doi:10.36849/JDD.S589B

April 2021 s10 Volume 20 • Issue 4 (supplement)

ORIGINAL ARTICLE

Journal of Drugs in Dermatology

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.

J Drugs Dermatol. 2021;20(4 Suppl):s10-16. doi:10.36849/JDD.S589B

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]

doi:10.36849/JDD.S589B

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

doi:10.36849/JDD.S589B

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

doi:10.36849/JDD.S589B

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

doi:10.36849/JDD.S589B

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

doi:10.36849/JDD.S589B

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