaction in white skin whereas dyspigmentation and lichenification is more common in black skin [41].

The response to irritation in Caucasian and African-American skin differs in the degree of severity. Caucasian skin has a lower threshold for cutaneous irritation than African-American skin [42]. Caucasian skin also has more severe stratum corneum disruption, parakeratosis, and detached corneocytes. Both groups have the same degree of intra-epidermal spongiosis epidermal (granular and spinous layer) vesicle formation.

The variability in human skin irritation responses sometimes creates difficulty in assessing the differences in skin reactivity between human subpopulations. There are conflicting results in studies comparing the sensitivity to irritants in Asian skin with that in Caucasian skin [32,43–46].

Dermis

The dermis lies deep to the epidermis and is divided into two layers: the papillary and reticular dermis. The papillary dermis is tightly connected to the epidermis via the basement membrane at the dermoepidermal junction. The papillary dermis extends into the epidermis with finger-like projections, hence the name "papillary." The reticular dermis is a relatively avascular, dense, collagenous structure that also contains elastic tissue and glycosaminoglycans. The dermis is made up of collagen fibers, elastic fibers, and an interfibrillar gel of glycosaminoglycans, salt, and water. Collagen makes up 77% of the fat-free dry weight of skin and provides tensile strength. Collagen types I, II, V, and VI are found in the dermis. The elastic fiber network is interwoven between the collagen bundles.

There are differences between the dermis of white and black skin. The dermis of white skin is thinner and less compact than that of black skin [47]. In white skin, the papillary and reticular layers of the dermis are more distinct, contain larger collagen fiber bundles, and the fiber fragments are sparse. The dermis of black skin contains closely stacked, smaller collagen fiber bundles with a surrounding ground substance. The fiber fragments are more prominent in black skin than in white skin. While the quantity is similar in both black and white skin, the size of melanophages is larger in black skin. Also, the number of fibroblasts and lymphatic vessels are greater in black skin. The fibroblasts are larger, have more biosynthetic organelles, and are more multinucleated in black skin [6]. The lymphatic vessels are dilated and empty with surrounding elastic fibers [47]. No racial differences in the epidermal nerve fiber network have been observed using laser-scanning confocal microscopy, suggesting that there is no difference in sensory perception between races, as suggested by capsaicin response to C-fiber activation [48].

Skin extensibility is how stretchable the skin is. Elastic recovery is the time required for the skin to return to its original state after releasing the stretched skin. Skin elasticity is elastic recovery divided by extensibility. Studies that investigated skin extensibility, elastic recovery, and skin elasticity between races yield conflicting results [31,49]. It is likely that elastic recovery and extensibility vary by anatomic site, race, and age.

Intrinsic skin aging in ethnic skin

The majority of literature regarding facial aging features Caucasian patients. Facial aging is result of the combination of photodamage, fat atrophy, gravitational soft tissue redistribution, and bone remodeling. Figure 4.1 demonstrates the morphologic changes of the face caused by aging. The onset of morphologic aging appears in the upper face during the thirties and gradually progresses to the lower face and neck over the next several decades [50].

Early signs of facial aging occur in the periorbital region. In the late thirties, brow ptosis, upper eyelid skin laxity, and descent of the lateral portion of the eyebrow ("hooding") lead to excess skin of the upper eyelids. During the mid-forties, "bags" under the eyes result from weakening of the inferior orbital septum and prolapse of the underlying intraorbital fat. Lower eyelid fat prolapse may occur as early as the second decade in those with a familial predisposition. Photodamage produces periocular and brow rhytides [50].

Brow ptosis in African-Americans appears to occur to a lesser degree and in the forties opposed to the thirties compared to that in whites [51]. Prolapse of the lacrimal gland may masquerade as lateral upper eyelid fullness in African-Americans [52]. For Hispanics, the brow facial soft tissues sag at an earlier age [53]. In Asians, the descent of thick juxtabrow tissues in the lateral orbit coupled with the absences of a supratarsal fold may create a prematurely tired eye [50].

The midface show signs of aging during the forties. The malar soft tissue adjacent to the inferior orbital rim descends, accumulating as fullness along the nasolabial fold. The malar

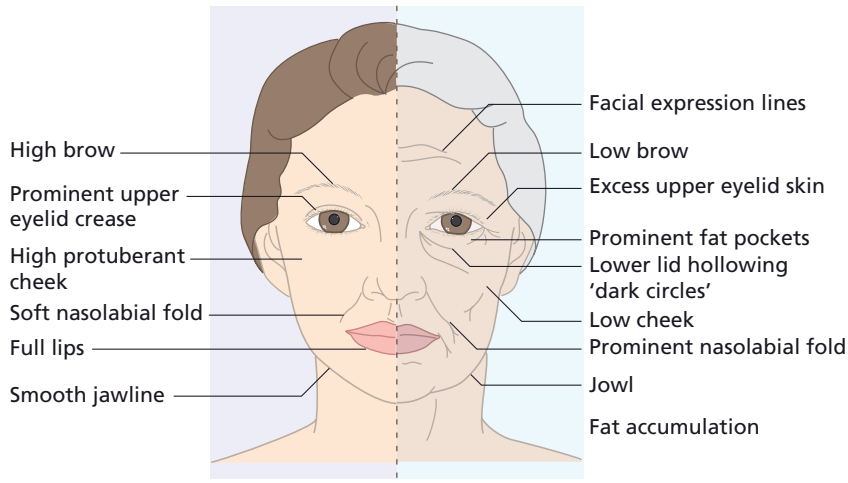

Figure 4.1 Morphologic signs of aging. (Adapted from figure by Cindy Luu. From Harris MO. (2006) Intrinsic skin aging in pigmented races. In: Halder RM, ed. *Dermatology and Dermatological Therapy of Pigmented Skins*. Taylor & Francis Group, pp. 197–209.)

soft tissue atrophy and ptosis result in periorbital hollowing and tear trough deformity. Early aging is evident in individuals of African, Asian, and Hispanic origin in the midface region more so than the upper or lower regions. Signs include tear trough deformity, infraorbital hollowing, malar fat ptosis, nasojugal groove prominence, and deepening of the nasolabial fold. This predisposition to midface aging is likely the result of the relationship of the eyes to the infraorbital rim, basic midface skeletal morphology, and skin thickness [50].

The soft tissue of the lower face is supported in a youthful anatomic position by a series of retaining ligaments within the superficial musculo-aponeurotic system (SMAS) [54]. The SMAS is a discrete fascial layer that envolps the face and forms the basis for resuspending sagging facial tissues [14]. The SMAS fascia envelope maintains tension on facial muscles and offsets soft tissue sagging. In the late thirties, gradual ptosis of the SMAS and skin elastosis sets the stage for jowl formation. Accumulation of submandibular fat and a sagging submandibular gland may have a role in interrupting the smooth contour of a youthful jaw line. Changes in the lower face lead to changes in the neck because the SMAS is anatomically continuous with the platysma muscle. Sagging of the SMAS–platysma unit and submandibular fat redistribution gradually blunts the junction between the jaw and neck. A "double chin" appears at any age as a result of cervicomental laxity with excess submental fat deposits. During the fifties, diastasis and hypertrophy of the anterior edge of the platysma muscle may produce vertical banding in the cervicomental area. During the sixth, seventh, and eighth decades, progressive soft tissue atrophy and bony remodeling of the maxilla and mandible create a relative excess of sagging skin, further exaggerating facial aging. Jowling is a sign of lower facial aging in black persons [50]. In some cases, a bony chin underprojection make create excess localized submental fatty deposits despite a smoothly contoured jaw line. However, in Asians, jowl formation may result from fat accumulation in the buccal space [50]. The "double chin" is more common in Caucasians under 40 years of age than Asians of the same age group, but more common in Asians over 40 years of age because of redundant cervical skin [55].

Extrinsic aging (photoaging) of ethnic skin

Sunlight is a major factor for the appearance of premature aging, independent of facial wrinkling, skin color, and skin elasticity. By the late forties, individuals with greater sun exposure appear older than those with less sun exposure. However, the perceived age of individuals in their late twenties is unaffected by sun exposure. Solar exposure greatly increases the total wrinkle length by the late forties. The extent of dermal degenerative change seen by the late forties correlates with premature aging. There is a high correlation between perceived age and facial wrinkles; perceived age and elastosis; and perceived age and the quantity of collagen. The grenz zone is a subepidermal band of normal dermis consisting of normal collagen fibers and thought to be a site of continual dermal repair. The grenz zone becomes visually apparent only after there is sufficient elastotic damage. With progressive elastosis, the grenz zone beomces thinner [56].

Histopathology

Epidermis

The absolute number of Langerhans cells vary from person to person but chronic sun exposure decreases their number or depletes them [57]. The severely sun-damaged skin has many vacuolated cells in the spinous layer, excessively vacuolated basal keratinocytes and melnanocytes, cellular atypia, and loss of cellular polarity. Apoptosis in the basal layer is increased. A faulty stratum lucidum and horny layer result from intracellur vesicles in the cells of the basal and spinous layers (sunburn cells), apoptosis, and dyskeratosis. There is focal necrobiosis in the epidermis and dermis in sun-exposed skin. While histologic findings of photoaging in white sun-exposed skin include a distorted, swollen, and distinctly cellular stratum lucidum, the stratum lucidum of African-American sun-exposed skin remains compact and unaltered [6]. The stratum lucidum in black skin is not altered by sunlight exposure [6].

With age, the dermoepidermal junction becomes flattened with multiple zones of basal lamina and anchoring fibril reduplication. Microfibrils in the papillary dermis become more irregularly oriented. Compact elastic fibers show cystic changes and separation of skeleton fibers with age. The area occupied by the superficial vascular plexus in specimens of equal epidermal surface length decreases from the infant to young adult (21–29 years) to adult (39–52 years) age groups, then increased in the elderly adult (73–75 years) age group [58]. With the exception of the vascularity in the elderly adult group, the above features are similar to those seen in aging white skin, and suggest that chronologic aging in white and black skin is similar. Oxytalan fibers are found in the papillary dermis of sun-exposed skin of white individuals in their twenties and early thirties but disappear in the forties. In black skin, the oxytalan fibers are still found in the dermis of individuals in their fifties. No solar elastosis is seen in specimens of black sun-exposed skin. Older black subjects have an increased number and thickness of elastic fibers that separate the collagenous fiber layer in the reticular dermis. The single-stranded elastic fibers in individuals <50 years of age resemble braids in those >50 years of age. Finally, the sun-exposed skin of a 45-year-old light-complexioned black female shared the same amount and distribution of elastic fibers as those in white sun-exposed skin [6].

The grenz zone consists of small fibers oriented horizontally and replaces the papillary dermis. When elastotic mate-

rial accumulates in the dermis, it crowds out all the collagenous fibers, which are resorbed. As the elastic material is resorbed, wisps of collagenous fibers form in its place. Widely spaced, larger collagenous fiber bundles lie between the waning elastotic masses. The total volume of the dermis gradually diminishes as the spaces between the remaining collagenous and elastic fibers are reduced. When the epidermis rests directly on top of the horizontally oriented, medium-sized collagenous fiber bundles of the intermediate dermis, the dermis lacks a papillary and grenz zone and the dermis cannot sufficiently support the epidermis. As a result, the shrinking dermis crinkles and small wrinkles form. This may be the reason for the absence of a structural basis in secondary wrinkles and may explain why wrinkles flatten out when fluids are injected into the skin or when edema occurs [57].

Photoaging in skin of color has variable presentations. Wrinkling is not as common a manifestation of photoaging in black persons, South Asians, or darker complexioned Hispanics as in white persons because of the photoprotective effects of melanin. All racial groups are eventually subjected to photoaging. Within most racial groups, the lighter complexioned individuals show evidence of photodamaged skin. Caucasian skin has an earlier onset and greater skin wrinkling and sagging signs than darker skin types. Visual photoaging assessments reveal that white skin has more severe fine lines, rhytides, laxity, and overall photodamage than African-American skin [41].

Photoaging is uncommon in black persons but is more often seen in African-Americans than in Africans or Afro-Caribbeans. The reason may be the heterogeneous mixture of African, Caucasian, and Native American ancestry often seen in African-Americans. In African-Americans, photoaging appears primarily in lighter complexioned individuals and may not be apparent until the late fifth or sixth decades of life [59]. Photoaging in this group appears as fine wrinkling and mottled pigmentation. In spite of the photoprotective effects of melanin, persons of skin of color are still prone to photoaging, but the reason is not completely known. Infrared radiation may also contribute to photodamage. There is evidence that chronic exposure to natural or artificial heat sources can lead to histologic changes resembling that of UV-induced changes, such as elastosis and carcinoma [60]. The pigmentary manifestations of photoaging common in skin of color include seborrheic keratoses, actinic lentigines, mottled hyperpigmentation, and solar-induced facial melasma [61]. However, African-American skin has greater dyspigmentation, with increased hyperpigmentation and uneveness of skin tone [40].

Hair

There are two types of hair fibers: terminal and vellus. Terminal hair is found on the scalp and trunk. Vellus hair is fine and shorter and softer than terminal hair. The hair fiber grows from the epithelial follicle, which is an invagination of the epidermis from which the hair shaft develops via mitotic activity and into which sebaceous glands open. The hair follicle is one of the most proliferative cell types in the body and undergoes growth cycles. The cycles include anagen (active growth), catagen (regression), and telogen (rest). Each follicle follows a growth pattern independent of the rest. The hair follicle is lined by a cellular inner and outer root sheath of epidermal origin and is invested with a fibrous sheath derived from the dermis. Each hair fiber is made up of an outer cortex and a central medulla. Enclosing the hair shaft is a layer of overlapping keratinized scales, the hair cuticle that serves as protective layers.

Racial differences in hair include the hair type, shape, and bulb. There are four types of hair: helical, spiral, straight, and wavy. The spectrum of curliness is displayed in Figure 4.2. The vast majority of black persons have spiral hair [62]. The hair of black persons are naturally more brittle and more susceptible to breakage and spontaneous knotting than that of white persons. The kinky form of black hair, the weak intercellular cohesion between cortical cells, and the specific hair grooming practices among black persons account for the accentuation of these findings [62]. The shape of the hair is different between races: black hair has an elliptical shape, Asian hair is round-shaped straight hair, and Caucasian hair is intermediate [63,64]. The bulb determines the shape of the hair shaft, indicating a genetic difference in hair follicle structure [30]. The cross-section of black hair has a longer major axis, a flattened elliptical shape, and curved follicles. Asian hair has the largest cross-sectional area and Western European hair has the smallest [64,65]. Black persons have fewer elastic fibers anchoring the hair follicles to the dermis than white subjects. Melanosomes were in the outer root sheath and in the bulb of vellus hairs in black, but not in

Figure 4.2 The spectrum of curliness in human hair. (This figure was published in: Loussouarn G, Garcel A, Lozano I, Collaudin C, Porter Crystal, Panhard S, *et al.* (2007) Worldwide diversity of hair curliness: a new method of assessment. *Int J Dermatol* **46** (Suppl 1), 2–6.)

white persons. Black hair also has more pigment and on microscopy has larger melanin granules than hair from light-skinned and Asian individuals. Similarities between white and black hair include: cuticle thickness, scale size and shape, and cortical cells [65].

While the curly nature of black hair is believed to result from the shape of the hair follicle [65], new research shows that the curliness of hair correlates with the distribution of cortical cells independent of ethnoracial origin [66]. Black hair follicles have a helical form, whereas the Asian follicle is completely straight and the Caucasian hair form is intermediate [65]. Mesocortical, orthocortical, and paracortical cells are the three cell types in the hair cortex. In straight hair, mesocortical cells predominate [66]. In wavy hair, the orthocortical and mesocortical cells are interlaced around paracortical cells. In tightly curled hair, the mesocortex disappears, making orthocortical cells the majority. Distinct cortical cells express the acidic hair keratin hHa8. Figure 4.3 displays the distribution of hHa8 cells in straight, wavy, and tightly curled hair. Straight hair has a patchy but homoge-

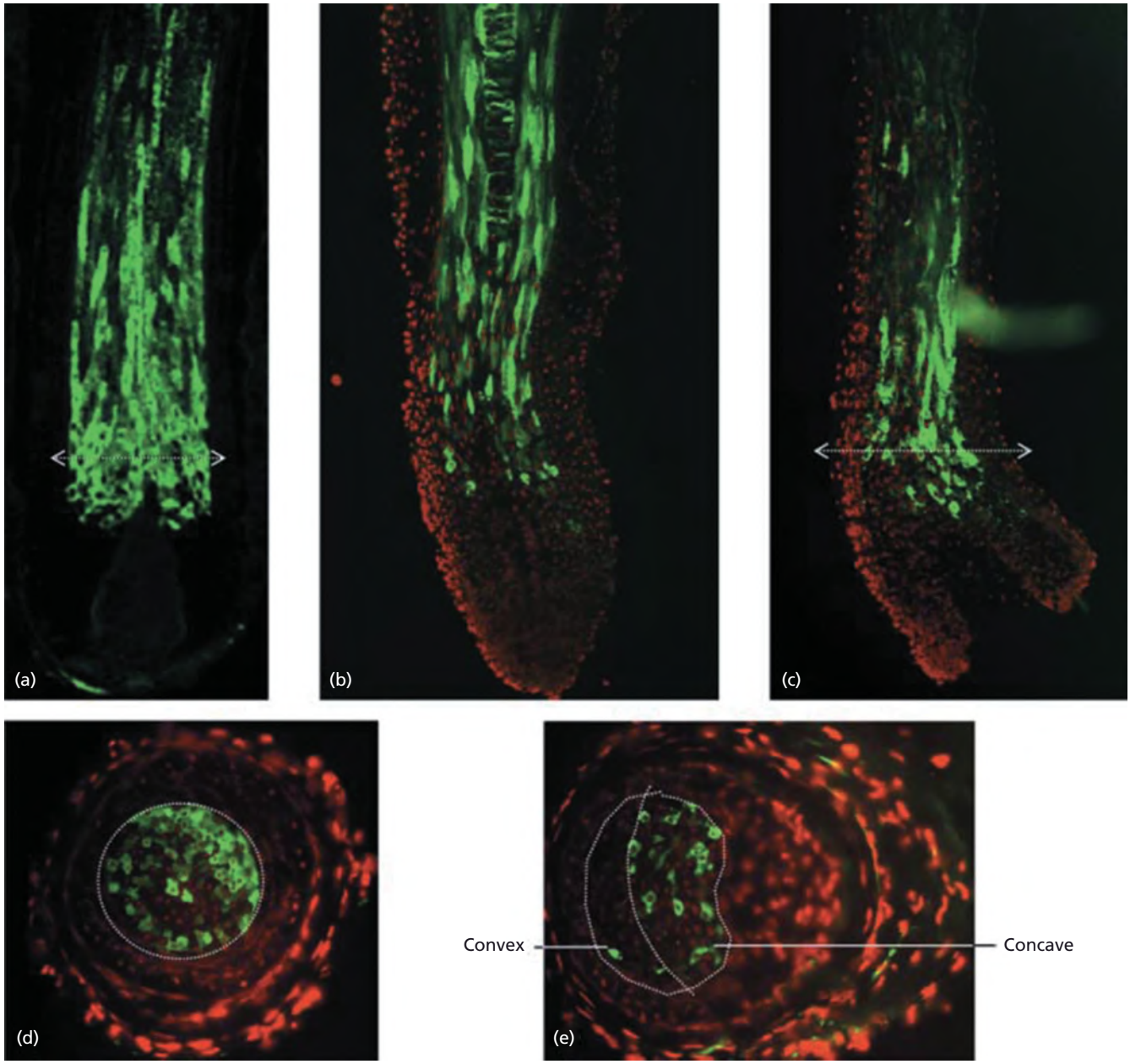

Figure 4.3 hHa8 hair keratin distribution in hair follicles. hHa8 pattern in (a) straight, (b) wavy, and (c) curly hair longitudinal sections. hHa8 pattern in (d) straight and (e) curly hair cross-sections. (From Thibaut *et al.* (2007) Human hair keratin network and curvature. *Int J Dermatol* **46** (Suppl 1), 7–10.)

nous pattern of positively charged hHa8 cells surrounding a core of negatively charged cells. As the degree of curl decreases, the hHa8 pattern becomes asymmetric, independent of ethnic origin. In tightly curled hair, hHa8 accumulates on the concave side of the hair fiber and the medulla compartment disappears.

There are no differences in keratin types between hair from different races and no differences in amino acid composition of hair from different races [67]. Among Caucasian, Asian, and Africans, there are no differences in the intimate structures of fibers, whereas geometry, mechanical properties, and water swelling differed according to ethnic origin [68]. One study [69] in 1941 did find variation in the levels of some amino acids between black and white hair. Black subjects had significantly greater levels of tyrosine, phenylalanine, and ammonia in the hair, but were deficient in serine and threonine.

The morphologic features of African hair were examined using the transmission and scanning electron microscopic (SEM) techniques in an unpublished study. The cuticle cells of African hair were compared with those of Caucasian hair. Two different electronic density layers were shown. The denser exocuticle is derived from the aggregation of protein granules that first appear when the scale cells leave the bulb region. The endocuticle is derived from the zone that contains the nucleus and cellular organites. The cuticle of Caucasian hair is usually 6–8 layers thick and constant in the hair perimeter, covering the entire length of each fiber. However, black hair has variable thickness; the ends of the minor axis of fibers are 6–8 layers thick, and the thickness diminishes to 1–2 layers at the ends of the major axis. The weakened endocuticle is subject to numerous fractures (Handjur C, Fiat, Huart M, Tang D, Leory F, unpublished data).

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Chapter 5: Sensitive skin and the somatosensory system

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BASIC CONCEPTS

- The primary sensory modality subserving the body senses is collectively described as the somatosensory system, and comprises all those peripheral afferent nerve fibers, and specialized receptors, subserving cutaneous, and proprioceptive sensitivity.
- Individuals with sensitive skin demonstrate heightened reactivity of the somatosensory system.
- A separate set of neurons mediates itch and pain. The afferent neurons responsible for histamine-induced itch in humans are unmyelinated C-fibers.
- Low threshold mechanoreceptors are responsible for the sensation of touch, a wide range of receptor systems code for temperature, and as the skin's integrity is critical for survival, there are an even larger number of sensory receptors and nerves that warn us of damage to the skin – the pain and itch systems.

Introduction

The primary sensory modality subserving the body senses is collectively described as the somatosensory system, and comprises all those peripheral afferent nerve fibers, and specialized receptors, subserving cutaneous and proprioceptive sensitivity. The latter processes information about limb position and muscle forces which the central nervous system uses to monitor and control limb movements and, via elegant feedback and feedforward mechanisms, ensure that a planned action or movement is executed fluently. This chapter focuses on sensory inputs arising from the skin surface – cutaneous sensibility – and describes the neurobiologic processes that enable the skin to "sense." Skin sensations are multimodal and are classically described as sensing the three submodalities of touch, temperature, and pain. We also consider the growing evidence for a fourth submodality, present only in hairy skin, which is preferentially activated by slowly moving, low force, mechanical stimuli.

This brief introduction to somatosensation starts with the discriminative touch system. Sensation enters the periphery via sensory axons that have their cell bodies sitting just outside the spinal cord in the dorsal root ganglia, with one ganglion for each spinal nerve root. Neurons are the building blocks of the nervous system and somatosensory neurons are unique in that, unlike most neurons, the electrical signal does not pass through the cell body but the cell body sits off to one side, without dendrites. The signal passes directly from the distal axon process to the proximal process which enters the dorsal half of the spinal cord, and immediately turns up the spinal cord forming a white matter column, the dorsal columns, which relay information to the first brain relay nucleus in the medulla. These axons are called the primary afferents, because they are the same axons that carry the signal into the spinal cord. Sensory input from the face does not enter the spinal cord, but instead enters the brainstem via the trigeminal nerve (one of the cranial nerves). Just as with inputs from the body, there are three modalities of touch, temperature, and pain, with each modality having different receptors traveling along different tracts projecting to different targets in the brainstem. Once the pathways synapse in the brainstem, they join the pathways from the body on their way up to the thalamus and higher cortical structures. Sensory information arising from the skin is represented in the brain in the primary and secondary somatosensory cortex, where the contralateral body surfaces are mapped in each hemisphere.

Peripheral nervous system

The skin is the most extensive and versatile organ of the body and in a fully grown adult covers a surface area approaching 2 m^2. This surface is far more than a just a passive barrier. It contains in excess of 2 million sweat glands and 5 million hairs that may be either fine vellous types covering all surfaces, apart from the soles of the feet and the palms of the hands (glabrous skin), or over 100 000 of the coarser type found on the scalp. Evidence is also emerging that non-glabrous skin contains a system of nerves that code specifically for the pleasant properties of touch. Skin consists

Cosmetic Dermatology: Products and Procedures. Edited by Z.D. Draelos.

of an outer, waterproof, stratified squamous epithelium of ectodermal origin – the epidermis – plus an inner, thicker, supporting layer of connective tissue of mesodermal origin – the dermis. The thickness of this layer varies from 0.5 mm over the eyelid to >5.0 mm over the palm and sole of the foot.

Touch

Of the three "classic" submodalities of the somatosensory system, discriminative touch subserves the perception of pressure, vibration, and texture and relies upon four different receptors in the digit skin:

1 Meissner corpuscles;
2 Pacinian corpuscles;
3 Merkel disks; and
4 Ruffini endings.

These are collectively known as low threshold mechanoreceptors (LTMs), a class of cutaneous receptors that are specialized to transduce mechanical forces impinging the skin into nerve impulses. The first two are classified as fast adapting (FA) as they only respond to the initial and final contact of a mechanical stimulus on the skin, and the second two are classified as slowly adapting (SA) as they continue firing during a constant mechanical stimulus. A further classification relates to the LTM's receptive field (RF; i.e. the surface area of skin to which they are sensitive). The RF is determined by the LTM's anatomic location within the skin, with those near the surface at the dermal–epidermal boundary, Meissner corpuscles and Merkel disks, having small RFs, and those lying deeper within the dermis, Pacinian corpuscles and Ruffini endings, having large RFs (Figure 5.1).

Psychophysical procedures have been traditionally employed to study the sense of touch where differing frequencies of vibrotactile stimulation are used to quantify the response properties of this sensory system. Von Bekesy [1] was the first to use vibratory stimuli as an extension of his research interests in audition. In a typical experiment participants were asked to respond with a simple button-press when they could just detect the presence of a vibration presented to a digit, within one of two time periods. This two alternative force choice paradigm (2-AFC) provides a threshold-tuning curve, the slopes of which provide information about a particular class of LTM's response properties.

Bolanowski *et al.* [2] proposed that there are four distinct psychophysical channels mediating tactile perception in the glabrous skin of the hand. Each psychophysically determined channel is represented by one of the four anatomic end organs and nerve fiber subtypes, with frequencies in the 40–500 Hz range providing a sense of "vibration," transmitted by Pacinian corpuscles (PC channel or FAI); Meissner corpuscles being responsible for the sense of "flutter" in the 2–40 Hz range (NPI channel or FAII); the sense of "pressure" being mediated by Merkel disks in the 0.4–2.0 Hz range (NPIII or SAI); and Ruffini end organs producing a "buzzing" sensation in the 100–500 Hz range (NPII or SAII). Neurophysiologic studies have, by and large, supported this model, but there is still some way to go to link the anatomy with perception (Table 5.1).

There have been relatively few studies of tactile sensitivity on hairy skin, the cat being the animal of choice for most of these studies. Mechanoreceptive afferents (Aβ fibers) have been described that are analogous to those found in human

Table 5.1 Main characteristics of primary sensory afferents innervating human skin.

Class	Modality	Axonal diameter (μm)	Conduction velocity ($m s^{-1}$)
Myelinated			
Aα	Proprioceptors from muscles and tendons	20	120
Aβ	Low threshold mechanoreceptors	10	80
Aδ	Cold, noxious, thermal	2.5	12
Unmyelinated			
C-pain	Noxious, heat, thermal	1	<1
C-tactile	Light stroking, gentle touch	1	<1
C-tutonomic	Autonomic, sweat glands, vasculature	1	<1

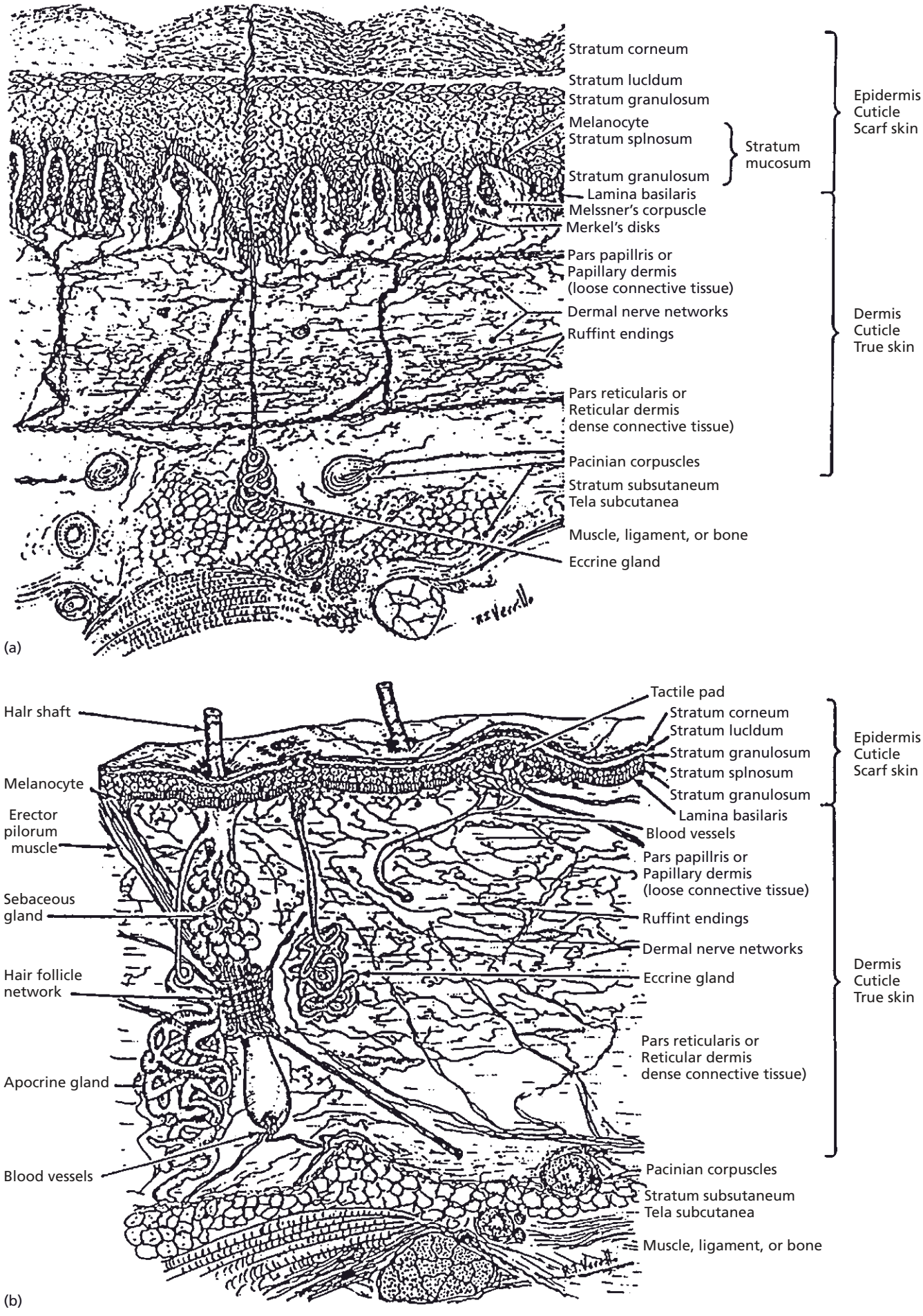

Figure 5.1 A cross-sectional perspective of (a) glabrous and (b) hairy skin. (This figure was published with permission of the artist, R.T. Verrillo.)

glabrous skin (FAI, FAII, SAI, SAII), and Essick and Edin [3] have described sensory fibers with these properties in human facial skin. The relationship between these sensory fibers and tactile perception is still uncertain.

Sensory axons are classified according to their degree of myelination, the fatty sheath that surrounds the nerve fiber. The degree of myelination determines the speed with which the axon can conduct nerve impulses and hence the nerves conduction velocity. The largest and fastest axons are called Aα and include some of the proprioceptive neurons, such as the muscle stretch receptors. The second largest group, called Aβ, includes all of the discriminative touch receptors being described here. Pain and temperature include the third and fourth groups, Aδ and C-fibers.

Electrophysiologic studies on single peripheral nerve fibers innervating the human hand have provided a generally accepted model of touch that relates the four anatomically defined types of cutaneous or subcutaneous sense organs to their neural response patterns [4]. The technique they employed is called microneurograpahy and involves inserting a fine tungsten microelectrode, tip diameter <5 μm, through the skin of the wrist and into the underlying median nerve which innervates the thumb and first two digits (Figure 5.2).

Temperature

The cutaneous somatosensory system detects changes in ambient temperature over an impressive range, initiated when thermal stimuli that differ from a homeostatic set-point excite temperature specific sensory nerves in the skin, and relay this information to the spinal cord and brain. It is important to recognize that these nerves code for temperature change, not absolute temperature, as a thermometer does. The system does not have specialized receptor end organs such as those found with LTMs but uses free nerve endings throughout skin to sense changes in temperature. Within the innocuous thermal sensing range there are two populations of thermosensory fibers, one that respond to warmth (warm receptors) and one that responds to cold (cold receptors), and include fibers from the Aδ and C range. Specific cutaneous cold and warm receptors have been defined as slowly conducting units that exhibit a steady-state discharge at constant skin temperature and a dynamic response to temperature changes [5,6]. Cold-specific and warm-specific receptors can be distinguished from nociceptors that respond to noxious low and high temperatures (<20 °C and >45 °C) [7,8], and also from thermosensitive mechanoreceptors [5,9]. Standard medical textbooks describe the cutaneous cold sense in humans as being mediated by myelinated A-fibers with CVs in the range 12–30 m s^{-1} [10], but recent work concludes that either human cold-specific afferent fibers are incompletely myelinated "BC" fibers, or else there are C as well as A cold fibers, with the C-fiber group contributing little to sensation (Figure 5.3) [11].

The free nerve endings for cold-sensitive or warm-sensitive nerve fibers are located just beneath the skin

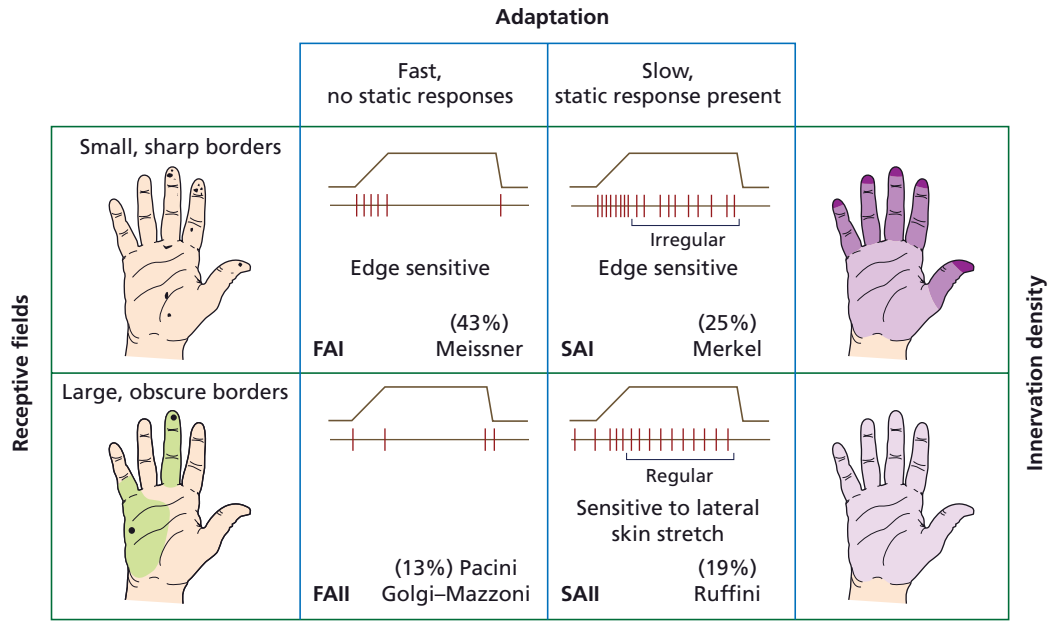

Figure 5.2 The four types of low threshold mechanoreceptors in human glabrous skin are depicted. The four panels in the center show the nerve firing responses to a ramp and hold indentation and the frequency of occurrence (%) and putative morphologic correlate. The black dots in the left panel show the receptive fields of type I (top) and type II (bottom) afferents. The right panel shows the average density of type I (top) and type II (bottom) afferents with darker area depicting higher densities. (From Westling GK. (1986) Sensori-motor mechanisms during precision grip in man. Umea University medical dissertation. New Series 171, Umea, Sweden.)

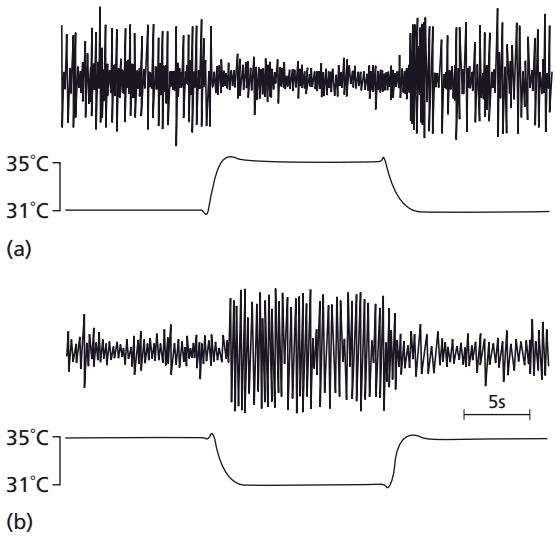

Figure 5.3 Resting discharge of a C cold fiber at room temperature [11]. (a) The resting discharge is suppressed by warming of the receptive field (RF) from 31 °C to 35 °C. (b) From a holding temperature of 35 °C, at which the unit is silent, activity is initiated by cooling the RF to 31 °C. (Time bar: 5 s.)

surface. The terminals of an individual temperature-sensitive fiber do not branch profusely or widely. Rather, the endings of each fiber form a small, discretely sensitive point, which is separate from the sensitive points of neighboring fibers. The total area of skin occupied by the receptor endings of a single temperature-sensitive nerve fiber is relatively small (approximately 1 mm in diameter), with the density of these thermosensitive points varying in different body regions. In most areas of the body there are 3–10 times as many cold-sensitive points as warm-sensitive points. It is well established from physiologic and psychologic testing that warm-sensitive and cold-sensitive fibers are distinctively different from one another in both structure and function.

Pain

Here we consider a system of peripheral sensory nerves that innervate all cutaneous structures and whose sole purpose is to protect the skin against potential or actual damage. These primary afferents include Aδ and C-fibers which respond selectively and linearly to levels of thermal, mechanical, and chemical intensity/strength that are tissue-threatening. This encoding mechanism is termed nociception and describes the sensory process detecting any overt, or impending, tissue damage. The term pain describes the perception of irritation, stinging, burning, soreness, or painful sensations arising from the skin. It is important to recognize that the perception of pain not only depends on nociceptor input, but also on other processes and pathways giving information about emotional or contextual components. Pain is therefore described in terms of an "experience" rather than just a simple sensation. There are again submodalties within the nociceptive system (Aδ and C) subserving nociception. Aδ fibers are thin (1–5 μm), poorly myelinated axons of mechanical nociceptors, thermal receptors, and mechanoreceptors with axon potential conduction velocities of approximately 12 m s^{-1}. C-fibers are very thin (<1 μm) unmyelinated slowly conducting axons of <1 m s^{-1}. Mechanical nociceptors are in the Aδ range and possess receptive fields distributed as 5–20 small sensitive spots over an area approximately 2–3 mm in diameter. In many cases activation of these spots depends upon stimuli intense enough to produce tissue damage, such as a pinprick. Aδ units with a short latency response to intense thermal stimulation in the range 40–50 °C have been described as well as other units excited by heat after a long latency – usually with thresholds in excess of 50 °C.

Over 50% of the unmyelinated axons (C-fibers) of a peripheral nerve respond, not only to intense mechanical stimulation, but also to heat and noxious chemicals, and are therefore classified as polymodal nociceptors [12] or C-mechano-heat (CMH) nociceptors [13]. Receptive fields consist of single zones with distinct borders and in this respect they differ from Aδ nociceptors that have multipoint fields. Innervation densities are high and responses have been reported to a number of irritant chemicals such as dilute acids, histamine, bradykinin, and capsaicin. Following inflammation some units can acquire responsiveness to stimuli to which they were previously unresponsive. Recruitment of these "silent nociceptors" implies spatial summation to the nociceptive afferent barrage at central levels, and may therefore contribute to primary hyperalgesia after chemical irritation and to secondary hyperalgesia as a consequence of central sensitization.

Nociceptors do not show the kinds of adaptation response found with rapidly adapting LTMs (i.e. they fire continuously to tissue damage), but pain sensation may come and go and pain may be felt in the absence of any nociceptor discharge. They rely on chemical mediators around the nerve ending which are released from nerve terminals and skin cells in response to tissue damage. The axon terminals of nociceptive axons possess no specialized end organ structure and for that reason are referred to as free nerve endings. This absence of any encapsulation renders them sensitive to chemical agents, both intrinsic and extrinsic, and inflammatory mediators released at a site of injury can initiate or modulate activity in surrounding nociceptors over an area of several millimeters leading to two kinds of sensory responses termed hyperalgesia – the phenomenon of increased sensitivity of damaged areas to painful stimuli. Primary hyperalgesia occurs within the damaged area; secondary hyperalgesia occurs in undamaged tissues surrounding this area.

One further sensation mediated by afferent C-fibers is that of itch. The sensation of itch has, in the past, been thought to be generated by the weak activation of pain nerves, but

with the recent finding of primary afferent neurons in humans [14] and spinal projection neurons in cats [15], which have response properties that match those subjectively experienced after histamine application to the skin, it is now recognized that separate sets of neurons mediate itch and pain, and that the afferent neurons responsible for histamine-induced itch in humans are unmyelinated C-fibers. Until relatively recently it was thought that histamine was the final common mediator of itch, but clinical observations where itch can be induced mechanically, or is not found with an accompanying flare reaction, cannot be explained by histamine-sensitive pruriceptors leading to evidence for the existence of histamine-independent types of itch nerves [16] in which itch is generated without a flare reaction by cowhage spicules. As with the existence of multiple types of pain afferents, different classes of itch nerves are also likely to account for the various experiences of itch reported by patients [17].

Pleasure

In recent years a growing body of evidence has been accumulating, from anatomic, psychophysical, electrophysiologic, and neuroimaging studies, that a further submodality of afferent, slowly conducting, unmyelinated C-fibers exists in human hairy skin that are neither nociceptive nor pruritic, but that respond preferentially to low force, slowly moving mechanical stimuli. These nerve fibers have been classified as C-tactile afferents (CT-afferents) and were first described by Nordin [18] and Johansson *et al.* [19]. Evidence of a more general distribution of CT-afferents have subsequently been found in the arm and the leg, but never in glabrous skin sites such as the palms of the hands or the soles of the feet [20]. It is well known that mechanoreceptive innervation of the skin of many mammals is subserved by A and C afferents but until the observations of Nordin and Vallbo C-mechanoreceptive afferents in human skin appeared to be lacking entirely.

The functional role of CT-afferents is not fully known, but their neurophysiologic response properties, fiber class, and slow conduction velocities preclude their role in any rapid mechanical discriminative or cognitive tasks, and point to a more limbic function, particularly the emotional aspects of tactile perception [21]. However, the central neural identification of low-threshold C mechanoreceptors, responding specifically to light touch, and the assignment of a functional role in human skin has only recently been achieved. In a study on a unique patient lacking large myelinated Ab-fibers, it was discovered that activation of CT-afferents produced a faint sensation of pleasant touch, and functional neuroimaging showed activation in the insular cortex but no activation the primary sensory cortex, identifying CT-afferents as a system for limbic touch that might underlie emotional, hormonal, and affiliative responses to skin–skin contacts between individuals engaged in grooming and bonding behaviors – pleasant touch [22]. If pain is elicited via sensory C- and Aδ-fibers then it is reasonable to speculate that the same system may be alternatively modulated to deliver a sensation of pleasure. A study employing the pan-neuronal marker PGP9.5 and confocal laser microscopy has identified a population of free nerve endings in the epidermis that may be the putative anatomic substrate for this submodality [23].

Sympathetic nerves

Although this chapter deals with sensory aspects of skin innervation it is important to acknowledge the role of a class of efferent (motor) nerves that innervate various skin structures: (a) blood vessels; (b) cutaneous glands; and (c) unstriated muscle in the skin (e.g. the erectors of the hairs). In sensitive skin conditions, and some painful neuropathic states, sympathetic nerves have a role in exacerbating inflammation and irritation (for review see Roosterman *et al.* [24]).

The central projections

The submodalties of skin sensory receptors and nerves that convey information to the brain about mechanical, thermal, and painful stimulation of the skin are grouped into three different pathways in the spinal cord and project to different target areas in the brain. They differ in their receptors, pathways, and targets, and also in the level of decussation (crossing over) within the CNS. Most sensory systems *en route* to the cerebral cortex decussate at some point, as projections are mapped contralaterally. The discriminative touch system crosses in the medulla, where the spinal cord joins the brain, the pain system crosses at the point of entry into the spinal cord.

Spinal cord

All the primary sensory neurons have their cell bodies situated outside the spinal cord in the dorsal root ganglion, there being one ganglion for every spinal nerve root.

Tactile primary afferents, or first order neurons, immediately turn up the spinal cord towards the brain, ascending in the dorsal white matter and forming the dorsal columns. In a cross-section of the spinal cord at cervical levels, two separate tracts can be seen: the midline tracts comprise the gracile fasciculus conveying information from the lower half of the body (legs and trunk), and the outer tracts comprise the cuneate fasciculus conveying information from the upper half of the body (arms and trunk). At the medulla, situated at the top of spinal cord, the primary tactile afferents make their first synapse with second order neurons where fibers from each tract synapses in a nucleus of the same name – the gracile fasciculus axons synapse in the gracile nucleus, and the cuneate axons synapse in the cuneate

nucleus. The neurons receiving the synapse provide the secondary afferents and cross immediately to form a new tract on the contralateral side of the brainstem – the medial lemniscus – which ascends through the brainstem to the next relay station in the midbrain, the thalamus.

As with the tactile system, pain and thermal primary afferents synapse ipsilaterally and then the secondary afferents cross, but the crossings occur at different levels. Pain and temperature afferents enter the dorsal horn of the spinal and synapse within one or two segments, forming the Lissauer tract as they do so. The dorsal horn is a radially laminar structure. The two types of pain fibers, C and Aδ, enter different layers of the dorsal horn. Aδ fibers enter the posterior marginalis and the nucleus proprius, and synapse on a second set of neurons. These are the secondary afferents which will relay the signal to the thalamus. The secondary afferents from both layers cross to the opposite side of the spinal cord and ascend in the spinothalamic tract. The C-fibers enter the substantia gelatinosa and synapse, but they do not synapse on secondary afferents. Instead they synapse on interneurons – neurons that do not project out of the immediate area but relay the signal to the secondary afferents in either the posterior marginalis or the nucleus proprius. The spinothalamic tract ascends the entire length of the cord and the entire brainstem and by the time it reaches the midbrain appears to be continuous with the medial lemniscus. These tracts enter the thalamus together.

It is important to note that although the bulk of afferent input adheres to the plan outlined above there is a degree of mixing that goes on between the tracts.

We have concentrated on somatosensory inputs from the body thus far, but as facial skin is often the source of sensitive reactions to topical applications, its peripheral and central anatomy and neurophysiology is briefly summarized here. The trigeminal nerve innervates all facial skin structures (including the oral mucosa) and, just as with the spinal afferents, these neurons have their cell bodies outside of the CNS in the trigeminal ganglion with their proximal processes entering the brainstem. Just as in the spinal cord, the three modalities of touch, temperature, and pain have different receptors in the facial skin, travel along different tracts, and have different targets in the brainstem – the trigeminal nucleus – a relatively large structure that extends from the midbrain to the medulla.

The large diameter (Aβ) fibers enter directly into the main sensory nucleus of the trigeminal and, as with the somatosensory neurons of the body, synapse and then decussate, the secondary afferents joining the medial lemniscus as it projects to the thalamus. The small diameter fibers conveying pain and temperature enter midbrain with the main Vth cranial nerve, but then descend down the brainstem to the caudal medulla where they synapse and cross. These descending axons form a tract, the spinal tract of V, and synapse in the spinal nucleus of V, so called because it reaches as far down as the upper cervical spinal cord. The spinal nucleus of V comprises three regions along its length: the subnucleus oralis, the subnucleus interpolaris, and the subnucleus caudalis. The secondary afferents from the subnucleus caudalis cross to the opposite side and join the spinothalamic tract where the somatosensory information from the face joins that from the body, entering the thalamus in a separate nucleus, the ventroposterior medial (VPM) nucleus.

Brain

The third order thalamocortical afferents (from thalamus to cortex) travel up through the internal capsule to reach the primary somatosensory cortex, located in the post-central gyrus, a fold of cortex just posterior to the central sulcus (Figure 5.4a).

The thalamocortical afferents convey all of the signals, whether from the ventroposterior lateral (VPL) or VPM nucleus, to primary somatosensory cortex where the sensory information from all body surfaces is mapped in a somatotopic (body-mapped) manner [25], with the legs represented medially, at the top of the head, and the face represented laterally (Figure 5.4b). Within the cortex there are thought to be eight separate areas primarily subserving somatosensation: primary somatosensory cortex, SI, comprised of four subregions (2, 1, 3a and 3b); secondary somatosensory cortex, SII, located along the superior bank of the lateral sulcus [26]; the insular cortex; and the posterior parietal cortex, areas 5 and 7b (Figure 5.5).

As with studies of the peripheral nervous system, outlined above, the technique of microneurography has again been employed, in this case to study the relationship between skin sensory nerves and their central projections, as evidenced by the use of concurrent functional magnetic resonance imaging (fMRI). Microstimulation of individual LTM afferents, projecting to RFs on the digit, produces robust, focal, and orderly (somatotopic) hemodynamic (BOLD) responses in both primary and secondary somatosensory cortices [27]. It is expected that this technique will permit the study of many different topics in somatosensory neurophysiology, such as sampling from FA and SA mechanoreceptors and C-fibers with neighboring or overlapping RFs on the skin, quantifying their spatial and temporal profiles in response to electrical chemical and/or mechanical stimulation of the skin areas they innervate, as well as perceptual responses to microstimulation.

Finally, the forward projections from these primary somatosensory areas to limbic and prefrontal structures has been studied with fMRI in order to understand the affective representations of skin stimulation for both pain and pleasure [28] and it is hoped that studies of this nature will help us to understand better the emotional aspects of both negative and positive skin sensations.

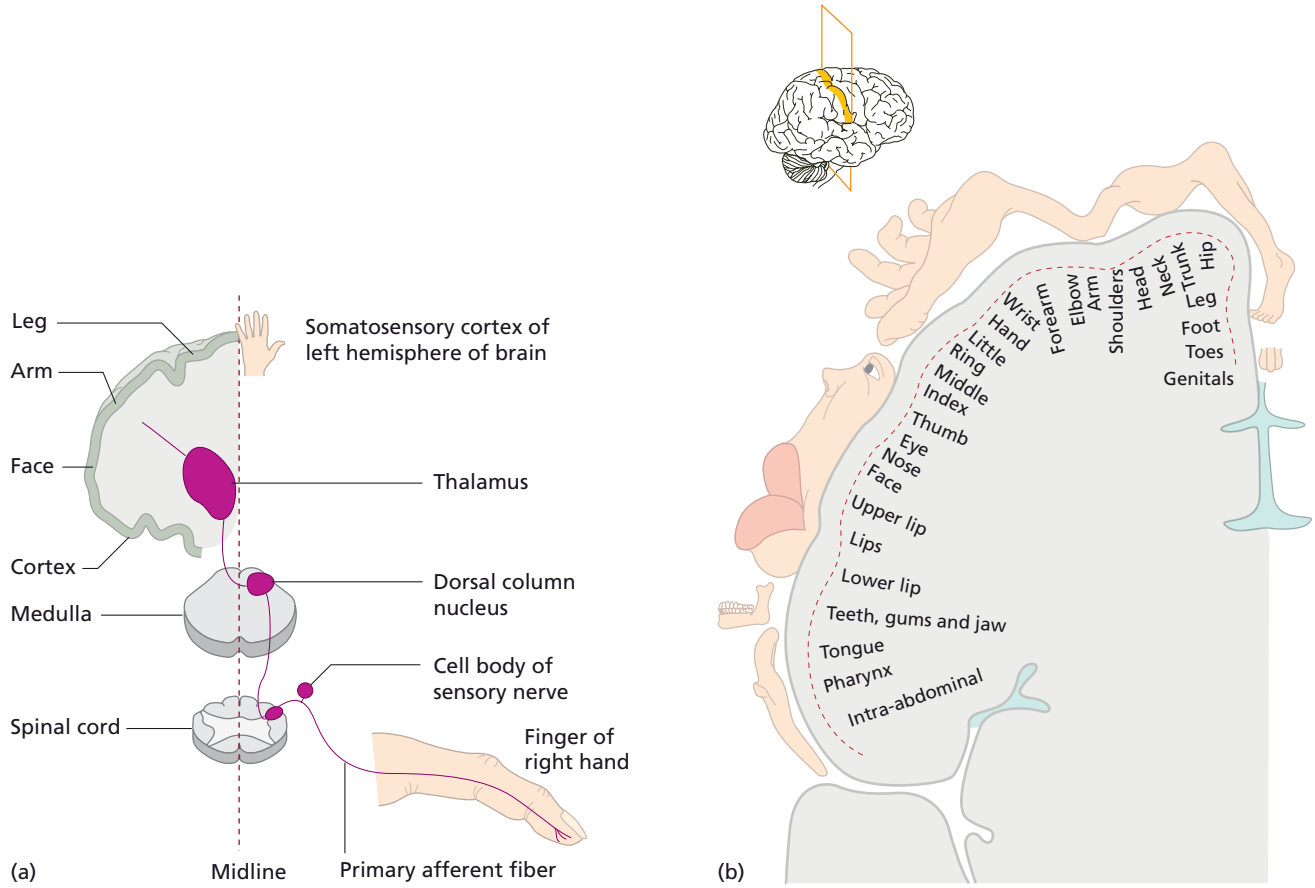

Figure 5.4 (a) Outline of the somatosensory pathways from the digit tip to primary somatosensory cortex, via the dorsal column nuclei and the thalamus. (b) Penfield's somatosensory homunculus. Note the relative overrepresentation of the hands and lips, and the relative underrepresentation of the trunk and arms.

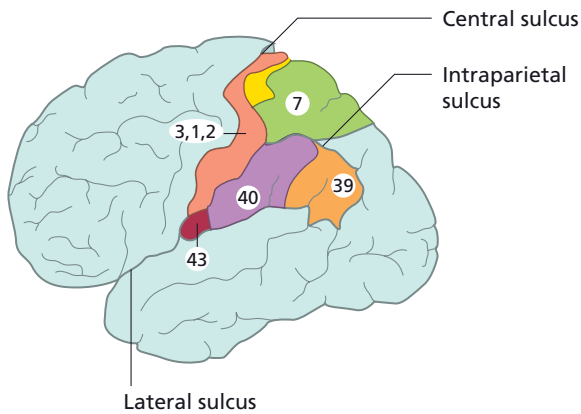

Figure 5.5 Cortical areas subserving somatosensation. Primary somatosensory cortex is located in the posterior bank of the central sulcus and the posterior gyrus and comprises areas 2, 1, 3a and 3b, secondary somatosensory cortex is located in the upper bank of the lateral sulcus with two further somatosensory regions in the posterior parietal cortex, areas 5 and 7b.

Conclusions

In this chapter we describe the neural architecture of the skin senses, where it has been shown that the skin surfaces we groom when applying cosmetic agents are receptive to a wide variety of physicochemical forms of stimulation. Low threshold mechanoreceptors are responsible for the sensation of touch, a wide range of receptor systems code for temperature, and, as the skin's integrity is critical for survival, there are an even larger number of sensory receptors and nerves that warn us of damage to the skin – the pain and itch systems. In addition to this "classic" description of the skin senses, we also provide recent evidence for the existence of another skin receptor system which shares many of the same characteristics as the pain system with one important distinction – this system of sensory nerves is excited by low force, slowly moving tactile stimulation – such as that employed when grooming the body surfaces. This C-fiber-based system of peripheral cutaneous sensory nerves is therefore serving both a protective and hedonic role in body grooming behaviors.

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Chapter 6: Novel, compelling, non-invasive techniques for evaluating cosmetic products

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BASIC CONCEPTS

- Skin care products must be studied for safety and efficacy.
- Non-invasive techniques were developed to assess the skin without a biopsy.
- Non-invasive techniques are used to evaluate visual appearance, moisturization, barrier integrity, oiliness, elasticity, firmness, erythema, and skin color.
- New photography techniques have been developed to detect changes in wrinkling of the face.

Introduction

Clinical trials for substantiation of cosmetic claims should be designed with good scientific rigor. In 1999, Rizer *et al.* [1] described an integrated, multidimensional approach for achieving this goal. The multistep process consisted of the following: careful subject selection, subject self-assessment of product performance, clinical grading, documentation photography, non-invasive bioengineering methods, and statistical analysis.

Recently, the use of digital photography combined with image analysis has provided clinical investigators with a powerful new tool for quantifying improvements in wrinkles, hyperpigmentation, pore size, skin tone, and other dermatologic conditions.

Unlike past years, in which photographs of subjects were used solely to document clinical changes, use of photographs of subjects has moved beyond simple study documentation. This chapter introduces dermatologists, cosmetic surgeons, and clinical researchers to the cost-effective, non-invasive methods for substantiating cosmetic claims. It includes an overview of commonly used, non-invasive methods in cosmetic studies and a description of various types of high-resolution digital photography and their application for evaluating changes in skin.

Cosmetic Dermatology: Products and Procedures. Edited by Z.D. Draelos.

Supporting cosmetic claims with bio-instrumentation

Most scientists would agree that the use of non-invasive methods is an objective way for generating quantitative data about a product's performance on skin. Does this mean that data from non-invasive instruments provide conclusive evidence to support a cosmetic claim?

Consider a topical lotion formulated to improve the appearance of facial wrinkles and moisturize skin. Now imagine that it is your responsibility to substantiate these claims in a clinical study using available non-invasive methods. Undoubtedly, you would choose proven methods such as replica profilometry to assess wrinkle changes and the Skicon™ (IBS Ltd., Tokyo, Japan) or Corneometer® (Courage & Khazaka Electronic GmbH, Köln, Germany) to assess changes in skin hydration. Would favorable data from both of these techniques provide conclusive evidence to support the claims? The answer may surprise you.

In many cases, non-invasive methods are more useful in providing indirect lines of evidence to support a cosmetic claim. In clinical research this is called a secondary endpoint. A primary endpoint refers to the most meaningful result in a clinical trial. In the example above, the primary endpoints would be a visible improvement in the appearance of wrinkles and reduction in the signs and symptoms of dry skin while the secondary endpoints would be improvements in wrinkle depth and high skin hydration values.

The fact that many non-invasive methods are secondary endpoints does not diminish their importance in clinical research. Non-invasive methods often provide valuable information about the mechanism of action of a cosmetic

ingredient or cosmetic product on skin and a more reliable method to quantify improvements in skin. The use of colorimetry, a combination of digital photography and image analysis, is a much better method to quantify changes in skin erythema than by clinical examination, even though the human eye is very sensitive to color shifts. This technique is more fully described at the end of this chapter.

Commonly used non-invasive methods in cosmetic studies

Approximately 90% or more of the cosmetic studies performed today are designed to support claims relating to improvements of fine lines or wrinkles, uneven skin pigmentation associated with sun exposure and/or hormonal changes, enlarged pores, skin radiance, skin roughness, skin tone, and skin dryness. Table 6.1 provides a listing of commonly used, non-invasive techniques that are used to help support these specific claims. For the reader who would like to learn more about these techniques or other non-invasive methods, there are a number of excellent books and articles available in the chapter's reference list [2–8].

Ideally, an investigator would like to see agreement between the clinical grading, non-invasive bio-instrumentation measurements and subject self-perception questionnaires. Occasionally, investigators obtain good concordance between clinical grading and self-perception questionnaires, but discordance with the non-invasive technique.

Table 6.1 Commonly used bio-instruments and non-invasive procedures.

Name	Use
NOVA Meter	Moisturization
SKICON	Moisturization
Corneometer	Moisturization
TEWA Meter	Skin barrier function assessment
Derma Lab	Skin barrier function assessment
Cutometer	Firmness and elasticity
ChromaMeter	Skin tone, erythema, skin lightening, brightness
Mexameter	Skin tone, erythema, skin lightening
Sebumeter	Oiliness (sebum)
Sebutapes	Oiliness
D-Squames	Scaling, exfoliation, and cell renewal
Silicone Replica Impressions	Skin texture, wrinkling

Let us return to the example of the topical product designed to improve the appearance of wrinkles. It is not uncommon to see visible improvements in wrinkles during clinical grading while failing to detect the improvements using silicone replica profilometry. The discordance is not a result of grader error, but of limitation of the replica impressions to fully detect changes over the entire periocular area. Replica impressions are usually taken by spreading the unpolymerized replica material a few millimeters from the corner of the eye with the subject's eyes closed. This is necessary in order to prevent the replica material from running into the eye itself. If the grader makes his or her judgment based on the appearance of wrinkling in the areas adjacent to the corner of the eye as well as the area under the eye with the subject's eyes open, there is chance the grader might see improvements in wrinkling that might not be detected by the replica impression. Additionally, having the eyes closed while the impression is being taken can occasionally result in situations in which the subject squints, resulting in deeper, more pronounced wrinkles. The end result is a replica impression that detects more or deeper wrinkles.

An alternative method, Raking Light Optical Profilometry (RLOP), which provides a newer, more novel approach for analyzing changes in wrinkling, is discussed below. The advantage of this technique is that the subject's eyes are open and the wrinkling appears in the same way as viewed by the clinical grader.

Application of digital photography as a non-invasive technique for assessing skin

The challenge for clinical documentation photography is twofold: to choose the best photographic technique relative to the aims of the study and to maximize consistency of the imaging at each clinic visit throughout the trial. The key to successful photography in clinical trials is the application of standardization, which includes the control subject's positioning, dress, lighting conditions, depth of field, background, and facial expression from visit to visit. The goal is to have images that accurately show treatment effects for use in medical and scientific journals. There is no place for misrepresenting clinical outcomes by changing viewing angles, altering lighting conditions, or having the subject apply facial makeup after using a product [9,10].

The first step to successful photography is to create the appropriate lighting and other photographic techniques specific to the skin conditions of interest in the clinical study. A study involving a product designed to reduce the appearance of fine lines and wrinkles demands significantly different lighting than would trials involving acne, photodamaged skin, skin dryness or flakiness, scars, wound healing, postinflammatory hyperpigmentation (PIH), or pseudofolliculitis

barbae. In order to ensure a high degree of color consistency in photographic technique, the photographer should include color standard chips in each documentation image. Typically, these standards include small reference chips of white, 18% reflectance gray, black, red, green, and blue, as well as a millimeter scale for size confirmation. In addition, a more comprehensive color chart such as a ColorChecker® (X-Rite America Inc., Grand Rapids, MI, USA) should be photographed under the exact standard lighting immediately before starting each photo visit.

Equally crucial is the careful and detailed recording of all aspects of lighting, camera, and lens settings in order to achieve maximum consistency of documentation photographs. Photographing each different photographic set-up provides more certainty that photographs at subsequent sessions are identical to the images made at baseline visit.

Prior to photography, all makeup and jewelry must be removed, and hair kept clear of the subject's face by use of a neutral-color headband. Clothing should be covered by a gray or black cloth drape to prevent errors caused by color reflected from colored clothing. At each subsequent visit in the study, it is necessary to display the baseline image on the computer monitor for side-by-side comparison with that visit's photograph. Subject position, size, color, and lighting can thus be checked to make sure that changes in the skin are brought about by product effect, and are not artifacts caused by careless photographic technique. When the study is over, the sequence of images should look similar to a time-lapse video, with the only difference from one image to another being changes in the condition of the subject's skin. At Stephens & Associates, Inc. we have designed fully equipped photographic studios within our clinics so that subjects can be photographed under standardized conditions from visit to visit (Figure 6.1). These studios are manned by experienced medical photographers who have been trained in the basic science of conducting a clinical trial. While it is not possible for many clinics to have fully equipped studios with medical photographers in their office, there are other off-the-shelf alternatives which will allow them to control the quality of the images in clinical research.

The VISIA, VISIA CR and VISIA CR2 are standardized camera systems that have been designed for use in clinical research. VISIA systems can be operated by individuals with little to no experience in photography. VISIA systems are composed of an oval-shaped plastic shell containing a digital camera and lighting system. Subject positioning is controlled by forehead and chin rests. VISIA contains proprietary software called VISIA Complexion Analysis Software System. The VISIA software system, developed by Procter and Gamble, counts the number of spots, pores, wrinkles, porphyrins, UV spots, red areas, and brown areas on the face of subjects.

The VISIA CR® (Canfield Scientific Inc., Fairfield, NJ, USA) system has an advantage over the VISIA system in that quality of the images are usually better, because the VISIA CR system is equipped with higher resolution cameras than the standard or first generation VISIA system. At the time of writing, the Complexion Analysis Software (Figure 6.2) is not available on the VISIA CR or VISIA CR 2. A simpler software, using the Canfield RBx system, is currently compatible with the VISIA CR machines. Images taken with either system must be exported from the

Figure 6.1 An example of a Stephens & Associates, Inc. photographic studio. The studio is equipped for taking photographs using standard lighting, parallel and polarized lighting, cross polarized lighting and raking light.

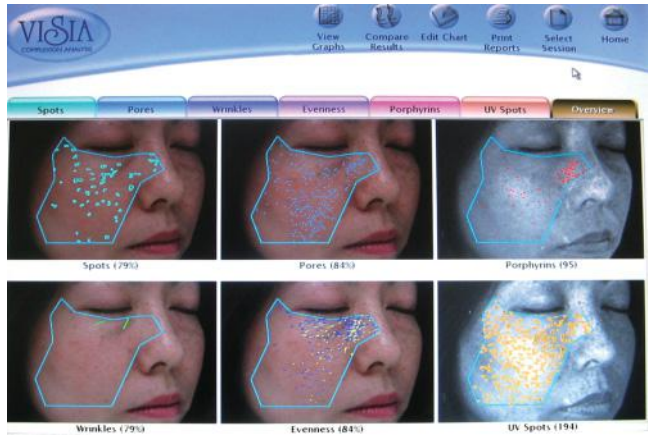


Figure 6.2 An example of the data reporting for the VISIA Complexion Analysis Software.

camera for more detailed image analysis of spots, lines, wrinkles, pores, and color changes.

VISIA systems, while easy to use, have limitations in certain situations. The chin and head rests are sometimes too small for individuals with large faces, resulting in "jammed in" appearance. Additionally, it is difficult to see skin details such as acne lesions or PIH marks on images taken of subjects with Fitzpatrick skin types V and VI because of the close proximity of the subject's face to the camera and lighting system. Unlike viewing software provided by Nikon and Canon, VISIA does not allow images to be displayed from previous treatment visits and the baseline visit for a side-by-side image comparison. Therefore, it is difficult to make sure the head position and facial expressions are the same in all photographs.

Review of terminology in clinical photography

Individuals incorporating digital photography into a clinical trial are often faced with the difficult task of understating the vocabulary used by staff at clinical research organizations (CROs). This section provides a concise description of commonly used terms and techniques in clinical photography.

Visible light photography

This refers to images made with unfiltered full-spectrum (white) light. It is the most common type of photography used in clinical trials. Proper positioning of the strobe flashes is a critical step for capturing various skin conditions in cosmetic clinical trials. Clinical studies involving evenness of color and skin tone require a more generalized, evenly distributed, visible lighting method while the imaging of fine lines, wrinkles, under eye bags, skin texture, and scaling is best achieved by placing the flashes in an off-axis direction. Off-axis lighting refers to lighting that is placed somewhat above and to the side to create small shadows and highlights on the skin thereby giving a three-dimensional quality to the image. Once the lighting conditions have been optimized, it is imperative that the photographer use documentation notes, setup photographs, light metering and color charts to prevent lighting changes from visit to visit.

Polarized photography

This involves the placement of linear polarizing filters on both the lighting flash head(s) and in front of the lens of the digital camera. This allows the documentation of skin in two different ways [11].

The parallel-polarized lighting technique accentuates the reflection of light from the skin and tends to obscure fine topical detail because of strong reflections from the lighting source(s). Parallel-polarized light minimizes subsurface details, such as erythema and pigmentation, while allowing for enhanced viewing of the surface features of the skin, such as sweat, oily skin, and pores.

The cross-polarized lighting technique involves fixing the transmission axis of the lens polarizer 90° to the axis of the lighting polarizer. This virtually eliminates the reflection of light (glare) from the surface of the skin and accentuates the appearance of inflammation from acne lesions, erythema, rosacea, and telangiectasia. Photodamaged skin becomes somewhat more apparent and some subsurface vascular features are made visible. Cross-polarized photography is useful for evaluating products designed to mitigate the appearance of dyschromic lesions, erythema, acne, and PIH resulting from acne. This technique is highly recommended for acne studies [12].

Examples of a parallel-polarized lighting technique and cross-polarized lighting technique can be found in Figure 6.3.

UV reflectance photography

This is a technique designed to highlight or enhance hyperpigmentation on the face. This is accomplished through filtering a flash source to only allow UV light to pass on to the subject's skin allowing visualization of subsurface melanin distribution. Figure 6.4 shows before and after UV reflectance photographs of a subject treated with a skin lightening product. A UV-blocking filter is placed in front of the lens of the digital camera. Note the improvement in the appearance and distribution of mottled and diffuse hyperpigmentation in the photograph on the right.

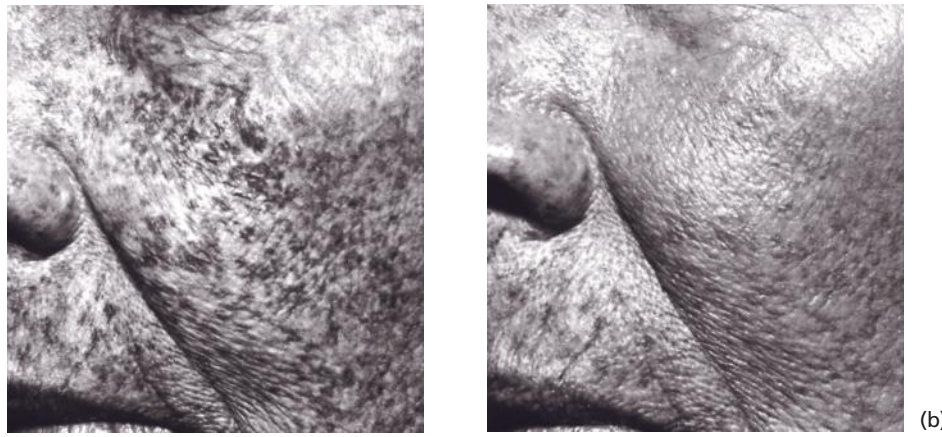
UV fluorescence photography

This is primarily used to visualize the locations of *Propionibacterium acnes* in the pores of subjects with acne. Porphyrins produced by *P. acnes* exhibit an orange–red fluorescence under UVA light. Excitation of *P. acnes* on skin is achieved using a xenon flash lamp equipped with an UVA bandpass filter. The resulting fluorescence can be recorded using a high-resolution digital camera equipped with an UV barrier filter. An example of this technique can be found in Figure 6.5.

Researchers have reported that UV fluorescence photography is a reliable, fast, and easy screening technique to demonstrate the suppressive effect of topical antibacterial agents on *P. acnes* [13]. Investigators need to be aware of a problem that can occur with using this technique to monitor *P. acnes* on the face. Many soaps, cosmetics, or sunscreen products contain quenching agents that can interfere with the accuracy of this imaging process. This can lead to an erroneous conclusion about the elimination of *P. acnes* from the face.

(a) (b)

Figure 6.3 Examples of a parallel-polarized lighting technique (a) and cross-polarized lighting technique (b).



(a) (b)

Figure 6.4 Before and after UV reflectance photographs of a subject treated with a skin lightening product. (a) Ultraviolet reflectance at baseline. (b) Ultraviolet reflectance at 12 weeks.

Digital fluorescence photography has other applications in dermatologic research. The technique can be used to detect salicylic acid in the skin and follicles of subjects participating in claim studies, as well as follow the migration of sunscreen products over the surface of face. Following the migration of sunscreen products over the surface can help explain why some sunscreen products find their way into the eyes producing stinging, burning, and ocular discomfort.

Guide photographs refer to photographs taken of mock subjects before the clinical trial begins to provide the sponsor and investigator with choices of techniques to best capture the dermatologic condition being studied. The chosen image becomes the guide, or standard, for photographing all subjects in the trial.

Use of RLOP to detect improvements in periocular fine lines and wrinkles

Optical profilometry refers to a technique in which photographic images of silicone rubber impressions taken of facial skin can be analyzed for changes in lines and wrinkles. Grove *et al.* [14] reported that optical profilometry provides an element of objectivity that can complement clinical assessment in the study of agents that are useful for treating photodamaged skin.

While no one would argue that optical profilometry is a time proven method for assessing textural changes, preparing quality silicone replicas can be quite challenging even

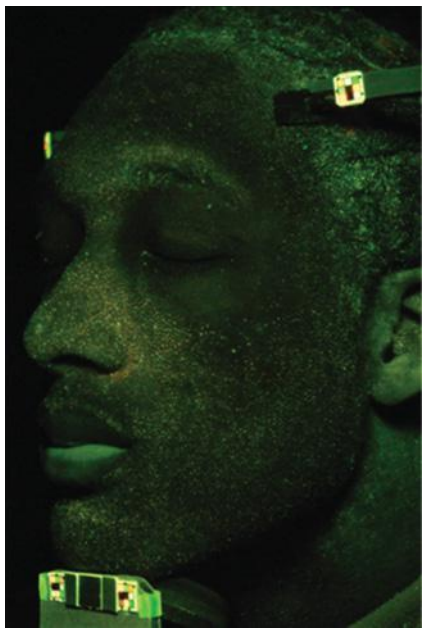


Figure 6.5 Ultraviolet fluorescence technique.

for veteran clinicians. Common problems include replica ring positioning errors, air bubbles in the replica impression, and controlling the polymerization process. Slight variations in temperature, humidity, and body temperature can produce unsuitable replica impressions.

In an effort to reduce the frustration level associated with preparing silicone replicas, we began investigations into using high-resolution digital photographs for quantifying changes in fine line and wrinkles on the face. Off-axial lighting, a common lighting technique used for clinical photography, could be used to create small shadows and highlights that could help define the surface texture of skin. Flash lighting can be placed above and at a 45° angle to the side of the face to create a three-dimensional effect of texture in a two-dimensional plane. The raw image files can be analyzed for fine lines and wrinkles on the face. The term to describe this technique is RLOP.

RLOP is designed to detect the number, length, width, and depth of horizontal wrinkles in the crow's feet area (coarse wrinkles) and the under eye area (fine lines). Wrinkles appear as dark lines on grayscale images. Deeper wrinkles appear darker because less light is present at the base of the wrinkle. An irregularly shaped area of interest is selected in the crow's feet area to avoid capturing the eyebrows or hairline, and a rectangular area of interest is used under the eye. Image Pro® v6 software (Media Cybernetics, Bethesda, MD, USA) is used for the analysis. A horizontal edge filter is used to locate the wrinkles and exclude any dark objects caused by hyperpigmentation or scars. Once the wrinkles are identified with the edge filter they are measured for size (length, width, and area) and grayscale density (where 0 = black) on the original grayscale image. Once the data are collected a paired *t*-test is used to check for significant changes from baseline or between groups.

As part of the validation process, RLOP has been included in several photoaging trials of cosmetic products involving several hundred subjects. The effectiveness of the products was evaluated using visual grading, digital photography with RLOP, bio-instrumentation, and subject self-assessment. The duration of these trials were typically 8 weeks, with clinic visits at 2, 4, and 8 weeks (Figure 6.6).

RLOP technology complements and supports the results of clinical grading of fine line and wrinkles. RLOP appears to have several advantages over traditional optical profilometry.

These advantages include:

- RLOP can be performed on multiple sites on the face using a single digital photograph.
- RLOP technology allows for precise location of the area of interest in each digital photograph through imaging software.
- Digital images can be archived electronically for an indefinite period of time.
- Results are expressed in meaningful units and endpoints.
- The area of interest is significantly larger than can be captured in a replica impression.
- RLOP can measure the full length of a wrinkle unlike traditional optical profilometry which limits the measured area to the size of the replica impression.

A non-invasive method for assessing the antioxidant protection of topical formulations in humans

It is well documented that the addition of antioxidants such as vitamins C, E, and A to skin care formulation can be beneficial in preventing and minimizing skin damage associated with UV light [15–17]. Manufacturers often face a difficult task when formulating with antioxidants, because they are easily destroyed or altered by oxidation which can occur during product manufacturing, filling, or storage.

To address these concerns, Pinnell and colleagues developed a human antioxidant assay which assesses the potential of topical antioxidants to enter into the skin and provide adequate protection against UV damage generated by a solar simulator. Antioxidants provide protection from UVR-induced damage by diminishing or blocking the formation of reactive oxygen species which is clinically manifested by erythema [17].

The technique involves the open applications of antioxidant products and a vehicle control to the demarcated areas on the lower back of subjects for four consecutive days. On day 3 the minimal erythema dose (MED) is determined for

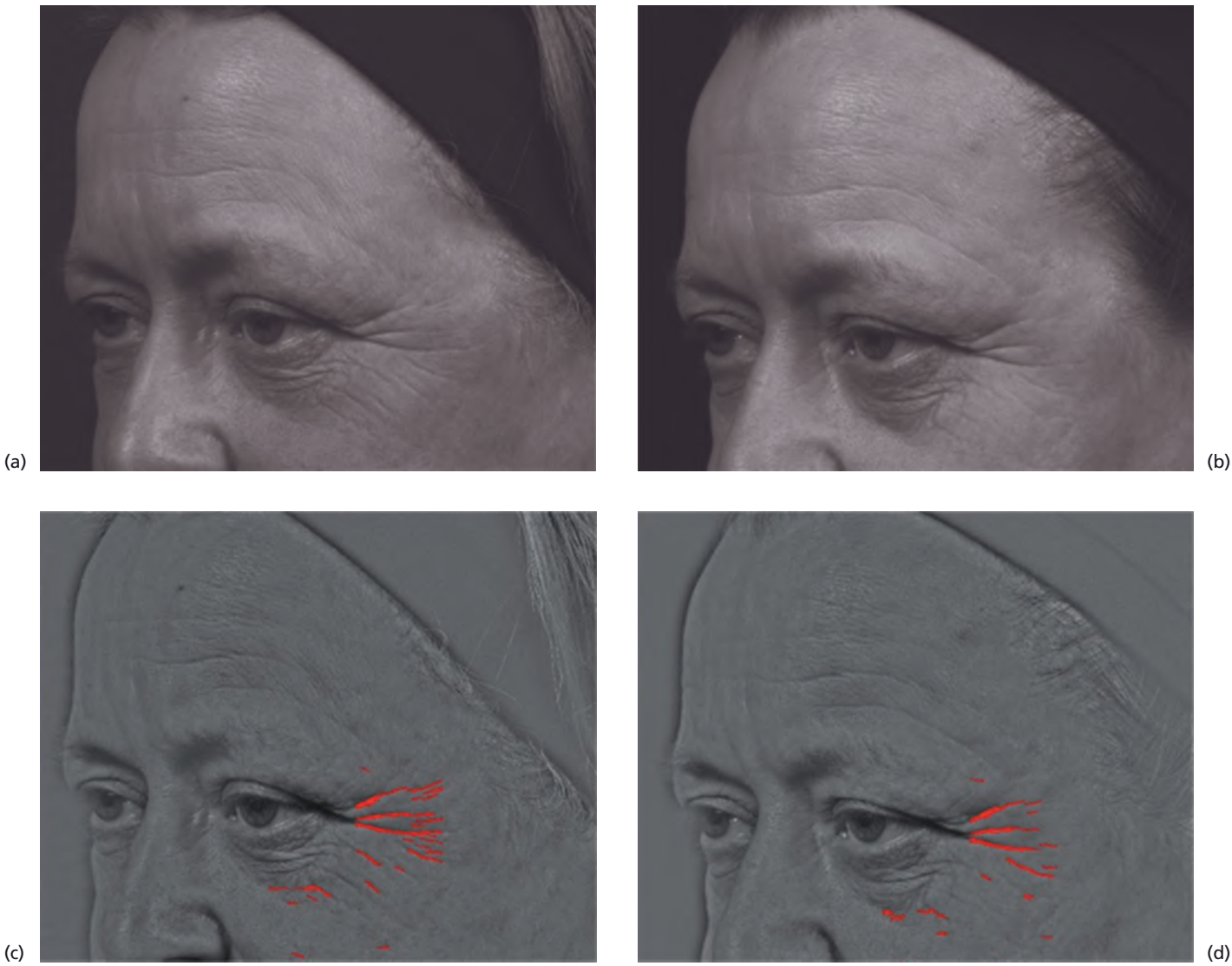


Figure 6.6 Before and after photographs using Raking Light Optical Profilometry. Top row: Digital photographs from a trial of a subject before (a) and 8 weeks after (b) treatment. Note the improvement in the appearance of wrinkling under the eye. Bottom row: Photographs shows the area of interest (AOI) in red. (c) Baseline. (d) Eight weeks after. The AOIs were precisely located in each digital image by using anatomic landmarks as anchors.

each subject. This is the dose of UV light that produces slight redness on fair-skinned individuals.

On day 4, the demarcated sites treated with the antioxidant product, vehicle control, and an untreated site receive solar-simulated UV irritation of 1–5X MED at 1X MED intervals. On day 5, digital images are taken and the investigator has the option of collecting punch biopsies at the treatment sites and analyzing the tissues for multiple bio-markers such as thymine dimers, interleukins, metaloproteins, Langerhans cells (CD1a), p53, and sunburn cells [13,14].

Figure 6.7 shows a pattern of UV responses for a site treated with an antioxidant and a site treated with a vehicle control. Using macro-programs written in Image Pro software, it is possible to determine accurately the a* (degree of redness according to the CIE color standard) of each spot and to calculate a protection factor for the antioxidant product relative to vehicle control treated site (Table 6.2).

Using this technique, Pinnell and associates have been able to formulate a third generation antioxidant product that provides protection against the damaging effects of UV light. The formulation containing 15% ascorbic acid, 1% alfa-tocopherol, and 0.5% ferulic acid was found to be effective in reducing thymine dimers known to be associated with skin cancer [18,19].

Conclusions

Photography and other non-invasive techniques are important to assess the efficacy and safety of cosmetic products.

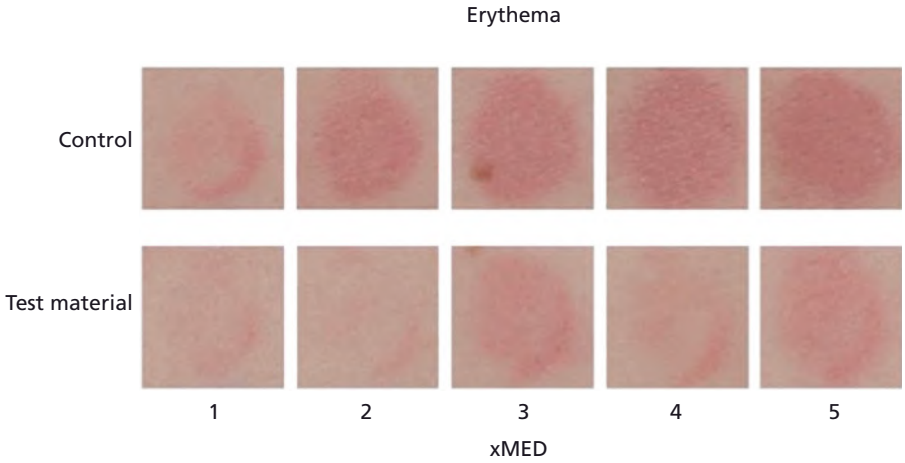


Figure 6.7 Pattern of UV responses for a site treated with an antioxidant and a site treated with a vehicle control.

Table 6.2 Results of theorectical antioxidant protection factor calculations.

	Increase from unexposed (adjusted for MED)	Protection factor (%)
No treatment (control)	10.50	0.0
Antioxidant	6.30	60.0
Vehicle control	0.53	2.6

MED, minimal erythema dose.

Often, the non-invasive assessments provide confirmation of the expert grader assessments. It is reassuring to see consistency within the data set to confirm a positive effect of cosmetics and skin care products. This validation technique is necessary to truly evaluate products. This chapter presents several cutaneous research tools.

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Chapter 7: Contact dermatitis and topical agents

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BASIC CONCEPTS

- Hypersensitivity reactions can occur in response to topical agents.
- Adverse reactions can be characterized by irritant contact dermatitis and allergtic contact dermatitis.
- Patch testing is a reliable method for determining the etiology of adverse reactions to topical products.
- Treatment of hypersensitivity reactions involves prompt recognition with identification and withdrawal of the offending agent.

Introduction

Topical cosmetic medications, cosmeceuticals, and minimally invasive procedures have always had an important role in dermatologic practice, but recent advances have led to a tremendous expansion in the repertory of treatment modalities available. In addition, the use of over-the-counter cosmetics is rising worldwide, along with potential exposure to irritants and allergenic substances [1]. Adverse skin reactions to cosmetics include irritant contact dermatitis, allergic contact dermatitis, contact urticaria, and foreign body reactions [2]. The clinician should be able to diagnose these cases, prescribe the correct treatment, and – most importantly – identify the causative agent. Most of these reactions are treatable without sequelae once the offending agent is identified and avoided [2].

Approximately 15 million Americans have been diagnosed with contact dermatitis [2]. The US Food and Drug Administration (FDA) regulations on cosmetics are based in two important laws: the Federal Food, Drug, and Cosmetic Act (FD&C) which prohibits the marketing of adulterated or misbranded cosmetics, and the Fair Packaging and Labeling Act (FPLA) which states that improperly labeled or deceptively packaged products are subject to regulatory action [3]. Ingredient labeling is mandatory in the USA and Europe, and compounds are listed in descending order of amount using the nomenclature format of the International Cosmetic Ingredient Dictionary [4,5]. However, with the exception of color additives, cosmetic products and ingredients are not subjected to FDA premarket approval and manufacturers' reporting of adverse reactions is a voluntary process [3]. In order to review the safety of the cosmetic ingredients, the Cosmetic, Toiletries and Fragrance Association (CTFA) sponsors the Cosmetic Ingredient Review (CIR). Reactions to cosmetics can manifest in a wide range of clinical signs, therefore it is important for the clinician to be familiar with the diversity of those presentations to enable prompt diagnosis and treatment.

Cosmetic Dermatology: Products and Procedures. Edited by Z.D. Draelos.

Hypersensitivity reactions: pathophysiology and clinical presentations

Irritant contact dermatitis

Most skin reactions to cosmetics are classified as irritant contact dermatitis [4]. Irritant contact dermatitis is caused by endogenous and environmental elements and it is defined as local inflammation that is not initially mediated by the immune system. Predisposing factors for the development of irritant dermatitis included the presence of a less effective stratum corneum, either from anatomic conditions (face, eyelids) or secondary to endogenous disorders, such as atopic dermatitis. The severity of the dermatitis depends on the amount and strength of the agent, and length and frequency of exposure. Repetitive exposures even to mild agents, such as soaps and detergents, will often result in irritant dermatitis. In addition, harsh scrubbing with mechanical assistance (brushes, synthetic sponges, or cosmetics containing microabrasive spheres) increases the risk for irritation. Psychiatric disorders, leading to compulsive repetitive behaviors of self-cleaning and handwashing, can sometimes be overlooked and a complete patient history must include cleaning habits, occupation, and a detailed list of all products used on both a daily and occasional basis.

Allergic contact dermatitis

Allergic dermatitis constitutes at least 10–20% of all cases of contact dermatitis and represents a true delayed-type (type IV) immune reaction. Previous exposure and sensitization

to the agent is necessary [2]. Chemical agents act as haptens, which are small electrophilic molecules that bind to carrier proteins and penetrate into the skin. HLA-DR or class II antigens act as the binding site in the surface of the antigen-presenting cells (APCs). These epidermal dentritic cells digest the allergen complex and display the antigenic site on their cell surfaces for presentation to T lymphocytes. If the individual has the genetic susceptibility to that allergen, clonal proliferation of T cells starts with the production of cytokines, further stimulating migration of inflammatory cells and keratinocyte proliferation.

Clinical distinction between irritant and allergic dermatitis can be challenging because both conditions manifest as eczematous reactions, ranging from mild erythema and scale with minimal itch to vesicular, bullous, and indurated plaques that are highly pruritic. Furthermore, the two conditions can be superimposed, because an irritated and broken epidermal barrier can facilitate the absorption of haptens and elicit an immune response in susceptible individuals.

Contact urticaria

Contact urticaria syndrome is divided into immunologic and non-immunologic subtypes. Non-immunologic contact urticaria is the most common form and occurs in the absence of previous exposure. Localized wheals appear within 30–60 minutes after exposure and are not followed by systemic symptoms. Allergic contact urticaria is an immediate-type (type I) hypersensitivity reaction and occurs in sensitized individuals within minutes to hours following the exposure to the allergen. The binding between allergens and immunoglobulin E (IgE) triggers mast cell degranulation and consequent release of inflammatory products, such as histamine, prostaglandins, leukotrienes, and cytokines. As a consequence, individuals experience erythema, swelling, and pruritus which may be localized (wheals and fares) or generalized (angioedema, conjunctivitis, bronchoconstriction, hypotension). Severe reactions may be fatal.

Foreign body reactions

Gel fillers are a group of exogenous substances used for soft tissue augmentation. Their mechanism of action is the addition of volume per se once injected and also the production of a collagen matrix. Fillers are supposed to be inert materials but the degree of the response elicited varies according to the material and the technique used, as well as the host immunologic pattern of reaction.

The normal initial host response to foreign body implantation is the formation of a blood-based matrix on and around the biomaterial, called the provisional matrix. The tissue injury may also lead to activation of the innate immune response and thrombus formation. The provisional matrix is rich in mitogens, chemoattractants, growth factors, and cytokines, proving an excellent medium both for wound healing and foreign body reaction. Acute inflammation is characterized by the presence of neutrophils, mast cell degranulation, and fibrinogen adsorption. The degree of the inflammation is highly dependent upon the injury produced, the site of injection, the material used, and the extent of the provisional matrix formed. The acute phase generally resolves within 1 week, and can be followed by chronic phase inflammatory response, which is characterized by the presence of monocytes, lymphocytes, and plasma cells. After resolution of acute and chronic phases of inflammation, a granulation tissue can be identified, rich in macrophages and fibroblasts which act to produce neovascularization and new healing tissue [6]. Prolonged duration of the inflammatory phase (i.e. longer than 3 weeks) should prompt an investigation to rule out complications, such as infection, allergic reaction, gel migration, abscesses formation, or granulomatous reaction. Foreign body granulomatous reactions with deleterious consequences have been previously described with the use of silicon, bovine collagen, hyaluronic acid, and other fillers [2,7–10].

Common allergens

Irritants

In the clinical setting, irritant substances are used for the purpose of selectively destroying the damaged superficial layers of the skin, and the depth of penetration is correlated with the agent used, concentration, and time of exposure. Examples of "peeling" agents include retinoic, glycolic, and salicylic acids, resorcinol, trichloroacetic acid, and phenol. Undesirable irritant reactions are commonly seen with daily use of topical retinoids, leading to erythema and fine scaling, which tend to improve with time.

A wide variety of substances may act as irritants when sufficient exposure in time and/or concentration is ensured (Table 7.1). Mechanical, chemical, and environmental factors can act alone or in combination to produce irritation in the skin. Mechanical factors include cosmetic procedures (shaving, waxing, laser therapy, dermoabrasion), habits (excessive rubbing of the skin with soaps, scrubs, usage of tight clothes or shoes, intense exercise), occupational exposure (latex gloves, microtrauma of the skin). Wet work (i.e. exposure of the skin to liquid), use of occlusive gloves for longer than 2 hours per day or frequent hand cleaning is one of the most common and important skin irritants [11]. Professions at risk include hairdressers, healthcare workers, and food handlers.

Almost all chemicals have the potential to cause skin irritation. The list of the chemical compounds capable of producing irritation of the skin is extensive and largely dependent on the concentration, volume, and time of exposure. Some substances are considered universal irritants, for example, strong acids (hydrofluoric, hydrochloric, sulfuric,

Table 7.1 List of common skin irritants: mechanic, chemical, and environmental factors known to cause skin irritation. The agents can act alone or in combination to produce contact dermatitis, therefore recognition of all factors involved is crucial for proper management of patients.

Mechanic	Chemical	Environmental
Shaving, waxing, laser treatment	Soaps	Excessive heat or sun exposure, sunburn
Dermoabrasion	Detergents	Food allergies
Rubbing of the skin (e.g. when using a soap or scrubbing)	Surfactants (cocamidopropyl betaine*)	Saunas and jacuzzis (chlorine*)
Friction and/or occlusion (tight clothes, certain fabrics: wool, synthetic fibers)	Chemical peelings	Extreme cold and windburn
Latex gloves	Alcohol	Stress
Intense exercise	Fragrances and color additives (musk*)	Dry air
Microtrauma	Preservatives (formaldehydes releasing substances: Quaternium 15*, imidazolidinyl urea, DMDM Hydantoin)	Hot and/or prolonged showers
Pressure	Sunscreens (para-aminobenzoic acid*)	Spicy foods, peppers, condiments
Wet work	Bleaches and whitening agents	Water

* Most common chemical compounds involved.

nitric acids) and strong caustics (sodium hydroxide, potassium hydroxide) produce severe burns even in brief and small exposures. Solvents, including alcohol, turpentine, ketones, and xylene, remove lipids from the skin, producing direct irritation and allowing other irritants, such as soap and water, to produce more damage on the exposed skin. Inappropriate skin cleansing with solvents to remove grease, paints, or oils is a common cause of skin irritation. Soaps are alkali substances and may produce irritation by disrupting the skin barrier; in contrast, cleansing agents with a pH of approximately 5.5 and alcohol-based hand-cleansing gels are less aggressive and should be preferred for sensitive skin.

Environmental elements may render the skin more susceptible to cutaneous irritants, and include dry air, extremes of temperature (cold, heat), or important weather variations. Food allergies may cause urticarial reactions; spicy foods and condiments may cause lip and perioral irritant dermatitis. Prolonged exposure to water can cause maceration and desiccation of the skin.

Acneiform eruptions refer to the presence of comedones, papules, pustules, and nodular cysts. Follicular plugging has been noticed secondary to the use of isopropyl myristate, an emollient and lubricant used in shaving lotions, shampoos, oils, and deodorants. Sodium lauryl sulfate (SLS) is a surfactant found in many topical medications, particularly for acne, and is a classic experimental cutaneous irritant. Pustular eruptions secondary to SLS have also been described. Bergamot oil (5-methoxypsoralen) induced phototoxic reactions in the past and it has subsequently been removed from the formulations of cosmetics. Photosensitivity reactions caused by topical retinoid preparations are common and patients should be advised to use sunscreens and avoid sun exposure during treatment.

Subjective irritation, described as a tingling, burning, stinging, or itching sensation without visible skin alteration is commonly observed with topical medications. Propylene glycol, hydroxy acids, and ethanol are capable of eliciting sensory irritation in susceptible individuals. Commonly used medications such as benzoic acid, azelaic acid, lactic acid, benzoil peroxide, mequinol, and tretinoin may have sensory irritation as a side effect. Sorbic acid is an organic compound used as a preservative in concentrations up to 0.2% in foods, cosmetics, and drugs. Subjective irritation has been demonstrated with 0.5% sorbic acid and to 1% benzoic acid in susceptible individuals [12].

"Sensitive skin" or cosmetic intolerance syndrome is a condition of cutaneous hyperreactivity secondary to substances that are not defined as irritants [13]. The condition encompasses a complex combination of objective and subjective irritative symptoms and may coexist with hidden allergic processes, urticarial reactions, and/or photodermatitis. Endogenous causes include seborrheic dermatitis, psoriasis, rosacea/perioral dermatitis, atopic dermatitis, and body dysmorphobia. Elimination of all cosmetic products for a prolonged period of time (6–12 months) followed by slow reintroduction (a new products every 2–3 weeks) is helpful when managing these cases.

Contact urticaria

Cinnamic acid is a white crystalline substance, slightly soluble in water, which is obtained from oil of cinnamon, or from balsams such as storax. Its primary use is in the

manufacturing of the methyl, ethyl, and benzyl esters for the perfume industry, producing the "honey, fruit" odor. Type I non-immunologic reactions can be triggered by fragrances that contain cinnamic acid and cinnamal.

Immunologic type I reactions can be triggered in susceptible individuals by parabens (preservatives), henna, and ammonium persulfate (oxidizing agent), leading to systemic symptoms and potentially fatal reactions [4]. Contact urticaria to latex is triggered by exposure to the proteins derived from *Hevea brasiliensis* tree. Risk factors include the presence of spina bifida, genitourinary tract abnormalities, previous contact to latex (from multiple surgical procedures, or occupational exposure) hand dermatitis, atopy, and specific food allergies (avocado, banana, chestnut, potato, tomato, kiwi, pineapple, papaya, eggplant, melon, passion fruit, mango, wheat, and cherimoya).

Allergic reactions

Fragrances

Allergic reactions to fragrances affect at least 1% of the population. The distribution of the eruption can be restricted to the areas of application (face, neck, hands, axillae) or it can present as generalized dermatitis. Products containing scents are ubiquitous and include cosmetics and toiletries, cleansers, and household goods. Common sensitizers are balsam of Peru, cinnamal, fragrance mix (eugenol, isoeugenol, oak moss absolute, geraniol, cinnamal, alfa-amyl cinnamic aldehyde, hydroxycitronellal and cinnamic alcohol), and colophony.

Patch testing to 26 fragrances was performed as a multicenter project in the European Union to further identify possible additional allergens and prevent adverse reactions by proper labeling of cosmetic products [14]. The compounds considered important allergens were defined as group I substances: tree moss, HMPCC (hydroxymethylpentylcyclohexene carboxaldehyde), oak moss, hydroxycitronellal, isoeugenol, cinnamic aldehyde, and farnesol. Group II included substances clearly allergenic, but less relevant regarding sensitization frequency: cinnamic alcohol, citral, citronellol, geraniol, eugenol, coumarin, lilial, amyl-cinnamic alcohol, and benzyl cinnamate. Rarely, substances in group III were sensitizers: benzyl alcohol, linalool, methylheptin carbonate, alfa-amyl-cinnamic aldehyde, alfa-hexyl-cinnamic aldehyde, limonene, benzyl salicylate, gamma-methylionon, benzyl benzoate, and anisyl alcohol [14].

Allergic reactions to *Myroxylon pereira* (balsam of Peru) have been correlated to scattered generalized dermatitis. Widespread involvement might also suggest a systemic exposure, and oral ingestion of balsam of Peru has been correlated with hand eczema [15].

Preservatives

Preservatives are low molecular weight, biologically active compounds that prevent product contamination by microorganisms, or degradation. The recent growing replacement of organic solvents and mineral oils to water-based products in the cosmetic industry has increased the need of preservatives. Distribution of the allergic rash includes face, neck, hands, axillae, or generalized. Common sensitizers include formaldehyde and formaldehyde releasers, thiomerosal, Kathon CG, parabens, glutaraldehyde, DMD-hydantoin, quaternium-15 and are widely present in water-containing products (e.g. shampoos, cosmetics, metalworking fluids, and soaps).

Formaldehyde allergy is common and is mostly caused by formaldehyde-releasing biocides in cosmetics, toiletries, and other products. In a recent review of 81 formaldehyde-allergic patients, allergic reaction to at least one of the 12 formaldehyde-releasing substances were detected in 79% of the cases and isolated reactions to releasers were rare [16]. Formaldehyde allergy is also reported as a common cause of occupational contact dermatitis and the professions at risk include hairdressers, healthcare workers, painters, photographers, housekeeping personnel, metalworkers, masseurs, and workers dealing with creams, liquid soaps, and detergents [16].

Cleansing agents

These are applied to remove sebum, desquamated cells, sweat, and microorganisms. Washout products are briefly in contact with the skin, therefore few cases of allergy have been reported. Allergens include surfactants (cocamidopropyl betaine), preservatives (methylchloroisothiozolinone), antimiocrobials (PCMX), and fragrances.

Moisturizers

Moisturizers inhibit transepidermal water loss by occlusion, and are composed of a mixture of substances such as petrolatum, lanolin, lanolin derivates, and fatty alcohols. Stasis dermatitis can be a predisposing factor for allergic contact dermatitis to lanolin. Self-tanning agents have become increasingly popular and are sold separately or in conjunction with moisturizers. Such agents may cause allergic contact reactions when dihydroxyacetone degrades to form formaldehyde, formic acid, and acetic acid.

Hydroquinone

Hydroquinone is a whitening agent present in up to 2% in over-the-counter creams and 4% in prescription bleaching creams. Irritant and allergic reactions, hypopigmentation and hyperpigmentation, and exogenous ochronosis are known side effects [17].

Shampoos and conditioners

Shampoos contain a combination of cleansing agents and surfactants that act to remove sebum, scales, and microorganisms from the hair and scalp. Conditioner agents neutralize static charge and soften the hair. Common ingredients

are moisturizers, oils, surfactants, lubricants, preservatives, and fragrances. Allergic reactions are uncommon because of the limited amount of time the substance is in contact with the skin, however, cocamidopropyl betaine (surfactant), formaldehyde, methylchloroisothiazolone and methylisothiazolone (preservatives) have been reported as causative agents of allergic contact dermatitis.

Hair dyes and bleaches

Hair dyes are classified in semi-permanent and permanent. Semi-permanent dyes are derivates from nitroanilines, nitrophenylenediamines, and nitroaminophenols which use low molecular weight elements that penetrate the hair cuticle. Permanent dyes act by the means of primary intermediates (*p*-phenylenediamine [PPD] or *p*-aminophenol) which are oxidized by hydrogen peroxide and react with different couplers to produce a wide range of colours. Once oxidized to para-benzo-quinone diamine, PPD is no longer allergenic [18]. A few exceptions include circumstances in which unreacted PPD remains in the skin, for instance with inadequate mixture of ingredients with the use of homemade coloring kits or poor rinsing. Distribution is on the hairline, scalp, face, and photo distributed. Consort dermatitis is defined as the presence of the allergic eruption in the partner of the subject using the allergenic substance. It has been described for cosmetics, including PPD [18].

Temporary henna tattooing and hair dying are common practices. Henna is a natural product derived from the leaves of *Lawsonia inermis* and rarely causes hypersensitivity reaction. The addition of PPD to henna causes contact sensitization to black henna and reported reactions include mild eczema to bullous reactions with scarring and pigmentation alterations [19].

Hair bleaches include hydrogen peroxide solutions that oxidize melanin and ammonium persulfate, a very strong oxidizing agent and a radical initiator, which can be used as a booster supplement in hair dyes. Type I and IV hypersensitivity reactions may arise from the use of ammonium persulfate.

Permanents

Permanents use mercaptans to cleave disufide bonds in hair; neutralizers are then added to reshape the configuration. Neutralizers contain hydrogen peroxide, bromates, perbromates, percarbromates, or sodium borate perhydrate. Ammonium thioglycolate, also known as perm salt, is a cleaving agent and if applied improperly can cause extensive hair damage and acute contact irritant dermatitis. Glycerol monothioglycolate (GMTG, "acid" permanents) can cause allergic contact dermatitis. Storrs [20] demonstrated positive allergic reactions to GMTG in concentrations as low as 0.25%, even when it was tested through glove fabric; however, household-weight neoprene gloves were proven to be protective.

Nail products

Nail polish and hardener contains nitrocellulose, resins, plasticizers, solvents and diluents, colors, and suspending agents. Most adverse reactions are secondary to tolysamide formaldehyde resin (toluene sulfonamide/formaldehyde resin). The dermatitis tends to affect places commonly reached by the fingers (e.g. face, eyelids, sides of the neck, mouth), sparing the hands and fingers. Nail elongation materials contain acrylics (ethy acrylate, 2-hydroxy ethyl acrylate, ethylene glycol dimethacrylate, ethyl cyanoacrylate, and triethylene glycol diacrylate) all previously reported as allergens.

Local anesthetics

Anesthetic agents can be divided in two groups: esters (benzocaine, tetracaine, and procaine) and amide derivates (lidocaine, mepivacaine, bupivacaine, etidocaine, and prilocaine). Cases of eczematous dermatitis have been reported secondary to the use of topical ester agents and rarely secondary to amide derivates. Contact sensitization to 2.5% lidocaine and 2.5% prilocaine emulsion (EMLA, Astra Zeneca Pharmaceuticals LP, Wilmington, DE, USA) is rare, and additional uncommon side effects reported include purpuric eruption, rash, redness, itching, and edema [2].

True IgE-mediated reactions to injectable anesthetics correspond to less than 1% of all adverse events. Although rare, such reactions may present as life-threatening events and prompt recognition of the symptoms and adequate management is imperative. In contrast, delayed-type reactions manifest within 12–48 hours and present as acute dermatitis (erythema, papules, vesicles and itching) [2,21].

The most common systemic adverse reactions to injectable anesthetics are psychosomatic responses, or exaggerated responses to epinephrine present in many products, caused by anxiety and vasovagal reflex. Patients may present with dyspnea, hyperventilation, and sympathetic responses, such as tachypnea, tachycardia, hypertension, and diaphoresis. Vasovagal syncope and peripheral paresthesias may also occur. Systemic toxicity occurs when excessive dosage is administered and manifest as light-headedness, tremors, restlessness, seizures, and depressed myocardial contractility. Methemoglobulinemia is an idiosyncratic reaction reported with local injectable anesthetics [21].

Topical corticosteroids

Non-halogenated topical steroids (hydrocortisone, budesonide) are the most common corticosteroids correlated with allergic reactions. Patients at risk are those with stasis dermatitis and chronic leg ulcers, followed by those with hand eczema, atopic dermatitis, anogenital, foot, and facial dermatitis. Patch testing with tixocortol pivalate and budesonide is useful to identify allergy to hydrocortisone and other steroids molecules that may cross-react [22].

Injectables

Botulinum toxin is a highly potent neurotoxin that inhibits acetylcholine release at the neuromuscular junction, blocking neuromuscular transmission and reversibly paralyzing striated muscle. Allergic reactions are rare and include generalized pruritus, psoriasiform eruption, urticaria, and erythema multiforme-type reactions [2,23].

Fillers can be classified as homogenous (polymer gels) and combination gels, which differ not only in composition, but also in duration of effect, tissue interaction properties, and type of adverse reactions evoked. Homogenous gels are the most commonly used and are subdivided into degradable (hyaluronic acid and collagen) and non-degradable gels (polyacrylamide and silicone). Degradable polymer gels resemble the elements commonly found in the tissues, therefore are degraded by naturally occurring enzymes, located in the extracellular matrix and/or within macrophages [8]. Hence, fibrous response generated by these hydrophilic gels is minimal. Although generally considered safe, affordable, and ease to use, degradable gels are not permanent, and rare complications include allergic reactions, transient swelling, and cystic swelling [2,7].

Collagen fillers are substances derived from bovine collagen, which become non-allergenic after enzymatic digestion with pepsin. Formulations available on the market are collagen I (Zyderm I and II, INAMED Corporation, Santa Barbara, CA, USA) or cross-linked collagen (Zyplast, INAMED Corporation, Santa Barbara, CA, USA). Transient swelling and erythema are the most common reactions and tend to resolve a few days after the procedure. Hypersensitivity allergic reactions involve localized humoral and cellular inflammatory processes. Such reactions can persist up to 1 year after the procedure and are strongly correlated with the presence of antibovine collagen antibodies, hence prophylactic testing of individuals is recommended.

Silicone is the term applied to describe the medical group of compounds derived from silicone-containing synthetics. Polydimethylsiloxanes are the most commonly substances used and contain silicon, oxygen, and methane [9]. The silicon gel is hydrophobic and once introduced in the tissues it is dispersed in vacuoles or droplets, which may be absorbed by macrophages and foreign body giant cells. The cells may then migrate to the reticuloendothelial system and/or evoke a local foreign body reaction in the surrounding tissue. Phagocytes enter and transverse the gel, followed by gradual replacement with connective tissue [8].

Adverse reactions to soft tissue augmentation include bacterial infections, abscesses, local inflammation, discoloration, ulceration, migration, and formation of silicon-type granulomas [2,8]. Deep-seated panniculitis can present early as a tingling sensation followed by local edema [8]. Late signs include the presence of a solid, painless tumefaction, with or without facial disfigurement and facial nerve paralysis [10].

Occupational hand eczema

Occupational hand eczema among hairdressers is a significant health problem and common sensitizers include hair dyes, ammonium persulfate, preservatives, amphoteric surfactants, fragrances, and glycerol thioglycolate. The use of gloves, mild soaps, and moisturizing creams alleviate the condition but severe refractory cases may require definitive interruption of the occupational activity. Gloves worn as protection may also constitute a source of allergens for hand dermatitis in hairdressers and healthcare professionals.

Diagnosis

Diagnostic evaluation of patients with hypersensitivity reactions should be directed towards identifying the causative agent. Prick tests and radio allergo-sorbent tests (RASTs) are available for detection of IgE antibodies against specific allergens, therefore indicated for patients with some type I hypersensitivity reactions.

Allergy to bovine collagen can be detected by intradermal challenge. The screening test is recommended for all cases prior to the procedure and consists of an intradermal injection of 0.1 mL of the filler substance in the volar forearm, with evaluation of the reaction within 48–72 hours. A positive test is defined as induration, erythema, tenderness, or swelling that persists or occurs longer than 6 hours after the injection. Positive subjects must be excluded from the procedure. A second test is recommended for non-reactive subjects to lower the chances of treatment-associated adverse reactions. The test should be performed within 2 weeks after the initial exam, in the contralateral forearm or periphery of the face [2].

Patch-testing is required to diagnose delayed type IV allergic reactions. Epicutaneous application of standardized concentrations of allergen chemicals on flat metal chambers are followed by occlusion and removal in 48 hours. The skin reaction is then graded and a second reading is performed in 1–5 days. The presence of induration, erythema, and/or vesicles denotes a positive reaction.

Treatment

Treatment is based on identifying the offending agent and lifetime avoidance. Type I reactions required blockage of histamine receptors. Severe anaphylactic reactions require immediate hospitalization for assessment of cardiorespiratory status and intravenous fluids, subcutaneous epinephrine, systemic steroids, and antihistaminic medication.

Mild forms of contact allergic dermatitis are readily treatable with avoidance of the offending agent. Topical steroids can be prescribed for a short period of time to hasten the process, whereas serious reactions may require addition of systemic immunosuppressant medication.

Conclusions

Cosmetic products are widely used and reactions to those products are commonly seen in daily dermatologic practice. Prompt recognition with identification and withdraw of the offending agent are key elements for successful management of such reactions.

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Part 2: Delivery of Cosmetic Skin Actives

Chapter 8: Percutaneous delivery of cosmetic actives to the skin

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BASIC CONCEPTS

- Percutaneous delivery is the penetration of substances into the skin.
- The goal of effective percutaneous delivery is to provide an effective amount of an active to the skin target site and thereby optimize efficacy while minimizing side effects.
- The main barrier of the active permeation through the skin is the stratum corneum. The active must cross this skin barrier and permeate transepidermally to be delivered to the target site.
- Molecules with a molecular weight of less than 500 Daltons penetrate the skin better than molecules with a larger molecular weight. The net charge of a molecule is important in enhancing penetration.

Introduction

Recent developments in new technologies combined with new knowledge in skin biology have advanced innovations in skin availability of actives and novel methods of substance delivery. The goal of this chapter is to review new advances in delivery of actives to the skin and the effects of penetration enhancers. An understanding of the structure of the skin is very important in managing active delivery.

The basics

The goal of percutaneous delivery is to provide an effective amount of an active to the skin target site and thereby optimize efficacy while minimizing side effects. This can be achieved by an understanding of the skin's complex structure and by relying on physical and chemical parameters of vehicles applied to the skin.

Skin physiology

There are defined compartments and biologic structures within the skin that provide opportunities to deliver actives (Figure 8.1). Within these compartments there are many chemical and biologic processes at work that may alter a given active or the physiology of skin target.

The main barrier of active permeation through the skin is the stratum corneum. The active must cross this skin barrier and permeate transepidermally to be delivered to the target site, and the penetration can be moderated by the secretion activity of the appendages. This structure is located at the outermost layer of the epidermis [1]. This transepidermal route can be further subdivided into transcellular and intercellular routes [2]. Delivery of hydrophilic substances can be achieved through sweat gland route; however, this is also minimal in total volume. Therefore, the principal pathway for skin penetration of actives is the transepidermal route (route 1 in Figure 8.1).

Active composition

One of the first steps in understanding the phenomenon of active delivery is to completely characterize the active that is intended for delivery to the skin. There are well-known physical and chemical parameters that are specific to all chemical compounds. The essentials for characterization of actives are typically described in the literature or can be measured in the laboratory. This includes the active's molecular weight, dissociation constant (pK), solubility, and octanol/water [O/W] partition coefficient (log P). These parameters, along with a thorough understanding of the net ionic charge (cationic, anionic, and amphoteric) of the active will help in understanding its penetration profile.

As general rule, molecules with a molecular weight of less than 500 Da penetrate the skin better than molecules with

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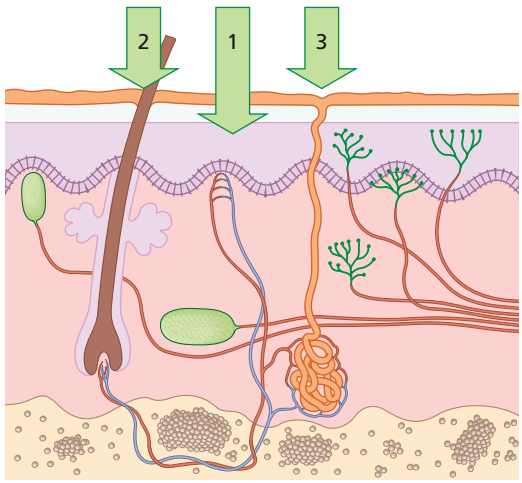

Figure 8.1 Possible pathways for a penetrant to cross the skin barrier. (1) across the intact horny layer; (2) through the hair follicles with the associated sebaceous glands; or (3) via the sweat glands. (This figure was published in: Daniels R. Strategies for skin penetration enhancement. *Skin Care Forum 37*, www.scf-online.com.)

a larger molecular weight. It is also known that the net charge of a molecule is important in enhancing penetration. An un-ionized molecule penetrates the skin better than an ionized molecule. A thorough understanding of the relationship between the dissociation constant and formulation pH is critical. In many cases it is advantageous to keep the pH of a formulation near the pK of the active molecule in an attempt to enhance penetration. When looking at the partition coefficient, molecules showing intermediate partition coefficients (log P O/W of 1–3) have adequate solubility within the lipid domains of the stratum corneum to permit diffusion through this domain while still having sufficient hydrophilic nature to allow partitioning into the viable tissues of the epidermis [3].

Fick's law

The permeation of active across the stratum corneum is a passive process, which can be approximated by Fick's first law:

$$J = \frac{DK}{L}(C) \qquad \text{(equation 8.1)}$$

This defines steady-state flux (J) is related to the diffusion coefficient (D) of the active in the stratum corneum over a diffusional path length or membrane thickness (L), the partition coefficient (K) between the stratum corneum and the vehicle, and the applied drug concentration (C) which is assumed to be constant.

Novel formulation strategies allow for manipulation of the partition coefficient (K) and concentration (C). Skin penetration can be enhanced by the following strategies:

1 Increasing drug diffusion in the skin;
2 Increasing drug solubility in the skin; and/or
3 Increasing the degree of saturation of the drug in the formulation [4].

Equation (1) aids in identifying the ideal parameters for the diffusion of the active across the skin. The influence of solubility and partition coefficient on diffusion across the stratum corneum has been extensively studied in the literature [5].

Vehicle effect

Delivery of actives from emulsions

The key for evaluation of the vehicle effect is to understand the dynamics between the vehicle and the active. Based on the physical and chemical nature of the active there are specific formulation strategies that can be designed to enhance delivery of actives.

The primary vector for topical delivery of actives is a semi-solid ointment or emulsion base. The main reason for selection of this dosage form is convenience and cosmetic elegance. Emulsions are convenient because they typically have two phases (hydrophilic and hydrophobic). The biphasic nature allows for placement of actives based on solubility and stability. This allows the formulator to bring lipophilic and hydrophilic actives into the dosage form while maintaining the optimized stability profile. The effect of the type of vehicle has been well described in the literature [6]. Numerous references are available for altering the delivery of actives from various emulsion forms (O/W, W/O, multiple emulsions, and nano-emulsions).

Formulation strategies

A basic formulation has many components. Table 8.1 provides an overview of these formula components and also provides a brief summary of the anticipated effect on active delivery. Some of these chemical functions are more clearly defined below in discussion on chemical penetration enhancers.

The ability of vehicles to deliver actives is tied to an understanding of diffusion of actives through various skin compartments (epidermal and dermal). Diffusion of actives across the skin is a passive process. Compounds with low solubility and affinity for the hydrophilic and lipophilic components of the stratum corneum would theoretically partition at a slow rate. These difficulties may be overcome by adding a chemical adjunct to the delivery system that would promote partitioning into the stratum corneum. Partitioning of actives from the dosage form is highly dependent on the relative solubility of the active in the components of the delivery system and in the stratum corneum. Thus, the formulation of the vehicle may markedly influence the degree

Table 8.1 Formulation components.

Ingredient	Chemical function	Effect on delivery
Water	Carrier/solvent	Hydration
Alcohol	Carrier/solvent	Fluidizes stratum corneum, alters permeability of stratum cornuem
Propylene glycol	Co-solvent/humectant	Alter permeability of stratum corneum Alter vehicle stratum corneum partition coefficient
Surfactant	Emulsifier/stabilizer	Emulsion particle size reduction, active solubilizer
Emollient	Skin conditioner, active carrier	Alter stratum corneum permeability Alter vehicle stratum corneum partition coefficient
Delivery system	Protect/target actives	Targeted/enhanced active penetration

of penetration of the active. Percutaneous absorption involves the following sequences:

- Partitioning of the molecule into the stratum corneum from the applied vehicle phase;
- Molecular diffusion through the stratum corneum;
- Partitioning from the stratum corneum into the viable epidermis; and
- Diffusion through the epidermis and upper dermis and capillary uptake [7].

One of the most effective formulation techniques to boost active penetration is supersaturation. This chemical process happens when an active's maximum concentration in solution is exceeded by the use of solvents or co-solvents. This type of solution state can happen during the evaporation of an emulsion on the skin. As water evaporates from a cream rubbed on the skin a superconcentrate depot of active forms on the skin. This creates a diffusional concentration gradient across the stratum corneum. One can attempt to boost this effect even further in the formulation by slightly exceeding the maximum solubility of the active in the formula using co-solvents. Supersaturation is an effective technique but the disadvantage is that active recrystallization can take place in this highly concentrated solution state. There are crystallization inhibitors that can be added to supersaturated solution but many experimental data need to be collected on this type of formulation strategy.

Eutectic blends are formulation techniques that can enhance penetration of actives. The melting point of an active influences solubility and hence skin penetration. According to solution theory, the lower the melting point, the greater the solubility of a material in a given solvent, including skin lipids. The melting point can be lowered by formation of a eutectic mixture. This mixture of two components which, at a certain ratio, inhibits the crystalline process of each other such that the melting point of the two components in the mixture is less than that of each component alone. In all cases, the melting point of the active is depressed to around or below skin temperature thereby enhancing solubility. This technique has been used to enhance the penetration of ibuprofen through the skin [8].

Manipulation of the vehicle skin partition coefficient of a formulation can be used as an overall formulation strategy to boost penetration of actives. This can be done by altering the solubility of the active in the vehicle via selection of different excipients. This change in the solubility parameter (δ) of the excipients can be tuned so that the active is more soluble in the stratum corneum than in the vehicle. Hence the diffusional gradient is altered towards the skin and thereby enhancing penetration. It has been shown that a solvent capable of shifting the solubility parameter (δ) of the skin closer to that of the activate will active flux rate [9]. Another strategy is to add a penetration enhancer that alters the membrane permeability of the skin. This strategy is discussed in more detail below.

Skin occlusion can increase stratum corneum hydration, and hence influence percutaneous absorption by altering partitioning between the surface chemical and the skin because of the increasing presence of water, swelling corneocytes, and possibly altering the intercellular lipid phase organization, also by increasing the skin surface temperature, and increasing blood flow [10].

The ultimate goal of penetration enhancement is to target the active in the stratum corneum and/or epidermis without allowing for systemic absorption. This remains the biggest

challenge for active penetration enhancement and it is one of the keys for targeted active delivery.

Penetration enhancers

In this section, the influence of penetration enhancers on the diffusion coefficient and solubility of the active in the stratum corneum is evaluated. The use of topically applied chemical agents (surfactants, solvents, emollients) is a well-known technique to modify the stratum corneum and also modify the chemical potential of selected actives. Collectively, these materials can be referred to as penetration enhancers (PEs). Based on the chemical structure, PEs can be categorized into several groups such as fatty acids, fatty alcohols, terpene fatty acid esters, and pyrrolidone derivatives [11]. PEs commonly used in skin care products have well-known safety profiles but their ability to enhance penetration of an active is challenging because of the manifold ingredients used in many formulations.

Solvents

A number of solvents (e.g. ethanol, propylene glycol, Transcutol® [Gattefossé, Saint-Priest, France] and *N*-methyl pyrrolidone) increase permeant partitioning into and solubility within the stratum corneum, hence increasing K_P in Fick's equation (equation 1). Ethanol was the first penetration enhancer co-solvent incorporated into transdermal systems [12]. Synergistic effects between enhancers (e.g. Azone® [PI Chemicals, Shanghai, China], fatty acids) and more polar co-solvents (e.g. ethanol, propylene glycol) have also been reported suggesting that the latter facilitates the solubilization of the former within the stratum corneum, thus amplifying the lipid-modulating effect. Similarly, solvents such as Transcutol are proposed to act by improving solubility within the membrane rather than by increasing diffusion. Another solvent, dimethylsulfoxide (DMSO), by contrast, is relatively aggressive and induces significant structural perturbations such as keratin denaturation and the solubilization of membrane components [13].

Physical enhancers

In addition to the chemical penetration enhancers discussed above, there is another class of penetration enhancers known as physical penetration enhancers. These materials stand between chemical enhancers and penetration enhancer devices. This unique classification is because in most cases the materials are particles of chemical origin (polyethylene, salt, sugar, aluminum oxide) but require physical energy to exert an action on the skin. These materials are used to physically débride or excoriate the stratum corneum by abrasive action. This is typically done by rubbing the particles by hand on the skin. New high-tech devices are now available that propel an abrasive against the skin thereby stripping away the stratum corneum.

Penetration enhancement vectors

There are customized carriers (vectors) for delivery of actives to the skin. These vectors are a type of vehicle that allow for enhanced penetration via their small size and unique physical chemical composition. These vectors are known as submicron delivery systems (SDS). Discussion focuses on liposomes, niosomes, lipid particles, and nanocapsules.

Liposomes

Liposomes are colloidal particles formed as concentric biomolecular layers that are capable of encapsulating actives. The lipid bilayer structure of liposomes mimics the barrier properties of biomembranes, and therefore they offer the potential of examining the behavior of membranes of a known composition. Thus, by altering the lipid composition of the bilayer or the material incorporated, it is possible to establish differences in membrane properties. Liposomes store water-soluble substances inside like biologic cells. The phospholipids forming these liposomes enhance the penetration of the encapsulated active agents into the stratum corneum [14].

There is debate on liposome formulations and their mode of action regarding penetration enhancement. Variation in performance may be caused by the variation in formulation and method of manufacture used to prepare this delivery form. Several factors such as size, lamellarity (unilamellar vs. multilamellar), lipid composition, charge on the liposomal surface, mode of application, and total lipid concentration have been proven to influence deposition into the deeper skin layers. It is reported by several authors that the high elasticity of liposome vesicles could result in enhanced transport across the skin as compared to vesicles with rigid membranes.

Liposomes have a heterogeneous lipid composition with several coexisting domains exhibiting different fluidity characteristics in the bi-layers. This property can be used to enhance the penetration of entrapped actives into the skin. It is supposed that once in contact with skin, some budding of liposomal membrane might occur. This could cause a mixing of the liposome bi-layer with intracellular lipids in the stratum corneum which may change the hydration conditions and thereby the structure of lipid lamellae. This may enhance the permeation of the lipophilic active into the stratum corneum and ease the diffusion of hydrophilic actives into the interlamellar spaces [15].

Niosomes

Niosomes are formed by blending non-ionic surfactants of the alkyl or dialkyl polyglycerol ether class and cholesterol

with subsequent hydration in aqueous media. These vesicles can be prepared using a number of manufacturing processes: ether injection, membrane extrusion, microfluidization, and sonication. Niosomes have an infrastructure consisting of hydrophilic, amphiphilic, and lipophilic moieties together and as a result can accommodate active molecules with a wide range of solubilities. They can be expected to target the active to its desired site of action and/or to control its release [16]. Niosomes are similar to liposomes in that they both have a bi-layer structure and their final form depends on the method of manufacture. There are structural similarities between niosomes and liposomes but niosomes do not contain phospholipids. This provides niosomes with a better stability profile because of improved oxidative stability.

Solid lipid nanoparticles

Solid lipid nanoparticles (SLNP) were developed at the beginning of the 1990s as an alternative carrier system to emulsions, liposomes, and polymeric nanoparticles. SLNP have the advantage of requiring no solvents for production processing and of relatively low cost for the excipients. SLNP represents a particle system that can be produced with an established technique of high-pressure homogenization allowing production on an industrial scale. This method also protects the incorporated drug against chemical degradation as there is little or no access for water to enter the inner area core of the lipid particle [17]. Lipid particles can be used as penetration enhancers of encapsulated actives through the skin because of their excellent occlusive and hydrating properties. SLNP have recently been investigated as carriers for enhanced skin delivery of sunscreens, vitamins A and E, triptolide, and glucocorticoids [18].

Nanocapsules

Nanocapsules are a type of submicron delivery system (SDS). This technology can segregate and protect sensitive materials and also control the release of actives. The more obvious opportunity for penetration enhancement of actives is because of their small size (20–1000 nm in diameter). Nanocapsules can be formed by preparing a lipophilic core surrounded by a thin wall of a polymeric material prepared by anionic polymerization of an alkylcyanoacrylate monomer. These very safe types of system have been proposed as vesicular colloidal polymeric drug carriers. Nanocapsules have the ability to enhance penetration but they can also control delivery of actives to the skin.

In a recent study, indomethacin was nano-encapsulated for topical use. This study compared cumulative release of indomethacin dispersed in gel base with indomethacin nano-encapsulated and indomethacin nano-encapsulated in a gel. The highest delivery was achieved with the nanoencapsulated indomethacin (Figure 8.2).

Devices for penetration enhancement

Devices for enhancing skin penetration of actives are at the leading edge of skincare technology. When utilizing devices for enhanced penetration of actives it is imperative to look into the regulatory classification of these instruments. The FDA has several guidelines and requirements for medical devices (510K). The 510K regulatory classification is important for safety and efficacy of any consumer device product and an understanding of the regulatory landscape in this area is essential. Four device technologies are reviewed. They range from moderately invasive to mildly invasive in terms of effect on the skin. In all cases, the goal is to reversibly alter the skin barrier function by physical or electroenergetic means.

Ultrasound waves

Ultrasound waves are sound waves that are above the audible limit (>20 kH). During ultrasound treatment the skin is exposed to mechanical and thermal energy which can alter the skin barrier property. Thermal and non-thermal characteristics of high-frequency sound waves can enhance the diffusion of topically applied actives. Heating from ultrasound increases the kinetic energy of the molecules in the

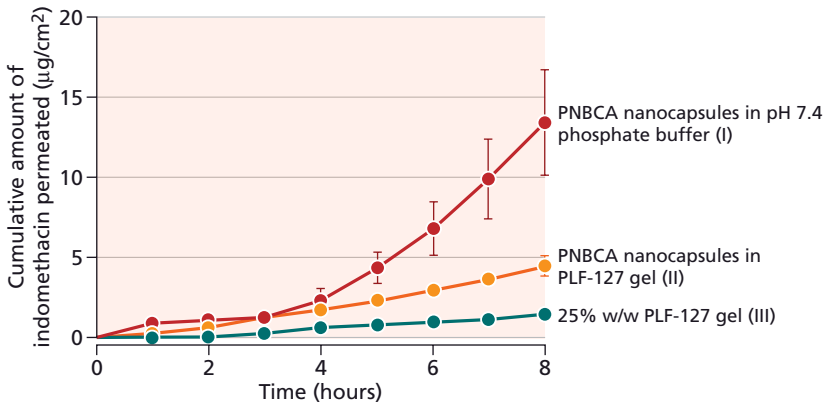

Figure 8.2 Cumulative amount of indomethicin (initial loading 0.5% w/v) per unit area, permeating through excised rat skin when released from PNBCA nanocapsule dispersion in pH 7.4 phosphate buffer, PNBCA nanocapsule dispersion in Pluronic F-127 gel and 25% w/w Pluronic F-127 gel. Each value is the mean ± SE of four determinations. (This figure was published in: Miyazaki S, Takahashi A, Kubo W, Bachynsky J, Löbenberg R. (2003) Poly n-butylcyanoacrylate (PNBCA) nanocapsules as a carrier for NSAIDs: *in vitro* release and *in vivo* skin penetration. *J Pharm Pharmaceut Sci* **6**, 238–45.)

active and in the cell membrane. These physiologic changes enhance the opportunity for active molecules to diffuse through the stratum corneum to the capillary network in the papillary dermis. The mechanical characteristics of the sound wave also enhance active diffusion by oscillating the cells at high speed, changing the resting potential of the cell membrane and potentially disrupting the cell membrane of some of the cells in the area [19].

A recent study on the use of ultrasound and topical skin lightening agents showed the effect of high-frequency ultrasound together with a gel containing skin-lightening agents (ascorbyl glucoside and niacinamide) on facial hyperpigmentation *in vivo* in Japanese women [20].

Patches

Delivery patches have been available for some time. One of the first applications of patch technology was in a transdermal motion sickness (scopolamine) patch. There are commercial products that provide actives in a patch formula. They utilize adhesive technology or a rate-limiting porous membrane to target and localize the actives. Some common patch applications are directed towards reduction of age spots or dark circles under the eye. The key delivery enhancement for patches is a combination of localized delivery and occlusion.

Microneedles

Another type of delivery device is the microneedle. Microneedles are similar to traditional needles, but are fabricated at the micro size. They are generally 1 μm in diameter and range 1–100 μm in length (Figure 8.3). The very first microneedle systems consisted of a reservoir and a range of projections (microneedles 50–100 mm long) extending from the reservoir, which penetrated the stratum corneum and epidermis to deliver the active. The microneedle delivery system is not based on diffusion as in other transdermal drug delivery products but based on the temporary mechanical disruption of the skin and the placement of the active within the epidermis, where it can more readily reach its site of action. Microneedles have been fabricated

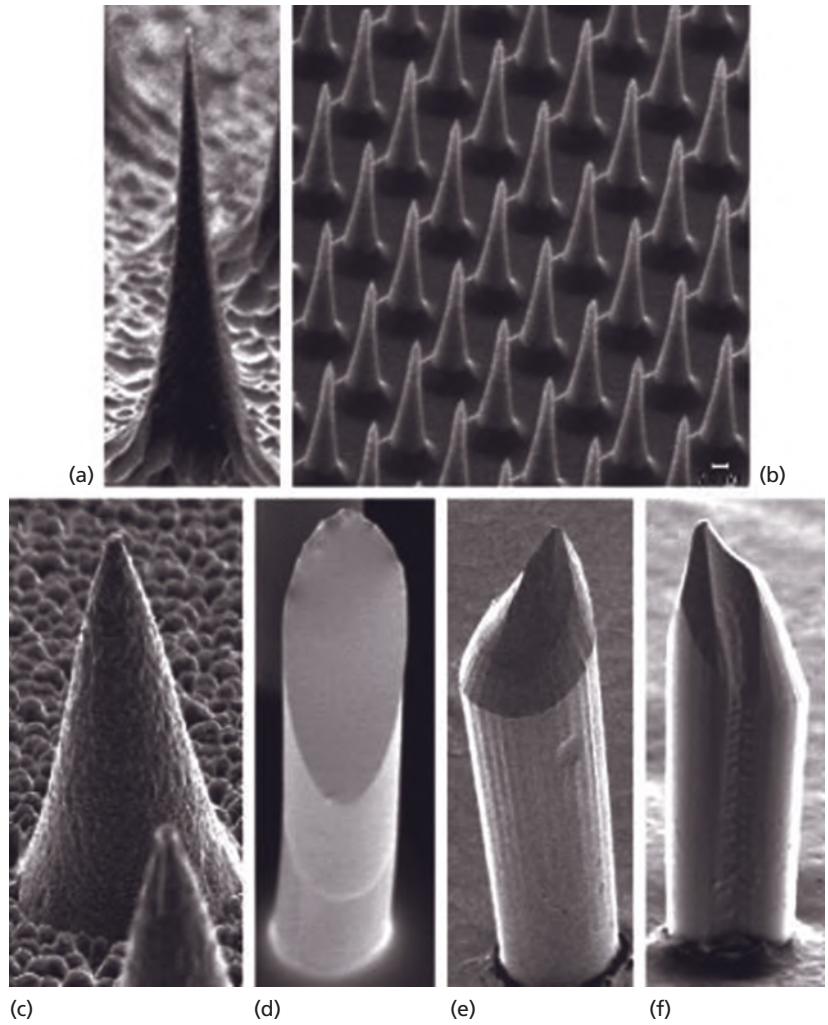

Figure 8.3 Solid microneedles fabricated out of silicon, polymer, and metal, imaged by scanning electron microscopy. (a) Silicon microneedle (150 μm tall) from a 400-needle array etched out of a silicon substrate. (b) Section of an array containing 160 000 silicon microneedles (25 μm tall). (c) Metal microneedle (120 μm tall) from a 400-needle array made by electrodepositing onto a polymeric mold. (d–f) Biodegradable polymer microneedles with beveled tips from 100-needle arrays made by filling polymeric molds. (d) Flat-bevel tip made of polylactic acid (400 μm tall). (e) Curved-bevel tip made of polyglycolic acid (600 μm tall). (f) Curved-bevel tip with a groove etched along the full length of the needle made of polyglycolic acid (400 μm tall). (This figure was published in: McAllister DV, Wang PM, Davis SP, Park JH, Canatella PJ, Allen MG, *et al*. (2003) Microfabricated needles for transdermal delivery of macromolecules and nanoparticles: fabrication methods and transport studies. *Proc Natl Acad Sci U S A* **100**, 13755–13760.)

with various materials such as metals, silicon, silicon dioxide, polymers, glass, and other materials. There are already patents granted for these types of moderately invasive delivery system [21].

Iontophoresis

Iontophoresis is a technology that has been brought to the cosmetic industry via the pharmaceutical development field. Iontophoresis passes a small direct current through an active-containing electrode placed in contact with the skin, with a grounding electrode to complete the circuit. Three important mechanisms enhance transport:

1 The driving electrode repels oppositely charged species;
2 The electric current increases skin permeability; and
3 Electro-osmosis moves uncharged molecules and large polar peptides [22].

There are limitations related to this technique. The active ingredient must be water-soluble, ionic, and with a molecular weight below 5000 Da. Even with all of these limitations, reported data show that the drug delivery effectiveness can be increased by one-third through iontophoresis [23].

In vitro and *in vivo* delivery assessment

A key in any evaluation assessment of skin bioavailability of actives is a quantitative measurement of activity by *in vitro* and *in vivo* methods. In early development phases *in vitro* methods provide a quick, reproducible way to identify promising formulations for next phase development studies. There are different techniques for evaluating percutaneous absorption of actives.

Franz cell

A well-known technique for measuring *in vitro* skin permeation is the Franz cell apparatus (Figure 8.4). The test apparatus and technique have been well documented for use within the pharmaceutical and cosmetic industries [24]. The technique utilizes a sampling cell which contains a solution reservoir and a sampling port, the top portion of the Franz cell is covered with a biologic membrane or skin substitute. The formulation is added to the top of the cell and periodic samples are taken from the cell reservoir and assays are plotted versus time to develop a time–penetration profile.

Tape stripping

Tape stripping is a technique used for *in vivo* active penetration evaluation. In this procedure, penetration of the active is estimated from the amount recovered in the stratum corneum by adhesive tape stripping at a fixed time point following application [25]. This technique is also recognized by FDA as a viable screening option for dermatologic evaluation [26].

Microdialysis

During the last decade, microdialysis has been shown to be a promising technique for the assessment of *in vivo* and *ex vivo* cutaneous delivery of actives. The technique is based on the passive diffusion of compounds down a concentration gradient across a semi-permeable membrane forming a thin hollow "tube" (typically, a few tenths of a millimeters in diameter), which – at least, in theory – functionally represents a permeable blood vessel (Figure 8.5.). Two kinds of probe are in common use: linear and concentric.

Confocal Raman microspectroscopy

Confocal Raman microspectroscopy (CRS) is a new, non-invasive technique which can be used for *in vivo* skin penetration evaluation. This technique combines Raman spectroscopy with confocal microscopy. CRS is a non-destructive and rapid technique that allows information to be obtained from deep layers under the skin surface, giving the possibility of a real-time tracking of the drug in the skin layers. The specific Raman signature of the active agent enables its identification within the skin [27].

There is a range of techniques of *in vitro* and *in vivo* evaluation for following penetration of actives through the skin.

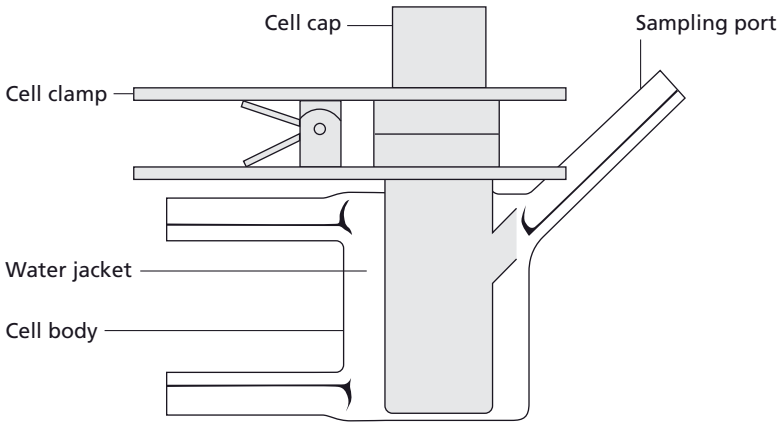

Figure 8.4 The Franz diffusion chamber.

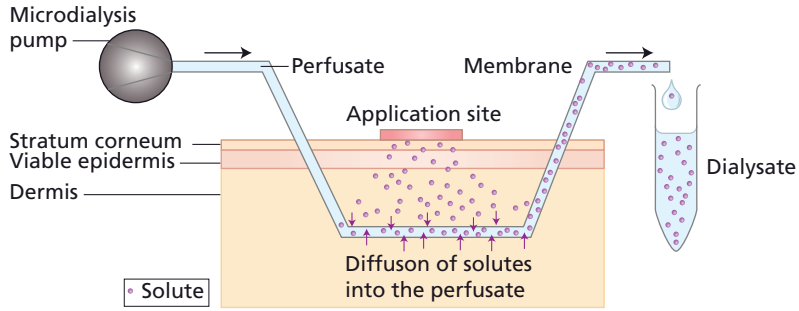

Figure 8.5 The microdialysis apparatus for the evaluation of penetration through the human skin barrier. (This figure was published in: Schnetz E, Fartasch M. (2001) Microdialysis for the evaluation of penetration through the human skin barrier: a promising tool for future research? *Eur J Pharm Sci* **12**, 165–74.)

Table 8.2 Methods to assess drug penetration into and/or across the skin. (From Herkenne C, et al. (2008) *In vivo* methods for the assessment of topical drug bioavailability. *Pharm Res* **25**, 87–103.)

	Method	Measure	Measurement site	Temporal resolution	Technical simplicity
In vitro	Diffusion cell	Q	Transport into and across skin	++	+
In vivo: non- or minimally invasive	Tape stripping	Q	Stratum corneum	0	+
	ATR-FTIR	Q	Stratum corneum	+	+
	Raman	Q/L	Upper skin	+	+
	Microdialysis	Q (free)	Dermis (or subdermis)	++	–
	Vasoconstriction	A	Microcirculation	+	±
In vivo: invasive	Blister	Q	Extracellular fluid	0	±
	Biopsy	Q	Skin	0	+
	Biopsy	Q + L	Skin (depth)	0	±

Q, quantity of drug; A, pharmacological activity of drug; L, drug localization.

Some are more invasive than others and some are more predictive across various dosage forms utilized on the skin. In Table 8.2 a summary chart shows a good comparison of the techniques based on strengths and weaknesses.

Conclusions and future trends

There are many formulation options available for delivering actives to targets within the skin. Understanding the skin and its interaction with various actives allows the chemist to select delivery options that provide safe and effective properties.

A good understanding of the physicochemical parameters of the active and the desired skin target are needed before deciding on a particular delivery option. Human studies are the "gold standard" against which all methods for measuring percutaneous absorption should be judged. The conduct of human volunteer experiments is well regulated. Study protocols and accompanying toxicologic data must be submitted to an ethics committee for approval [28].

Next generation delivery technologies are being developed and in some cases are already on the way to the market. Researchers from device and skincare companies are already in collaboration to bring combinations of devices and actives to the field of cosmetic dermatology. The approach can vary from non-invasive LEDs all the way to more invasive, laser-based enhanced penetration of actives. There are many home-use devices coming to the market today. These advances in delivery technology will likely culminate in a commercially available topical product that has its efficacy boosted by some type of chemical or physical delivery device as demonstrated in the delivery of estradiol using either a delivery vesicle (ultra-deformable liposomes) or a device (iontophoresis) [29].

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Chapter 9: Creams, lotions, and ointments

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BASIC CONCEPTS

- Creams, lotions, and ointments are both vehicles and delivery systems for dermatologic products.
- Creams and lotions are emulsions, which are colloidal dispersions comprising two immiscible liquids (e.g. oil and water), one of which is dispersed as droplets representing the internal or discontinuous phase within the other external phase.
- Ointments are semi-solid preparations used topically for protective emollient effects or as vehicles for the local administration of medicaments.
- Ointments are mixtures of fats, waxes, animal and plant oils, and solid and liquid hydrocarbons.

Introduction

This chapter examines creams, lotions, and ointments as both vehicles and delivery systems for dermatologic products. Creams, lotions, and ointments have a unique composition that can alter the ability of ingredients to reach the skin surface while also influencing product esthetics. The construction of the cream or ointment is an important determining factor in patient compliance, because if patients do not like the feel, smell, or color of a dermatologic they will not properly follow directions for its use.

Definition of creams, lotions, and ointments

Creams and lotions

Creams and lotions are classified as emulsions. There are several different types of emulsions that function as a vehicle and delivery system for cosmetic and drug materials. The classic definition of an emulsion is a colloidal dispersion comprising two immiscible liquids (e.g. oil and water), one of which is dispersed as droplets representing the internal or discontinuous phase within the other external phase [1]. All emulsions require the inclusion of an emulsifier or dispersing agent responsible for keeping the two immiscible phases together for an extended period of time. All emulsions are unstable and will eventually separate into two or more phases.

Cosmetic Dermatology: Products and Procedures. Edited by Z.D. Draelos.

Emulsions can be classified as a cream or lotion. There are no legal definition differences between a cream and a lotion. The determination of what constitutes a cream or lotion emulsion is determined by viscosity. If an emulsion can be poured from a bottle or pumped from a jar, it is labeled a lotion. If the emulsion requires a jar or a tube for dispensing and does not readily flow, it is labeled a cream. The term emulsion will be used for the remainder of this chapter to indicate a cream or lotion.

The other important part of the definition of an emulsion is based on the materials that comprise the internal phase and the materials that comprise the external or continuous phase. The two categories of emulsions are oil-in-water (O/W) and water-in-oil (W/O). The names describe the composition of the emulsion (Figure 9.1).

Emulsions can also be described by their emulsifier type as anionic, cationic, and non-ionic. This terminology refers to the ionic charge, or lack of charge, on the emulsifier system. Emulsions have also been developed that are based on polymeric emulsifiers and liquid crystal emulsifiers. These emulsions are different from traditional emulsions, because the two phases are held together by different mechanisms. Sophisticated emulsion technology is beyond the scope of this chapter; however, additional information can be found in Bloch [1].

Ointments

Ointments can be defined as semi-solid preparations used topically for protective emollient effects or as vehicles for the local administration of medicaments. They are mixtures of fats, waxes, animal and plant oils, and solid and liquid hydrocarbons [2]. Ointments are traditionally anhydrous bases, meaning they do not contain water, and therefore pose fewer microbial contamination issues than emulsions, which is a distinct advantage. In addition, because ointments

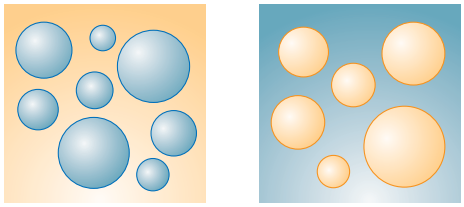

Figure 9.1 Different emulsion types. (This figure is from "Emulsions" presentation from Cognis Corp. August 2004.)

Table 9.1 Generic composition of a typical oil-in-water emulsion.

Ingredients	% (weight/weight)
Water phase	
Deionized water	60.0–90.0
Humectant	2.00–7.0
Preservative*	0.05–0.5
Water-soluble emulsifier†	0.25–2.5
Thickener(s)	0.1–1.0
Water-soluble emollient	0.5–2.0
Chelating agent	0.05–0.20
Oil phase	
Emollient system – oils, esters, silicones, etc.	3.0–15.0
Oil-soluble emulsifiers	2.0–5.0
"Active ingredients"	As required by regulations
Oil-soluble antioxidants	0.05–0.5
Fragrance/essential oil, etc.	0.1–2.0
Color	As required
*Preservative**	0.05–1.0
pH adjustments	As required

* Preservatives are frequently added in two places in the formulation.
† May also be added into the oil phase.

are anhydrous in nature, they tend to be more water-resistant than emulsions. However, ointments have less esthetic appeal for skin care and dermatology products as they are frequently described as oily, waxy, greasy, sticky, tacky, and heavy. Ointments are used more commonly for the delivery of medications than for skin care products because of their undesirable esthetics.

Table 9.2 A typical "non-ionic" oil-in-water emulsion base.

Ingredients	Function	% weight/weight
Water phase		
Deionized water	External phase vehicle	82.95
Carbomer	Thickener	0.20
Disodium EDTA	Chelating agent	0.10
Butylene glycol	Humectant	2.00
Oil phase		
Cetearyl alcohol (and) ceteaeth-20	Emulsifier	2.00
Cyclopentasiloxane	Silicone emollient	4.00
Dimethicone	Silicone emollient	1.00
Caprylic/capric triglyceride	Organic emollient	5.00
Glyceryl stearate (and) PEG100 stearate	Emulsifier	1.25
Triethanolamine (99%)	Neutralizing agent and pH adjuster	0.50
Preservative	Antimicrobial	1.00

The pH of this cream would be 5.5–6.5.
The viscosity would be approximately 15 000–25 000 centipoise.

Composition of creams and ointment

Oil-in-water creams

The most popular type of emulsion used in skin care products and cosmeceuticals is oil-in-water (O/W). A generic composition for an O/W emulsion is presented in Table 9.1 [3]. Each of the ingredient classes are discussed in detail to aid in the understanding of O/W formulations. A typical "non-ionic" oil-in-water emulsion composition is shown in Table 9.2.

Emulsifiers

Emulsifiers are important to keep the oil and water ingredients miscible. The choice of emulsifier will also determine the emulsion pH and effect the application and stability of the emulsion, as well as the delivery of materials into the skin. Emulsifiers can damage the skin barrier by emulsifying the sebum and intercellular lipids. This has led to the need to develop "skin friendly emulsifiers." These emulsifiers do not adversely affect the barrier properties of the skin and in some cases even help maintain barrier properties. Because the route of delivery into the skin is primarily through the lipid layer, which constitutes the mortar in the "brick and

mortar" model of the skin, the selection of an emulsifier can determine whether the disruption of the lipid layer.

Liquid crystal forming emulsifiers are being used more frequently because they maintain the skin barrier. These emulsifiers function like the phospholipids and ceramides found in the skin and therefore do not disrupt barrier function because of their skin lipid compatibility. A popular liquid crystal forming emulsifier is lecithin or hydrogenated lecithin [4].

Another recent trend is the use of emulsifiers as part of the emollient system in the product. Emollients are substances that make the skin feel smooth and soft, which is important to consumer acceptability. The most popular of this emulsifier type are "cationic" emulsifiers, which possess a net positive charge. The skin has a net negative charge because of its amino acid composition. A positively charged emulsifier will be attached to the skin and remain on the skin because of electrostatic attraction. Examples of these emulsifiers are behentrimonium methosulfate and dicetyldimonium chloride. Cationic emulsifiers are also very effective when there is a need to formulate low pH emulsions (less than pH 4.5) as cationic emulsifiers are very stable in low pH environments.

Emollients

The choice of emollient or combination of emollients will have a dramatic effect on the feel, application, and delivery of the active to the skin. Matching solubility of active with the oil phase has a big effect in determining the material to be used. Matching the solubility parameter of an organic sunscreen to the solubility parameter of the oil phase has a significant effect on the sunscreen performance.

The emollient category has been greatly expanded because of the increased use of silicones and the increasing number of "natural" emollients. The selection of emollient combinations is where art and science are combined. Selecting the right combination which provides the proper initial, middle, and end feel is one of the biggest challenges affecting the successful development of a cream. Concepts such as "cascading effect" describe this type of change which occurs as you apply an emollient system.

Active ingredients

Examples of active ingredients are sunscreen materials (e.g. octinoxate, titanium dioxide, avobenzone), antiacne actives (i.e. salicylic acid, benzoyl peroxide), skin lighteners (hydroquinone), etc.

Humectants

The humectant, usually a glycol or polyol, will have an effect on "skin cushion" and can also be part of the solvent system for an active ingredient. Glycols, such as propylene glycol and butylene glycol, are very good solvents for salicylic acid (an FDA approved over-the-counter active ingredient used to treat acne) and are frequently used for this purpose in an emulsion system. In addition, they also function to help with freeze–thaw stability.

Thickeners

The thickener(s) are used to control the viscosity and the rheology of the emulsion and can also help in maintaining the stability or product integrity of the emulsion, especially at elevated temperatures. Even in W/O creams thickeners are used for viscosity control. The viscosity of a cream is primarily determined by the thickener used and the viscosity of the external phase.

The choice of thickeners, to a large extent, depends upon the compatibility of the thickener with the rest of the ingredients in the formulation, the pH of the formulation, and the desired feel that is trying to be achieved.

The predominant thickeners used in O/W emulsions are acrylic-based polymers. The most popular materials are carbomers and its derivatives. Carbomers are a cross-linked polyacrylate polymer and their derivatives which are high molecular weight homopolymer and co-polymers of acrylic acid cross-linked with a polyalkenyl polyether [4]. These polymeric thickeners are very effective in stabilizing emulsions at elevated temperatures. (In W/O emulsions the predominant thickeners for the external phase are waxes – natural or synthetic.)

Water-in-oil creams

The composition of a W/O emulsion may not look much different on paper than an O/W emulsion except that the emulsifier system would be different and would be designed to make a W/O emulsion. The ratio of the two phases is not an indication of the type of emulsion. There are many O/W emulsions in which the oil phase may be at a higher persentage than the water phase and in a W/O emulsion the water phase is frequently at a higher percentage than the oil phase.

Ointments

There are different types of ointments. The traditional type of ointment contains very high levels of petrolatum as this material is a very good water-resistant film former and serves as very effective delivery system for drug actives on the skin. An example of a traditional petrolatum-based ointment is shown in Table 9.3.

In reviewing this formulation you will notice that there is no antimicrobial preservative present. Some ointment formulations put in low levels of antimicrobial preservatives for added protection during consumer use, but anhydrous ointments are hostile environments for bacteria and are generally "self-preserving." The use of an oil-soluble emulsifier helps with the application properties of the ointment as well as the ability to wash it off the skin.

Recently, there has been an increased interest in "natural ointments" – ointments that do not use petrochemicals (i.e.

Table 9.3 An example of a traditional petrolatum-based ointment.

Ingredients	% (weight/weight)
White petrolatum USP	50.0–80.0
Lanolin	1.0–5.0
Natural and/or synthetic waxes	2.0–10.0
Oil-soluble emulsifier	1.0–3.0
"Drug actives"	As required
Antioxidants	0.1–0.5
Fragrance/essential oils	0.1–1.0
Skin feel modifiers	1.0–5.0

Table 9.4 A typical "natural ointment" composition.

Ingredients	% (weight/weight)
Soy bean oil (and) hydrogenated cottonseed oil	50.0–80.0
Lanolin	1.0–5.0
Natural waxes	2.0–10.0
Oil vegetable-soluble emulsifier	1.0–3.0
"Drug actives"	As required
Natural antioxidants	0.1–0.5
Natural fragrance/essential oils	0.1–1.0
Natural skin feel modifiers	1.0–5.0

petrolatum) and are primarily based on plant-derived materials. The primary difference is in the use of the material that replaces petrolatum in the formulation. There are a number of hydrogenated oil/wax mixtures that are offered and used as "natural petrolatums." A typical "natural ointment" composition is shown in Table 9.4).

"Natural ointments" generally do not have the same unctuous, heavy feel that petrolatum-based ointments have and they usually do not leave as much residual feel on the skin. As with petrolatum-based ointments, little or no antimicrobial preservative is needed because of the anhydrous nature of the ointment. However, antioxidants are a very important components, as these "natural oil-based" ointments have a tendency to turn color and go rancid (similar to what you would see in a vegetable oil) without adequate protection.

While the number of different ingredients that can be used in an emulsion or an ointment can sometimes seem overwhelming, once you break down the product into the attributes and benefits and esthetics that are desired, the choices become less daunting.

Cream and ointment stability

Once the formulations have been put together and evaluated, the next step is stability testing. This testing is carried out to determine what happens to the product once it is on the market. The ideal test would be to store the product at ambient temperature for 2–3 years and observe any changes that may occur in product integrity and determine the stability of the active ingredient(s) that are present. Because this timeframe is not practical, accelerated stability testing is conducted to predict the long-term stability of the product.

For most emulsions, this testing involves storage of the finished product at 5 °C, 25 °C (RT), and 40 °C and sometimes at 50 °C. Stability at 40 °C is traditionally carried out for 3 months [1]. This testing is accepted by the US FDA for expiration dating until a full 2–3 year study is complete. Its purpose is not to ascertain product integrity but to establish the stability of the drug actives in the product.

Elevated temperature testing (40 °C for 3 months) is conducted so that a determination can be made in a reasonable amount of time as to the integrity and stability of the product and to allow the product to be marketed in a reasonable amount of time from the completion of formulation development.

Conclusions

The development of the final formulation is a combination of art and science, and both have an important role in the use of the product by the patient or consumer.

Once the type of formulation is determined, the ingredients have been selected, the formulation developed, and the appropriate safety, efficacy, preservative and stability testing completed, the product is ready to introduce to the market.

References

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