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Skin Barrier Insights: From Bricks and Mortar to Molecules and Microbes

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ABSTRACT

Recent advances in genomics, spectroscopy, and immunology have increased our understanding of the skin barrier. A new model of barrier lipid organization has emerged owing to the application of advanced modeling and microscopy techniques. The contribution of filaggrin gene mutations to atopic dermatitis has increased our appreciation of the role barrier perturbations play in inflammatory dermatoses. Next generation sequencing techniques have led to a greater understanding of the diversity of resident skin microorganisms and the close association between microbes and the host immune system. This paper reviews the basics of stratum corneum structure and function, with an emphasis on recent advances in our understanding of barrier perturbations and their effect on skin health.

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INTRODUCTION

The skin has evolved a unique structure that is essential for survival; the stratum corneum (SC). The SC prevents excess water loss and forms a formidable barrier to the ingress of microorganisms and exogenous materials. Morphologically, the SC is comprised of corneocytes interspersed in lipid bilayers, the so called "brick and mortar model".[1] However, the analogy suggests that the SC is an inert, unchanging structure and fails to convey the extent to which metabolism and remodeling continue as the corneocytes transit from the base of the SC to the surface. The explosion in microbiome research has demonstrated that the SC harbors a rich diversity of microbes[2] that form an additional barrier to the colonization of pathogens. Moreover, the microflora communicates with and *directs* the host immune system and can be considered an integral part of the skin's immunity. The intimate association of microbes and skin is such that, perhaps, the bricks and mortar analogy should be updated to include skin's microbiota as a third component, wherein the microbes form the shingles on the roof of a bricks and mortar house.

Stratum Corneum Formation

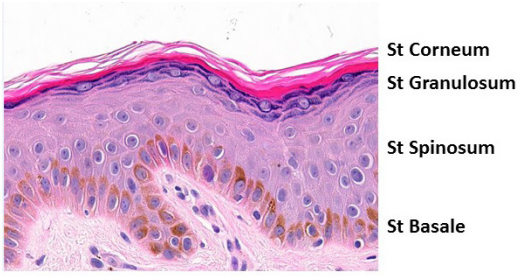
The epidermis has several distinct layers. At the dermal-epidermal junction sits the basal layer of proliferating keratinocytes. Within this layer reside both epidermal stem cells and transit amplifying cells that are destined to detach from the basement membrane and begin the process of terminal differentiation. As keratinocytes differentiate, they enlarge and progressively flatten. This progression is accompanied by a bewilderingly complex set of changes in the expression of specialized proteins and lipids. Terminal differentiation culminates in the transition of the granular layer cells to corneocytes, anucleate, and proteinaceous sacs, surrounded by a lipid envelope, and interspersed in lipid lamellae. This amazing conversion from viable keratinocyte to corneocyte occurs in a highly ordered fashion within the space of *one cell layer*, cued by mechanisms that are still not clearly understood (Figure 1). It is truly a remarkable transformation.

Cornified Envelope

Keratins are cytoskeletal proteins that form intermediate filaments and serve as the scaffolding for cornified envelope (CE) formation. Commitment to differentiation is evidenced by a switch to a predominance of K1 & K10 in the spinous layer where the synthesis of proteins, such as involucrin, also begins. In the granular layer, these proteins are crosslinked into the CE by the action of transglutaminases. Specialized lipids are secreted into the extracellular space and will form the lipid coat that surrounds each corneocyte, the cornified lipid envelope (CLE). The stratum granulosum (SG) is so-named because of the presence of keratohyalin granules (KHG) containing loricrin and small proline-rich proteins (SPR), which further reinforce the CE. KHGs also contain filaggrin. This protein aggregates keratin filaments and promotes the collapse of the keratinocyte. The lamellar granules (LG) are also extruded from granular cells releasing their precious cargo of lipids and proteins.

As the keratinocyte transitions to a corneocyte, nuclei, intracellular organelles, and plasma membrane components are degraded. Desmosomes, the molecular rivets between adjacent keratinocytes, are converted to corneodesmosomes (CD) by the addition of corneodesmosin[3] strengthening cell:cell adhesion. Maturation continues as the corneocyte transits through the SC. Crosslinking of proteins such as loricrin, trichohyalin, and SPRs into the CE continues and this facilitates the switch from a "fragile" to a "rigid" phenotype.[4] In dry or photoexposed skin, the ratio of rigid:fragile in the outer SC is decreased indicat-

FIGURE 1. H&E stained section of epidermis.



ing the presence of immature corneocytes.[5] Desquamation is the loss of corneocytes from the surface at a rate that balances (ideally) the rate of proliferation, and CD degradation is crucial for proper desquamation. In the lower SC, CDs may be found on all sides of the corneocyte, however, in the upper half, many CDs have been degraded and only peripheral CDs persist. CD degradation requires the activity of proteases and the kallikreins, a family of serine proteases, are the key enzymes in CD degradation.[6,7]

The activity of these degradative enzymes needs to be tightly controlled to prevent premature or over-activation and that is accomplished, in part, by enzyme inhibitors that are synthesized in and secreted by LG. Dysregulation of enzyme:inhibitor balance has been reported in phenotypes as distinct as dandruff and peeling skin disorders.[8,9] The protease inhibitor, lymphocyte-epithelial Kazal-type related inhibitor (LEKT1) maintains a block on the activity of several KLKs, dissociating as the SC acidifies.[10] Netherton syndrome, a rare autosomal recessive mutation in the gene encoding LEKT1, is characterized by overactivation of KLKs, excessive desquamation, and barrier defects. Tight junction (TJ) proteins were identified in close association with CDs[11] and it was hypothesized that TJ may limit access of proteases to CDs thus controlling the site-specific degradation of CD.[12]

Filaggrin proteolysis also takes place in SC[13] and generates hydrophilic amino acids that are key components of the skin's natural moisturizing factor (NMF).[14] Water is an absolute requirement for the activity of enzymes thus the water-holding capacity of SC is critical in supporting the activity of SC enzymes. Xerosis and scaling disorders such as icthyosis vulgaris (IV) and atopic dermatitis (AD) are characterized by defects in NMF production leading to reduced hydration and desquamation and impaired barrier function. Mutations in the filaggrin gene are a strong risk factor for development of IV and AD.[15-17] Because of the compromised barrier, these individuals are more susceptible to entry of allergens that may contribute to prolonged inflammation. However, some individuals who are homozygous for loss-of function mutations exhibit no symp-

FIGURE 2. Ceramides consist of a sphingoid base bonded to a fatty acid via an amide linkage. Ceramides are denoted by "Cer-fatty acid-sphingoid base". The sphingoid base can be either sphingosine (S), 6-hydroxy sphingosine (H), dihydroxy sphinganine (T), dihydrosphingosine (dS), or phytosphingosine (P). The fatty acid chain is denoted as non-hydroxy (N), α-hydroxy (A), ω-hydroxy (O). ω-Hydroxy ceramides contain an additional fatty acid, usually linoleic acid, esterified to the fatty acid denoted by "E".

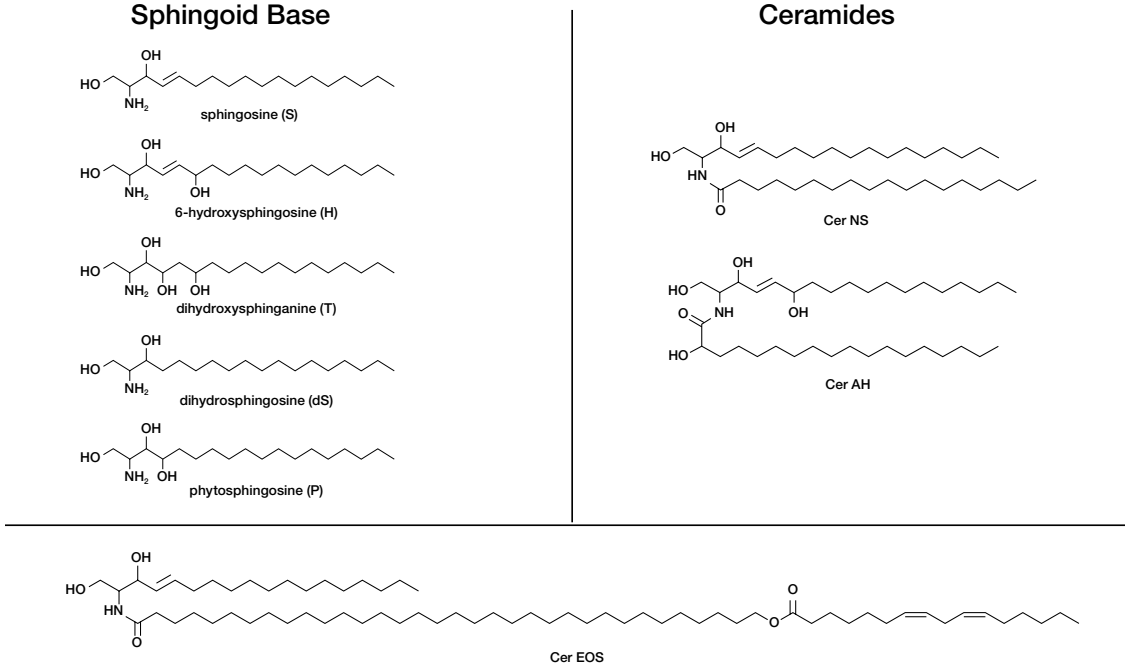
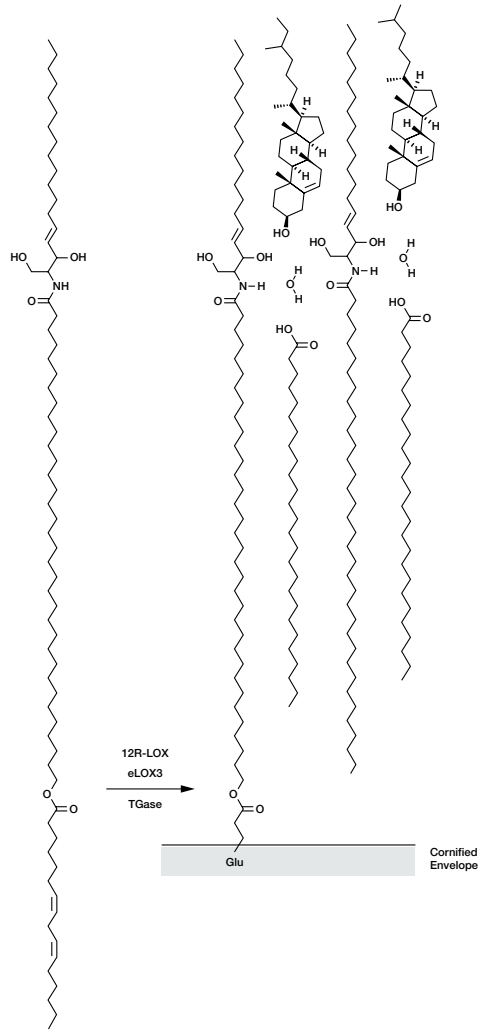


FIGURE 3. Schematic of lamellar lipids demonstrating the organization of ceramides (Cer), cholesterol (Chol), and free fatty acids (FFA).[43] Ceramides are represented in the splayed conformation. Very long chain ω-hydroxy ceramides are enzymatically processed prior to incorporation into the cornified envelope.



toms suggesting that other factors are also important in disease progression.[18] Furthermore, filaggrin expression is downregulated in *all* AD patients, even those with no demonstrable filaggrin gene mutations,[19] and this may be secondary to the enhanced Th2 cytokine milieu that suppresses the expression of differentiation-associated proteins.[20,21] Moisturizers can help restore enzyme activity by supplying humectants, to augment skin's natural moisturizing systems, and occlusives, to retard water loss thus promoting desquamation and reduced scaling.

Lamellar Lipids

Lamellar lipids are stored as precursors in LG and secreted at the interface of the SG and SC.[22] Enzymes that process these lipids to mature forms are also packaged in and secreted by LG.[23] Comprised primarily of ceramides, cholesterol, and fatty acids, (in an approximate molar ratio of 1:1:1) with small amounts of cholesterol sulfate and cholesterol esters, the extracellular lipids form a highly ordered structure that resists the flux of water. The effectiveness of this lipid matrix is highly dependent on the relative proportions of the major lipid classes[24] and is altered in xerosis,[25,26] aging,[26,27] and AD.[28,29] The structure of the individual lipid species also affects barrier quality. Ceramides and free fatty acids with longer acyl chains tend to form highly ordered, orthorhombic phases while shorter acyl chain variants form less ordered hexagonal phases.[30] The acyl chain length of SC ceramides in AD is shorter and correlates with decreased lipid order and barrier function.[31] Lipid order as measured by FTIR is also significantly decreased in dry skin subjects.[32]

Perhaps as many as 1000 species of free ceramides and corneocyte-bound ceramides have been identified in SC[33-35] (Figure 2). While most ceramide-derived fatty acids are saturated with acyl chain lengths of between 16 and 32 carbons, there exist some very long carbon chain lengths (30 to 34) that are particularly important in SC function[36]; these are the ω-hydroxy ceramides that contain the unsaturated essential fatty acid (EFA), linoleic acid, esterified to the fatty acid. It is the ω-hydroxy ceramides that become covalently bound to the CE forming the CLE and act as scaffolding for the free ceramides that comprise the intercellular lipid bilayers. Two lipoxygenases process the ω-hydroxy ceramides prior to incorporation into the CLE.[37] Lipoxygenases are usually associated with arachidonic acid metabolism and inflammation. However, 12RLOX and 3eLOX3 sequentially oxidize the linoleic acid moiety and this processing is required for ester formation between ceramide and CE proteins (Figure 3). Ultimately degradation of ceramides may also be required for reduced cohesivity and desquamation and this is accomplished by the action of acid and alkaline ceramidases in the SC.[38]

A novel model of SC lipid organization was proposed by Norlen and colleagues in which the ceramides are fully extended or "splayed'[39] (Figure 3) and this conformation was confirmed by infrared spectroscopy.[40] The proposed structure contrasts with the previously described sandwich model in which ceramides were represented in a folded position.[41]

The question of how fatty acids contained in moisturizers and cleansers interact with SC and affect lipid organization is pertinent. Bouwstra and colleagues demonstrated that topically applied C16 fatty acids are elongated to C24 and 26. Furthermore, these fatty acids integrated into the lamellar structure and increased lipid order.[42] This suggests that topical application of the appropriate fatty acid can be used to improve the permeability barrier in compromised skin.

Acid Mantle

The pH of healthy SC is acidic and several potential acidifying mechanisms have been identified.[43] Acidification is important

for antimicrobial defense, enzyme activity, and cytokine activation. Elevated SC pH is associated with perturbations in barrier function as many enzymes involved in lipid metabolism and CD degradation require an acidic environment.[44] Elevated pH is also associated with shifts in the microbial composition toward potentially pathogenic organisms.[45] These findings provide support for use of acid-neutral pH cleansers and moisturizers in the prevention and treatment of barrier deficiencies.[46]

Microbiome

The skin harbors a diverse population of microbes whose composition is largely determined by site-specific factors such as moisture and sebum content.[47] The skin's invaginations and appendages harbor a large number of microorganisms but microbial DNA has also been found deep within the dermis.[48] This surprising finding challenges many existing concepts of skin, its microflora, and the mechanisms by which the host immune system is educated by the microbiome.

The SC provides a formidable barrier to microbial colonization due to the physical barrier, low water and nutrient content, acidic pH, and antimicrobial lipids (AML) and peptides (AMP). Sphingosine and free fatty acids are potent AMLs and are found in ample concentrations in SC.[49] AMPs are a diverse, yet highly conversed set of proteins belonging to the host innate immune system that kill microbes primarily through disruption of the cell membrane. The predominant AMPs of skin belong to the cathecidin and defensin classes and are delivered to the SC via LGs or glandular secretions.[50] However, microbes can also produce AMP-like proteins. *S. epidermidis* produces AMPs that inhibit colonization of *S. aureus,*[51] however strain variation is significant, and the commensals in some AD patients lack the ability to produce AMPs selective for *S. aureus*.[52] Microbial AMPs also synergize with host AMPs, thus the secretome of commensals could be viewed as an integral part of most innate defense system.[53]

There exists a complex interplay between the microbiome and the host immune system. The innate and adaptive arms of the immune system modulate the composition of the microbiome and in turn, the microflora communicates with and directs the host immune response.[54] *Propionibacteria acne*s induces expression of AMPs and inflammatory cytokines in keratinocytes in a toll-like receptor-dependent (TLR) fashion.[55] Staphylococcal lipoteichoic acid blunts inflammation via interaction with TLR3 and stimulates wound healing.[56] These are but a few examples that illustrate how the microflora directly modulates both the innate and adaptive arms of the host immune response. The complexity of this interaction suggests a coevolution of host and microbes and this balance may be disrupted by our rapidly changing environment; diet, hygiene, and antibiotics could affect the microbiome at a faster rate than the microbiota can adapt. This has led some researchers to speculate that these changes are responsible for the dramatic increase in the incidence of inflammatory diseases such as AD.

Barrier perturbations can drive changes in the skin's microbiota, ie, dysbiosis. There is now a strong recognition that barrier dysfunction is a driving element in many inflammatory skin disorders and not merely a bothersome sequela. Most notably patients with AD tend to be at increased risk of infection and colonization by *S. aureus*.[57] In contrast to the many microbiome studies on AD patients, definitive studies on microbial changes that might accompany cosmetic dry skin are yet to be published.[58] On the other hand, mild cleansers have been shown to help restore normal flora.[59] The use of mild cleansers that protect the skin's barrier lipids[60,61] would be expected to help maintain a "healthy" microbiome.

CONCLUSIONS

Barrier impairment, whether the result of genetic or environmental influences, increases the flux of water out of and exogenous materials in to the skin. The consequences of this increased permeation will depend, in large part, on the host response to that stimulus. In some instances, the host response can further degrade barrier function leading to a vicious cycle of stimulus and response. Optimizing a skin care regime to support the skin's own barrier repair mechanisms is an integral part of any successful therapy. Continued research efforts are necessary to provide the scientific foundation for identifying new therapies and optimal skin care regimes.

DISCLOSURES

Carol Bosko is an employee of Unilever.

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