

CHAPTER 1

Epidermal Barrier

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BASIC CONCEPTS

- The outermost structure of the epidermis is the stratum corneum (SC), and it forms the epidermal permeability barrier which prevents the loss of water and electrolytes.
- Understanding the structure and function of the stratum corneum and the epidermal barrier is vital because it is the key to healthy skin.
- Novel delivery systems play 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.
- Cosmetic companies will exploit this new knowledge in developing more efficacious products for strengthening the epidermal barrier and to enhance the functional and aesthetic 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, 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), and it forms the epidermal permeability barrier which prevents the loss of water and electrolytes. Other protective/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, that 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, SC is made of anucleated cells called corneocytes that are derived from keratinocytes. 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. SC is a structurally heterogeneous tissue composed of nonnucleated, 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 play 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 contains 70% water as do most tissues, yet the SC contains 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 proteinaceous core containing keratins, other structural proteins, free amino acids, and amino acid derivatives, and melanin particles that 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 cornified cell envelope, formed from cross-linked cytoskeletal proteins [3].

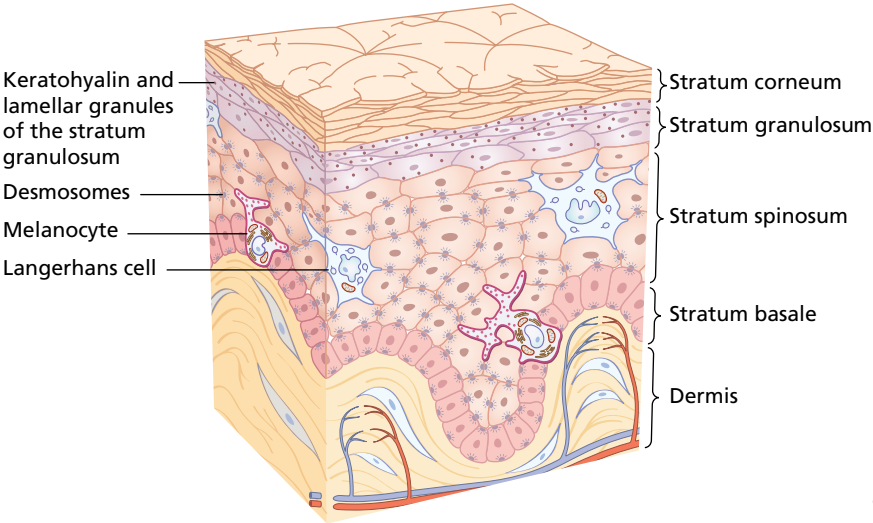


Figure 1.1 Diagram of the epidermis indicating the different layers of the epidermis and other structural components of the epidermal barrier.

Table 1.1 Structural and functional components of the stratum corneum.

Components	Function	Location
Stratum corneum (SC)	Protection	Topmost layer of epidermis
Cornified envelope (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 the SC
Lamellar granules (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 bilayers
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
Natural moisturizing factor (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, and 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

Proteins of the cornified envelope

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 CE. 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 that is detectable histochemically in the granular cell layer and lower segments of

the SC. 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 nonpolar epidermal permeability barrier lipids, namely, cholesterol, free fatty acids (FFAs), 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 bilayer. It is these lipids along with the corneocytes that constitute the bulk of the water barrier property of the SC [4, 6].

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 SC to release the omega hydroxyl ceramide that gets cross-linked to the amino groups of the CE 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 [6, 7]. In addition, other FFAs 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-to-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 (SP), for the skin to shed in a process called desquamation [8].

Keratohyalin granules

Keratohyalin granules are irregularly shaped granules present in the granular cells of the epidermis, thus providing these cells the granular appearance (Figure 1.1). These organelles contain 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 SC is the formation of the epidermal permeability barrier [1, 4, 9]. SC limits the transcutaneous movement of water and electrolytes, a function that is essential for terrestrial survival. Lipids, particularly ceramides, cholesterol, and FFAs, together form lamellar membranes in the extracellular spaces of the SC that limit the loss of water and electrolytes. Corneocytes are embedded in this lipid-enriched matrix, and the CE, 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, 10, 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.

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

Mechanical barrier

CE 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 CE. 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. Microflora that normally inhabit human skin can contribute to the barrier defenses by competing for nutrients and niches that more pathogenic organisms require, by expressing antimicrobial molecules that kill or inhibit the growth of pathogenic microbes and by modulating the inflammatory response [13]. Desquamation that 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. 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. They contain common secondary structures that vary from α helical to β sheets, and their unifying characteristic is the ability to kill microbes or inhibit them from growing. Pathways that generate and regulate the antimicrobial barrier of the skin are closely tied to pathways that modulate the permeability barrier function. Expression of endogenous AMPs coincides with the presence of a number of epidermal structural components that may become part of the permeability barrier. For instance, murine cathelin-related antimicrobial peptide CRAMP and mBD-3 are essential for permeability barrier homeostasis. In addition, acute and chronic skin barrier disruption lead to increased expression of murine β -defensins (mBDs)-1, -3, and -14, and this increase in expression is diminished when the barrier is artificially restored [13].

NMF and skin hydration/moisturization

NMF is a collection of water-soluble compounds that are found in the SC (Table 1.4). These compounds compose approximately 20–30% of the dry weight of the corneocyte. Many of the components of the NMF are derived from the hydrolysis of filaggrin, a histidine-, and glutamine-rich basic protein of the keratohyalin granule. 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 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, 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 that contributes skin hydration. Glycerol is produced locally within 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 [14]. Other humectants in the NMF include urea, sodium, and potassium lactates and PCA [9].

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	% levels
Amino acids and their salts (over a dozen)	30–40
Pyrrolidine carboxylic acid (PCA) sodium salt, urocanic acid, ornithine, citrulline (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

Protection from environmental toxins and topical drugs penetration

The SC also has the important task of preventing toxic substances and topically applied drugs from penetrating the skin. SC acts as a protective wrap due to 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. 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 [8]. In addition, SC also contains protease inhibitors that keep these proteases in check and the balance of protease – protease inhibitors play 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 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 play specific roles in the desquamatory process at different layers of the epidermis.

Melanin and UV barrier

Although melanin is not typically considered a functional component of 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 melanin, 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 ultraviolet radiation is very damaging to proteins, lipids, and nucleic acids and causes oxidative damage to these macromolecules. The SC absorbs some ultraviolet 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 play a critical role in the decreased photocarcinogenesis seen in individuals with darker skin [15]. 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. Depletion of atmospheric “ozone layer” allows most energetic UV wavelength of sun radiation, i.e. UVC and short UVB to reach earth level. This high-energy UV radiation penetrates deep into papillary dermis. UVA radiation in addition to damaging 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 [16]. 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 [17]. 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 [18]. Skin naturally uses antioxidants to protect itself from photodamage. UV depletes antioxidants from outer SC. A gradient in the antioxidant levels (alpha-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 the SC from the oxidative stress [19]. Depletion of antioxidant protection leads to UV-induced barrier abnormalities. Topical application of antioxidants would support these physiological mechanisms and restore a healthy skin barrier [6, 20].

Regulation of barrier homeostasis

Epidermal barrier is constantly challenged by environmental and physiological factors. Since 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. Continuous renewal process is balanced by desquamation that 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 that 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 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. 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 that 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/ligands for the nuclear hormone receptors (example: PPAR – peroxisome proliferation-activated receptor and vitamin D receptor) that influence keratinocyte terminal differentiation are endogenous lipids synthesized by the 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 FFAs (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, as explained in the previous section, 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 physiological 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. Physiological factors can also have influence on barrier function. High stress (chronic as well as acute) increases corticosteroid levels and causes disruption of barrier homeostasis. During periods of psychological stress, the cutaneous homeostatic permeability barrier is disturbed, as is the integrity and protective function of the SC. Many skin diseases, including atopic dermatitis and psoriasis, are precipitated or exacerbated by psychological stress [24]. Circadian rhythmicity also applies to skin variables related to skin barrier function. Significant circadian rhythmicity has been observed in transepidermal water loss (TEWL), skin surface pH, and skin temperature. These observations suggest skin permeability is higher in the evening than in the morning [25]. 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.

Skin conditions and disease states

Epidermal lipid profiles and barrier architecture are altered in many common skin conditions. Atopic dermatitis, or eczema, is associated with significantly lower levels of ceramides with abnormal lipid organization, reduced barrier function, and skin hydration [26, 27]. Table 1.5 summarizes the changes in SC lipid composition and barrier function associated with common pathological skin conditions. The abnormal lipid profile and

Table 1.5. Changes in stratum corneum lipid composition and barrier function associated with common skin conditions.

Skin condition	Change in SC lipid composition	Effect of skin barrier function
Atopic dermatitis [26, 27]	Decreased total ceramide level	Reduced barrier function and hydration
Psoriasis [28, 29]	Decreased total ceramide levels in lesional skin compared to nonlesional skin Severity of psoriatic lesions correlated with a reduction in ceramide synthesis	Reduced barrier function and hydration
Acne vulgaris [30]	Reduced total ceramides	Reduced barrier function
Keratosis pilaris [31]	No change to lipid composition reported Disrupted epidermal lipid bilayers	Reduced barrier function

impaired barrier function contribute to the clinical symptoms of xerosis, disordered desquamation, and pruritus typical associated with eczema and psoriasis.

Neonates

Skin developments start *in utero* during the first trimester, with a well-defined SC appearing around 34 gestational weeks [32]. At birth, skin is covered in a hydrophobic biofilm called the vernix caseosa. The vernix layer forms during the third trimester and can be thought of as a “mobile phase” SC, composed of sebaceous and epidermal lipids combined with desquamation of maturing fetal corneocytes (~80% water, ~10% protein, and ~10% lipids). The vernix serves a protective role for the fetus and neonate, thought to assist with water loss, temperature regulation, acid mantle formation, and antimicrobial and antioxidant defenses [33, 34]. Infant skin is not fully mature at birth; its structure, composition, and function continues developing after birth and continues through the first years of life [32] (Table 1.6).

Physiological differences in infant skin reflect a developing barrier. This can leave children in a vulnerable state, which could be associated with the proneness for children to develop pathological conditions, such as atopic dermatitis. In recent years, several studies have explored the prophylactic use of emollients in reducing the incidence of atopic dermatitis in “high risk” infants, defined as those with a parent or full sibling with physician-diagnosed atopic dermatitis, asthma, or allergic rhinitis. In a pilot study with 124 newborns in the United States and the United Kingdom, daily emollient application

versus typical infant care without emollient application found a reduced cumulative incidence of atopic dermatitis (relative risk reduction of 50%) for the treatment group after six months [35].

Aging

Skin aging is characterized by profound functional changes. In the epidermis, these include reduced capacities for protection against mechanical and chemical insults, maintenance of hydration and osmotic balance, immunological defense, and toxin elimination [36]. While there are multifactorial etiologies for these changes, age-related deterioration of the skin barrier is a primary cause. Skin barrier abnormalities can be attributed, in large part, to reduced delivery of secreted lipids to the SC, and a resultant decrease in the number of extracellular lamellar bilayers it contains [37, 38]. Although these bilayers are healthy and intact, the extracellular matrix may be more permeable in aged than in the young epidermis. If aged skin becomes dehydrated or damaged, its dysfunction may be exacerbated and the barrier more easily compromised. Studies of TEWL demonstrate that the barrier of aged skin recovers significantly more slowly than young skin after damage from repeated tape stripping [37].

Hormones

Barrier homeostasis/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 alpha, beta, and gamma) and liver X receptor (LXR). The activators for these receptors are endogenous lipids and lipid intermediates or metabolites such as certain FFAs, 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.

pH and calcium

Outermost SC pH is maintained in the acidic range, typically in the range of 4.5–5.0 by a variety of different mechanisms. This acidity is maintained by formation of FFAs 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, plays a major role in maintaining the acid pH of the SC. Maintenance of an acidic pH in the SC is important for the integrity/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

Table 1.6. Summary of infant skin structure, composition, and function in comparison to normal adult skin [32].

Infant skin compared to adult skin	
<i>Skin structure</i>	
Corneocyte size	Smaller
Stratum corneum thickness	30% thinner
Dermal structure	Flatter dermal papillae No distinction between papillary and reticular dermis
<i>Skin composition</i>	
Water content	Drier at birth
NMF concentration	Lower
Surface lipid concentration	Lower
Melanin concentration	Lower
<i>Skin function</i>	
Barrier function/TEWL	Lower at birth
pH	Higher
Cell proliferation rate	Higher

Source: Data from Stamatas et al. 2011 [*Int J Cosmetic Sci*, **33**, 17–24.].

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 the neutral pH. Under conditions 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 [39]. The calcium gradient regulates barrier properties by controlling the maturation of the corneocytes, regulating the enzymes that process lipids, and modulating the desquamatory process. Calcium stimulates a variety of processes including the formation and secretion of lamellar bodies, differentiation of keratinocytes, formation of CE 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 CE, 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. Disruption of permeability barrier leads to activation of cytokine cascade (increased levels of primary cytokines, interleukin-1, and tumor necrosis factor- α) which in turn activates the synthesis of antimicrobial peptides of the SC. In addition, 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 that release the free amino acids that form the NMF (histidine, glutamine arginine, and their byproducts) and tUCA (deamination of histidine) that serves as UV barrier.

Methods for studying barrier structure and function

Physical methods

SC integrity/desquamation can be measured using tape stripping methods. Under dry skin conditions, when 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 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 or TEWL) can be determined using an evaporimeter [40]. 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 epidermal barrier (TEWL) after barrier disruption using physical methods (e.g. tape strips) or chemical methods (organic solvent washing) provides valuable information on the epidermal barrier properties [41].

Skin hydration can be measured using Corneometer®. The measurement is based on capacitance of a dielectric medium. Any change in the dielectric constant due to skin surface hydration variation alters the capacitance of a precision measuring capacitor. The measurement can detect even slightest changes in the hydration level. Another important recent development in skin capacitance methodology is using SkinChip®. Skin capacitance imaging of skin surface can be obtained using SkinChip. This method provides information regarding 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 [42].

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 [43]. Other more sophisticated (although not easily portable) instrumentation techniques such as ultrasound, optical coherence tomography, and the magnetic resonance imaging (MRI) can provide useful information on internal structures of SC/epidermis and its improvements with treatment. MRI has been successfully used to evaluate skin hydration and water behavior in aging skin [44].

Biological methods

Ultrastructural details of SC and the intercellular spaces of the SC can be visualized using transmission electron microscopy (TEM) of thin vertical sections and freeze-fracture replicas, field emission scanning electron microscopy (SEM), and

immunofluorescence confocal laser scanning microscopy [45]. The ultrastructural details of the lipid bilayers within the SC can be visualized by EM after fixation using ruthenium tetroxide. The existence of corneodesmosomes in the SC and their importance in desquamation can be measured by scanning electron microscopy (SEM) 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 detergent solution. The suspension so obtained can be analyzed by microscopy, biochemical, or immunological techniques.

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

Relevance of skin barrier to cosmetic product development

Topical products that influence barrier functions

The human skin is constantly exposed to hostile environment. These include 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. 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.

In particular, cleansing products including alkaline soaps and synthetic detergents are associated with the disruption of the skin barrier. Soaps, the alkaline salts of fatty acids, leave the skin dry and feeling tight. This is attributed to their high pH and protein binding leading to swelling and hyperhydration that quickly evaporates leaving behind a dehydrated and damaged barrier. In addition to efficiently cleaning the skin's surface, soaps also remove NMF epidermal lipids, damaging the skin barrier. Synthetic surfactants typically have a lower pH and range in degree of harshness. Surfactants have the potential to bind to and denature proteins and remove both NMF and lipids like soaps; however, they are a diverse class of ingredient and the degree to which a synthetic surfactant disrupts the barrier is associated to its properties such as charge density and pH [46, 47].

Cosmetics that restore skin barrier properties

Water is the most important plasticizer of SC. Cracking and fissuring of skin develop 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. Moisturizers affect the SC

architecture and barrier homeostasis, that is, topically applied ingredients are not as inert to the skin as one might expect. A number of different mechanisms behind the barrier-influencing effects of moisturizers have been suggested, such as simple deposition of lipid material outside the skin. Ingredients in the moisturizers may also change the lamellar organization and the packing of the lipid matrix and thereby change skin permeability [48]. 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 either or 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 [49]. A novel, cholesterol-dominant topical formulation containing high concentrations of ceramides (2%), natural cholesterol (4%), and omega fatty acids (2%) has established its ability to improve barrier function and specific indices of skin aging. This novel cholesterol-dominant physiological lipid formulation demonstrated significant improvements in clinical signs of aging including skin tone and texture, radiance, clarity, fine lines and wrinkles, firmness, laxity, appearance of pores, and global appearance [50]. Lipid analysis showed that the same formulation induced significant increases in the epidermal content of total ceramides, cholesterol, and triglycerides after eight weeks of treatment. Furthermore, in a clinical model of skin barrier repair, where the formula was applied for one week prior to skin injury by tape stripping, skin treated with the formulation achieved significantly more rapid and greater recovery of barrier function than untreated skin [50].

A complementary approach is the topical application of ingredients that stimulate the *de novo* synthesis of epidermal lipids. The daily application of nicotinamide, also called niacinamide, increased the level of ceramides and FFAs in skin and improved skin's water barrier [51]. 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 [6, 20].

Topically applied substances may penetrate deeper into the skin and interfere with the production of barrier lipids and the maturation of corneocytes. Creams may influence the desquamatory proteases and change the thickness of the SC. The increased understanding of the interactions between topically applied substances and epidermal biochemistry will enhance the possibilities to tailor skin care products for various SC abnormalities [48].

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 skin 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 due to its increased penetration into deeper layers of the skin.

Summary 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 the SC and has characterized the major components and their importance in providing protection for the organism 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 are an area being actively researched.

New knowledge in 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 into barrier homeostasis mechanisms.

Novel delivery systems play 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.

Interest and research on the relationship between skin conditions and barrier functions are expected to grow, as existing and ongoing research suggests the barrier to be a relevant therapeutic target for helping to address the symptoms, and potentially the development, of pathological skin conditions.

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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