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Recent Advances in Skin Barrier Research: From Basic Science to Clinical Discovery

Qian Zheng MD PhD, Charbel Bouez PhD

L'Oréal Research and Innovation, Clark, NJ

The human skin barrier, as the first line of defense against external aggressors and the essential modulator of our body's internal homeostasis, has been studied extensively. During the past few years, with the emergence of new discoveries and scientific evidence, this field has been updating in a highly dynamic manner. The shift of biochemical compositions, especially epidermal ceramides and essential lipids, the alteration of key biomarkers under environmental damage, and the clinical manifestation of compromised skin barrier (eg, xerosis, atopic dermatitis, etc) are all at the center stage for investigation. More importantly, it has become extremely critical to provide effective prevention and treatment routines for patients of all skin types who are suffering from impaired skin barrier.

Sun exposure, as one of the most impactful environmental aggressors on human skin, poses significant risks for skin health; For example, photoaging, skin pigmentary disorders such as melasma, as well as UV-induced skin cancers (eg, basal cell carcinoma, squamous cell carcinoma, and melanoma). Its impact on viable epidermis, melanocytes, and dermal cells and extracellular matrix has long been studied. However, the direct impact of sun exposure on skin barrier disruption is yet to be fully elucidated. This will remain an important topic in skin barrier-related research programs.

This JDD supplement is aimed at providing the most recent updates in skin barrier discovery for both healthy and pathological conditions, summarizing new developments in the evolution of the understanding of barrier ultrastructure, novel scientific models for studying skin barrier, compromised skin barrier and sensitive skin in diverse population, signaling pathways, and clinical representations of barrier damage and restoration. There is a focus on demonstrating the direct impact of UV on barrier alteration and providing evidence on the efficacy of barrier restoration through ceramide-containing sunscreen formulations. Ultimately, it is essential for the personal care industry to collaborate with medical practitioners to provide effective and complementary long-term care strategy for patients seeking barrier health and overall skin quality improvement.

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ORIGINAL ARTICLE

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Evolution of Skin Barrier Science for Healthy and Compromised Skin

Marek Haftek MD PhD,[a] Daniel C. Roy PhD,[b] I-Chien Liao PhD[b]

[a]CNRS UMR5305 LBTI - Laboratory for Tissue Biology and Therapeutic Engineering, Lyon, France

[b]L'Oréal Research and Innovation, Clark, NJ

ABSTRACT

Skin is a complex organ comprised of multiple cell types and microstructures that work in concert to serve critical functions and support the body's homeostasis. It is the outermost, cornified layer of our body that is primarily responsible for the permeability barrier, protecting against external aggressors and preventing water loss from within. The understanding of the organization, functionality, and underlying mechanisms of the skin barrier has evolved greatly through the years. The formation of an intact and well-maintained stratum corneum (SC), where the permeability barrier resides, relies heavily on the differentiation of epidermal keratinocytes and the synthesis, release, localization, and binding of lipids that include principally ceramides, cholesterol, and free fatty acids. The in-depth research on SC barrier, its disruption in the pathogenesis of diseases, as well as on barrier responses to environmental insults, has enabled the development of modern therapeutics and topical care routines. Among them, ceramide-containing moisturizers have clinically demonstrated the ability to support the management of skin conditions such as atopic dermatitis and psoriasis by reducing the disease severity and recurrence and improving the patients' perception of overall skin quality and health. This review focuses on the contributions of various barrier constituents to skin barrier function in health and pathological conditions, and how topical interventions containing essential barrier lipids support barrier restoration and provide relief.

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INTRODUCTION

Organization and Function of Epidermis Providing an Efficient Skin Barrier

The epidermis maintains its homeostasis and serves critical functions through a dynamic, self-renewing process in which the basal keratinocytes divide and migrate through the stratum spinosum and granulosum while progressively differentiating (Figure 1). When the keratinocytes reach the top of the granular layer, the process of terminal differentiation occurs in which the keratinocytes undergo programmed cell death and flatten out to form the stratum corneum (SC).[1] During this process, the lamellar bodies of granular layer keratinocytes merge with the plasma membranes and release their predominantly lipid contents into the intercellular spaces of the nascent horny layer. An interplay of hydrolytic enzymes and their inhibitors, also excreted via the lamellar bodies, participate in elaboration of the intercellular layered lipid structure and, ultimately, are involved in cell desquamation at the top of the skin.[2] Simultaneous to the extracellular lipid build-up, important changes occur within the keratinocytes upon the formation of SC. Transglutaminase-1 –mediated cross-linking of cytoplasmic proteins at the cell periphery results in the formation of highly insoluble cornified envelopes of the SC cells, thereafter called corneocytes.[3] It is followed by a covalent binding to these structures of a monolayer of ceramides, replacing phospholipid plasma membranes of the living cells. These newly formed cornified lipid envelopes constitute the scaffold for further stacking and organization of the intercellular lipids. The composite structure of the SC, made of corneocytes intercalated by polar lipids, can be compared to a brick and mortar wall constituting SC permeability barrier.[4]

In order to perform its function as a permeability barrier, the epidermis must remain mechanically resistant while sufficiently flexible to accommodate skin movements and the treadmill-like flow of keratinocytes through the successive layers. Cell-cell and cell-substrate junctions play central roles in the maintenance of mechanical properties of the epidermis. Desmosomes, which interconnect individual cell cytoskeletons into a superstructure, evolve throughout the epithelial tissue, and change their location, protein composition, and glycan distribution according to the stage of the cell differentiation and the occurrence of mechanical constraints.[5–7] In this process, actin cytoskeleton-bound adherens junctions participate in the dynamics of desmosome and tight junction expression. Upon the SC formation, these junctions become cross-linked to cornified envelopes and contribute to the enhanced physical resistance of the functional SC barrier.[8] Mechanical properties of the SC show a significant increase in stiffness between the deep and superficial corneocytes.[9] The mechanical integrity of the SC also depends on the direction of the applied shearing forces since lateral, side to side adhesion between the cells is stronger compared to that between the successive corneocyte layers.[9–12]

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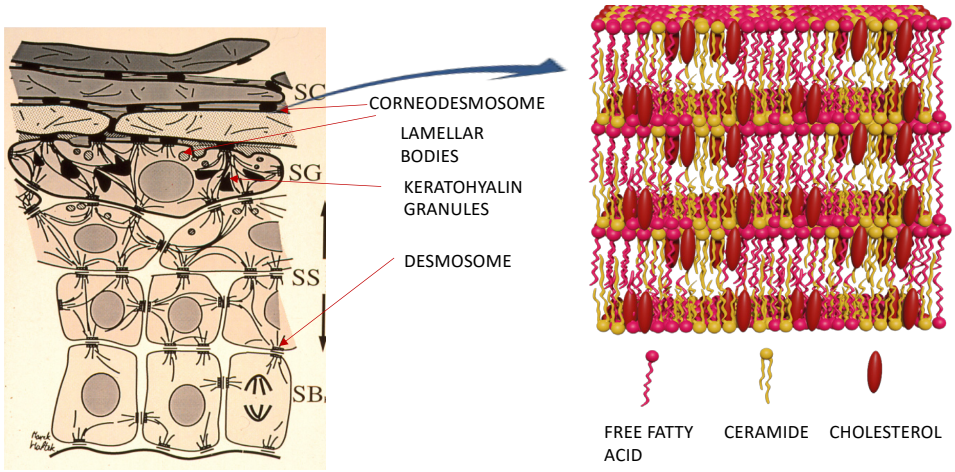
FIGURE 1. Schematic anatomy of the epidermis and its stratum corneum.



SB: *stratum basale*; SS: *stratum spinosum*; SG: *stratum granulosum*; SC: *stratum corneum*.

The relative impermeability of the SC and, thus, its barrier function, rely essentially on the intercellular lipids, even though they account for only 15% of the SC weight. Quasi equimolar proportions of ceramides, cholesterol, and free fatty acids appear to be prerequisite for the correct auto-assembly of the intercellular lipid multilayers within the SC. The composition of the lipids of the SC further subdivides into free fatty acids (FFA; 10%), cholesterol (CHOL; 27%), cholesterol esters (10%), cholesterol sulfate (3%), and ceramides (CER; 50%).[13] These lipids organized in multiple bi-layers parallel to the corneocyte surfaces may assemble within the layers into domains presenting different densities. A dense orthorhombic lateral packing of lipid molecules and a more fluid hexagonal format predominate in normal human skin.[14] Efficient filling of SC interstices is essential for preventing excessive water loss and penetration of environmental contaminants/aggressors.

Ceramides are structurally heterogeneous sphingolipids that can be classified by their molecular structures and their polarity into 12 classes of unbound ceramides and 3 classes of covalently bound ceramides.[15] Names of these families of molecular structures reflect the differences in their (i) sphingoid bases (S: Sphingosine, DS: Sphinganine, and P: Phytosphingosine) and (ii) acyl chains (N: Non-hydroxy FA, A: α-hydroxyl FA, EO: esterification of ω-hydroxyl FA with linoleic acid, and O: ω-hydroxyl-FA). Within the different classes of ceramides, CER [NP] (22%), CER [NH] (14.5%), CER -[H] (10.8%), AS (9.6%), CER [NDS] (9.8%), CER [AP] (8.8%), and CER [NS] (7.4%) compose the majority of the free and bound ceramides.[16,17] Free FA chain length is most commonly 18, 22, or 24 carbon atoms. The differences in chain length and the different subclasses of ceramides are regulated by different biosynthesis pathways (*de novo*, sphingomyelinase, and salvage via late endosomes) and are subjected to change in various skin disease conditions.[18] In particular, the dynamic changes to ceramides CER[EOS], CER[NP], and CER[NP] in atopic dermatitis and psoriasis patients are clues for the design of different product options to alleviate the symptoms at the lesional skin sites.[13] In addition to their crucial structural functions within the SC, ceramides are also able to influence keratinocyte differentiation and apoptosis. Glycosylated, short, and long chain ceramides, all have been demonstrated to enhance differentiation of keratinocytes. This suggests an additional explanation of how ceramides improve the barrier function: through influencing the proliferation/differentiation balance within the living epidermal layers, resulting in enhanced formation of SC.

New Players in the Epidermal Barrier Function

Since the middle of the past century, the views on the place of the horny layer in epidermal biology have changed dramatically. SC has ceased to be considered not more than a kind of Saran® wrap and acquired the status of a complex and highly interactive biosensor.[19] The fundamental role of the intercellular lipids for the SC relative impermeability has been put forward and elaborated upon by various groups.[20] Peter Elias' 'brick and mortar' concept of the barrier has been widely accepted and studied in detail using various physical-chemical-structural and experimental approaches, each contributing to a better understanding of the barrier function.[21–25] As more studies have emerged, it has become clear that the establishment and maintenance of a healthy skin barrier relies on coordinated processes from keratinocyte proliferation to desquamation that must constantly adapt to the environmental conditions (for in-depth reviews, please consult "Skin barrier", Elias & Feingold, Eds., 2005).[26]

The initially ignored epidermal tight junctions (TJ) and their structural remnants that persist in the SC were shown

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to contribute to the barrier's natural development and degradation.[1,27–29] Indeed, in human epidermis, occlusive TJ are present in the upper stratum granulosum (SG) but appear to be expressed in a patchy pattern, usually not fully circumventing the flattened cell outlines. Nevertheless, these TJ strands, most frequently encountered in the last three living cell layers are able to hinder the outward penetration of tracers experimentally applied to the dermal side of the skin. As TJ expression coincides with the apically oriented migration of lamellar bodies, it may be speculated that epidermal TJ contribute to the SG cell polarization.[30] All the transmembrane and cytoplasmic proteins necessary for formation of functional TJ are present in the SG and the functional junctions may be created instantly, eg, in case of acute abrogation of the principal permeability barrier in the SC.[31] In human skin, TJ may thus participate in a regulatory mechanism of SC barrier formation and constitute an instantly available backup system when the SC barrier fails. The fact that these riveting structures become immobilized at the cell periphery during the process of cornification further underlines their importance for the SC barrier homeostasis. Increased number of TJ-like contacts may be observed in the SC after chemical challenge or in pathologies provoking abnormal SC formation, thus indicating a possible compensatory effect.[32]

Most recently, various signaling pathways involved in the epidermal development and maturation continue to be studied and still new molecular mechanisms contributing to normal and pathological barrier function are being discovered.[33–37] A new exciting field of investigation concerns epigenetic regulation of the homeostatic mechanisms of epidermal proliferation/differentiation leading to the barrier formation. Involvement of the non-coding micro-RNAs and lncRNAs in stabilization of these processes through modulation of the gene transcription adds a supplementary level to the complex mechanisms of the barrier control.[38] Together, these findings highlight the dependence and synergy between different processes and behaviors within the epidermis to create a healthy, intact skin barrier. As such, irregularities to intrinsic mechanisms of the epidermis (eg, keratinocyte differentiation or tight junction formation), as well as SC disruptions through external means can trigger a chain of events that lead to prolonged barrier disorders.

Many of the data on molecular mechanisms underlying epidermal barrier function have been obtained using rodent models, either submitted to acute barrier disruption and/or bearing laboratory-induced genetic modifications. In many instances, conclusions drawn from these experiments remain fully valid as far as human skin is concerned. Nevertheless, the existing notable differences in skin morphology and physiology between the species make rather controversial some animal-derived observations. Human pathology, instead, provides a wide spectrum of situations where defined gene mutations result in abnormal expression of skin barrier's constitutive or regulatory elements.[8] These correlations may be advantageously exploited for a better understanding of the permeability barrier function and be a source of ideas for therapeutic intervention.[37,39]

Epidermis, Compromised Barrier, and Disease

Epidermal impairment can result from acute injury or exposure, or be linked to lifelong, chronic conditions that require daily attention. Virtually all dysfunctions of the epidermis, whether inborn or acquired, are associated with notable modifications of the permeability barrier. It is particularly evident in dermatoses with an important inflammatory component.[40,41] In many cases, barrier dysfunction may be at the origin of a skin disease, like it is the case in atopic dermatitis (AD), and contributes to the vicious circle of a given pathology via induction of an inflammatory response.[35,40] Deficient expression of an epidermal protein filaggrin, due to the loss-of-function gene mutations, has been found responsible for AD occurrence in up to 50% of the northern European cases.[42] Filaggrin is elaborated in the granular layer keratinocytes and its catabolic processing in the SC leads to the abundance of hydrophilic amino acids constituting the bulk of so-called natural moisturizing factor (NMF).[43] Absence or a marked reduction of the NMF compromises SC hydration and, thus, barrier function. Interestingly, the same filaggrin mutations present on both gene alleles result in ichthyosis vulgaris phenotype, most frequently associated with atopy. In the case of ichthyosis, the epidermis must compensate for the leaky barrier by hyperkeratosis. Accumulation of the corneocytes is likely promoted by a particularly low degree of SC hydration, possibly impeding activity of hydrolytic SC enzymes.[25] This putative mechanism could overdrive the desquamation -favorable context of serine protease activation due to a more basic (optimal) intracellular pH in the amino acid-deficient tissue.[44] Nano-mechanical and ultrastructural investigations of elastic properties of filaggrin deficient corneocytes demonstrate a significant reduction in the cell stiffness and a delayed degradation of corneodesmosomes, both being potential indicators of SC functionality.[45,46] In addition to an alteration of filaggrin expression, AD epidermis also exhibits a significant reduction in key TJ proteins and, most importantly, ceramides, including CER1[EOS].[13,14] Regarding the changes to ceramides, their decreased levels and shortening of their acyl chains have been observed in non-involved skin of AD, independent of filaggrin mutations, which may have etiologic significance. Altered ceramide expression levels and both their lamellar and lateral organization correlate with the disease activity (SCORAD).[14] Even more depressed ceramide levels, mainly CER[EOS], CER[NP], and free sterols, have been reported in AD lesions, with concomitant increase of sphingosine (CER[S]) and sphinganine (CER[DS])-based ceramides.[13] The observed changes may be due to modifications in pH and inflammatory cytokine-sensitive enzymes involved in lipid biosynthesis

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and processing, thus opening potential new windows for pharmacologic intervention.

In psoriasis, inflammatory skin lesions induced by interleukin 23-recruitedTh17 lymphocytes are characterized by keratinocyte hyperproliferation and incomplete terminal differentiation leading to inefficient permeability barrier function.[47] Although the immune cell subsets and cytokines involved in AD and psoriasis pathogenesis differ notably, the deleterious vicious circle of barrier disruption/inflammation is still present in the latter. The incomplete terminal differentiation of psoriatic lesional keratinocytes is induced byT-lymphocyte mediated skin inflammation, which has significant impact on the ceramide expression compared to normal or non-involved skin.[13] Similar to AD, in psoriasis lesions, ceramide species show shorter fatty acid chains and the reduced levels of CER[EOS], CER[NP], CER [EOH]. CER [AS] and CER [AP].[48] Clinical observations of improvement of psoriasis vulgaris lesions under simple occlusion and of AD lesions with topical emollient therapy alone clearly indicate that restoration of / compensation for the SC barrier helps to interrupt the vicious circle of pathogenic self-propagation.[49,50] Medical doctors were first to study the question given the abundance of clinical examples, including rare dermatological syndromes, and the impact of the barrier integrity on disease history, and often, patients' fate, eg, in severe burns or generalized blistering diseases.

Environmental Stressors

In order to perform its protective functions, epidermis must adapt continuously to the changes in environmental conditions. These encompass climate/season–related factors such as relative humidity, ambient temperature, and sun exposure, as well as environmental aggressions due to the wide-spread use of chemicals, presence of atmospheric pollutants and changes in the composition and importance of skin surface microbiota, the latter being largely related to the aforementioned factors.

Prolonged natural ultraviolet (UV) radiation induces increased epidermal and perifollicular keratinization, resulting in flares in patients suffering from acne, that occur after discontinuation of inflammation-suppressing sun baths. Instead, acute, high-dose exposure to UVB, and also UVA, promotes permeation of the SC barrier. Yet, barrier disruption produced by UV does not necessarily result in enhanced skin absorption. It depends on such factors as the UV wavelength, irradiation energy, and physicochemical properties of the permeants.[51] In a hairless mice model,Takagi et al investigated the effects of UVB induced perturbation of skin barrier.[52] In their experiment, 75 mJ/cm^2 UVB induced significant increase in transepidermal water loss (TEWL) and reduction in the level of covalently bound ceramide and of transglutaminase-1. Tight junctions were also shown to be disrupted by UVB irradiation in human skin xenografts and skin equivalent models.[53] The deleterious effects of UVB on the mechanical properties of human frozen/thawed SC, ie, SC cohesiveness, were only observed with non-physiological energy doses, greater than 160 J/cm^2.[54] The impact of physiologically relevant doses of UV irradiation in terms of barrier structure, ceramide profiles, and consumer perceivable changes remains to be further investigated.

Moisture influences SC turnover by changing the rate of corneocyte desquamation. Indeed, it promotes a rapid rise in the SC pH, resulting in an increase of activity of kallikreins, the major SC serine proteases involved in desquamation.[44] Also, water exposure facilitates accessibility of corneodesmosomes to the proteolytic enzymes, which stay otherwise encased within the largely hydrophobic extracellular spaces, and thus promotes release of the cells at the skin surface.[25] Conversely, there is an observed persistence of corneodesmosomes in the outer SC of xerotic winter skin compared to normal skin.[7] A recent review of the literature indicated that low humidity and low temperatures lead to a general decrease in skin barrier function and to increased susceptibility towards mechanical stress.[55] These findings remain in line with the clinical observations of winter xerosis and of skin dryness in the elderly. Moreover, cold and dry weather are known to increase the prevalence and risk of flares in patients with atopic dermatitis.

Environmental factors causing impairment of skin barrier function include exposure to irritants and allergens. In the industrialized societies, the skin barrier is affected by the everyday use of detergents and disinfectants, in combination with the deleterious action of atmospheric pollutants that vary with geographic location and source. These pollutants contain solid and liquid particles suspended in the air and various gases such as ozone, nitrogen oxides, volatile organic compounds, and carbon monoxide. Particles vary in number, size, shape, surface area, and chemical composition, while both particles and gases may vary in solubility and toxicity. Occupational factors also play a role since they increase the risks in specific subpopulations. In health care professions, the extensive use of gloves results in occlusion, which significantly worsens the negative effect on skin barrier function of detergents/soaps. The published data indicate that a dose–response relationship is important with respect to duration of occlusion. This is particularly relevant for workplaces where shifting between wearing of gloves and hand washing is common. In the present "COVID era", the problems once encountered by medical and paramedical staff may spread into lay populations due to the widespread and highly repetitive use of hydrogels and other protective means.

The growth of skin flora is favored by increased temperature and humidity and modified by body location, age, sex, and chronic diseases such as diabetes. Occupation, hospitalization, use of soaps, disinfectants, and medications exert promoting and

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inhibiting influences, as well. Misbalance between commensals and pathogens often appears with elevation of skin surface pH and thereto related barrier dysfunction. Bacterial proteases worsen the situation by further impacting SC cohesiveness and the TJ system.

Topical Approaches to Manage Epidermal Barrier Disruption

When the epidermal barrier is compromised, as is the case for many common skin conditions including AD, eczema, and psoriasis, the skin is susceptible to excessive water loss, xerosis, and infection.[56] These same skin conditions are characterized by an inflammatory response which manifests in pain, redness, irritation, and pruritus. The increased understanding of the complex pathology associated with diseases that impact the skin barrier has shown that barrier disruption and inflammatory events most often coincide.[40,57] Therefore, effective treatment approaches should address both the recovery of the epidermal barrier and suppression of the underlying inflammatory conditions that, if left untreated, can further impede barrier repair.[58]

Factors that influence the therapeutic intervention include chronicity and severity of the disease, age, and general health of the individual.[56] When considering topical versus systemic administration of therapeutic actives, topical administration is generally preferred for less severe cases due to potential risks associated with systemic exposure.[58] Penetration of actives through an intact epidermis can vary greatly based on factors including anatomical location and surface area, the nature of active ingredient, and environmental factors.[59] Such complexity has necessitated models and imaging modalities to accurately predict and visualize penetration.[59] However, in the case of skin barrier-associated diseases, improved penetration of topically-applied active ingredients through the compromised barrier is expected.[60] This, in combination with the reduced risk of systemic exposure, have made topical therapies the common first-line approach to manage disease symptoms associated with impaired skin barrier.

The mechanisms of action for many topical, pharmacologic approaches for skin conditions such as AD involve anti-inflammatory and immunomodulatory interventions.[61] Corticosteroids have been used for more than 50 years to reduce inflammation. They act on T lymphocytes, monocytes, macrophages, and dendritic cells, suppressing pro-inflammatory cytokine release and leading to a reduction of redness, swelling, and itching.[62] The incidence of negative side effects is low; however, there are concerns linked to skin discoloration and atrophy following prolonged use of corticosteroids.[61,62] Calcineurin inhibitors, including tacrolimus and pimecrolimus, have been in use since 2000 as a more targeted approach that reduces T-cell activation and subsequent cytokine release.[57,58,61,62] An association with malignancy resulted in a black box warning related to cancer risk from the United States Food and Drug Administration (FDA) in 2005, although it is not clear whether there is a causal relationship.[58,61] Examples of other topical treatments currently in development and testing include modulators of the Janus kinase/signal transducer and activator of transcription (JAK/STAT) pathway, which is activated by pro-inflammatory cytokines and downregulates the expression of structural skin proteins, and phosphodiesterase-4 (PDE-4) inhibitors aimed at reducing production of pro-inflammatory cytokines and signals.[37,58]

In addition to anti-inflammatory and immunomodulatory approaches, there is a continued role for topical products that restore, reinforce, and maintain the barrier function of the SC. Without effective barrier recovery, the skin is susceptible to prolonged and repeated inflammatory flares. In the case of chronic conditions such as AD and psoriasis, daily application of products that support skin barrier maintenance between flares can reduce the onset of symptoms and improve general quality of life.[62–64] Moisturizers, creams, and lotions, including cosmetics, are safe, readily-available, and inexpensive products that have been mainstays among the skin care community for years. Moisturizers, alone or in combination with other anti-inflammatory/immunomodulatory agents, have demonstrated clinical benefit to reduce the onset, symptoms, and progression of diseases characterized by compromised barrier.[50,61–64] Clinical benefits have been observed in cohorts ranging from adults to neonates. A preventive role of such approach against declaration of AD has been evidenced in a study in which neonates benefited from daily moisturizer application for 32 weeks after birth.[65]

The ingredient list, complexity, and overall understanding of moisturizers has evolved in order to provide coverage to the SC, reduce water loss and hydrate the skin. Standard ingredients of many commercially-available moisturizers include emollients to soften the skin, humectants to attract and bind water (eg, glycerin), and/or occlusive agents (eg, dimethicone) that physically prevent liquid from leaving the skin.[56,62] This approach to maintain the protective and hydrating function of the skin barrier has made frequent and routine use of the skin care products the recommendation of many health care professionals.[64] Given the role of ceramides in epidermal barrier function, many moisturizers include ceramides to help support the restoration of the skin barrier.

Several clinical reports have demonstrated the ability of lipid-based emollients and ceramide-containing moisturizers to support accelerated repair, reduce symptom intensity, and promote soft, healthy-looking skin when applied alone or in combination with other therapies to skin conditions linked to impaired barrier. When used in combination with topical corticosteroids and/or calcineurin inhibitors, pediatric AD

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patients that replaced standard moisturizers with ceramide-dominate lipid-based emollients experienced reduced injury severity, decreased TEWL, and increased hydration.[66] In cases of mild-to-moderate eczema, moisturizers and cleansers containing ceramides outperformed mild bar soap, when each was paired with a topical corticosteroid, by reducing severity scores within the first week of application.[67] Similarly, twice-daily application of a ceramide-containing cleanser and moisturizer reduced dryness, itching, and other AD symptoms in both adult (>12 year old) and child (<12 year old) populations after 42 days compared to baseline.[68] When used in combination with the corticosteroid mometasone furoate, ceramide-linoleic acid-containing moisturizer accelerated the reestablishment of the epidermal permeability barrier, increased capacitance, reduced TEWL, and reduced pruritus in AD patients compared to mometasone furoate alone.[69] When applied to psoriasis vulgaris, a similar combination treatment reduced pruritus, accelerated the reduction in TEWL, and increased capacitance compared to mometasone furoate cream alone.[70] Consumer perception following application of ceramide-containing moisturizers is also improved, as one study found that ~70% of subjects with mild-to-moderate psoriasis self-reported improved appearance and when a ceramide-containing cream was used in combination with a ceramide-containing cleanser, 85% reported relief of psoriasis, and ~90% experienced soft and smooth skin.[71] While it is important to acknowledge that these studies do not suggest that the improved clinical outcomes are solely due to the inclusion of ceramides, they nonetheless highlight the positive impact of regular application of ceramide-containing moisturizers to support recovery from skin conditions associated with compromised barrier.

CONCLUSION

Formation and restoration of abolished SC barrier is a dynamic, finely regulated process prone to the influences from intrinsic and environmental factors. In addition to disease conditions (eg, AD and psoriasis) and severe environmental exposures from ultraviolet rays or pollution, events that occur in everyday life can also negatively impact the skin barrier. The importance of the SC in maintaining skin homeostasis, coupled with the prevalence and severity of internal and external factors that can alter its permeability, highlight the need for topical products to support the skin barrier. Fortunately, continued progress in the understanding of the epidermal permeability barrier structure, composition, and function provides sound foundations for knowledge-based elaboration of topical treatments aimed at the maintenance and improvement of patients' skin in health and disease. This advanced understanding is evidenced by the inclusion of essential lipids (eg, ceramides) into moisturizers and skin protectants. Whether applied alongside a topical drug for disease management (eg, corticosteroids for AD) or as part of one's daily skin care routine, ceramide-containing topical products are an effective way to help restore and maintain the skin barrier.

DISCLOSURE

Dr. Marek Haftek has received honoraria for consultancy from L'Oréal Research and Innovation.

REFERENCES

1. Haftek M. 'Memory' of the stratum corneum: exploration of the epidermis' past. *Br J Dermatol.* 2014;171:6-9.
2. Menon GK, Feingold KR, Elias PM. Lamellar body secretory response to barrier disruption. *J Invest Dermatol.* 1992;98(3):279-289.
3. Eckert RL, Sturniolo MT, Broome A-M, et al. Transglutaminase function in epidermis. *J Invest Dermatol.* 2005;124(3):481-492.
4. Elias PM. Epidermal lipids, barrier function, and desquamation. *J Invest Dermatol.* 1983;80(1):S44-S49.
5. Brandner JM, Haftek M, Niessen CM. Adherens junctions, desmosomes and tight junctions in epidermal barrier function. *Open Dermatol J.* 2010;4(1):14-20.
6. Danzberger J, Donovan M, Rankl C, et al. Glycan distribution and density in native skin's stratum corneum. *Skin Res Technol.* 2018;24(3):450-458.
7. Rankl C, Zhu R, Luengo GS, et al. Detection of corneodesmosin on the surface of stratum corneum using atomic force microscopy. *Exp Dermatol.* 2010;19(11):1014-1019.
8. Haftek M. Epidermal barrier disorders and corneodesmosome defects. *Cell Tissue Res.* 2015;360(3):483-490.
9. Milani P, Chlasta J, Abdayem R, et al. Changes in nano-mechanical properties of human epidermal cornified cells depending on their proximity to the skin surface. *J Mol Recognit.* 2018;31(9):e2722.
10. Guo S, Domanov Y, Donovan M, et al. Anisotropic cellular forces support mechanical integrity of the stratum corneum barrier. *J Mech Behav Biomed Mater.* 2019;92:11-23.
11. Potter A, Luengo G, Santoprete R, Querleux B. Stratum Corneum Biomechanics. In: Skin Moisturization. *Informa Healthcare.* 2009:259-278.
12. Luengo GS, Potter A, Ghibaudo M, et al. Stratum Corneum Biomechanics (Mechanics and Friction): Influence of Lipids and Moisturizers. In: Agache's Measuring the Skin. *Springer International Publishing.* 2017:373-387.
13. Coderch L, López O, de la Maza A, Parra JL. Ceramides and skin function. *Am J Clin Dermatol.* 2003;4(2):107-129.
14. Janssens M, van Smeden J, Gooris GS, et al. Increase in short-chain ceramides correlates with an altered lipid organization and decreased barrier function in atopic eczema patients. *J Lipid Res.* 2012;53(12):2755-2766.
15. Wertz PW, Miethke MC, Long SA, Strauss JS, Downing DT. The composition of the ceramides from human stratum corneum and from comedones. *J Invest Dermatol.* 1985;84(5):410-412.
16. Breiden B, Sandhoff K. The role of sphingolipid metabolism in cutaneous permeabilitybarrier formation. *Biochim Biophys Acta - Mol Cell Biol Lipids.* 2014;1841(3):441-452.
17. van Smeden J, Janssens M, Gooris GS, Bouwstra JA. The important role of stratum corneum lipids for the cutaneous barrier function. *Biochim Biophys Acta - Mol Cell Biol Lipids.* 2014;1841(3):295-313.
18. Kitatani K, Idkowiak-Baldys J, Hannun YA. The sphingolipid salvage pathway in ceramide metabolism and signaling. *Cell Signal.* 2008;20(6):1010-1018.
19. Kligman AM. Corneobiology and corneotherapy– a final chapter. *Int J Cosmet Sci.* 2011;33(3):197-209.
20. Madison KC. Barrier function of the skin: "la raison d'être" of the epidermis. *J Invest Dermatol.* 2003;121(2):231-241.
21. Bouwstra JA, Gooris GS, Ponec M. Skin lipid organization, composition and barrier function. *Int J Cosmet Sci.* 2008;30(5):388-388.
22. Yu G, Zhang G, Flach CR, Mendelsohn R. Vibrational spectroscopy and microscopic imaging: novel approaches for comparing barrier physical properties in native and human skin equivalents. *J Biomed Opt.* 2012;18(6):061207.
23. Guy RH. Skin – 'that unfakeable young surface'. *Skin Pharmacol Physiol.* 2013;26(4-6):181-189.
24. Celli A, Crumrine D, Meyer JM, Mauro TM. Endoplasmic reticulum calcium regulates epidermal barrier response and desmosomal structure. *J Invest Dermatol.* 2016;136(9):1840-1847.
25. Haftek M, Teillon MH, Schmitt D. Stratum corneum, corneodesmosomes and ex vivo percutaneous penetration. *Microsc Res Tech.* 1998;43(3):242-249.
26. Elias PM, Feingold KR, eds. *Skin Barrier.* CRC Press; 2005. doi:10.1201/b14173
27. Brandner J, Zorn-Kruppa M, Yoshida T, et al. Epidermal tight junctions in health and disease. *Tissue Barriers.* 2015;3(1-2):e974451.
28. Haftek M, Callejon S, Sandjeu Y, et al. Compartmentalization of the human stratum corneum by persistent tight junction-like structures. *Exp Dermatol.* 2011;20(8):617-621.
29. Bergmann S, von Buenau B, Vidal-y-Sy S, et al. Claudin-1 decrease impacts epidermal barrier function in atopic dermatitis lesions dose-dependently. *Sci*

doi:10.36849/JDD.S589A

Rep. 2020;10(1):2024.
30. Kirschner N, Haftek M, Niessen CM, et al. CD44 regulates tight-junction assembly and barrier function. *J Invest Dermatol.* 2011;131(4):932-943.
31. Abdayem R, Formanek F, Minondo AM, Potter A, Haftek M. Cell surface glycans in the human stratum corneum : distribution and depth-related changes. *Exp Dermatol.* 2016;25(11):865-871.
32. Haftek M., Callejon S., Pirot F., Traupe H. O V. Ultrastructural evaluation of the stratum corneum in peeling skin disease suggests a compensatory tight junction upregulation. *J Invest Dermatol.* 2012;132:suppl 2:S77.
33. Urwyler-Rösselet C, Tanghe G, Leurs K, et al. Keratinocyte-specific ablation of RIPK4 allows epidermal cornification but impairs skin barrier formation. *Invest Dermatol.* 2018;138(6):1268-1278.
34. Huebner AJ, Dai D, Morasso M, et al. Amniotic fluid activates the Nrf2/Keap1 pathway to repair an epidermal barrier defect in utero. *Dev Cell.* 2012;23(6):1238-1246.
35. Bhattacharya N, Ganguli-Indra G, Indra AK. Transcriptional control and transcriptomic analysis of lipid metabolism in skin barrier formation and atopic dermatitis (AD). *Expert Rev Proteomics.* 2019;16(8):627-645.
36. Konieczny P, Lichawska-Cieslar A, Kwiecinska P, et al. Keratinocyte-specific ablation of Mcpip1 impairs skin integrity and promotes local and systemic inflammation. *J Mol Med.* 2019;97(12):1669-1684.
37. Amano W, Nakajima S, Kunugi H, et al. The Janus kinase inhibitor JTE-052 improves skin barrier function through suppressing signal transducer and activator of transcription 3 signaling. *J Allergy Clin Immunol.* 2015;136(3):667-677.e7.
38. Das Mahapatra K, Pasquali L, Ziegler C, et al. LncRNA ELDAR acts as a key regulator of late epidermal differentiation program in the human epidermis. 2020. https://openarchive.ki.se/xmlui/handle/10616/47313.
39. Chiang A, Tudela E, Maibach HI. Percutaneous absorption in diseased skin: an overview. *J Appl Toxicol.* 2012;32(8):537-563.
40. Elias PM, Schmuth M. Abnormal skin barrier in the etiopathogenesis of atopic dermatitis. *Curr Opin Allergy Clin Immunol.* 2009;9(5):437-446.
41. Guttman-Yassky E, Nograles KE, Krueger JG. Contrasting pathogenesis of atopic dermatitis and psoriasis--part II: immune cell subsets and therapeutic concepts. *J Allergy Clin Immunol.* 2011;127(6):1420-1432.
42. Irvine AD, McLean WHI, Leung DYM. Filaggrin mutations associated with skin and allergic diseases. *N Engl J Med.* 2011;365(14):1315-1327.
43. Cau L, Méchin M-C, Simon M. Peptidylarginine deiminases and deiminated proteins at the epidermal barrier. *Exp Dermatol.* 2018;27(8):852-858.
44. Hachem J-P, Man M-Q, Crumrine D, et al. Sustained serine proteases activity by prolonged increase in pH leads to degradation of lipid processing enzymes and profound alterations of barrier function and stratum corneum integrity. *J Invest Dermatol.* 2005;125(3):510-520.
45. Riethmuller C, McAleer MA, Koppes SA, et al. Filaggrin breakdown products determine corneocyte conformation in patients with atopic dermatitis. *J Allergy Clin Immunol.* 2015;136(6):1573-1580.e2.
46. Haftek M, McAleer MA, Jakasa I, et al. Changes in nano-mechanical properties of human epidermal cornified cells in children with atopic dermatitis. *Wellcome Open Res.* 2020;5:97.
47. Hawkes JE, Chan TC, Krueger JG. Psoriasis pathogenesis and the development of novel targeted immune therapies. *J Allergy Clin Immunol.* 2017;140(3):645-653.
48. Kim B, Shon J, Liu K, et al. 122 Changes in fatty acid lengths of ceramides toward shorter chain dominance in human psoriasis skin. *J Invest Dermatol.* 2017;137(10):S213.
49. Friedman SJ. Management of psoriasis vulgaris with a hydrocolloid occlusive dressing. *Arch Dermatol.* 1987;123(8):1046. doi:10.1001/archderm.1987.01660320088018
50. Grimalt R, Mengeaud V, Cambazard F. The steroid-sparing effect of an emollient therapy in infants with atopic dermatitis: a randomized controlled study. *Dermatology.* 2007;214(1):61-67.
51. Hung C-F, Fang C-L, Al-Suwayeh SA, et al. Evaluation of drug and sunscreen permeation via skin irradiated with UVA and UVB: comparisons of normal skin and chronologically aged skin. *J Dermatol Sci.* 2012;68(3):135-148.
52. Takagi Y, Nakagawa H, Kondo H, et al. Decreased levels of covalently bound ceramide are associated with ultraviolet B-induced perturbation of the skin barrier. *J Invest Dermatol.* 2004;123(6):1102-1109.
53. Yuki T, Hachiya A, Kusaka A, et al. Characterization of tight junctions and their disruption by UVB in human epidermis and cultured keratinocytes. *J Invest Dermatol.* 2011;131(3):744-752.
54. Biniek K, Levi K, Dauskardt RH. Solar UV radiation reduces the barrier function of human skin. *Proc Natl Acad Sci.* 2012;109(42):17111-17116.
55. Engebretsen KA, Johansen JD, Kezic S, et al. The effect of environmental humidity and temperature on skin barrier function and dermatitis. *J Eur Acad Dermatol Venereol.* 2016;30(2):223-249.
56. Giam YC, Hebert AA, Dizon MV, et al. A review on the role of moisturizers for atopic dermatitis. *Asia Pac Allergy.* 2016;6(2):120-128.
57. Chovatiya R, Silverberg JI. Pathophysiology of atopic dermatitis and psoriasis: implications for management in children. *Child (Basel, Switzerland).* 2019;6(10).
58. Nygaard U, Deleuran, M, Vestergaard C. Emerging treatment options in atopic dermatitis: topical therapies. *Dermatology.* 2017;233(5):333-343.
59. Grégoire S, Luengo GS, Hallegot P, et al. Imaging and quantifying drug delivery in skin - Part 1: Autoradiography and mass spectrometry imaging. *Adv Drug Deliv Rev.* 2020;153:137-146.
60. Gattu S, Maibach HI. Modest but increased penetration through damaged skin: an overview of the in vivo human model. *Skin Pharmacol Physiol.* 2011;24(1):2-9.
61. Catherine Mack Correa M, Nebus J. Management of patients with atopic dermatitis: the role of emollient therapy. *Dermatol Res Pract.* 2012;2012:836931.
62. Eichenfield LF, Tom WL, Chamlin SL, et al. Guidelines of care for the management of atopic dermatitis: section 2. Management and treatment of atopic dermatitis with topical therapies. *J Am Acad Dermatol.* 2014;71(1):116-32.
63. van Zuuren EJ, Fedorowicz Z, Christensen R, et al. Emollients and moisturisers for eczema. *Cochrane Database Syst Rev.* February 2017. doi:10.1002/14651858.CD012119.pub2
64. Hebert AA, Rippke F, Weber TM, Nicol NH. Efficacy of nonprescription moisturizers for atopic dermatitis: an updated review of clinical evidence. *Am J Clin Dermatol.* 2020;21(5):641-655.
65. Horimukai K, Morita K, Narita M, et al. Application of moisturizer to neonates prevents development of atopic dermatitis. *J Allergy Clin Immunol.* 2014;134(4):824-830.e6.
66. Chamlin SL, Kao J, Frieden IJ, et al. Ceramide-dominant barrier repair lipids alleviate childhood atopic dermatitis: changes in barrier function provide a sensitive indicator of disease activity. *J Am Acad Dermatol.* 2002;47(2):198-208.
67. Draelos ZD. The effect of ceramide-containing skin care products on eczema resolution duration. *Cutis.* 2008;81(1):87-91.
68. Lynde CW, Andriessen A. A cohort study on a ceramide-containing cleanser and moisturizer used for atopic dermatitis. *Cutis.* 2014;93(4):207-213.
69. Yang Q, Liu M, Li X, Zheng J. The benefit of a ceramide-linoleic acid-containing moisturizer as an adjunctive therapy for a set of xerotic dermatoses. *Dermatol Ther.* 2019;32(4):e13017.
70. Liu M, Li X, Chen X-Y, et al. Topical application of a linoleic acid-ceramide containing moisturizer exhibit therapeutic and preventive benefits for psoriasis vulgaris: a randomized controlled trial. *Dermatol Ther.* 28(6):373-382.
71. Del Rosso JQ. Ceramide- and keratolytic-containing body cleanser and cream application in patients with psoriasis: outcomes from a consumer usage study. *J Clin Aesthet Dermatol.* 2019;12(7):18-21.

AUTHOR CORRESPONDENCE

I-Chien Liao PhD

Email:..... ichien.liao@rd.loreal.com

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ORIGINAL ARTICLE

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Models to Study Skin Lipids in Relation to the Barrier Function: A Modern Update on Models and Methodologies Evaluating Skin Barrier Function

Rebecca Barresi, Hawasatu Dumbuya PhD, Xue Liu PhD, I-Chien Liao PhD

L'Oréal Research and Innovation, Clark, NJ

ABSTRACT

The skin barrier is a multifaceted microenvironment, comprised not only of structural and molecular components that maintain its integrity, but also a lipid matrix comprising an equimolar ratio of cholesterol, free fatty acids, and ceramides. Lipid abnormalities induced by environmental or pathological stimuli are often associated with impaired skin barrier function and integrity. Incorporation of skin lipids in skincare formulations to help fortify barrier function has become widespread. While there are resources available to study the barrier, a comprehensive evaluation of skin models, from in situ to in vivo, that focus on alterations of the lipid content, seems to be lacking. This article reviews current methods to evaluate the skin lipid barrier and touches upon the significance of using such models within the cosmetic field to study formulations that incorporate barrier lipids.

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INTRODUCTION

The human skin is critical in protecting internal organs from exogenous factors to maintain homeostasis by contributing to a multifaceted structure known as the skin barrier. The stratum corneum (SC), or the skin's first line of barrier protection, is comprised of corneocytes embedded within a lipid matrix. The lipid matrix in healthy skin tissue comprises an equimolar ratio of cholesterol (CHOL), free fatty acids (FFAs), ceramides (CERs), and sterol/wax esters.[1] Functionally, CERs maintain and influence the barrier integrity of the skin by forming the skin lipid membrane and regulating cellular processes.[1]

Impaired barrier function relating to changes in skin CER concentration can be a direct result of environmental or pathological factors. The incorporation of CERs and skin lipids into formulas for moisturizers has become increasingly popular across the cosmetics and skin care field to enforce barrier integrity. Knowledge surrounding the skin barrier is continuously developing through the use of novel models and studies. This review addresses the field's lacking comprehensive evaluation of skin models to study barrier function, particularly with application for barrier restoration and proper delivery of essential skin lipids in the cosmetic field.

In Situ Models

Lipid Model Membranes

Lipid Composition Mixtures

Lipid model membranes study the functionality of particular CERs in relation to the skin barrier. Such membranes are prepared using synthetic CERs or CERs isolated from native SC. Synthetic CERs have been shown to mimic the lipid organization of native human skin through small and wide angle x-ray diffraction.[2] The function of particular lipids in barrier function is elucidated by different types of lipid mixture models. For example, ternary and quaternary lipid mixtures incorporate one or two specific CER types in conjunction with a fatty acid and CHOL. These types of models have demonstrated phase separation, whereas in vivo, the CER subclass and chain length variety protects proper structure. Simple lipid mixtures are not ideal for studying lipid phase structures, as demonstrated by mixtures lacking CER[EOS], which cannot form long phase periodicity.[3]

Multicomponent lipid mixtures allow for the evaluation of lipid phase behaviors in addition to studying the function of specific CERs. Short periodicity of lipid mixtures was previously studied using neutron diffraction methods.[4] Multi-component lipid mixtures have shown the significance of FFAs in forming the short phase periodicity and promoting orthorhombic packing.[5]

The function of specific CERs incorporated the lipid mixtures helps to link specific CERs with diseased-state skin or an impaired barrier. Low and wide angle x-ray diffraction demonstrated that although the removal of some CER subclasses, like CER[EOS], is responsible for phase changes, exclusion of other subclasses does not necessarily affect the lipid organization.[2] Infrared spectroscopy has also been used to study the effect of lipid ratios on crystalline lattices by varying the FFA levels in lipid mixtures. It was found that lower FFA levels favored a combination of hexagonal and orthorhombic packing, while the equimolar ratio favors solely orthorhombic packing.[6]

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Stratum Corneum Substitutes

Stratum corneum substitutes (SCS) allow for the in vitro evaluation of barrier function through biophysical, in situ, and permeation studies. These substitutes are made by coating a porous substrate with a uniform composition and thickness of synthetic lipids.[7] SCS can be used to predict the permeability of the membrane and allow for the modification of lipid composition in order to study the relationship of barrier function with lipid composition and organization in healthy and diseased-state skin.[7,8] To mimic diseased skin structure, an altered lipid composition can be coated on the membrane. To date, there is limited information using modified SCS models to evaluate the effect of supplemented CERs in an impaired barrier state.

Groen et al used a SCS model to evaluate altered FFA composition impact on lamellar and lateral organization through the use of FTIR and small angle x-ray diffraction. It was demonstrated that such changes resulted in hexagonal packing and a disrupted lamellar organization.[9] SCS linked shifts in lipids with altered skin permeability.[10] SCS models can also be utilized in studying the effect of short chain CERs and FFAs on barrier permeability. Short chain CERs have been found to increase the permeability of the SCS membranes as demonstrated through electrical impedance and flux of small and large molecules.[8] It was also found that these short chain CERs induce phase separation and inefficient lipid packing resulting in impaired barrier properties, as compared to native long chain CERs.[8]

Lipid membrane mixtures and SCS have demonstrated to be an informative means of studying lipid composition and structure on barrier integrity. In respect to the cosmetics, these models can be extended to evaluate supplemental CERs or additional FFAs that may mimic topical application of a skincare product to better understand barrier integrity. One limitation to this model is that it solely demonstrates barrier impairment as a function of lipid changes. It is important to note that the integrity of the skin barrier entails additional structural and molecular changes that occur in conjunction with lipid changes that would not be demonstrated in such models.

Computational Lipid Membrane Simulations

Biological systems have a high level of complexity that have been successfully captured using modern computational and bioinformatics systems. Such systems remove the constraints of in vivo models and permit for a more comprehensive evaluation of essential skin barrier elements and interactions. Different model types, ranging from cellular models to full lipid membranes, can be used to study particular functions of the skin barrier. Each of these simulation types vary in resolution regarding computing time and system size.

Cell-centered agent-based models have been used to study the epidermis in models as simple as mimicking keratinocyte cultures ranging to epidermal homeostasis in full-thickness tissue.[11] Atomistic simulations break down systems into subsets to be used with molecular dynamics to better understand SC lipids.[12] One simplified atomistic model, lacking lipids such as CHOL and FFAs, elucidated the relationship of CER tail chain length with water permeability.[13]

Coarse-grained models are ideal for studying skin lipids, as it can undertake long simulation run times with a larger system size needed for visualizing significant lipid rearrangements.[14,15] Complex systems are simplified into subsystems of different granularity levels. Unlike atomistic models, this model combats the constraints of system run-time and size by approximating atoms as a group. This reduces molecular detail but permits for the study of more complex systems. Interactions between FFAs with CER[NS] head groups and the self-assembly of large membranes using a CER and FFA mixture have also been studied.[14]

Computer simulation models aid in understanding SC lipid behavior in relation to barrier integrity. Because of its efficient computational power and run time, coarse-grained models can be used to study larger scale systems, such as more complex lipid membranes, but lack molecular detail evident within atomistic simulations. The level of detail that all-atom models provide can still only be studied with small systems. It is possible to combine these two model types into a multiscale model in order to utilize the benefits of both: high accuracy and molecular detail from atomistic simulations with the computational speed and power of the coarse-grained models. This can be used to not only understand large scale applications of changes to the lipid membrane, but also the specific molecular changes such as the lateral and lamellar lipid organization. Although there is limited incorporation of such models in the cosmetics field, computer simulations can be extended to better understand skin in a diseased state by limiting particular CERs or altering the lipid composition to assess the impact on barrier function.

In Vitro Models

Principle of Generating Biofabricated Skin Tissue Models

Biofabricated tissue is used in research and industry to understand biological mechanisms and develop products. Successful tissue engineering generally includes the following components: keratinocytes or fibroblasts, use of scaffold that recreates the in vivo extracellular matrix to provide mechanical and biological support for epidermis growth, evaluating the tissue quality at all scales.[16] Optimized tissue has been used to study skin physical barrier, chemical barrier, immunological barrier, and microbial skin barrier.[17] The incorporation of bioengineering further allows the tissue to have specific disease phenotypes. Diseased skin models can be used in developing and evaluating compounds that target specific disease and

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barrier compromised tissue conditions. Simulating different types of disease based on inflammatory reaction (psoriasis and atopic dermatitis), trauma (wound healing, photodamage), or abnormal cell behavior (melanoma, squamous cell carcinoma) have been explored to study site of action or drug efficacy. The cellular phenotype can be achieved by using patient donor cells, or adding cytokine cocktail to the tissue culture media.[16] For the models mentioned, one of the critical readouts is the restoration of epidermal barrier function.

Barrier Function Measurements Used in Biofabricated Skin Models

The integrity of barrier function is critical when the tissue is used for assessment of dermal chemicals.[18] There are multiple aspects to evaluate a biofabricated skin tissue barrier functionality. Table 1 lists the readouts used to determine the skin barrier function in biofabricated skin models.

Studying Skin Barrier Function in Human Inflammatory Diseased Models

Both psoriasis and atopic dermatitis (AD) are complex immune mediated skin disorders.[24] Skin barrier dysfunction is a common feature among the patients. The barrier impairments include mutations in corneocytes, reduced lipids content and tight junction proteins, and increased transepidermal water loss.[16]

Psoriasis Models

Psoriasis is a chronic autoimmune condition whereby immune cells activate skin cells to secrete pro-inflammatory cytokines that intensifies the pro-inflammatory signaling cascade. From recent clinical studies, the secretion of INF-γ, IL-1, IL-6, IL-17, IL-22, and TNFα by polarized Th1 and Th17 cells are elevated in the plaque site.[25] Adding these cytokines to the tissue culture media can generate typical phenotype of psoriasis skin including parakeratosis, reduced barrier differentiation protein (eg, filaggrin and loricrin), and increased level of hBD1 and SKALP.[24,26] A psoriatic skin model generated by adding IL-22 to the media showed increased thickness of epidermis and used matrix-assisted laser desorption/ionization mass spectrometry imaging (MALDI-MSI) technique to evaluate drug penetration with normal and diseased tissue.[27] Fourier transform infrared spectroscopy (FTIR) and small angle x-ray diffraction (SAXD) have been frequently used in quantifying the SC lipid composition. MALDI-MSI is also a powerful technique which has the ability to detect the actives applied topically as well as the spatial location of lipids in the tissue.[27,28] Some of the studies included all-trans retinoic acid (ATRA) in the treatment and demonstrated that this classic compound for treating psoriasis also has efficacy in rescuing filaggrin expression and improving the barrier function of the diseased model.[24] This aligns with clinical studies, which proves that the diseased models can to be used for skin barrier research.

Engineered psoriatic skin model can also be developed by using patient donor cells.[29] The self-assembly tissue showed disease phenotypes including reduction in keratinocyte differentiation, expression of CXCR2, and upregulated proinflammatory genes.[30] Skin models that used psoriatic patient cells showed higher lipid disorder and change of protein conformation in the SC compared to the control tissue.[31]

Atopic Dermatitis Models

Similar to psoriasis, the tissue cultured with Th2 related cytokine IL-4 and IL-13 cytokines can also resemble AD.[32] Several studies added IL-4 or IL-13 into the tissue culture media and the tissue carried impaired barrier features including intra-epidermal intercellular edema, abnormal expression of important differentiation proteins, and reduction in tight

TABLE 1.

Readouts to Determine Skin Barrier Function in Biofabricated Skin Models

	Parameter	Principle	Assay	Example Application
Tight Junction	**Tight Junction Proteins** **Basal Cell Layer:** Cldn-1 **Stratum Spinosum:** Cldn-4, ZO-1 **Stratum Granulosum:** Cldn-4, ZO-1, Occludin	Tight junction protein presents in the specific layer of the viable epidermis.	IHC, Western blot, qPCR	19
Water Barrier	Electrical resistance	--	TEER, TEWL	20
Barrier to Large Molecules	Large dye location in the tissue by adding systemically	The compromised barrier allows large dye molecule pass the TJ.	Biotin tracers, Dextran	21
Barrier to Permeation	Tracking molecules penetrate from the SC	Molecules will penetrate the compromised SC to the viable epidermis. The speed of diffusion also indicates the barrier function integrity.	Lucifer yellow, Small molecule diffusion studies	22
Lipids Composition	Lipid analysis of the SC	Lipids composition and structure consist with native skin tissue.	HPTLC, LC-MS, MALDI-MS, FTIR, SAXD, Lipids dye (eg, Nile Red)	23

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junction proteins.[33,34] A common AD drug was evaluated together with JAK inhibitors by using 3D bioprinted tissue from a recent study.[35] They demonstrated that AD-related drugs have great efficacy to restore barrier function such as increased TEER and increased expression of filaggrin and Claudin-1. The proinflammatory cytokines and chemokines that present in AD patients are reduced significantly in the tissue culture system within the JAK inhibitor treated tissue. This again emphasized that skin barrier function can be a secondary event in chronic skin inflammation.[17]

Ex Vivo Models

Ex vivo human skin models are used to not only alleviate ethical constraints of in vivo studies, but to provide a more comprehensive and accurate representation of the skin's natural response to a variety of stimuli, including inflammation, wound closure, anti-aging effects, or skin barrier function. Tissue for such models is collected typically during abdominoplasty or other reductive surgeries with donor consent and can be cultured for 7–10 days. Compared to in situ and reconstructed human epidermis models, these tissue explants have an intact physical and biochemical barrier. Culture conditions have been demonstrated to not only affect regenerated barrier integrity, but also proliferative activity.[36,37] This not only allows for components of experimentally damaged barriers to be evaluated but full formulations as well. Skin explant barriers can be disrupted by both physical, via stripping, or chemical, such as SLS, methods. SLS treatment has been shown to increase trans-epidermal water loss.[37]

Skin-stripping is a minimal invasive method, used both in vitro and in vivo, which removes the SC cell layers using glue or adhesive films. The condition and area of the skin being stripped can influence the thickness of SC, the number and nature of corneocytes, as well as the composition and levels of SC lipids being removed.[38–40] These factors are important in order for the successful homogeneity and uniform removal of the SC. In an ex vivo model, regenerated SC reached an identical thickness as native skin in addition to expressing terminal differentiation proteins following 8 days in culture.[36] Regenerated SC in this model also demonstrated a shift towards hexagonal lateral lipid organization and an increase in ceramide quantity.[36] In efforts to elucidate the relationship between regenerated stratum corneum post-tape stripping in both an in vivo and ex vivo model, one study analyzed shifts in the ceramide profile. Ceramides were quantified using liquid chromatography combined with mass spectrometry. It was found that both ceramide composition and lipid organization was identical in both models.[41]

Barrier repairing formulations can also be evaluated through the use of an impaired ex vivo skin model. One study tape stripped skin explants and applied a ceramide-containing ceramide to determine if the lipid barrier was restored.[42] Lipid organization was studied using FTIR and small angle x-ray diffraction. The application of the formulation containing a single ceramide resulted in a shift to a more dense orthorhombic packing and no change to the lamellar organization.[42] Application of a fatty acid-containing formula on stripped skin also increase the fraction of lipids forming a dense orthorhombic packing.[43]

Although this model is not high-throughput, learnings are more clinically relevant. Mechanical means of inducing barrier damage are well established, but information on other extrinsic factors, such as the relationship pollution or UV damage with barrier function, is limited. The relation between extrinsic factors and cosmetic formulas have yet to be elucidated as well.

Clinical Models

Mechanical Models

Skin Stripping

Under clinical settings, skin-stripping is often used to assess skin lipid and protein compositions and levels. For instance, super glue skin-strip was used to evaluate the differences in SC lipid compositions in healthy individuals from three different ethnic groups, revealing that African subjects had the lowest CER and CHOL ratio compared to Asian, who were similar to Danish subjects.[44] Another study with subjects of skin phototype IV–V demonstrated to have a faster barrier recovery, enhanced SC integrity, and increase epidermal lipid content and lamellar body density compared to phototype I–II.[45]

Skin-stripping is also widely used to determine the distribution of barrier creams applied topically, along with sunscreen filter's protective efficacy, by measuring and optimizing sun absorption spectra and distribution homogeneity onto the skin.

Skin-stripping is an effective and basic method to study skin barrier integrity, skin lipid composition, in addition to penetration depth of various formulations after topical application. Moreover, the removed SC layers can further be examined by other methods, including histochemical, genetic, lipodomic, and proteomic means.

Suction Blister

Blister-induction model is another invasive technique used to clinically study skin barrier integrity. The most popular method is suction-blister, which involves the use of pumps to induce blisters that apply a constant negative pressure onto the skin, leading to the separation of the epidermis from the dermis and causing the formation of a blister.[47] Once fully shaped and filled with interstitial or tissue fluid, the blister is excised, revealing an epidermal wound.[47]

In clinical settings, suction blister is used to examine wound healing pathways in relation to skin barrier. It was shown that

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epidermal thickness of the wounded lesions in healthy subjects correlated with a decreased TEWL, suggesting epidermal and SC restoration after wounding.[48]

The use of the suction blister model has enabled studying skin barrier function in relations to epidermal wound healing, immune, and microvascular responses.[48–50] However, it is a technique that can lead to the formation of uneven and different blister sizes, which have the potential to not heal properly and ultimately leave a scar.

Skin Irritants Model

Cutaneous irritation, as the natural skin response to an exogenous stimulus that elicits an inflammation reaction, is widely used as a clinical model to study acute barrier disruption. At the cellular level, anionic surfactants, such as sodium lauryl sulfate (SLS), are shown to cause damage to nucleated cells of the epidermis and to result in a dose-dependent inflammatory response.[51,52] While organic solvents, such as acetone, disrupt the cohesion between the epidermal lipids lamellae at all levels of the SC.[51]

Under clinical conditions, the SLS-induced irritation model can be used to study skin sensitization in relations to skin barrier integrity under both physiological and pathological conditions. For instance, one study demonstrated that repeated SLS application over 3 weeks in non-atopic dermatitis (AD) subjects led to a decrease TEWL overtime and an increase in CER 1, suggesting a protective role for this CER species against chronic irritation.[53] Another study showed that SLS skin sensitivity is only seen in patients with active AD, with an AD history or with atopic asthma, who have a tendency to have a higher basal TEWL level.[54] These results are consistent with compromised SC barrier as the major contributing factor to skin irritation.

Skin irritants are effective methods to induce and study acute barrier impairment and lipids, plus inflammation in the clinics. The drawback of this model is depending on the skin condition, the irritants nature and concentrations used, length of exposure, and time at which each endpoint is assessed, will immensely vary the epidermal disruption and restoration processes.

Environmental Models

Solar radiation is one of the most prominent environmental skin stressors. The human skin is exposed to ultraviolet radiation (UVR), comprising of ~95% UVA (320–400 nm) and ~5% UVB (280–320 nm).[55] Numerous models were developed to clinically evaluate the impact of UVR on skin barrier function.

For instance, single exposure to UVB and UVA was shown to increase the amount of SC triglycerides, FFAs, alkanes, and squalene in subjects with skin phototype II–III.[56] Interestingly, UVB exposure alone can decrease intracellular lipid cohesion and change SC lipids and keratin structures.[57] One research group evaluated the impact of acute UV exposure on skin biophysical properties on healthy Korean subjects with skin phototype II–IV. Although no information on skin barrier proteins and lipids were presented, they found that exposure to UV increased TEWL and decreased skin hydration in a dose-dependent manner within 24 hours.[58]

In regard to repeated sun exposures, one research group evaluated the biological effects induced by semi-chronic exposure to simulated standard ultraviolet daylight (UV-DL) on subjects with skin phototype II–III; they showed that 9 doses of 0.25, 0.5, and 0.75 MED over 2 weeks with UV-DL caused significant decrease in skin hydration, but observed increased epidermal thickness only at 0.75 MED.[59] Another popular method to study the impact of chronic UVR exposure is under real life conditions. For example, one group demonstrated that the chronic sun-exposed hands of middle-aged Japanese golfers were photodamaged and showed reduction in skin hydration, but interestingly no difference in TEWL compared to the glove-protected hands.[60]

UV exposure is shown to affect skin barrier integrity by increasing epidermal thickness, decreasing skin hydration, plus increasing skin lipids and proteins levels and modifying their structures. Due to different doses and sources of irradiations used in the literature, the impact of UV on TEWL and skin lipid in particular is inconclusive. Although not highlighted here, previous studies have also investigated the impact of age, seasons, and climate on skin barrier.[61] It is important to note that the wavelength, length of exposure, intensity used, and subjects skin phototype and history are all important factors to take into consideration when studying the influence of environmental factors on skin barrier function.

Skincare Application

Intercellular SC lipids, CHOL, CERs and FFAs, are essential to maintain epidermal barrier homeostasis. Prior studies have shown that application of an equal ratio of SC lipids promotes normal repair and increasing the ratio of any of these lipid classes accelerated the recovery process.[62] As discussed above, many models from in situ to clinical have since been used to study skin barrier function and to evaluate the efficacy of various natural and synthetic lipid mixtures for optimized barrier health.

As lipid abnormalities are often associated with impaired skin barrier integrity in several dermatologic conditions, SC intercellular lipids, particularly CERs, are now commonly used as main ingredients in moisturizers and other products for managing different skin disorders. For instance, it was observed that AD patients have decreased levels of CER 1 and 3, which was associated with an increased skin susceptibility

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to irritants and increased TEWL, indicating that these two CER species are essential for barrier function.[63] One research group demonstrated that a twice-daily regimen of a synthetic CER-containing cleanser and moisturizer in AD patients significantly improved skin condition, clinical outcome, and quality of life after 42-day treatment.[64] Similarly, combining synthetic skin-identical CERs 1, 3 6-II with multi-lamellar vesicular emulsion (MVE) technology can effectively deliver these lipids within the skin layers over a sustained period of time.[65] This unique approach to CER-containing formulation was shown to restore skin barrier integrity and improve clinical appearance of rosacea, eczema, skin dryness, and more recently in conjunction with combination therapy, for treating facial acne vulgaris.[65–69] While there is clear evidence of barrier restoration following the mentioned routines of ceramide-infused skin products, continuous research is essential in identifying the correct combination of skin essential lipids in conjunction with a proper delivery system in order to improve and maximize skin health.

CONCLUSION

Lipid abnormalities, stemmed from inherited, exogenous, or pathological factors, are often associated with impaired skin barrier function and integrity. To achieve optimal results and bring the right strategy for skin care formulation, it is crucial to utilize models to not only understand skin barrier function but the role of lipids in maintaining barrier integrity. In situ, ex vivo, and RHE models are value tools in understanding the relationship of lipids with skin barrier integrity without the ethical constraints present in clinical evaluations. However, such models exist in controlled and simplified conditions that may not hold true for real-world applications. Ex vivo models are able to provide a comprehensive and clinically relevant understanding to barrier components because of its intact barrier. As the models grow in complexity, the full barrier function, especially lipid composition, can be thoroughly evaluated; however, there is an increasing limitation in study size and biological variability.

Because of their benefits in promoting health barrier function, improving appearance of lesions, minimizing skin irritation, and increasing patients' compliance and treatment efficacy, incorporation of CERs and other barrier-enforcing lipids into formulas have become increasingly popular across the skincare field. In the future, it would be critical to determine the benefits of these lipids-containing formulations on compromised skin barrier caused by daily skin stressors, such as pollution and UVR.

REFERENCES

1. Vavrova K, Kovačik A, Opalka L. Ceramides in the skin barrier. *Eur Pharm J.* 2017. doi:10.1515/afpuc-2017-0004
2. De Jager MW, Gooris GS, Ponec M, Bouwstra JA. Lipid mixtures prepared with well-defined synthetic ceramides closely mimic the unique stratum corneum lipid phase behavior. *J Lipid Res.* 2005. doi:10.1194/jlr.M500221-JLR200
3. Groen D. Stratum corneum model membranes: molecular organization in relation to skin barrier function. 2011.
4. Groen D, Gooris GS, Barlow DJ, et al. Disposition of ceramide in model lipid membranes determined by neutron diffraction. *Biophys J.* 2011. doi:10.1016/j.bpj.2011.02.001
5. Bouwstra JA, Gooris GS, Dubbelaar FER, Ponec M. Phase behavior of lipid mixtures based on human ceramides: Coexistence of crystalline and liquid phases. *J Lipid Res.* 2001.
6. Gooris GS, Bouwstra JA. Infrared spectroscopic study of stratum corneum model membranes prepared from human ceramides, cholesterol, and fatty acids. *Biophys J.* 2007. doi:10.1529/biophysj.106.094292
7. de Jager M, Groenink W, van der Spek J, et al. Preparation and characterization of a stratum corneum substitute for in vitro percutaneous penetration studies. *Biochim Biophys Acta - Biomembr.* 2006. doi:10.1016/j.bbamem.2006.04.001
8. Školová B, Januìššová B, Zbytovská J, et al. Ceramides in the skin lipid membranes: Length matters. *Langmuir.* 2013. doi:10.1021/la4037474
9. Groen D, Poole DS, Gooris GS, Bouwstra JA. Is an orthorhombic lateral packing and a proper lamellar organization important for the skin barrier function? *Biochim Biophys Acta - Biomembr.* 2011. doi:10.1016/j.bbamem.2010.10.015
10. Groen D, Poole DS, Gooris GS, Bouwstra JA. Investigating the barrier function of skin lipid models with varying compositions. *Eur J Pharm Biopharm.* 2011. doi:10.1016/j.ejpb.2011.05.007
11. Sun T, McMinn P, Coakley S, et al. An integrated systems biology approach to understanding the rules of keratinocyte colony formation. *J R Soc Interface.* 2007. doi:10.1098/rsif.2007.0227
12. Das C, Olmsted PD. The physics of stratum corneum lipid membranes. *Philos Trans R Soc A Math Phys Eng Sci.* 2016. doi:10.1098/rsta.2015.0126
13. Gupta R, Dwadasi BS, Rai B. Molecular dynamics simulation of skin lipids: effect of ceramide chain lengths on bilayer properties. *J Phys Chem B.* 2016. doi:10.1021/acs.jpcb.6b08059
14. Moore TC, Iacovella CR, Leonhard AC, Bunge AL, McCabe C. Molecular dynamics simulations of stratum corneum lipid mixtures: A multiscale perspective. *Biochem Biophys Res Commun.* 2018. doi:10.1016/j.bbrc.2017.09.040
15. Kmiecik S, Gront D, Kolinski M, et al. Coarse-grained protein models and their applications. *Chem Rev.* 2016. doi:10.1021/acs.chemrev.6b00163
16. Marques AP, Reis RL, Pirraco RP, Cerqueira M. *Skin Tissue Models.*
17. Niehues H, Bouwstra JA, El Ghalbzouri A, et al. 3D skin models for 3R research: The potential of 3D reconstructed skin models to study skin barrier function. *Exp Dermatol.* 2018;27(5):501-511. doi:10.1111/exd.13531
18. Desprez B, Barroso J, Griesinger C, et al. Two novel prediction models improve predictions of skin corrosive sub-categories by test methods of OECD Test Guideline No. 431. *Toxicol Vitr.* 2015;29(8):2055-2080. doi:10.1016/j.tiv.2015.08.015
19. Smits JPH, Niehues H, Rikken G, et al. Immortalized N/TERT keratinocytes as an alternative cell source in 3D human epidermal models.
20. Wei Z, Liu X, Ooka M, et al. Two-dimensional cellular and three-dimensional bio-printed skin models to screen topical-use compounds for irritation potential. *Front Bioeng Biotechnol.* 2020;8:109. doi:10.3389/FBIOE.2020.00109
21. Ramadan Q, Ting FCW, Thyssen JP, et al. In vitro micro-physiological immune-competent model of the human skin. *Lab Chip.* 2016;16(10):1899-1908. doi:10.1039/C6LC00229C
22. Sriram G, Bigliardi PL, Bigliardi-Qi M. Full-thickness human skin equivalent models of atopic dermatitis. *Methods Mol Biol.* 2019;1879:367-383. doi:10.1007/7651_2018_163
23. Boehnke K, Mirancea N, Pavesio A, Fusenig NE, Boukamp P, Stark HJ. Effects of fibroblasts and microenvironment on epidermal regeneration and tissue function in long-term skin equivalents. *Eur J Cell Biol.* 2007;86(11-12):731-746. doi:10.1016/j.ejcb.2006.12.005
24. Smits JPH, Niehues H, Rikken G, et al. Immortalized N/TERT keratinocytes as an alternative cell source in 3D human epidermal models. *Sci Rep.* 2017;7(1):11838. doi:10.1038/s41598-017-12041-y
25. Hawkes JE, Chan TC, Krueger JG, York N. Psoriasis pathogenesis and the development of novel targeted immune therapies. 2017. doi:10.1016/j.jaci.2017.07.004
26. Bernard F-X, Morel F, Camus M, et al. Keratinocytes under fire of proinflammatory cytokines: bona fide innate immune cells involved in the physiopathology of chronic atopic dermatitis and psoriasis. *J Allergy.* 2012;2012:1-10. doi:10.1155/2012/718725
27. Harvey A, Cole LM, Day R, et al. MALDI-MSI for the analysis of a 3D tissue-engineered psoriatic skin model. *Proteomics.* 2016;16(11-12):1718-1725. doi:10.1002/pmic.201600036
28. Mieremet A, Rietveld M, Absalah S, et al. Improved epidermal barrier formation in human skin models by Chitosan modulated dermal matrices. *PLoS One.* 2017;12(3). doi:10.1371/journal.pone.0174478

doi:10.36849/JDD.S589B

29. Barker CL, Mchale MT, Gillies AK, et al. The development and characterization of an in vitro model of psoriasis. 2004. doi:10.1111/j.0022-202X.2004.23435.x
30. Jean J, Lapointe M, Soucy J, Pouliot R. Development of an in vitro psoriatic skin model by tissue engineering. doi:10.1016/j.jdermsci.2008.07.009
31. Bernard G, Auger M, Soucy J, Pouliot R. Physical characterization of the stratum corneum of an in vitro psoriatic skin model by ATR-FTIR and Raman spectroscopies. *Biochim Biophys Acta - Gen Subj.* 2007;1770(9):1317-1323. doi:https://doi.org/10.1016/j.bbagen.2007.06.014
32. Kamsteeg M, Bergers M, de Boer R, et al. Type 2 helper T-cell cytokines induce morphologic and molecular characteristics of atopic dermatitis in human skin equivalent. *Am J Pathol.* 2011;178(5):2091-2099. doi:10.1016/j.ajpath.2011.01.037
33. Gschwandtner M, Mildner M, Mlitz V, et al. Histamine suppresses epidermal keratinocyte differentiation and impairs skin barrier function in a human skin model. *Allergy Eur J Allergy Clin Immunol.* 2013;68(1):37-47. doi:10.1111/all.12051
34. Niehues H, Schalkwijk J, van Vlijmen-Willems IMJJ, et al. Epidermal equivalents of filaggrin null keratinocytes do not show impaired skin barrier function. *J Allergy Clin Immunol.* 2017;139(6):1979-1981.e13. doi:10.1016/j.jaci.2016.09.016
35. Liu X, Michael S, Bharti K, Ferrer M, Song MJ. A biofabricated vascularized skin model of atopic dermatitis for preclinical studies. *Biofabrication.* 2020;12(3):35002. doi:10.1088/1758-5090/ab76a1
36. Danso MO, Berkers T, Mieremet A, et al. An ex vivo human skin model for studying skin barrier repair. *Exp Dermatol.* 2015. doi:10.1111/exd.12579
37. Döge N, Avetisyan A, Hadam S, et al. Assessment of skin barrier function and biochemical changes of ex vivo human skin in response to physical and chemical barrier disruption. *Eur J Pharm Biopharm.* 2017. doi:10.1016/j.ejpb.2016.12.012
38. Berardesca E, Pirot F, Singh M, Maibach H. Differences in stratum corneum pH gradient when comparing white caucasian and black African-American skin. *Br J Dermatol.* 1998. doi:10.1046/j.1365-2133.1998.02513.x
39. Greene RS, Downing DT, Pochi PE, Strauss JS. Anatomical variation in the amount and composition of human skin surface lipid. *J Invest Dermatol.* 1970. doi:10.1111/1523-1747.ep12280318
40. Ya-Xian Z, Suetake T, Tagami H. Number of cell layers of the stratum corneum in normal skin relationship to the anatomical location an the body, age, sex and physical parameters. *Arch Dermatol Res.* 1999. doi:10.1007/s004030050453
41. Berkers T, Boiten WA, Absalah S, et al. Compromising human skin in vivo and ex vivo to study skin barrier repair. *Biochim Biophys Acta - Mol Cell Biol Lipids.* 2019. doi:10.1016/j.bbalip.2019.04.005
42. Berkers T, Visscher D, Gooris GS, Bouwstra JA. Topically Applied Ceramides Interact with the Stratum Corneum Lipid Matrix in Compromised Ex Vivo Skin. *Pharm Res.* 2018. doi:10.1007/s11095-017-2288-y
43. Berkers T, van Dijk L, Absalah S, van Smeden J, Bouwstra JA. Topically applied fatty acids are elongated before incorporation in the stratum corneum lipid matrix in compromised skin. *Exp Dermatol.* 2017. doi:10.1111/exd.13116
44. Jungersted JM, Hiøgh JK, Hellgren LI, Jemec GBE, Agner T. Ethnicity and stratum corneum ceramides. *Br J Dermatol.* 2010. doi:10.1111/j.1365-2133.2010.10080.x
45. Gunathilake R, Schurer NY, Shoo BA, et al. PH-Regulated mechanisms account for pigment-type differences in epidermal barrier function. *J Invest Dermatol.* 2009. doi:10.1038/jid.2008.442
46. Jacobi U, Weigmann HJ, Ulrich J, Sterry W, Lademann J. Estimation of the relative stratum corneum amount removed by tape stripping. *Ski Res Technol.* 2005. doi:10.1111/j.1600-0846.2005.00094.x
47. Wilhelm KP, Wilhelm D, Bielfeldt S. Models of wound healing: an emphasis on clinical studies. *Ski Res Technol.* 2017. doi:10.1111/srt.12317
48. Ahlström MG, Gjerdrum LMR, Larsen HF, et al. Suction blister lesions and epithelialization monitored by optical coherence tomography. *Ski Res Technol.* 2018. doi:10.1111/srt.12391
49. Larsen HF, Ahlström MG, Gjerdrum LMR, et al. Noninvasive measurement of reepithelialization and microvascularity of suction-blister wounds with benchmarking to histology. *Wound Repair Regen.* 2017. doi:10.1111/wrr.12605
50. Smith TJ, Wilson MA, Young AJ, Montain SJ. A suction blister model reliably assesses skin barrier restoration and immune response. *J Immunol Methods.* 2015. doi:10.1016/j.jim.2015.01.002
51. Fartasch M. Ultrastructure of the epidermal barrier after irritation. *Microsc Res Tech.* 1997. doi:10.1002/(SICI)1097-0029(19970501)37:3<193::AID-JEMT4>3.0.CO;2-P
52. Ponec M, Kempenaar J. Use of human skin recombinants as an in vitro model for testing the irritation potential of cutaneous irritants. *Skin Pharmacol Physiol.* 1995. doi:10.1159/000211330
53. Heinemann C, Paschold C, Fluhr J, et al. Induction of a hardening phenomenon by repeated application of SLS: Analysis of lipid changes in the stratum corneum. *Acta Derm Venereol.* 2005. doi:10.1080/00015550410026362
54. Löffler H, Effendy I. Skin susceptibility of atopic individuals. *Contact Dermatitis.* 1999. doi:10.1111/j.1600-0536.1999.tb06056.x
55. Amaro-Ortiz A, Yan B, D'Orazio JA. Ultraviolet radiation, aging and the skin: Prevention of damage by topical cAMP manipulation. *Molecules.* 2014. doi:10.3390/molecules19056202
56. Wefers H, Melnik BC, Flür M, et al. Influence of UV irradiation on the composition of human stratum corneum lipids. *J Invest Dermatol.* 1990. doi:10.1111/1523-1747.ep12476124
57. Biniek K, Levi K, Dauskardt RH. Solar UV radiation reduces the barrier function of human skin. *Proc Natl Acad Sci U S A.* 2012. doi:10.1073/pnas.1206851109
58. Lim SH, Kim SM, Lee YW, Ahn KJ, Choe YB. Change of biophysical properties of the skin caused by ultraviolet radiation-induced photodamage in Koreans. *Ski Res Technol.* 2008. doi:10.1111/j.1600-0846.2007.00272.x
59. Seité S, Medaisko C, Christiaens F, et al. Biological effects of simulated ultraviolet daylight: A new approach to investigate daily photoprotection. *Photodermatol Photoimmunol Photomed.* 2006. doi:10.1111/j.1600-0781.2006.00209.x
60. Kikuchi-Numagami K, Suetake T, Yanai M, et al. Functional and morphological studies of photodamaged skin on the hands of middle-aged Japanese golfers. *Eur J Dermatology.* 2000.
61. Rogers J, Harding C, Mayo A, et al. Stratum corneum lipids: the effect of ageing and the seasons. *Arch Dermatol Res.* 1996. doi:10.1007/s004030050138
62. Mao-Qiang M, Feingold KR, Thornfeldt CR, Elias PM. Optimization of physiological lipid mixtures for barrier repair. *J Invest Dermatol.* 1996. doi:10.1111/1523-1747.ep12340135
63. Di Nardo A, Wertz P, Giannetti A, Seidenari S. Ceramide and cholesterol composition of the skin of patients with atopic dermatitis. *Acta Derm Venereol.* 1998. doi:10.1080/00015559850135788
64. Lynde CW AAA. A cohort study on a ceramide-containing cleanser and moisturizer used for atopic dermatitis. Randomized Control Trial. 2014.
65. Draelos ZD. The effect of ceramide-containing skin care products on eczema resolution duration. *Cutis.* 2008.
66. Danby SG, Andrew P V, Brown K, et al. An investigation of the skin barrier restoring effects of a cream and lotion containing ceramides in a multi-vesicular emulsion in people with dry, eczema-prone, skin: the RESTORE Study Phase 1. *Dermatol Ther (Heidelb).* 2020. doi:10.1007/s13555-020-00426-3
67. James Q Del Rosso 1. The use of moisturizers as an integral component of topical therapy for rosacea: clinical results based on the Assessment of Skin Characteristics Study. *Cutis.* 2009.
68. Draelos ZD, Baalbaki NH, Raab S, Colón G. The effect of a ceramide-containing product on stratum corneum lipid levels in dry legs. *J Drugs Dermatol.* 2020. doi:10.36849/JDD.2020.4796
69. Cannizzaro M V., Dattola A, Garofalo V, Del Duca E, Bianchi L. Reducing the oral Isotretinoin skin side effects: Efficacy of 8% omega-ceramides, hydrophilic sugars, 5% niacinamide cream Compound in acne patients. *G Ital di Dermatologia e Venereol.* 2018. doi:10.23736/S0392-0488.17.05742-X

AUTHOR CORRESPONDENCE

Rebecca Barresi
Email:..... rebecca.barresi@rd.loreal.com

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Compromised Skin Barrier and Sensitive Skin in Diverse Populations

Yan Wu MD PhD,[a] Janet Wangari-Olivero PhD,[b] Yaxian Zhen MD PhD[b]
[a]Department of Dermatology, Peking University, First Hospital, Beijing, China
[b]L'Oréal Research and Innovation, Clark, NJ

ABSTRACT

The most important function of the stratum corneum (SC), the uppermost layer of the human epidermis, is the formation of the epidermal permeability barrier. Lipids, particularly ceramides, cholesterol, and free fatty acids, together form lamellar membranes in the extracellular spaces of the SC that limit the loss of water and electrolytes. In addition to preventing water and electrolyte loss, the SC as a permeability barrier prevents the entry of harmful irritants, allergens, and microorganisms into the skin. Disruption of the epidermal barrier leads to skin that is irritated, more reactive, and more sensitive than normal skin. SC thickness, lipid profile, and barrier function vary with different ethnic groups, which is also reflected the differences in prevalence and manifestation of diverse skin conditions related to the skin barrier function such as atopic dermatitis and sensitive skin. In addition to these compromised skin barrier related conditions, we are just now starting to understand the direct and indirect impact of COVID-19 on the skin and how current preventative measures are contributing to skin barrier disorders. Our understanding of various approaches for restoration of skin barrier, especially the role of topically applied mixtures of cholesterol, ceramides, and essential/nonessential free fatty acids (FFAs) allows for the strengthening of the compromised skin barrier and alleviation of symptoms and discomfort associated with skin barrier disorders. Ceramide containing products on the market are commonly available and offer protection and reparative benefits to the skin barrier.

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INTRODUCTION

"A pivotal point of terrestrial adaptation is prevention of desiccation and maintenance of internal water homeostasis".[1] The critical role of the stratum corneum (SC) permeability barrier is the protection of the human body from desiccation by limiting transcutaneous movement of water and electrolytes, as well as preventing the entry into the skin of harmful substances like irritants, allergens, and microorganisms. The SC lipids, particularly ceramides, cholesterol, and free fatty acids together forming lamellar membranes in the extracellular spaces of the SC, play a key role in the integrity of SC permeability barrier commonly referred to as epidermal barrier, or skin barrier.[1] The intercellular lipids of the SC together with intracellular humectants (natural moisturizing factor, NMF) endow the SC with softness and flexibility by their water holding capacity.[2,3] Disruption of the epidermal barrier leads to alterations of SC proteins and lipids, increased transepidermal water loss (TEWL), decreased skin hydration status (clinically seen as dry skin), decreased skin elasticity and smoothness, increased skin reactivity to external stimuli,[2,3] and even skin diseases. This review summarizes current understanding of skin barrier integrity and function, clinical consequence of impaired skin barrier integrity, impact of COVID-19 on skin health, sensitive skin in diverse populations, and management strategies.

Ethnicity and Skin Barrier Function

Several methods have been used to understand differences in skin barrier among Caucasian, Asian, and African American ethnic groups including the measurement of TEWL, tape stripping to examine stratum corneum layers, lipid content analysis, and irritation with sodium lauryl sulfate.[4,5,6] For TEWL, the evidence indicates that African American skin has greater TEWL than Caucasian skin.[5] However, for Asians, the data is inconsistent, with some studies showing TEWL similar to African American skin[7] and some showing TEWL lower than Caucasian or Hispanic skin. Other studies have compared differences of skin barrier in different skin pigmentation types (Fitzpatrick phototypes) instead of ethnicity. The study by Reed et al comparing Fitzpatrick skin type II and III of Asians and Caucasians to types IV and VI of Asian, Hispanic, and African American backgrounds with TEWL measured after tape stripping, showed that phototypes IV and V required more tape stripping than phototypes II and III to achieve the same TEWL.[8] This led to the conclusion that darker skin may have better barrier integrity and is thus able to withstand insults more than lighter skin. Other studies have supported this theory by demonstrating that African American skin has more corneocyte layers, with a more compactly packed stratum corneum due to increased intercellular cohesiveness.[9,10] This connection of

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barrier function to epidermal pigmentation is thought to have emerged with evolution to ensure human survival in Africa where ambient humidity was in decline and where there is high exposure to ultraviolet B (UVB).[11]

In a study comparing African American, Hispanic, and Caucasian skin, ceramide levels were found to be highest in Asian skin, followed by Caucasian and Hispanic skin, and lowest in African American skin.[12] In addition, the study also showed that Asian skin had more water content than Caucasian and African American skin. This is supported by other evidence that African American skin is more prone to dryness,[8] suggesting that this may be as a result of the lipid differences between the ethnic backgrounds. Similar results showing lower ceramide to protein ratio have also been reported in comparison to Caucasian and Asian skin.[13] From these findings, it is clear that enhancing the lipids and especially ceramide levels in the skin can help in the recovery of barrier function and increased water content in the SC.

Skin Barrier Disorders in Diverse Populations

The skin barrier is impaired or dysfunctional in some skin conditions such as atopic dermatitis (AD), psoriasis, xerosis, ichthyosis, and in diabetetics.[14] The compromised skin barrier leads to excess loss of water, increased pH, susceptibility to infection, and accelerated penetration of antigens and microbes, which cause contact sensitization and inflammation.[15] Without repair to the compromised barrier, clinical signs of barrier disruption become more evident and progressive, which presents as increased desquamation, clumping of corneocytes leading to scaling, flaking, and decrease in elasticity, therefore causing cracking of the skin and hyperkeratosis as a hallmark of increased keratinocyte proliferation.[16] These can cause the skin to be cosmetically disfigured or unappealing, which creates social stigma, increased anxiety, and social distress in affected individuals.

Skin barrier disorders show differences in prevalence and manifestations in different skin types. AD is the most common, representative skin barrier disease affecting 3–10% of the population, globally.[17] AD has been shown to be 1.7 times more prevalent in African Americans than Caucasians, even with adjustment of social economic factors and environment.[18] Additionally, Africa and Oceania show higher rates of AD than India and Northern and Eastern Europe.[19] Evidence shows that there is a genetic component to AD with genome-wide association studies identifying 31 risk loci with ethnic variations between African, Hispanic, Asian, and Caucasian patients.[20]

COVID-19 in Dermatology and Barrier Disruption

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which causes the novel coronavirus disease COVID-19, emerged in 2019 as a global healthcare threat. While initially presenting as a disease of the lower respiratory system, it is now known to be asymptomatic or symptomatic affecting the gastro-intestinal, cardiovascular, neurological, and dermatological systems, and can result in multi-system inflammatory syndrome in children.[21,22] Various dermatological conditions associated with COVID-19 have been noted such as pernio-like inflammatory skin reactions consisting of red or purple itchy bumps on the toes, heels, or fingers commonly referred as "COVID toes", measles-like rashes – morbilliform exanthema, chilblains, erythematous macules, or papules and petechial eruptions (Freeman et al 2020). Differences in manifestation of COVID-19 cutaneous diseases vary with skin color and it is especially apparent that darker skin types are challenging in examination and diagnoses of erythema and pernio-like lesions, which can lead to inaccurate diagnosis.

Protective measures against COVID-19 include frequent hand washing and use of personal protective equipment (masks, gloves, shields, eye wear) leading to a high prevalence of occupational dermatoses among healthcare workers and in the general population.[23] Frequent handwashing has been reported to cause xerosis, irritant contact dermatitis, and even allergic contact dermatitis, as a result of frequent exposure to water, soaps, detergents that strip the skin of lipids, and use of hand sanitizers with high alcohol content.[24] Recommendations to alleviate xerosis and hand dermatitis include liberal application of moisturizers and ointments after handwashing and especially those that contain humectants such as urea, occlusive emollients, such as petrolatum, lanolin, and vegetable oils, or physiological lipids such as ceramides that replenish the depleted skin lipids and prevent dehydration.[25] Additionally, mask usage has been reported to exacerbate acne flare ups. This type of acne, acne *mechanica* or "maskne", is multifactorial and occurs as a result of the mechanical insult to the skin barrier, increased sweating causing a buildup in humidity and blockage in the pilosebaceous unit, with symptoms that include burning, itching, and scratching, which can reduce the efficacy of mask wearing.[26] With dermatologists increasingly seeing patients with acne mechanica, the recommendations have been to wear properly fitting masks, wash reusable masks often, use mild cleansers that are gentle on the skin, and use non-comodogenic moisturizers.[27]

Sensitive Skin

Sensitive skin is a complex problem with genetic, individual, environmental, occupational, and ethnic implications. "The role of biological (ethnic differences), social, economic, and psychological (ethnic variations) factors for the skin sensitivity are reflected in the concept of 'ethnic sensitive skin'".[28]

Although initially believed to be an unusual reaction to common products, evidenced in only a small subset of consumers, epidemiological surveys surprisingly found a high prevalence

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TABLE 1.

Worldwide Prevalence of Sensitive Skin

Country	Year	Number		% Sensitive Skin		Reference
		Female	Male	Female	Male	
U.K.	2001	2046	260	51.4	38.2	Willis C M, et al. Sensitive skin: An epidemiological study. *British Journal of Dermatology,* 2001, 145(2):258-263
8 Europe countries all together	2009	4506		49.4 38.4 sensitive or very sensitive skin 61.6 slightly or not sensitive skin	37	Misery L, et al. Sensitive skin in Europe. *Journal of the European Academy of Dermatology and Venereology,* 2009, 23(4):6
Belgium		500		25.8		
France		1006		51.8		
Greece		500		29.8		
Germany		500		35.6		
Italy		500		53.8		
Portugal		500		27.4		
Spain		500		31.6		
Switzerland		500		30.8		
U.S.	2011	499	495	50.9	38.2	Misery L, et al. Sensitive skin in the American population: prevalence, clinical data, and role of the dermatologist. *International Journal of Dermatology,* 2011, 50(8):961-967
China	2011	1272		31.9	18.2	Ling-ling Y, et al. Epidemiological study of sensitive skin in Shanghai. *Journal of Clinical Dermatology,* 40(7), 2011
Japan	2013	1500		55.98	52.8	Kamide R, et al. Sensitive skin evaluation in the Japanese population. *The Journal of Dermatology,* 2013, 40(3):177-181
Brazil	2014	1022		45.7	22.3	Taieb C, et al. Sensitive skin in Brazil and Russia: An epidemiological and comparative approach. *European Journal of Dermatology,* 2014, 24(3):372-376
Russia	2014	1500		50.1	25.4	
South Korea	2017	1000		56.8		Kim Y R, et al. Sensitive skin in Korean population: An epidemiological approach. *Skin Research & Technology,* 2017
France	2018	5000		66	51	Misery L, et al. Sensitive skin in France: a study on prevalence, relationship with age and skin type and impact on quality of life. *Journal of the European Academy of Dermatology and Venereology.* 2018;32(5):791-795

of self-perceived sensitive skin across the industrialized world. In fact, most women in the United States, Europe, and Japan believe they have sensitive skin.[29]

The term sensitive skin was initially introduced by Bernstein in 1947 as one of the factors contributing to soap induced dermatitis[30] and further reintroduced and described by Frosch and Kligman[31] in the 1970s. Later on, the terms Cosmetic Intolerance Syndrome (CIS),[32] Status Cosmeticus, and Sensitive Skin Syndrome (SSS),[33] were also introduced in several literatures. In 2017, a group of international experts published a position paper defining sensitive skin as *"a syndrome defined by the occurrence of unpleasant sensations (stinging, burning, pain, pruritus, and tingling sensations) in response to stimuli that normally should not provoke such sensations. These unpleasant sensations cannot be explained by lesions attributable to any skin disease. The skin can appear normal or be accompanied by erythema. Sensitive skin can affect all body locations, especially the face".*[34] Sensitive skin may occur with people with seemingly normal skin, or as a part of the symptoms associated with underlying dermatological conditions.

Epidemiological Differences in Diverse Populations

Due to the fact that the diagnosis of sensitive skin is mainly based on individuals' subjective description of the symptoms, most epidemiological studies use questionnaire surveys.[35,36] As summarized in Table 1, the first large scale questionnaire survey was conducted in 2001 in the United Kingdom with 2046 out of 3300 women and 260 out of 500 men responding to the sensitive skin questionnaire. Among those respondents, 51.4% women and 38.2% men reported having experienced sensitive skin symptoms.[35] In another multinational study comprised

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of eight European countries, 49.4% women and 37% men declared having sensitive skin. Italy and France had the highest prevalence rate (Table 1). In Asia, women more frequently complaining about sensitive skin than men, and South Korea has the highest prevalence of sensitive skin compared to Japan and China (Table 1).

Questions have been raised as to whether there are differences between ethnic groups. A study conducted in the US on four ethnic groups (African Americans, Asians, Euro-Americans, and Hispanics) found a high prevalence of sensitive skin in the US, mainly associated with fair skin phototype, despite no statistical differences between these four ethnic groups.[36] The study also found some differences in triggering factors and clinical symptoms; Asians expressed greater reactivity to spicy food, sudden temperature changes, wind, and experienced more frequent itchiness, while African Americans expressed moderate skin reactivity to the environmental factors and less frequency of recurring facial redness, which may be due to less visibility of erythema on darker skin (Table 1).

Different Associating Factors in Diverse Populations

Numerous internal and external factors either contribute to or trigger sensitive skin. Studies have found that sensitive skin has a higher prevalence in individuals with fair skin phototypes (Fitzpatrick skin type I and II in Caucasians; type III in Asians),[36,37,38] but overall prevalence is similar across different ethnic groups with some differences regarding what triggers skin discomfort.[36] The most reported triggering factors are weather conditions (cold, heat, humidity), air pollution, air conditioning, dry air, psychological stress, personal hygiene products, personal care products, and rough fabric clothing.[39] Sun exposure also plays a role in triggering sensitive skin.[37,40] In terms of gender, women have higher prevalence compared with men globally based on current epidemiology studies. However, a study conducted in 2018 with 5000 people in France has shown an increase in prevalence of sensitive skin with the increase larger in men than women in comparison to a study conducted in 2009 (Table 1). Regarding the body location, face is the most reported site of sensitive skin because of its dense nervous network and higher frequency of exposure to triggering factors. The clinical signs and symptoms associated with sensitive skin have been also reported to occur in conjunction with the menstrual cycle and have been shown to be correlated with high concentrations of estradiol or luteinizing hormone,[39] this may in part explain the differences in skin sensitivity between women and men. Dry skin and susceptibility to blushing and flushing are also more likely to be associated with sensitive skin.[35]

Skin Barrier Impairment and Sensitive Skin

One of the leading hypotheses is that impaired epidermal barrier leads to increased trans-cutaneous penetration of substances and less protected cutaneous nerve endings, which results in heightened neurosensory response when experiencing environmental challenges or in contact with substances that normally do not cause irritation.[41,42,43] In recent years, researchers have suggested that keratinocytes may act as a stimulus sensor that processes and transfers information to the C-fiber terminals.[44] One of the receptor families present in keratinocytes is transient receptor potential (TRP), which acts as sensors for temperature or other physical or chemical factors.[45]

It has been confirmed that the impaired epidermal barrier leads to an increase in TEWL, a decrease in SC hydration status, which clinically manifests as dry skin, and sensitive skin is frequently reported by people with dry skin. Furthermore, people with skin barrier disorders such as AD, rosacea, acne, seborrheic dermatitis, irritant contact dermatitis, and allergic contact dermatitis, tend to experience some degree of sensitive skin symptoms.[35]

Studies also suggest people with sensitive skin may have a thinner SC with a reduced corneocyte area,[46] an imbalance of intercellular lipid of SC,[47] and lower SC ceramides contents;[48] all of these can have a strong impact on epidermal barrier integrity. A study conducted in South Korea compared the amount of SC ceramides between the sensitive skin group and the non-sensitive group, and found that the amount of SC ceramides was significantly lower on facial skin in the sensitive skin group than in the non-sensitive skin group, and lower on the forearms, thighs, legs, and back skin in the sensitive skin group than in the non-sensitive skin group.[48]

Recently, a role of cutaneous microbiota in skin sensitivity had been hypothesized, and more studies are needed to demonstrate the link between skin sensitivity and skin microbiota.[49]

Sensitive skin as a dermatological condition can have a significant impact on affected individuals' quality of life.[50] The management of sensitive skin sometimes can be very challenging due to its complicated contributing and triggering factors and pathogenesis.

Skin Barrier Restoration

The importance of lipids that form the epidermal barrier (equimolar ratios of sphingolipids, cholesterol, and free fatty acids) is demonstrated by the fact that disruption of skin barrier using physical (tape stripping) or chemical (acetone extraction) stimulates epidermal proliferation and lipid biosynthesis.[51] In addition, it has been reported that topical application of ceramides, cholesterol, and essential/nonessential free fatty acids (FFAs) mixture in an equimolar ratio facilitates normal skin barrier recovery.[52] These evidences strongly suggest utilization of physiologic lipids is an effective approach for compromised epidermal barrier-associated dermatological conditions (eg,

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acne, rosacea, psoriasis, atopic dermatitis, irritant dermatitis, and sensitive skin, etc) and relief of skin symptoms.

Of these skin barrier lipids, ceramides occupy a central and essential role. Topical application of a ceramide-dominant, barrier repair emollient in children with AD has been demonstrated to be a safe, useful adjunct to the treatment of childhood AD.[53] In a large multicenter, open-label study, the investigator evaluated the efficacy of ceramide-dominant lipid barrier repair emulsion in 207 AD patients after three weeks either using a ceramide-dominant emulsion only or in combination with another AD treatment. The ceramide-dominant product provided clinical efficacy with patient satisfaction and improvement of pruritus and quality of life.[54] AD and other impaired epidermal barrier-associated dermatological conditions have provided clear rationale for the use of ceramides as topical agent in restoring epidermal barrier integrity and function.

In addition, exposure to hot water, soaps, certain chemicals, and other environmental factors can also cause a decrease in SC lipids, especially ceramides. Currently, a variety of products are available in the market containing ceramides for moisturization, protection, and restoration of skin barrier. Products that feature at least three types of essential ceramides (Ceramides 1, 3, 6) can help restore the skin barrier integrity and function, and improve the quality of life more efficiently.[55,56]

CONCLUSION

It is clear that skin properties and barrier vary considerably between healthy and compromised skin. Although much progress has been achieved in understanding physiological differences between these two skin states, recent developments are allowing us to better understand them and especially in relation to skin health, reactivity, and sensitivity. Since we already known how to deal with compromised skin barrier-related conditions such as AD and sensitive skin, we can apply these learnings in the management of emerging conditions such as the cutaneous manifestation of COVID-19 and those associated with PPE and hand washing dermatoses. While ceramides have long existed in the field of dermatology, new emerging science on how ceramides are affected by daily activities such as sun exposure and skincare habits will lead us to optimize their usage in daily life.

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REFERENCES

1. Elias PM, Menon GK. Structural and Lipid Biochemical Correlates of the Epidermal Permeability Barrier. In: ELIAS PMBT-A in LR, ed. *Skin Lipids*. Vol 24. Elsevier; 1991:1-26. doi:https://doi.org/10.1016/B978-0-12-024924-4.50005-5
2. Elias PM. Epidermal lipids, barrier function, and desquamation. *J Invest Dermatol*. 1983;80(1 Suppl):44s-9s. doi:10.1038/jid.1983.12
3. Tagami H, Kobayashi H, Zhen XS, Kikuchi K. Environmental effects on the functions of the stratum corneum. *J Investig Dermatol Symp Proc*. 2001;6(1):87-94. doi:10.1046/j.0022-202x.2001.00016.x
4. Wilson D, Berardesca E, Maibach HI. In vitro transepidermal water loss: differences between black and white human skin. *Br J Dermatol*. 1988;119(5):647-652. doi:10.1111/j.1365-2133.1988.tb03478.x
5. Wesley NO, Maibach HI. Racial (ethnic) differences in skin properties: the objective data. *Am J Clin Dermatol*. 2003;4(12):843-860. doi:10.2165/00128071-200304120-00004
6. Rawlings A V. Ethnic skin types: Are there differences in skin structure and function? *Int J Cosmet Sci*. 2006;28(2):79-93. doi:10.1111/j.1467-2494.2006.00302.x
7. Kompaore F, Marty JP, Dupont C. In vivo evaluation of the stratum corneum barrier function in blacks, Caucasians and Asians with two noninvasive methods. *Skin Pharmacol*. 1993;6(3):200-207. doi:10.1159/000211136
8. Reed JT, Ghadially R, Elias PM. Skin type, but neither race nor gender, influence epidermal permeability barrier function. *Arch Dermatol*. 1995;131(10):1134-1138.
9. Muizzuddin N, Hellemans L, Van Overloop L, Corstjens H, Declercq L, Maes D. Structural and functional differences in barrier properties of African American, Caucasian and East Asian skin. *J Dermatol Sci*. 2010;59(2):123-128. doi:10.1016/j.jdermsci.2010.06.003
10. Weigand DA, Haygood C, Gaylor JR. Cell layers and density of Negro and Caucasian stratum corneum. *J Invest Dermatol*. 1974;62(6):563-568. doi:10.1111/1523-1747.ep12679412
11. Elias PM, Menon G, Wetzel BK, Williams JW. Barrier requirements as the evolutionary "driver" of epidermal pigmentation in humans. *Am J Hum Biol*. 2010;22(4):526-537. doi:10.1002/ajhb.21043
12. Sugino K, Imokawa G, Maibach HI. Ethnic difference of varied stratum corneum function in relation to stratum corneum lipids. *J Dermatol Sci*. 1993;6(1):108. doi:10.1016/0923-1811(93)91343-s
13. Hellemans L, Muizzuddin N, Declercq L, Maes D. Characterization of stratum corneum properties in human subjects from a different ethnic background. *J Invest Dermatol*. 2005;124.
14. Augustin M, Wilsmann-Theis D, Körber A, et al. Diagnosis and treatment of xerosis cutis – a position paper. *J Ger Soc Dermatology*. 2019;17(S7):3-33. doi:10.1111/ddg.13906
15. Eberting C. Repairing a compromised skin barrier in dermatitis: leveraging the skin's ability to heal itself. *J Allergy Ther*. 2014;05(05). doi:10.4172/2155-6121.1000187
16. Rosso J Del, Zeichner J, Alexis A, Cohen D, Berson D. Understanding the epidermal barrier in healthy and compromised skin: clinically relevant information for the dermatology practitioner: proceedings of an expert panel roundtable meeting. *J Clin Aesthet Dermatol*. 2016;9(4 Suppl 1):S2-S8. http://www.ncbi.nlm.nih.gov/pubmed/28936279%0Ahttp://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC5608132
17. Noda S, Suárez-Fariñas M, Ungar B, et al. The Asian atopic dermatitis phenotype combines features of atopic dermatitis and psoriasis with increased TH17 polarization. *J Allergy Clin Immunol*. 2015;136(5):1254-1264. doi:10.1016/j.jaci.2015.08.015
18. Kaufman BP, Guttman-Yassky E, Alexis AF. Atopic dermatitis in diverse racial and ethnic groups—Variations in epidemiology, genetics, clinical presentation and treatment. *Exp Dermatol*. 2018;27(4):340-357. doi:10.1111/exd.13514
19. Odhiambo JA, Williams HC, Clayton TO, Robertson CF, Asher MI. Global variations in prevalence of eczema symptoms in children from ISAAC Phase Three. *J Allergy Clin Immunol*. 2009;124(6):1251-8.e23. doi:10.1016/j.jaci.2009.10.009
20. Paternoster L, Standl M, Waage J, et al. Multi-ancestry genome-wide association study of 21,000 cases and 95,000 controls identifies new risk loci for atopic dermatitis. *Nat Genet*. 2015;47(12):1449-1456. doi:10.1038/ng.3424
21. Geng YJ, Wei ZY, Qian HY, Huang J, Lodato R, Castriotta RJ. Pathophysiological characteristics and therapeutic approaches for pulmonary injury and cardiovascular complications of coronavirus disease 2019. *Cardiovasc Pathol*. 2020;47(January). doi:10.1016/j.carpath.2020.107228
22. Jiang L, Tang K, Levin M, et al. COVID-19 and multisystem inflammatory syndrome in children and adolescents. *Lancet Infect Dis*. 2020;20(11):e276-88. doi:10.1016/S1473-3099(20)30651-4
23. Trepanowski N, Larson AR, Evers-Meltzer R. Occupational dermatoses among frontline healthcare workers during the COVID-19 pandemic: a cross-sectional survey. *J Am Acad Dermatol*. Published online 2020. doi:10.1016/j.jaad.2020.08.126
24. Beiu C, Mihai M, Popa L, Cima L, Popescu MN. Frequent hand washing for

doi:10.36849/JDD.S589C

COVID-19 prevention can cause hand dermatitis: management tips. *Cureus.* 2020;12(4). doi:10.7759/cureus.7506
25. Rundle CW, Presley CL, Militello M, et al. Hand hygiene during COVID-19: recommendations from the American Contact Dermatitis Society. *J Am Acad Dermatol.* Published online 2020:1-8. doi:10.1016/j.jaad.2020.07.057
26. Han C, Shi J, Chen Y, Zhang Z. Increased flare of acne caused by long-time mask wearing during COVID-19 pandemic among general population. *Dermatol Ther.* 2020;33(4). doi:10.1111/dth.13704
27. Gomolin T, Cline A, Russo M. Maskne: Exacerbation or eruption of acne during the COVID-19 pandemic. *Natl Soc Cutan Med.* 2020;4(5):438-439. doi:10.1111/dth.13704
28. Fluhr JW, Darlenski R, Berardesca E. Ethnic groups and sensitive skin: two examples of special populations in dermatology. *Drug Discov Today Dis Mech.* 2008;5(2):e249-e263. doi:https://doi.org/10.1016/j.ddmec.2008.06.004
29. Kligman AM. Human models for characterizing "sensitive skin." *Cosmet Dermatology.* 2001;14:15-19.
30. Bernstein ET. Cleansing of sensitive skin; with determination of the pH of the skin following use of soap and a soap substitute. *J Invest Dermatol.* 1947;9(1):5-9. doi:10.1038/jid.1947.61
31. Frosch P, Kligman AM. A Method for appraising the stinging capacity of topically applied substances. *Soc Cosmet Chem.* 1977;28:197-209.
32. Maibach HI. The cosmetic intolerance syndrome. *Ear Nose Throat J.* 1987;66(1):29-33.
33. Fisher AA. "Status cosmeticus": a cosmetic intolerance syndrome. *Cutis.* 1990;46(2):109-110.
34. Misery L, Ständer S, Szepietowski JC, et al. Definition of sensitive skin: an expert position paper from the Special Interest Group on Sensitive Skin of the International Forum for the Study of Itch. *Acta Derm Venereol.* 2017;97(1):4-6. doi:10.2340/00015555-2397
35. Willis CM, Shaw S, De Lacharrière O, et al. Sensitive skin: an epidemiological study. *Br J Dermatol.* 2001;145(2):258-263. doi:10.1046/j.1365-2133.2001.04343.x
36. Misery L, Sibaud V, Merial-Kieny C, Taieb C. Sensitive skin in the American population: prevalence, clinical data, and role of the dermatologist. *Int J Dermatol.* 2011;50(8):961-967. doi:10.1111/j.1365-4632.2011.04884.x
37. Falcone D, Richters RJH, Uzunbajakava NE, van Erp PEJ, van de Kerkhof PCM. Risk factors associated with sensitive skin and potential role of lifestyle habits: a cross-sectional study. *Clin Exp Dermatol.* 2017;42(6):656-658. doi:10.1111/ced.13133
38. Xu F, Yan S, Wu M, et al. Self-declared sensitive skin in China: a community-based study in three top metropolises. *J Eur Acad Dermatol Venereol.* 2013;27(3):370-375. doi:10.1111/j.1468-3083.2012.04648.x
39. Berardesca E, Farage M, Maibach H. Sensitive skin: an overview. *Int J Cosmet Sci.* 2013;35(1):2-8. doi:10.1111/j.1468-2494.2012.00754.x
40. Pons-Guiraud A. Sensitive skin: a complex and multifactorial syndrome. *J Cosmet Dermatol.* 2004;3(3):145-148. doi:10.1111/j.1473-2130.2004.00082.x
41. Buhé V, Vié K, Guéré C, et al. Pathophysiological study of sensitive skin. *Acta Derm Venereol.* 2016;96(3):314-318. doi:10.2340/00015555-2235
42. Seidenari S, Francomano M, Mantovani L. Baseline biophysical parameters in subjects with sensitive skin. *Contact Dermatitis.* 1998;38(6):311-315. doi:10.1111/j.1600-0536.1998.tb05764.x
43. Raj N, Voegeli R, Rawlings AV, et al. A fundamental investigation into aspects of the physiology and biochemistry of the stratum corneum in subjects with sensitive skin. *Int J Cosmet Sci.* 2017;39(1):2-10. doi:10.1111/ics.12334
44. Denda M, Nakatani M, Ikeyama K, Tsutsumi M, Denda S. Epidermal keratinocytes as the forefront of the sensory system. *Exp Dermatol.* 2007;16(3):157-161. doi:10.1111/j.1600-0625.2006.00529.x
45. Dhaka A, Viswanath V, Patapoutian A. Trp ion channels and temperature sensation. *Annu Rev Neurosci.* 2006;29:135-161. doi:10.1146/annurev.neuro.29.051605.112958
46. Berardesca E, Cespa M, Farinelli N, Rabbiosi G, Maibach H. In vivo transcutaneous penetration of nicotinates and sensitive skin. *Contact Dermatitis.* 1991;25(1):35-38. doi:10.1111/j.1600-0536.1991.tb01770.x
47. Ohta, M., Hikima, R. and Ogawa T. Physiological characteristics of sensitive skin classified by stinging test. *J Cosmet Science Soc Japan.* 2000;23:163-167.
48. Cho HJ, Chung BY, Lee HB, Kim HO, Park CW, Lee CH. Quantitative study of stratum corneum ceramides contents in patients with sensitive skin. *J Dermatol.* 2012;39(3):295-300. doi:10.1111/j.1346-8138.2011.01406.x
49. Seite S, Misery L. Skin sensitivity and skin microbiota: is there a link? *Exp Dermatol.* 27;2018:1061-1064. doi:10.1111/exd.13686
50. Misery L, Jourdan E, Huet F, et al. Sensitive skin in France: a study on prevalence, relationship with age and skin type and impact on quality of life. *J Eur Acad Dermatol Venereol.* 2018;32(5):791-795. doi:10.1111/jdv.14837
51. Proksch E, Holleran WM, Menon GK, Elias PM, Feingold KR. Barrier function regulates epidermal lipid and DNA synthesis. *Br J Dermatol.* 1993;128(5):473-482. doi:10.1111/j.1365-2133.1993.tb00222.x
52. Zettersten EM, Ghadially R, Feingold KR, Crumrine D, Elias PM. Optimal ratios of topical stratum corneum lipids improve barrier recovery in chronologically aged skin. *J Am Acad Dermatol.* 1997;37(3 Pt 1):403-408. doi:10.1016/s0190-9622(97)70140-3
53. Chamlin SL, Kao J, Frieden IJ, et al. Ceramide-dominant barrier repair lipids alleviate childhood atopic dermatitis: changes in barrier function provide a sensitive indicator of disease activity. *J Am Acad Dermatol.* 2002;47(2):198-208. doi:10.1067/mjd.2002.124617
54. Kircik LH, Del Rosso JQ, Aversa D. Evaluating clinical use of a ceramide-dominant, physiologic lipid-based topical emulsion for atopic dermatitis. *J Clin Aesthet Dermatol.* 2011;4(3):34-40.
55. Draelos ZD. The effect of ceramide-containing skin care products on eczema resolution duration. *Cutis.* 2008;81(1):87-91.
56. Lynde CW, Andriessen A. A cohort study on a ceramide-containing cleanser and moisturizer used for atopic dermatitis. *Cutis.* 2014;93(4):207-213.

AUTHOR CORRESPONDENCE

Yaxian Zhen MD PhD

Email:..... yaxian.zhen@rd.loreal.com

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Alteration to the Skin Barrier Integrity Following Broad-Spectrum UV Exposure in an Ex Vivo Tissue Model

Rebecca Barresi, Emily Chen, I-Chien Liao PhD, Xue Liu PhD, Nada Baalbaki PhD, Stephen Lynch PhD, Patricia Brieva PhD, Miao Wang, Qian Zheng MD PhD, Charbel Bouez PhD
L'Oréal Research and Innovation, Clark, NJ

ABSTRACT

Dynamic changes to the skin barrier's molecular structure and ceramide profile are well-documented in skin conditions such as atopic dermatitis and psoriasis. Pathological and environmental factors have been shown to impair barrier integrity and demonstrate shifts in ceramide composition in the skin. However, the relationship between acute and prolonged sun exposure and its effects on skin barrier homeostasis is insufficiently investigated. This study aims to uncover new scientific evidence to elucidate the relationship of UV irradiation with the skin barrier using an ex vivo tissue model following simulated UVA/UVB exposure.

Fresh ex vivo human skin pretreated either with or without a broad-spectrum sunscreen was exposed to either a physiological or elevated UV condition. Following eight days in culture, structural and molecular changes were evaluated. UV irradiated skin displayed epidermal cell death and altered expression of key barrier proteins. TEM analysis demonstrated disruption to adherens junctions and dissociation between tissue layers following both physiological and extensive UV exposures. An effective broad-spectrum sunscreen containing essential skin ceramides completely protected the skin from such changes. This is one of the first works demonstrating a clear correlation of altered skin barrier integrity using a physiologically relevant dose in an ex vivo tissue model. Our findings also further support the additional importance and benefits of sun protection among the consumers.

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INTRODUCTION

The human skin barrier is a complex structure comprising of physical, chemical, immunological, and microbial components that maintain skin homeostasis whilst protecting the body from external irritants.[1] The outermost layer of the skin, or the stratum corneum (SC), is the skin's first line of defence against external irritants and comprises of corneocytes embedded in a lipid matrix composed of an equimolar ratio of cholesterol (CHOL), free fatty acids (FFAs), ceramides (CERs), and sterol/wax esters.[2] Pathological conditions, such as atopic dermatitis (AD) or psoriasis, are known to trigger barrier disturbance through disruption of the natural ceramide profile, adherens junctions, and key barrier proteins.[3–6] Furthermore, a direct correlation was observed in AD patients, where decreased FFA and CER chain lengths in AD skin caused less dense lipid organization increased transepidermal water loss (TEWL).[7]

The health concerns of prolonged UV exposure are well-documented; it is shown to induce premature photoaging, altered pigmentation, inflammation, and carcinoma.[8–10] In addition, there are several articles investigating the effects of ultraviolet (UV) radiation on skin barrier function.[11–13] UV exposure was reported to induce epidermal barrier damage by altering the tight junction protein expression, disruption to the basement membrane junctions, decrease in the level of covalently bound ceramides, increase stratum corneum (SC) thickness & increase stratum corneum (SC) thickness and TEWL, and induce degradation to the structural and mechanical integrity of the skin.[11,13–22] While each of the studies has furthered the understanding on UV on barrier function, the connection between a physiologically relevant UV dose with the molecular changes to the barrier of the skin remains unclear. This study is the first of two parts that utilized a physiologically relevant, fresh ex vivo skin model to understand how broad-spectrum UV affects the composition of epidermal barrier and illustrate how the application of sunscreen can provide barrier protection. This study also investigated the effects of a physiologically relevant dose, which mimics a chronic exposure to the maximum level of daily UV condition, and an elevated non-physiological exposure dose to serve as a contrast group.

Experimental Design

Ex Vivo Tissue Culture

Fresh ex vivo human skin was acquired from BioIVT LLC (Westbury, NY) one day post-abdominoplasty. A total of seven lots of fresh ex vivo skin was utilized in this study, (Caucasian (n=6), Hispanic (n=1), Male (n=1), Female (n=6), 29–51 years old). Tissue was defatted, cleaned of blood residue, and 1.2mm skin biopsy punches were created. Broad-spectrum CeraVe Hydrating Sunscreen SPF 50 Face Lotion, which contains Ceramides 1, 3, 6, in addition to other essential skin lipids, was applied (4.42 µL/cm^2) to the respective biopsy punches fifteen minutes prior to irradiation. At this time, the study solely studied ceramide-incorporated formulas. These biopsy punches were then exposed to one-time exposure of 20J/cm^2, a five-time exposure of 20J/cm^2 over 1 week or one-time exposure of 100J/

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cm² of 96% UVA/4% UVB using a solar simulator (Sol3A Class AAA Solar Simulator, Newport Corporation, CA). The 20J/cm² and 100J/cm² UV doses falls into an average range of a 3MED and 13MED respectively based on skin phototypes II-III.[23] The 20J/cm² condition simulated the effects of a 1-week exposure to the maximal level of daily UV condition, while the 100J/cm² demonstrated an elevated and severe non-physiological level of UV exposure and serves as a contrast group.[24] Following irradiation, skin explants were cultured in a 12-well transwell at an air-liquid interface in Dulbecco's Modified Eagle's Medium (DMEM) with 10% Fetal Bovine Serum and 1% Penicillin-Streptomycin at 37°C and 5% CO2. The group receiving 20J/cm²/5x over 1 week were subjected to daily UV-exposure conditions and returned to the incubator. Following the 8-day culture period, all biopsies were processed for histological and transmission electron microscopy analysis.

H&E and Immunofluorescence Staining

Skin explants were processed for hematoxylin and eosin staining (Tejas Pathology, Trumbull, TX) and frozen sectioning. Frozen section samples were fixed in methanol/acetone and blocked with 10% normal goat serum for 1 hour at room temperature. Tissue sections were then incubated with TUNEL (Reveal Biosciences, San Diego, CA), Rabbit Polyclonal to Anti-Transglutaminase 1 (Novus Biologicals, Centennial, CO), Rabbit Polyclonal to Anti-Involucrin (Abcam, Cambridge, MA), Mouse Monoclonal to Anti-Desmoglein 1 (Abcam, Cambridge, MA), Rabbit Polyclonal to Anti-Claudin 4 (Abcam, Cambridge, MA), or Anti-Laminin 5 γ2 chain (Millipore Sigma, Temecula, CA) primary antibody. Following the primary staining, tissue sections were then incubated with secondary antibody Goat Anti-Rabbit IgG H&L Alexa Fluor 594 or Goat Anti-Mouse IgG H&L Alexa Fluor 594 (Invitrogen, Carlsbad, CA) and counterstained with DAPI. Frozen sections were stained with Rabbit Polyclonal to Anti-Filaggrin (Abcam, Cambridge, MA) according to the Rabbit Specific HRP/DAB (ABC) Detection IHC Kit (Abcam, Cambridge, MA). All sections were then imaged with a fluorescent microscope (Leica DM500, Wetzlar, Germany).

Transmission Electron Microscopy (TEM)

Tissue explants were fixed in 5% glutaraldehyde solution and prepared for TEM as previously described by Van den Bergh et al.[25] Sections were imaged on Jeol 1200 EX Transmission Electron Microscope (Robert Wood Johnson Medical School, Rutgers University, Piscataway, NJ) at 80kV.

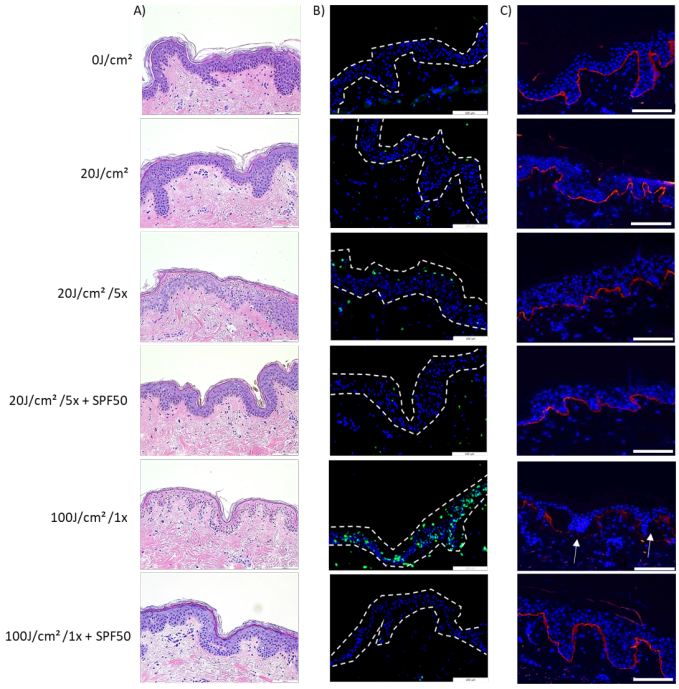
RESULTS/DISCUSSION

The health risks associated with prolonged UV exposure on unprotected skin are well-documented. While the shorter wavelength of UVB light is less able to penetrate the skin, excessive exposure to solar UVB irradiation induces DNA damage and inflammation.[26] The longer wavelength of UVA light penetrates deeper into the skin and is one of the key exogenous factors promoting premature skin aging and inducing oxidative stress. To date, there are limited studies that investigated the relationship of prolonged sun exposure with skin barrier function.[11,14,22] The objective of this study was to elucidate the effects of exposure to high doses of UV irradiations on structural and molecular properties of skin barrier using ex vivo human skin, a model that is able to closely represent physiologically relevant changes. To accomplish this, a solar simulator was utilized with a filter that allowed a balanced ratio of 4% UVB/96% UVA in order to provide physiologically relevant UV energies. The doses elected for this study are 1) 5x exposure to 20J/cm² for 1 week and 2) 1x exposure to 100J/cm². The 20J/cm² group is designed to simulate the effects of 1-week exposure to the maximal level of daily UV condition, while the 100J/cm² group serves as a contrast group when an extreme, above-physiological level of UV irradiation is applied. Multiple skin lots from different donors have provided consistent results.

Structural Changes in UV-Irradiated Tissue

The relationship of UV-exposure with tissue structure was evaluated by hematoxylin and eosin (H&E), Laminin 5 staining, and TUNEL staining to assess epidermal apoptosis. One-time exposure of 20J/cm² UV dose displayed no structural changes while a daily exposure of 20J/cm²/5x demonstrated localized apoptosis in the stratum granulosum (Figure 1A). A one-time

FIGURE 1. Representative images of H&E (A), TUNEL (B), and Laminin 5 (C) staining of ex vivo tissue following various UV energy exposure with/without the application of sunscreen. White arrows denote disruption of Laminin 5 expression following elevated UV exposure (100J/cm²).



Blue-DAPI, Green-TUNEL, Red-Laminin 5

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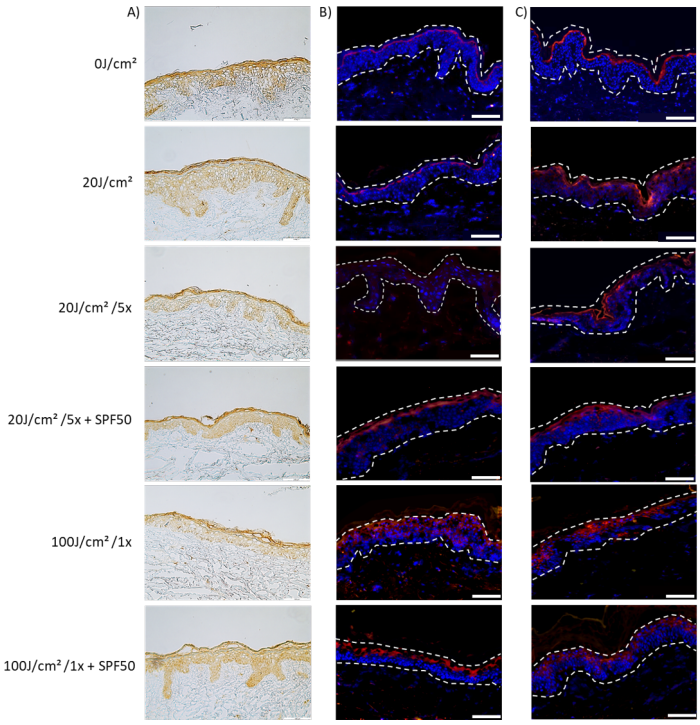
exposure to an elevated UV dose (100J/cm²) demonstrated epidermal cell death and separation of the dermis-epidermis junction (DEJ). Through evaluation of TUNEL expression, there was no significant apoptosis in a one-time 20J/cm² exposure, but there was an observed accumulated expression in the 20J/cm²/5x and 100J/cm² exposure conditions (Figure 1B). One-time and cumulative exposure of 20J/cm² maintains identical Laminin-5 expression to untreated control tissue, while a one-time elevated UV-exposure (100J/cm²) induced interrupted Laminin-5 expression (Figure 1C). The cumulative effect of UV in regard to epidermal cell death and disruption to the DEJ aligned with changes to tissue structure in histology. The application of sunscreen prior to UV irradiation demonstrated a clear protective benefit in the 20J/cm²/5x and 100J/cm² conditions as illustrated by the preservation of tissue morphology in H&E staining, reduction in the number of apoptotic cells and minimized DEJ disruption (Figure 1).

Molecular Changes in UV-Irradiated Tissue

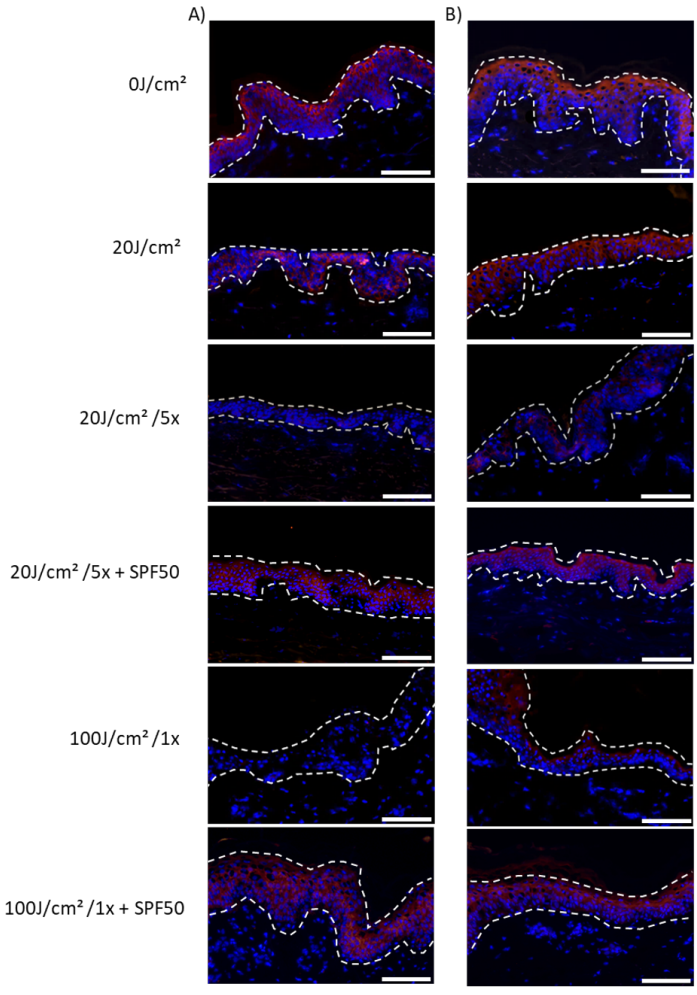
To understand how different doses of UV irradiation can compromise the skin barrier, this study evaluates some of key biomarkers related to barrier function. Immunostaining against Filaggrin (Figure 2A) demonstrated that UV exposure did not negatively influence the expression at all of the doses evaluated in this study, suggesting non-obvious impact on the external skin barrier. Additionally, staining against DSG1 (Figure 3A) and Claudin 4 (Figure 3B) evaluated the impact on the adherens junctions within the stratum granulosum layer. Figure 2B illustrates a significant decrease in expression for 20J/cm²/5x and 100J/cm²/1x treatment groups but not for 20J/cm²/1x, suggesting a cumulative and a dose-dependent effect on disrupting the adherens junctions. The tissue receiving sunscreen prior to UV irradiation showed normal level of DSG1 and Claudin 4 staining, suggesting the role of sunscreen in preventing disruption of the adherens junctions (Figure 3A and 3B). Immunostaining against TGM1 (Figure 2B) increased expression in the extreme UV condition (100J/cm²/1x), which does not hold true for the daily UV exposure condition (20J/cm²/1x and 20J/cm²/5x). This elevated expression in the higher UV dose suggests that the skin was entering a reparative state to address the altered barrier. The increase in TGM1 induced

FIGURE 2. Representative images of Filaggrin (A), Transglutaminase 1 (B) and Involucrin (C) staining of ex vivo tissue following various UV energy exposure with/without the application of sunscreen.



Scale bar = 100µm. Blue-DAPI, Red-TGM1(B)/INV(C)

FIGURE 3. Representative images of Desmoglein 1 (A) and Claudin 4 (B) staining of ex vivo tissue following UV exposure with/without the application of sunscreen.



Scale bar = 100µm. Blue-DAPI, Red-DSG1(A)/CLD4(B)

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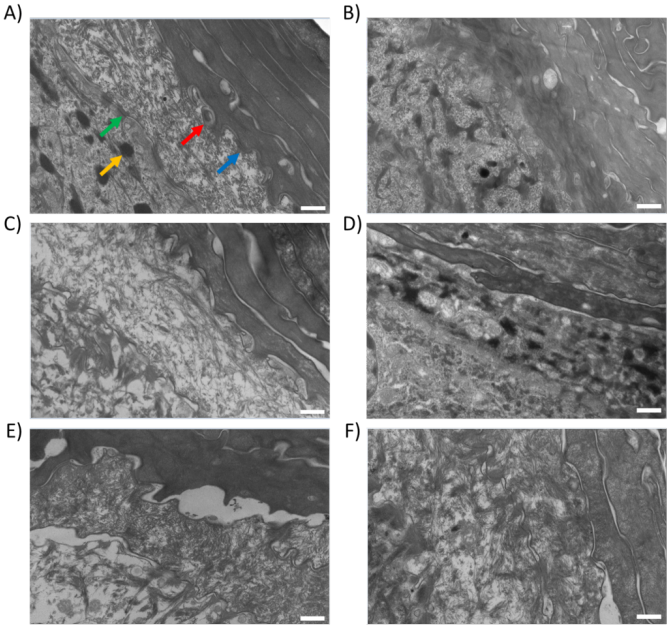
by 100J/cm² is also significantly reduced by the application of sunscreen (Figure 2B). Immunostaining against Involucrin (Figure 2C) demonstrates a modest increase in the expression level only for the 100J/cm² conditions and is ameliorated by the application of sunscreens. Altogether, these stains displayed a cohesive understanding that the skin barrier is compromised not only in the elevated UV dose (100J/cm²) but in some physiological UV conditions (20J/cm²) as well.

TEM Analysis of Ex Vivo Skin Following UV-Exposure

Figure 4 demonstrates TEM images captured to evaluate changes in tissue ultrastructure following UV irradiation. Figure 4A is able to illustrate the presence of intact corneodesmosomes (blue arrow), desmosomes (green arrow), lamellar bodies (red arrow), and keratohyalin granules (yellow arrow) in the stratum corneum and transition layer of untreated control tissue. Untreated tissue also has abundance of keratohyalin granules, an essential component of the keratinocyte cornification process, in the stratum granulosum layer. In agreement with histological and immunostaining analysis, TEM analysis illustrated limited disruption to tissue structure as a result of one-time irradiation at 20J/cm² level (Figure 4B). When the tissue was exposed to a daily irradiation of 20J/cm² over the course of 5 days, alterations to the keratin fibers and disruption to the adherens junctions can be observed. Keratinocytes in the stratum granulosum also became more apoptotic (Figure 4C). The UV irradiation effects on barrier disruption were amplified in the 100J/cm² conditions, where the tissue displayed severe disruption to the adherens junctions, disassociation between cellular layers, and marked reduction of keratohyalin granules and lamellar bodies (Figure 4E). The benefits of photoprotection were observed through TEM images, where the application of sunscreen in the daily irradiation conditions showed better preservation of the adherens junctions and the keratohyalin granules (Figure 4D). At the high UV dose (100J/cm²), photoprotection applied prior to irradiation helped maintain tissue structure, although some regions still demonstrated disrupted corneodesmosomes and reduction of the keratohyalin granules (Figure 4E).

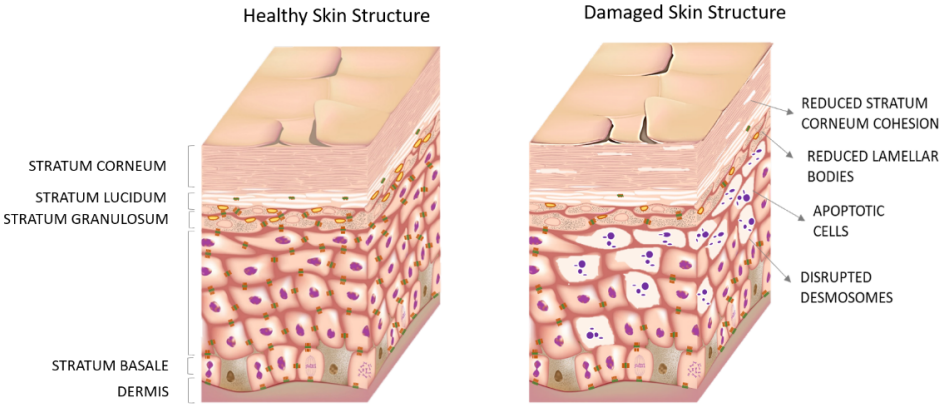
FIGURE 4. Representative TEM images of Untreated Control Tissue (A), 20J/cm² 1x (B), 20J/cm² 5x (C), 20J/cm² 5x with SPF50 Sunscreen (D), 100J/cm² 1x (E), and 100J/cm² 1x with SPF50 Sunscreen (F) treatment groups.



Scale bar = 500nm.

In this study, fresh ex vivo skin was utilized as a physiologically relevant model to understand the dynamic changes to skin barrier as a result of high-level UV irradiation. Our findings can be summarized by Figure 5, which illustrates that prolonged sun exposure significantly impacted the inside-out skin barrier, referring to cell junctions that prevent loss of water, electrolytes, and proteins, while being less potent in altering the outside-in barrier.[27] Our study demonstrated a reduction in proteins that contribute to adherens junctions (Claudin 4 and

FIGURE 5. The relationship of prolonged UV exposure on the skin barrier.



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Desmoglein 1) with no effect on Filaggrin. This observation suggests a potential mechanism in which UV penetrates through the stratum corneum to induce apoptosis at the stratum granulosum layer, which in turn reduces the integrity of the adherens junctions. Since adherens junctions provide the mechanical cohesion between the cells in the epidermal layers and the key signaling cues to cytoskeletal dynamics and polarity, the disruption to the adherens junction have significant function implications.[28] In skin diseases such as psoriasis vulgaris, Occludin and ZO-1 are up-regulated and Claudins are down-regulated, suggesting that the compromised skin is undergoing active repair.[28] The irradiated skin at elevated levels also had increased levels of transglutaminase, which is a hallmark of compromised skin barrier as previously demonstrated in a SLS-challenged skin model.[29] The functional implications to the changes to transglutaminase has also been illustrated in patients suffering from atopic dermatitis (AD) and psoriasis, where lesional tissues marked increase in both TGM1 and TGM3.[5,6] The observed similarities in skin barrier changes following UV exposure with patients suffering from skin disorders further illustrates the importance of adequate photoprotection to prevent the alteration of barrier structure after receiving extended sunlight exposure. This is also one of the first works that clearly demonstrates how sunscreen can have direct benefits to the protection from UV-induced barrier damage, as previous studies have focused on the fundamental science of barrier alterations.[14,30]

Although this study has revealed very interesting structural and molecular changes the skin goes through as a result of UV irradiation, there are still many unanswered questions in the topic of barrier protection against UV irradiation. This study investigated the effect of balanced physiologically relevant UVA/UVB doses but did not explore whether the contribution of barrier disruption is driven by UVB or UVA wavelengths. One study had demonstrated that while suberythemal doses of UVB can be used as a therapeutic treatment for atopic dermatitis, elevated levels can induce barrier disruption.[31] This enables our model to be used to evaluate the therapeutic use of UV by monitoring structural and molecular changes within the tissue at varying UVB doses. While we demonstrated the benefit of sunscreen protection, further work is recommended to compare varying sunscreens with and without ceramides. Furthermore, the relationship between the structural disruption with alterations of ceramides and lipids has yet to be clarified and will be addressed in our future work. One of the key unanswered questions is the clinical and consumer relevance of the observed structural disruption and lipid ratio modification as a result of UV irradiation. The following article in this supplement will unpack the potential changes to skin barrier in a clinical study and also illustrate the benefits of a sunscreen and moisturizer routine following sun exposure. We believe through these efforts, the relationship between UV exposure and the alteration of the skin barrier can be explained, therefore illustrating the importance of barrier restoring photoprotection products.

CONCLUSION

In this study, we have introduced the use of ex vivo skin to identify additional parameters in studying barrier damage following UV exposure. Our results demonstrated that prolonged UV exposure induces epidermal cell death in addition to disruption of key basement membrane proteins. This exposure also altered expression of key differentiation proteins (TGM1, INV) and adherens junction proteins (DSG1, CLD4). Such effects can be ameliorated by the application of a ceramide containing sunscreen.

UV exposure not only induces detrimental effects on skin health and photoaging but has been demonstrated to have acute and prolonged impacts on both the structural and molecular components responsible for maintaining barrier integrity. Further work, which will be an upcoming second part to accompany this study, will be aimed to explore the relationship of the observed results with alterations to the skin ceramide composition following UV exposure. This future study permits for the understanding as to possible alteration of skin ceramide composition following UV exposure and would highlight the importance of introducing proper ceramide blends in sun care and skin care routines to help combat the UV-induced barrier damage and restore skin health following daily sun exposure. Replenishment of essential skin ceramides can not only help to recover the ideal lipid ratio within the skin but to also accelerate recovery of barrier impaired skin. Continued research in the field is needed to not only identify proper lipid combinations that lead to clinical and consumer-perceived benefits following UV exposure, but to also recognize any further benefits of photoprotection associated with UV-induced barrier disruption.

DISCLOSURE

The authors declare no conflict of interest. This research is sponsored by L'Oreal Research & Innovation.

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REFERENCES

1. Niehues H, Bouwstra JA, El Ghalbzouri A, Brandner JM, Zeeuwen PLJM, van den Bogaard EH. 3D skin models for 3R research: The potential of 3D reconstructed skin models to study skin barrier function. *Exp Dermatol.* 2018;27(5):501-511. doi:10.1111/exd.13531
2. van Smeden J., Janssens M., Gooris G.S. BJA. The important role of stratum corneum lipids for the cutaneous barrier function. *Biochim Biophys Acta.* 2014;1841:295-313.
3. Berdyshev E, Goleva E, Bronova I, et al. Lipid abnormalities in atopic skin are driven by type 2 cytokines. *JCI Insight.* 2018;3(4):1-15. doi:10.1172/jci.insight.98006

doi:10.36849/JDD.S589D

4. Pietrzak A, Chabros P, Grywalska E, et al. Serum lipid metabolism in psoriasis and psoriatic arthritis – An update. *Arch Med Sci.* 2019;15(2):369-375. doi:10.5114/aoms.2018.74021
5. Liedén A, Winge MCG, Sääf A, et al. Genetic variation in the epidermal transglutaminase genes is not associated with atopic dermatitis. Brandner JM, ed. *PLoS One.* 2012;7(11):e49694. doi:10.1371/journal.pone.0049694
6. de Koning HD, van den Bogaard EH, Bergboer JGM, et al. Expression profile of cornified envelope structural proteins and keratinocyte differentiation-regulating proteins during skin barrier repair. *Br J Dermatol.* 2012;166(6):1245-1254. doi:10.1111/j.1365-2133.2012.10885.x
7. Smeden J Van, Janssens M, Kaye ECJ, et al. The importance of free fatty acid chain length for the skin barrier function in atopic eczema patients. 2014:45-52. doi:10.1111/exd.12293
8. Morganti P, Morganti G, Colao C. Biofunctional Textiles for Aging Skin. *Biomedicines.* 2019. doi:10.3390/biomedicines7030051
9. Escoffier C, de Rigal J, Rochefort A, Vasselet R, Lévêque JL, Agache PG. Age-related mechanical properties of human skin: An in vivo study. *J Invest Dermatol.* 1989. doi:10.1111/1523-1747.ep12280259
10. Wlaschek M, Tantcheva-Poór I, Naderi L, et al. Solar UV irradiation and dermal photoaging. *J Photochem Photobiol B Biol.* 2001. doi:10.1016/S1011-1344(01)00201-9
11. Biniek K, Levi K, Dauskardt RH. Solar UV radiation reduces the barrier function of human skin. *Proc Natl Acad Sci USA.* 2012. doi:10.1073/pnas.1206851109
12. Bak H, Hong S phil, Jeong SK, et al. Altered epidermal lipid layers induced by long-term exposure to suberythemal-dose ultraviolet. *Int J Dermatol.* 2011;50(7):832-837. doi:10.1111/j.1365-4632.2010.04789.x
13. Pirot F, Falson F. *Skin Barrier Function.* Vol 49; 2017. doi:10.1007/978-3-319-32383-1_139
14. Yuki T, Hachiya A, Kusaka A, et al. Characterization of tight junctions and their disruption by UVB in human epidermis and cultured keratinocytes. *J Invest Dermatol.* 2011;131(10):744-752. doi:10.1038/jid.2010.385
15. Jungersted JM, Høgh JK, Hellgren LI, Jemec GBE, Agner T. The impact of ultraviolet therapy on stratum corneum ceramides and barrier function. *Photodermatol Photoimmunol Photomed.* 2011;27(6):331-333. doi:10.1111/j.1600-0781.2011.00618.x
16. Lipsky ZW, German GK. Ultraviolet light degrades the mechanical and structural properties of human stratum corneum. *J Mech Behav Biomed Mater.* 2019;100(Dsg 1):1-28. doi:10.1016/j.jmbbm.2019.103391
17. Wefers H, Melnik BC, Flür M, Bluhm C, Lehmann P, Plewig G. Influence of UV irradiation on the composition of human stratum corneum lipids. *J Invest Dermatol.* 1990. doi:10.1111/1523-1747.ep12476124
18. Yamamoto T, Kurasawa M, Hattori T, Maeda T, Nakano H, Sasaki H. Relationship between expression of tight junction-related molecules and perturbed epidermal barrier function in UVB-irradiated hairless mice. *Arch Dermatol Res.* 2008;300(2):61-68. doi:10.1007/s00403-007-0817-y
19. Johnson JL, Koetsier JL, Sirico A, et al. The desmosomal protein desmoglein 1 aids recovery of epidermal differentiation after acute UV light exposure. *J Invest Dermatol.* 2014;134(8):2154-2162. doi:10.1038/jid.2014.124
20. Lavker RM. Structural alterations in exposed and unexposed aged skin. *J Invest Dermatol.* 1979;73(1):59-66. doi:10.1111/1523-1747.ep12532763
21. Amano S. Possible involvement of basement membrane damage in skin photoaging. *J Investig Dermatology Symp Proc.* 2009;14(1):2-7. doi:10.1038/jidsymp.2009.5
22. Takagi Y, Nakagawa H, Kondo H, Takema Y, Imokawa G. Decreased levels of covalently bound ceramide are associated with ultraviolet B-induced perturbation of the skin barrier. *J Invest Dermatol.* 2004;123(6):1102-1109. doi:10.1111/j.0022-202X.2004.23491.x
23. Marionnet C, Tricaud C, Bernerd F. Exposure to non-extreme solar UV daylight: Spectral characterization, effects on skin and photoprotection. *Int J Mol Sci.* 2015. doi:10.3390/ijms16010068
24. Rigel EG, Lebwohl MG, Rigel AC, Rigel DS. Ultraviolet radiation in alpine skiing: Magnitude of exposure and importance of regular protection. *Arch Dermatol.* 2003. doi:10.1001/archderm.139.1.60
25. Van den Bergh BAI, Swartzendruber DC, Bos-van der Geest A, et al. Development of an optimal protocol for the ultrastructural examination of skin by transmission electron microscopy. *J Microsc.* 1997;187(2):125-133. doi:10.1046/j.1365-2818.1997.2200779.x
26. D'Orazio J, Jarrett S, Amaro-Ortiz A, Scott T. UV radiation and the skin. *Int J Mol Sci.* 2013. doi:10.3390/ijms140612222
27. 27. Brandner JM, Zorn-Kruppa M, Yoshida T, Moll I, Beck LA, De Benedetto A. Epidermal tight junctions in health and disease. *Tissue Barriers.* 2015. doi :10.4161/21688370.2014.974451
28. Brandner JM, Haftek M, Niessen CM. Adherens junctions, desmosomes and tight junctions in epidermal barrier function. *Open Dermatol J.* 2010;4(1):14-20. doi:10.2174/1874372201004010014
29. Törmä H, Lindberg M, Berne B. Skin barrier disruption by sodium lauryl sulfate-exposure alters the expressions of involucrin, transglutaminase 1, profilaggrin, and kallikreins during the repair phase in human skin in vivo. *J Invest Dermatol.* 2008;128(5):1212-1219. doi:10.1038/sj.jid.5701170
30. Biniek K, Levi K, Dauskardt RH. Solar UV radiation reduces the barrier function of human skin. *Proc Natl Acad Sci.* 2012;109(42):17111-17116. doi:10.1073/pnas.1206851109
31. Permatasari F, Zhou B, Luo D. Epidermal barrier: Adverse and beneficial changes induced by ultraviolet B irradiation depending on the exposure dose and time (Review). *Exp Ther Med.* 2013. doi:10.3892/etm.2013.1175

AUTHOR CORRESPONDENCE

Rebecca Barresi
Email:..... rebecca.barresi@rd.loreal.com

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Efficacy of Ceramide-Containing Formulations on UV-Induced Skin Surface Barrier Alterations

Hawasatu Dumbuya PhD, Xi Yan MD PhD, Ying Chen PhD, Janet Wangari-Olivero PhD, Stephen Lynch PhD, Patricia Brieva PhD, Qian Zheng MD PhD, Charbel Bouez PhD
L'Oréal Research and Innovation, Clark, NJ

ABSTRACT

The human skin, particularly the stratum corneum, serves as a protective barrier against exogenous factors, including ultraviolet radiation (UVR) and pathogen invasions. The impact of UVR on skin cancer and photoaging has been extensively studied. However, the direct impact of UVR on skin barrier integrity under clinical settings remains poorly explored. Due to their benefits in reducing inflammation and promoting skin barrier repair, ceramide-containing formulations can provide added photoprotection benefits. In this study, the efficacy of a ceramide-containing sunscreen and moisturizer were evaluated in preventing UV-induced skin surface barrier changes. Expert grading, instrumental, and tape-stripping assessments demonstrated that UVR induced erythema and hyperpigmentation and caused changes in skin cells surface morphological organization and maturation. Treatment with a ceramide-containing sunscreen and moisturizing cream routine reduced erythema and hyperpigmentation, improved skin hydration, and maintained normal superficial skin cells morphology and turnover after UVR. Our results indicate that barrier-enforcing lipids formulations can provide additional benefits in patient's daily routine by strengthening the barrier and improving skin health overall against chronic sun exposure.

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INTRODUCTION

The epidermis, the skin's outermost layer, functions as a barrier against environmental aggressors through the cornification of keratinocytes to form the stratum corneum (SC). Embedded within a lipid matrix that mainly comprises cholesterol (CHOL), free fatty acids (FFAs), and ceramides (CERs), corneocytes undergo a maturation process that is essential to maintain proper SC barrier integrity and function.[1]

Solar ultraviolet radiation (UVR), comprised of ~95% UVA (320–400 nm) and ~5% UVB (280–320 nm), is a prominent environmental skin stressor.[2] Numerous in vivo and ex vivo models have been developed to evaluate the impact of UVR on skin barrier, revealing mixed results. For instance, UVB and UVA irradiations were shown to increase SC triglycerides, FFAs, alkanes, and squalene levels in subjects with skin phototype II–III.[3] Interestingly, UVB exposure alone decreases SC lipid cohesion and damages intercellular barrier permeability formed by tight-junctions.[4,5] One research group evaluated the impact of UVR on skin biophysical properties on subjects with skin phototype II–IV. They found that UV exposure at various minimal erythema (MED) doses increased trans-epidermal water loss (TEWL) and decreased skin hydration in a dose-dependent manner within 24 hours, suggesting impaired barrier function.[6] Another study demonstrated that 1.5 MED did not affect skin hydration, but increased TEWL after 72 hours. They also observed a decrease in total CERs and increase in CHOL, indicating that alterations in SC lipid content in response to UV may disrupt barrier integrity.[7]

Under real life conditions, chronic sun-exposed hands of middle-aged Japanese golfers were shown to be photodamaged and to have reduced skin hydration, but interestingly, no difference in TEWL compared to the glove-protected hands.[8] It was also demonstrated in Chinese subjects that skin barrier recovery after tape-stripping was not as efficient for body sites exposed to sunlight compared to non-exposed.[9] Overall, these findings indicate that depending on the nature of exposure and skin phototypes, UV-induced changes in skin barrier-related endpoints will greatly vary and may contradict one another. Thus, further studies are needed.

Prior studies have shown that application of an equal ratio of SC lipids promotes barrier repair, and increasing their ratios accelerate recovery.[10] Because of their skin benefits, intercellular lipids, particularly CERs, are now commonly used as prominent ingredients in moisturizers for managing several dermatologic conditions.[11–13] Moreover, many models have been used to evaluate the efficacy of various natural lipid mixtures for optimizing barrier repair in response to exogenous stimuli. For example, Byun et al reported that topical application of CHOL decreased elicited inflammatory response on tape-stripped human skin irradiated with UV while linoleic acid and N-oleoyl-phytosphingosine promoted cell death and inflammation, respectively.[14] Additionally, synthetic CERs are also shown to promote faster barrier recovery after various stimuli, including UV and tape-stripping.[15]

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Despite our growing understanding of the benefits of lipids-containing formulations in promoting skin barrier repair, there is limited knowledge on the clinical efficacy of these formulations following UV exposure. In our study, we investigated the impact of UVR on skin surface barrier properties and evaluated the protective efficacy of a ceramide-containing sunscreen and moisturizing cream.

METHODS

Study Participants

The study was monocentric, randomized, and double-blinded, and performed in accordance with Good Clinical Practices and the principles of the Declaration of Helsinki. The procedures used in this study were approved by IntegReview IRB (Texas, USA). Before any study procedures, the subjects received the necessary information and provided informed consent. Eligibility was determined by physical examination and confirmation of all inclusion and exclusion criteria. Sixteen healthy men and women aged 18–50 years (mean age, 33 years) with Fitzpatrick skin phototype III and an average individual typology angle (ITA°) of 34.9 completed the study.

Solar Stimulator

An ORIEL solar simulator, model 92292-1000 sn 115, was used (Newport, USA). Its artificial luminous source consisted of a 1500 Watts xenon arc lamp, giving a continuous spectrum covering UV (240 nm) until infrared (>800 nm). It included a dichroic mirror that passes 280–400 nm to greatly reduce visible to infrared output. Schott WG 320/2.6 mm filter was used to obtain UVR spectrum (290–400 nm). For each test zone, light intensity was measured just prior to irradiation with a calibrated PMA 2100 radiometer (Solar Light Co., USA), equipped with dUVA and erythema optimal sensors.

UV Irradiation

UV exposure was performed in two consecutive procedures. First, the MED of individual (MEDi) subjects was determined during screening. Six areas of 2.25 cm^2 on the back of each subject were exposed with UV doses using a 1.25 geometrical progression. The starting UV dose was calculated according to the ITA° mean measured on the six areas. MEDi of each subject, with an average of 0.06 J/cm^2, were evaluated 24 hours after irradiation. Secondly, at baseline (day 0), all test zones, excluding MED sites, were irradiated with a single dose of 2 MED.

Test Materials

Test materials consisted of a currently marketed multilamellar vesicular emulsion ceramide-containing sunscreen SPF 25 (SPF) and moisturizing cream (Moisturizer), which were applied at 4 mg/cm^2.

Study Design

On day 0, five test zones of 16 cm^2 were delineated on the middle section of each subject's back: one negative control (untreated and UV-irradiated), one positive control (UV-irradiated only), and three treated and UV-irradiated. The four irradiated zones, excluding the negative control, were exposed to 2 MED. According to a randomization plan, out of the three treated and UV irradiated zones, one received the SPF 15 minutes before exposure on day 0; another received the Moisturizer immediately after exposure on day 0, plus once a day for another nine days (day 1 to day 4 and day 7 to day 11); and the third zone received both the SPF and Moisturizer, as respectively described.

All evaluations were conducted in a room under controlled temperature (22 °C) and relative humidity (40%) after subjects acclimated for at least 15 minutes. Clinical grading for skin pigmentation and erythema, plus standardized photographs were performed at baseline (before product application and UV exposure), day 1, day 7, and day 14; TEWL and skin hydration measurements from day 1 to day 4, day 7 to day 11, and on day 14; and tape-stripping at baseline, day 1, and day 14.

Pigmentation and Erythema Assessments

Skin pigmentation and erythema were visually assessed by expert grading using an internally validated scale, ranging 0 (absence) to 13 (pronounced brown or pink). The scale is based on the visual comparison of the skin color of the test zone with that of the surrounding unexposed control skin. Scoring was performed by the same clinical expert throughout the study. Standardized photographs were taken using a Canon EOS Rebel T5 camera with standard cross polarized filters under the same source of artificial light.

Transepidermal Water Loss (TEWL) and Skin Hydration Measurements

TEWL was assessed to evaluate skin barrier function using a Tewameter (Model TM300; Courage-Khazaka, Germany). Results were expressed in grams of water per unit area of skin per unit of time ($g/m^2/h$), as mean values of the measurement performed on three different areas within the test zone. Skin hydration was assessed using a Corneometer (Model CM825; Courage-Khazaka, Germany). Results were expressed in arbitrary units, as mean values of the measurement performed on five different areas within the test zone.

Tape-stripping Procedure

Tape stripping was performed using 22 mm D-Squame disc (CuDerm Corporation, USA). Six consecutive tapes were placed onto cleaned test sites with even pressure using a pressure plunger before being slowly removed with forceps. The first two tapes were disregarded and the four subsequent tapes from the same location were collected and stored at -80 °C. Tape strips from six subjects out of sixteen who completed the study and whom we considered best responders based on clinical

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assessments were chosen for further analysis.

Corneocyte Cornified Envelope (CE) Maturation

Corneocyte CE maturation technique is based on the double-staining of CE-bound lipids with Nile red and CE structural protein with involucrin. CE maturation was evaluated from the fourth D-Squame of the six mentioned subjects. Briefly, half of the tapes were extracted following Sylnevia laboratory (Labège, France) isolation protocol. Isolated CEs in suspension were placed onto microscope slides and incubated with involucrin primary and respective secondary antibodies before being washed and mounted with Nile red. Images of both Nile red-stained and involucrin immunostained corneocytes were taken separately with a fluorescence microscope (ZEISS, ApoTome). IMAGEJ image analysis software was used to analyze the red pixels obtained from the Nile red stained mature cells and the green pixels from the involucrin immunostained immature cells. The ratio of red /green pixels corresponds to the CE maturation.

Skin Surface Isotropy Assessment by Scanning Electron Microscopy (SEM)

The other half of the fourth D-Squame of the six mentioned subjects were prepared for visualization with SEM (Quanta 250 FEG FEI; ThermoFisher Scientific, USA) by Sylnevia laboratory. Briefly, after being coated with a thin layer of gold, the samples were placed in the microscope, where 36 images per group were taken (6 subjects; 2 timepoints; 3 magnifications: x50, x250, and x500), for a total of 180 images. High-resolution pictures were taken and evaluated in a blinded fashion by one scientist. Adapting the semiquantitative scoring system of Fluhr et al, for SC surface isotropy (ie, micromorphology organizational patterns), three parameters were assessed: cellular clusters at x50, dispersion at x250, and differentiated single cells appearance at x500.[16] Scoring for each parameter according to defined criteria was translated into a quantitative scale from 0 to 3. The sum of individual scores obtained after evaluation of the three parameters gives a skin surface isotropy score. Lower score corresponds to a more disorganized SC surface morphology (low isotropy).

Statistical Analysis

For pigmentation and erythema clinical scores, TEWL and hydration index, linear mixed models were used to analyze longitudinal data with change from baseline as response vector; baseline, time, treatment and treatment-time interaction as fixed effect; and subject as random effect. *P* values were adjusted with Benjamini-Hochberg approach for TEWL and Hydration Index, and a signed-rank Wilcoxon test for pigmentation and erythema scores.

For tape-stripping analysis endpoints, data were analyzed to determine mean, and standard error with normality not assumed according to the number of samples per group. Bonferroni's multiple comparison test was first performed, followed by a Wilcoxon matched-pairs signed rank test to compare each condition at each time points. *P* values <0.05 were considered statistically significant.

RESULTS

Skin Color Change after UVR

Clinical assessment for erythema and skin pigmentation are illustrated in Figure 1A and 1B, respectively. UVR elicited a perceivable and statistically significant increase in erythema, peaking at day 1 and recovering to baseline by day 7. Treatment with SPF or SPF+Moisturizer routine presented with a significantly less-marked increase in erythema; while treatment with Moisturizer showed no significant effect and was similar to UV only (Figure 1A). For skin pigmentation, UV induced a noticeable and statistically significant skin darkening response, which persisted up to day 14. Treatment with SPF or SPF+Moisturizer routine presented a statistically significant, but less-pronounced increase in pigmentation, which was maintained at minimal level following irradiation until day 14; whereas treatment with Moisturizer showed no significant effect (Figure 1B and 1C). Pairwise comparisons reveal no statistical difference between UV only and Moisturizer for erythema and pigmentation. Treatment with SPF or SPF+Moisturizer routine showed similar performance and were most effective in reducing both erythema and hyper-pigmentation after UVR at all timepoints (Table 1).

Skin Barrier Properties (Hydration and TEWL) after UVR

Next, we investigated the impact of UV on skin barrier by assessing skin hydration and TEWL. There was no statistical difference in skin hydration between control and UV only zones (Figure 2A). Compared to UV only, SPF+Moisturizer routine showed an increasing statistical trend in skin hydration at day 1, and demonstrated significant higher hydration levels by day 3, day 7 and day 14. Treatment with Moisturizer alone showed an increasing trend in skin hydration at day 3 and significant improvement by day 14 compared to UV only, while treatment with SPF showed improved skin hydration only at day 14 (Figure 2A and Table 1). These results suggest that SPF+Moisturizer routine and Moisturizer alone, to a lesser extent, were both effective in promoting skin hydration following UVR.

TEWL showed smaller variations over time following UV, inducing no significant change in all conditions (Figure 2B). Table 1 illustrates no statistical differences in performance between treatments, except at day 3, where SPF+Moisturizer routine showed significant reduced TEWL compared to UV only.

Corneocyte Visualization and Maturation after UVR

To further elucidate the impact of UV on skin barrier integrity, we determined whether UVR affects the superficial SC surface

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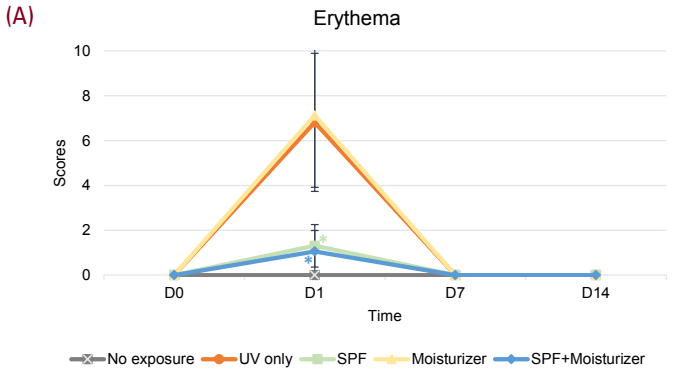
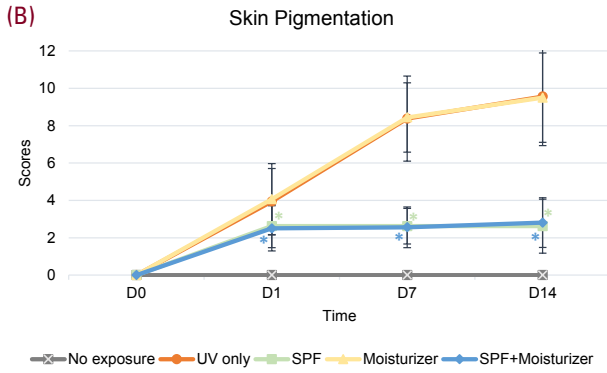
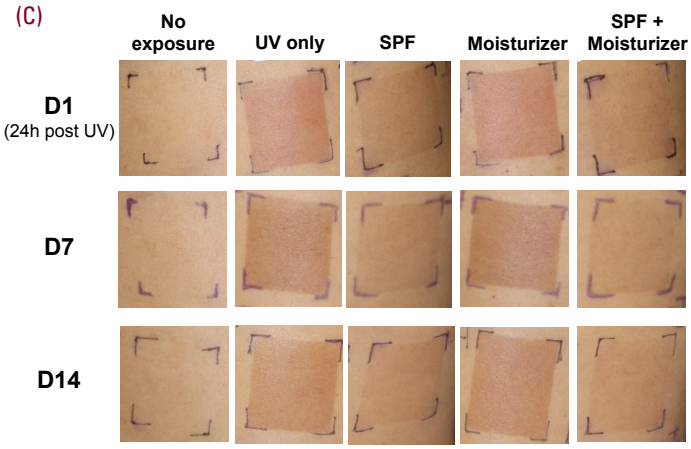
FIGURE 1. Sunscreen alone or in combination with moisturizer decrease UV-induced erythema and hyperpigmentation, while treatment with moisturizer alone was similar to UV only site. (A) Clinical grading of erythema and (B) pigmentation scores for each condition following UV exposure. (C) Representative images of UV-induced erythema and pigmentation responses for each condition at indicated timepoints.







isotropy by assessing corneocyte micromorphology, using SEM on tape-stripped skin samples from six subjects, whom we considered best responders based on clinical assessments. Similar to Fluhr et al., the skin surface isotropy was obtained by evaluating three corneocyte microstructural parameters: cellular clusters at magnification x50, dispersion at x250, and differentiated single cell appearance at x500 (Figure 3A).[16] We found that the UV only zone tended to exhibit a lower SC surface isotropy compared to control at day 1 and day 14 after irradiation, indicating disruption of superficial SC barrier organization patterns (Figure 3A and 3B). At day 1 following UVR, the appearance of both regular clusters and well differentiated corneocytes were significantly reduced in UV only, which the latter tended to be prevented by SPF+Moisturizer routine. By day 14, treatment with SPF or SPF+Moisturizer routine significantly preserved the appearance of well differentiated cornecoytes comparable to control, while weakly differentiated cells persisted in UV only (Figure 3C and 3D). Together, these results suggest that the SPF+Moisturizer routine tended to be most effective in maintaining SC barrier morphological features after UV exposure.

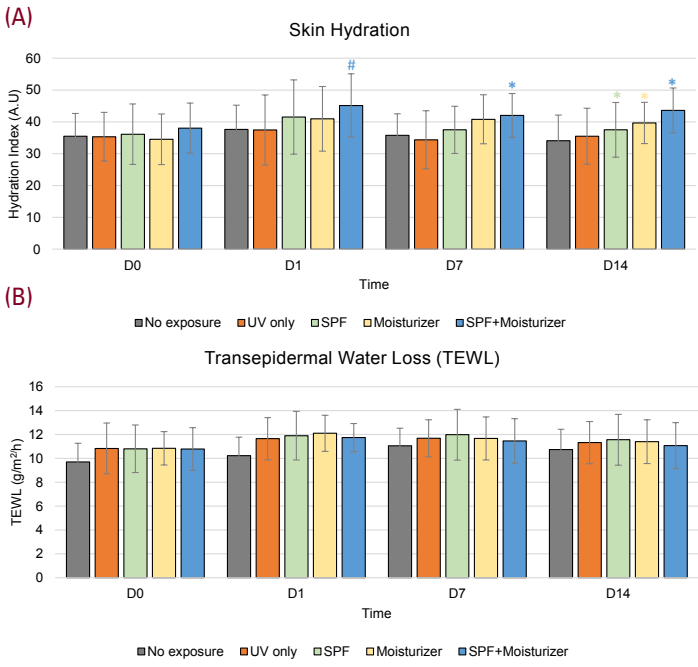
TABLE 1.

Efficacy of Each Product for Indicated Endpoints and Timepoints

Parameters (Compared to UV only)	SPF				Moisturizer				SPF+Moisturizer			
	D1	D3	D7	D14	D1	D3	D7	D14	D1	D3	D7	D14
Erythema	↓				↔				↓			
Hyperpigmentation	↓		↓	↓	↔	↔	↔	↔	↓		↓	↓
Skin Hydration	↔	↔	↔	↑	↔	↑	↔	↑	↑	↑	↑	↑
Barrier Function Impaired (TEWL)	↔	↔	↔	↔	↔	↔	↔	↔	↔	↓	↔	↔
Irregular Skin Cells Appearance	↔			↓	↔			↓	↓			↓

↑ denotes statistical significance, $P<0.05$; ↑ denotes trend, P value between 0.1–0.05;
↔ denotes not significant

FIGURE 2. Following UV exposure, treatment with sunscreen and moisturizer alone or in combination improve skin hydration but cause no relevant change in TEWL. Change in (A) skin hydration and (B) TEWL at indicated timepoints for each condition following UV exposure.



*denotes $P<.05$ and # denotes P value between 0.1–0.05 vs UV only.

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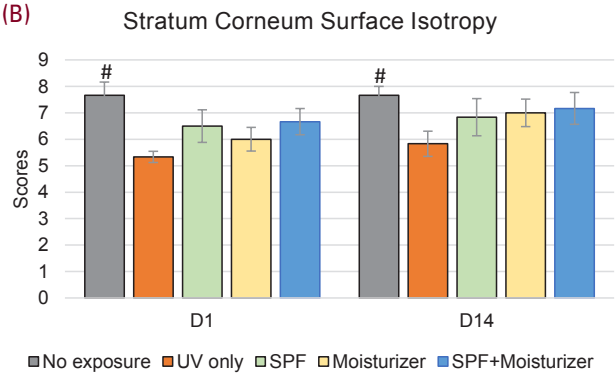
FIGURE 3. Following UV exposure, treatment with sunscreen and moisturizer alone or in combination tend to promote regular stratum corneum (SC) surface isotropy by maintaining normal appearance of differentiated superficial corneocytes. (A) SC surface isotropy scores observed between conditions at day 1 and day 14 post UV, (B) resulting from the sum of individual scores obtained after evaluation of clusters (x50), dispersion (x250) and differentiated single cells appearance (x500). (C) Representative scanning electron images of superficial SC corneocytes obtained by tape-stripping at day 14 post UV for each condition at magnification x500, scale bars = 50 µM. Arrows pointing to cells appearing weakly differentiated. (D) Mean scores of differentiated single cells appearance observed between conditions at day 14 (x500) post UV.

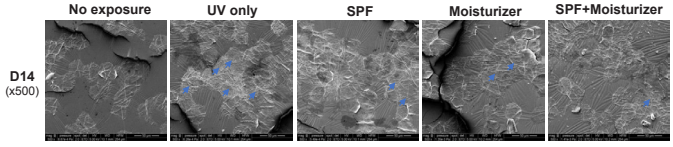
(A)

	Clusters Appearance (x50)		Dispersion Form (x250)		Differentiated Single Cells Appearance (x500)		SC Surface Isotropy (sum score)	
	D1	D14	D1	D14	D1	D14	D1	D14
No exposure	2.67*	2.5	2.33	2.5	2.67*	2.67*	**7.67#**	**7.67#**
UV only	1.67	2.33	2	1.83	1.67	1.67	**5.34**	**5.83**
SPF	2.17	2.17	2.17	2	2.17	2.67*	**6.51**	**6.84**
Moisturizer	2.17	2.5	2	2.17	1.83	2.33#	**6.00**	**7.00**
SPF+Moisturizer	2.17	2.17	2.17	2.5	2.33#	2.5*	**6.67**	**7.17**

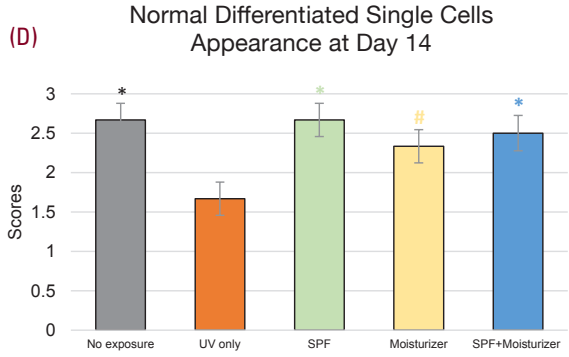
(B)



(C)



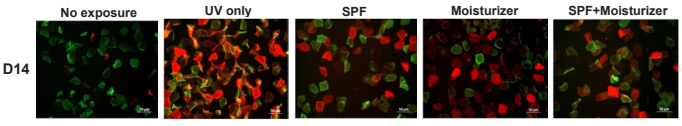
(D)



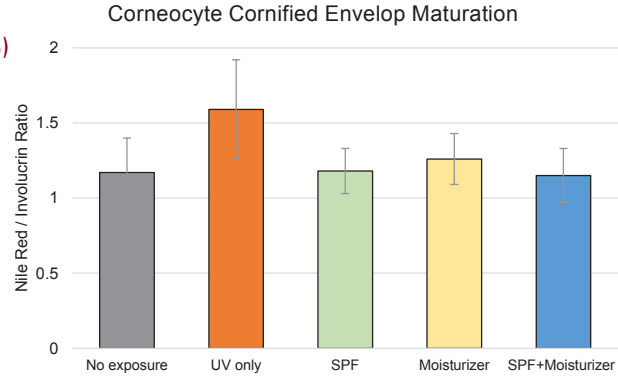
*denotes *P*<.05 and # denotes *P* value between 0.1–0.05 vs UV only.

FIGURE 4. UV exposure tends to alter corneocyte cornified envelop (CE) maturation, which is minimized by treatment with sunscreen and moisturizer alone or in combination. (A) Representative images of double staining patterns for each treatment at day 14 post UV of corneocyte CE maturation with Nile red (red) and antiinvolucrin (green); scale bars = 50 µM. (B) Quantification of Nile red/Involucrin ratio between conditions at day 14 post UV.

(A)



(B)



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To identify the possible mechanism of UV-induced superficial SC barrier alterations, we investigated whether UVR disrupts corneocyte cornified envelope (CE) maturation properties in the same six subjects. Double staining with Nile red and anti-involucrin revealed no clear trend at day 1 (data not shown). By day 14, UVR tended to stimulate CE maturation (increase in Nile red) in UV only zone, while treatment with SPF or Moisturizer alone or in combination tended to reduce response comparable to control (Figure 4A and 4B). However due to the variation in small sample size, no statistically significant difference was detected.

DISCUSSION

Due to their benefits in promoting skin barrier strength and repair, incorporation of SC lipids into formulas has become increasingly popular across the skincare field.[17] Since our skin is constantly exposed to UVR, it is crucial to delineate its influence on skin barrier, plus to assess the potential benefits of barrier-enforcing lipids formulations for solar protection. Here, we demonstrate the clinical efficacy of a ceramide-containing sunscreen and moisturizer routine in preventing UV-induced skin surface barrier changes.

Solar radiation leads to an immediate skin inflammatory response followed by a protective process, which clinically manifest as erythema and hyper-pigmentation respectively.[18] We showed that treatment with ceramide-containing sunscreen alone or in combination with moisturizing cream prevented the initial erythema response, as well as reduced skin darkening at all timepoints compared to UV only and moisturizer alone (Figure 1A and 1B). The protective effects provided by the ceramide-containing sunscreen is likely due to its UV-filter capability, as the photoprotective clinical efficacy of sunscreens against UV-induced cutaneous responses are well documented.[19] Although the exact mechanism awaits further investigation, growing evidence indicates that CERs also have anti-pigmentation properties.[20] Future studies are needed to expand on these premises and determine the mechanisms of CERs depigmentation capabilities, and added benefits when combined with UV filters.

UV is shown to disrupt skin barrier integrity by increasing TEWL, decreasing skin hydration, promoting SC and epidermal thickness, plus changing skin lipids and proteins levels and structures.[4,6,21] Despite the differences in study conditions, the variation of UVR effects on skin barrier-related endpoints remains poorly understood. Haratake et al, demonstrated that UVR (7.5 MED) can lead to a delayed impaired barrier response followed by rapid recovery, which was dependent on epidermal hyperproliferation and inflammation.[22] Subsequently, Holleran et al, reported that the same UV dose after 24 hours caused incidence of damaged lamellar bodies (LBs) only at the stratum granulosum (SG) and SC interface, which contributed to the delayed in abnormal barrier permeability and no change in TEWL. Following 72 hours, he observed a deficient lamellar membrane in the lower SC and an increase in impaired LBs at SG/SC interface, causing elevated TEWL and compromised barrier. By 120 hours, there was a hyperproliferative response promoting thickening of the SG and arrival of normal lamellar membranes in the lower SC, which in turn resulted in the restoration of the epidermal barrier.[23]

In our study, UVR (2 MED) did not cause a drastic change in either TEWL nor hydration (Figure 2A and 2B), which could be attributed to the skin's ability to delay barrier deficiency and rapidly recover from superficial damage. Nevertheless, treatment with ceramide-containing sunscreen in combination with moisturizer (SPF+Moisturizer) improved skin hydration over time, indicating that the skin water content, which is essential for maintaining barrier function, was both maintained and ameliorated. Moreover, we observed that UVR tended to alter skin surface organization patterns and promote corneocyte maturation (Figure 3 and 4). Out of the three corneocyte microstructural parameters evaluated, UVR significantly increased the appearance of weakly differentiated cells in untreated skin, which persisted up to day 14 and was prevented by treating with the sunscreen or moisturizer alone or in combination (Figure 3C and 3D). This phenomenon is consistent with the ability of UVR to decrease SC cohesion by altering intracellular lipids and corneodesmosomes to compromise barrier integrity.[4] Altogether, our findings suggest that an increase in corneocyte maturation was a result of some degree of UV-induced skin barrier damage, disrupting superficial SC morphology. Thus, increased SC turnover or epidermal hyperplasia, as shown in prior studies, are all compensatory mechanisms that the skin barrier utilizes to adapt in response to UV stress to prevent subsequent damage.[21–23] Our results indicate that a skincare routine combining a ceramide-containing sunscreen and moisturizer may prevent early UV-induced skin barrier damage and the consequent skin physiological alterations. However, some limitation should be noted. We were unable to compare the efficacy of our ceramide-containing products with non-ceramide containing sunscreen and moisturizer due to limited test sites on subjects. Future studies will expand on our findings and determine the exact mechanism of CERs capabilities, plus added benefits when combined with UV filters and other ingredients for promoting skin barrier health in response to UV-induced stress.

CONCLUSION

Collectively, our results show that a ceramide-containing sunscreen and moisturizer routine protects against UV-induced skin surface barrier changes by preventing erythema and hyperpigmentation, improving skin hydration, and maintaining normal superficial skin cells morphology and turnover. In addition to improving appearance of lesions and minimizing

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skin irritation, our findings highlight that delivering skin-identical SC lipids could add benefits to patients' daily routine by strengthening the barrier and improving skin health overall against chronic sun exposure.

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REFERENCES

1. Feingold KR, Elias PM. Role of lipids in the formation and maintenance of the cutaneous permeability barrier. *Biochim Biophys Acta - Mol Cell Biol Lipids.* Published online 2014. doi:10.1016/j.bbalip.2013.11.007
2. Amaro-Ortiz A, Yan B, D'Orazio JA. Ultraviolet radiation, aging and the skin: Prevention of damage by topical cAMP manipulation. *Molecules.* Published online 2014. doi:10.3390/molecules19056202
3. Wefers H, Melnik BC, Flür M, Bluhm C, Lehmann P, Plewig G. Influence of UV irradiation on the composition of human stratum corneum lipids. *J Invest Dermatol.* Published online 1990. doi:10.1111/1523-1747.ep12476124
4. Biniek K, Levi K, Dauskardt RH. Solar UV radiation reduces the barrier function of human skin. *Proc Natl Acad Sci U S A.* Published online 2012. doi:10.1073/pnas.1206851109
5. Yuki T, Hachiya A, Kusaka A, et al. Characterization of tight junctions and their disruption by UVB in human epidermis and cultured keratinocytes. *J Invest Dermatol.* Published online 2011. doi:10.1038/jid.2010.385
6. Lim SH, Kim SM, Lee YW, Ahn KJ, Choe YB. Change of biophysical properties of the skin caused by ultraviolet radiation-induced photodamage in Koreans. *Ski Res Technol.* Published online 2008. doi:10.1111/j.1600-0846.2007.00272.x
7. Yoon SH, Park J Il, Lee JE, Myung CH, Hwang JS. In vivo change of keratin-bound molecules in the human stratum corneum following exposure to ultraviolet radiation. *Skin Pharmacol Physiol.* Published online 2019. doi:10.1159/000501132
8. Kikuchi-Numagami K, Suetake T, Yanai M, Takahashi M, Tanaka M, Tagami H. Functional and morphological studies of photodamaged skin on the hands of middle-aged Japanese golfers. *Eur J Dermatology.* Published online 2000.
9. Liu Z, Fluhr JW, Song SP, et al. Sun-Induced changes in stratum corneum function are gender and dose dependent in a chinese population. *Skin Pharmacol Physiol.* Published online 2010. doi:10.1159/000314138
10. Zhang Z, Lukic M, Savic S, Lunter DJ. Reinforcement of barrier function – skin repair formulations to deliver physiological lipids into skin. *Int J Cosmet Sci.* Published online 2018. doi:10.1111/ics.12491
11. Lynde CW AAA. A cohort study on a ceramide-containing cleanser and moisturizer used for atopic dermatitis. *Randomized Control Trial.* Published online 2014.
12. Cannizzaro M V., Dattola A, Garofalo V, Del Duca E, Bianchi L. Reducing the oral isotretinoin skin side effects: efficacy of 8% omega-ceramides, hydrophilic sugars, 5% niacinamide cream compound in acne patients. *G Ital di Dermatologia e Venereol.* Published online 2018. doi:10.23736/S0392-0488.17.05742-X
13. Draelos ZD, Baalbaki NH, Raab S, Colón G. The effect of a ceramide-containing product on stratum corneum lipid levels in dry legs. *J Drugs Dermatol.* Published online 2020. doi:10.36849/JDD.2020.4796
14. Byun HJ, Cho KH, Eun HC, et al. Lipid ingredients in moisturizers can modulate skin responses to UV in barrier-disrupted human skin in vivo. *J Dermatol Sci.* Published online 2012. doi:10.1016/j.jdermsci.2011.12.005
15. Oh MJ, Nam JJ, Lee EO, Kim JW, Park CS. A synthetic C16 omega-hydroxyphytoceramide improves skin barrier functions from diversely perturbed epidermal conditions. *Arch Dermatol Res.* Published online 2016. doi:10.1007/s00403-016-1674-3
16. Fluhr JW, Lachmann N, Baudouin C, et al. Development and organization of human stratum corneum after birth: Electron microscopy isotropy score and immunocytochemical corneocyte labelling as epidermal maturation's markers in infancy. *Br J Dermatol.* Published online 2014. doi:10.1111/bjd.12880
17. Kahraman E, Kaykın M, Şahin Bektay H, Güngör S. Recent advances on topical application of ceramides to restore barrier function of skin. *Cosmetics.* Published online 2019. doi:10.3390/cosmetics6030052
18. Casetti F, Miese A, Mueller ML, Simon JC, Schempp CM. Double trouble from sunburn: UVB-induced erythema is associated with a transient decrease in skin pigmentation. *Skin Pharmacol Physiol.* Published online 2011. doi:10.1159/000323274
19. Young AR, Claveau J, Rossi AB. Ultraviolet radiation and the skin: Photobiology and sunscreen photoprotection. *J Am Acad Dermatol.* Published online 2017. doi:10.1016/j.jaad.2016.09.038
20. Kagotani K, Nakayama H, Zang L, et al. Lecithin-based dermal drug delivery for anti-pigmentation maize ceramide. *Molecules.* Published online 2020. doi:10.3390/molecules25071595
21. Seité S, Medaisko C, Christiaens F, et al. Biological effects of simulated ultraviolet daylight: A new approach to investigate daily photoprotection. *Photodermatol Photoimmunol Photomed.* Published online 2006. doi:10.1111/j.1600-0781.2006.00209.x
22. Haratake A, Uchida Y, Schmuth M, et al. UVB-induced alterations in permeability barrier function: Roles for epidermal hyperproliferation and thymocyte-mediated response. *J Invest Dermatol.* Published online 1997. doi:10.1111/1523-1747.ep12292163
23. Holleran WM, Uchida Y, Halkier-Sorensen L, et al. Structural and biochemical basis for the UVB-induced alterations in epidermal barrier function. *Photodermatol Photoimmunol Photomed.* Published online 1997. doi:10.1111/j.1600-0781.1997.tb00214.x

AUTHOR CORRESPONDENCE

Hawasatu Dumbuya PhD
Email:..... hawasatu.dumbuya@rd.loreal.com

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