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Review

Recent advances in skin 'barrier' research

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Abstract

Objectives Our knowledge on the complexity of stratum corneum biology, chemistry and biophysics has grown over the last decade. This and the intricate control mechanisms in the stratum corneum that bring about its full and proper structural maturity will be reviewed.
Key findings The importance of the total architecture of the stratum corneum in relation to desquamation and barrier function, the role of the corneodesmosomes and their degrading enzymes, new insights into the importance of natural moisturising factor and the emerging knowledge on the chemical antimicrobial barrier of the stratum corneum are discussed.
Summary Despite our increasing knowledge of the complexity of stratum corneum, we are still far from understanding its intricate control mechanisms that bring about its maturity and desquamation.
Keywords barrier; ceramide; desquamation; kallikrein; stratum corneum

Introduction

This short review gives a historical overview of what I think has been important in this area over the last 10 years of research conducted by both academic and industry scientists on the 'barrier' function of the stratum corneum. I use inverted commas as we need to consider barrier function in its broadest context including hydration, desquamation and its antimicrobial shield. However, I will also set the scene with a few selected older references to complement the newer research conducted over the last decade.

The original 'bricks and mortar' model has been refined over the years and it is now recognised as a continuous poly-proteinaceous structure of varying thickness (the bricks are tightly-interconnected by corneodesmosomes in all layers of the stratum corneum; but the bricks are actually more like natural moisturising factor (NMF)-containing keratin sponges as they hydrate extensively) interspersed between a continuous highly-ordered lamellar and largely orthorhombically-packed lipid phase (Figure 1).[1–10]

Corneocyte maturation

The structure, however, is not a homogeneous brick wall. The corneocytes change in dimension and biochemistry as they transit from the 'stratum compactum' to the 'stratum disjunctum'. Increased transglutaminase-mediated protein crosslinks and increased levels of corneocyte envelope-bound ceramides and fatty acids aid the transition of a 'fragile' corneocyte (CE_f) to a 'rigid' phenotype (CE_r). During this journey the CE_fs lose their many non-peripheral corneodesmosomes and the transformed CE_rs only have peripheral corneodesmosomal attachments with the corneocytes interdigitating within each consecutive layer (Figure 2).[11]

The presence of the CE_fs are reported to be increased in both diseased and cosmetic barrier compromised conditions.[12–14] The loss of the non-peripheral corneodesmosomes and the initial transglutaminase-mediated corneocytic changes seem to appear at the same time as another important epidermal protein is catabolised, namely (pro)filaggrin.

(Pro)filaggrin biology

Profilaggrin is an NH_2-terminal Ca^{2+}-binding protein of the S-100 family, linked to 10–20 tandem filaggrin monomer repeats.[15,16] Furin, a member of the proprotein convertase family, has been proposed to cleave the N-terminus of profilaggrin, facilitating the release of the S-100 protein. Calpain I and profilaggrin endopeptidase (PEP-I) have been implicated in the processing of the linker regions between the filaggrin monomer repeats to

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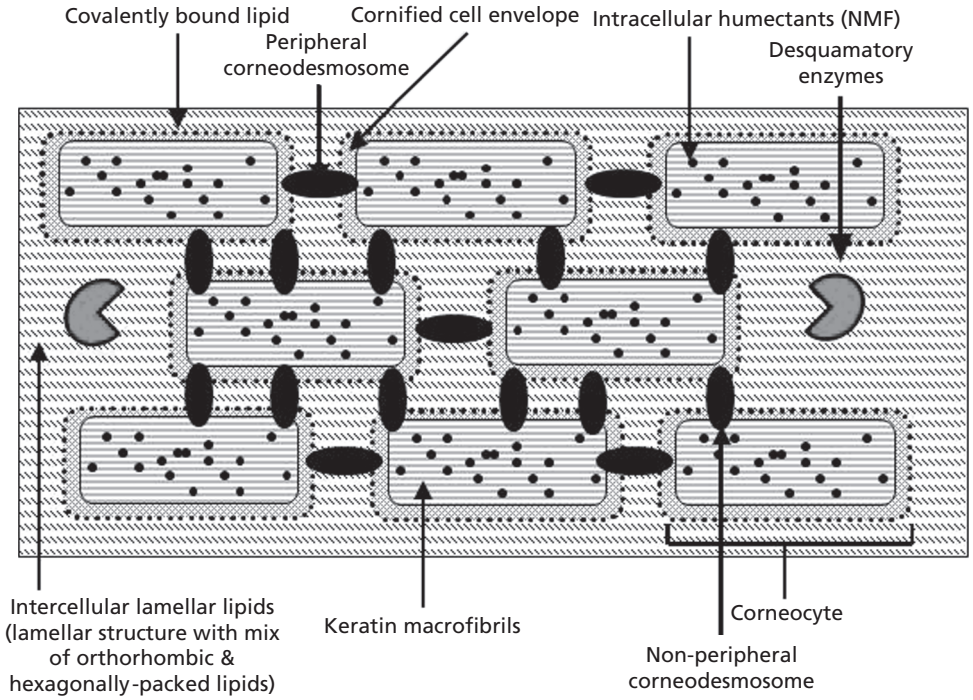

Figure 1 Refined 'bricks and mortar' representation of the structural components of the stratum corneum. Modified from Harding.[10] NMF, natural moisturising factor

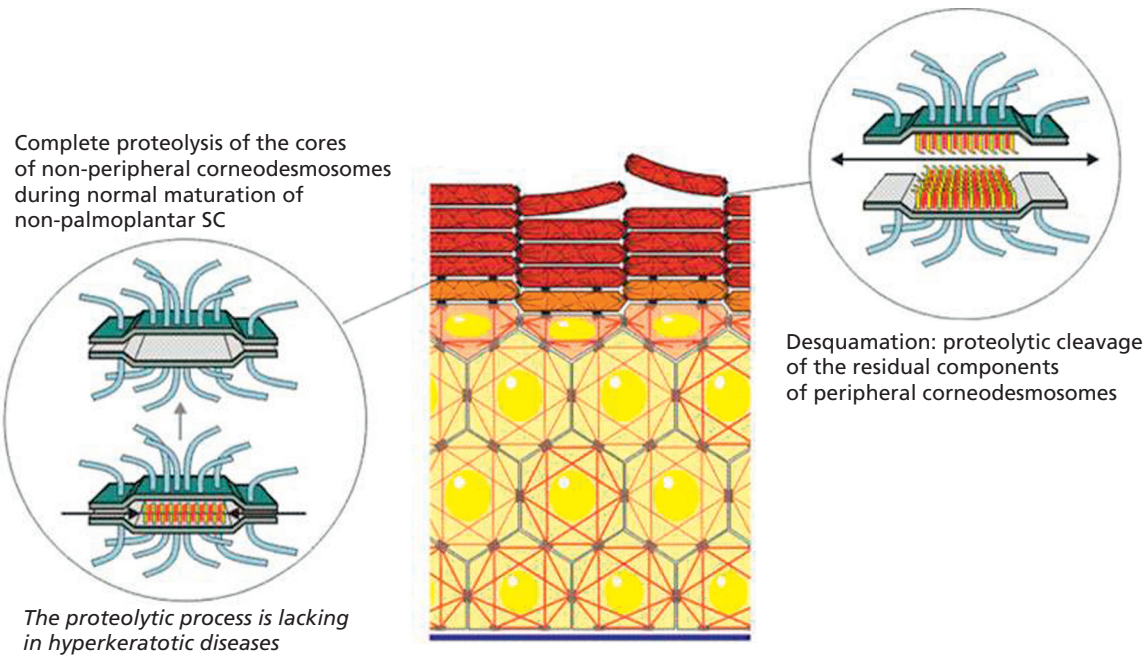

Figure 2 Representation of initial degradation of non-peripheral corneodesmosomes. This representation of the initial degradation of non-peripheral corneodesmosomes shows the early stages of desquamation in the lower layers of the stratum corneum (SC) followed by the later degradation of the peripheral corneodesmosomes in the upper layers of the stratum corneum to facilitate corneocyte cell loss. Figure courtesy of Professor G. Serre, University of Toulouse, France

generate the filaggrin monomer. Matriptase/MT-SP1, prostatin/channel activating serine protease I/PRSS 8 and caspase 14 have also been implicated in profilaggrin conversion. Each individual repeat is completely removed by proteolysis to generate the mature filaggrin monomer, which is then deiminated by peptidylargininedeiminase 1 or 3 before being degraded in the uppermost layer of the stratum corneum to natural moisturising factor (NMF). Limited caspase

14-generated peptides are produced from the deiminated filaggrin (Cit-F) but the cysteine protease calpain I, a calcium-activated protease, preferentially degrades Cit-F into smaller peptides, which are acted on by a neutral cysteine protease: bleomycin hydrolase.[17] It has been demonstrated that bleomycin hydrolase knockout mice developed an ichthyosis-like phenotype that resembled the phenotype of the filaggrin-deficient flaky tail (ft/ft) mouse mutant.[18] However, the processing of filaggrin is very humidity dependent, where high humidities reduce its biotransformation.[19]

Role of natural moisturising factor

NMF, as it was originally named, is a plasticiser of the stratum corneum under basal conditions.[20,21] At high humidity, or after the application of water, the NMF-bound water aids the swelling of corneocytes above the 'stratum compactum'.[22] The corneocyte swelling characteristics are naturally greatest where the greater concentration of NMF is located, but also where the mechanical constraints have been removed i.e. the non-peripheral corneodesmosomes.[11,15] As a gradation of NMF occurs towards the surface layers of the stratum corneum, due to it being washed from the skin during bathing, and the corneocytes are greatly mechanically strengthened, the swelling of the corneocytes reduces towards the skin's surface.[23,24]

Filaggrin, natural moisturising factor and problem skin

Profilaggrin and NMF synthesis are compromised in problem skin and especially in subjects with atopic dermatitis. It has been known for decades that NMFs are reduced in the stratum corneum in subjects with atopic dermatitis. However, mutations in the filaggrin gene have now been shown to be the major predisposing factor for atopic dermatitis.[16] A dose relationship between filaggrin deficiency and disease severity is known such that patients with double allele mutations display a greater severity of the disease and express an earlier onset. The flaky tail mouse model, which exhibits reduced filaggrin expression from a lack of processing of profilaggrin, yields a phenotype similar to ichthyosis vulgaris (another disease associated with filaggrin loss).[25] These mice, however, have abnormal barrier function and low grade inflammation. Structural defects in the lamellar body secretory system also occur and these have a hapten-induced atopic dermatitis-like dermatosis at lower challenge concentrations than normal mice. Thus filaggrin deficiency yields a paracellular barrier abnormality that favours induction of atopic dermatitis by allowing hapten penetration.[26] Even human carriers of the null mutations, who do not have the disease, still have elevated transepidermal water loss as well as reduced NMF levels, further highlighting the important role of filaggrin in stratum corneum barrier formation.[27] Other NMF precursors such as hornerin and filaggrin-2 have recently been identified but their full impact on stratum corneum functioning is not yet known.[28,29]

Stratum corneum lipids, structure and biophysics

No review, even a short one, could ignore the recent advances that have been made in understanding stratum corneum lipid biophysics. Although new ceramide species are still being identified and the control of the synthesis pathways of the long acylceramides in particular are being further elucidated, the last 10 years has been the decade of increased knowledge of stratum corneum lipid biophysics.[30–33] The electron microscopic work originally identified the unusual lamellar packing of stratum corneum lipids, which was further refined by X-ray diffraction studies and the exquisite structures were further elucidated by cryoelectron microscopy in the single gel phase model.[3,5,8,34] However, a new 'sandwich' lipid model was also proposed to take into account both the periodicity frequencies in lamellar packing that had been observed, the long (LPP) and short (SPP) periodicity phase, together with the presence of a fluid phase (Figure 3).[4]

The long acylceramides, critical to barrier function, were shown to be essential for the LPP but their omega-esterified lipid dictated the presence of the fluidity phase, further highlighting the importance of linoleic acid for these properties.[35] However, using cryoelectron microscopy of vitreous human skin the trilamellar conformation LPP could not be observed.[8] Nevertheless, this was not the whole story. It had been known for a long time that the lamellar phase was missing from the outer layers of the stratum corneum even in healthy skin, but other structural changes are now known to occur.[36,37] The most tightly packed lipid barrier is known as the orthorhombically-packed state. The presence of long chain fatty acids are needed to induce the formation of the orthorhombic lattice in ceramide and cholesterol mixtures.[38] The orthorhombic packing together with the presence of the LPP defines ultimate lipid barrier functionality (Figure 4).[39]

However, a transition to a less tightly packed hexagonal phase occurs towards the surface of the skin, which can be induced by sebum lipids.[40,41] An amorphous state occurs after bathing, especially with soap or syndets.[23] The outer layers of the stratum corneum lipids are definitely 'broken'.

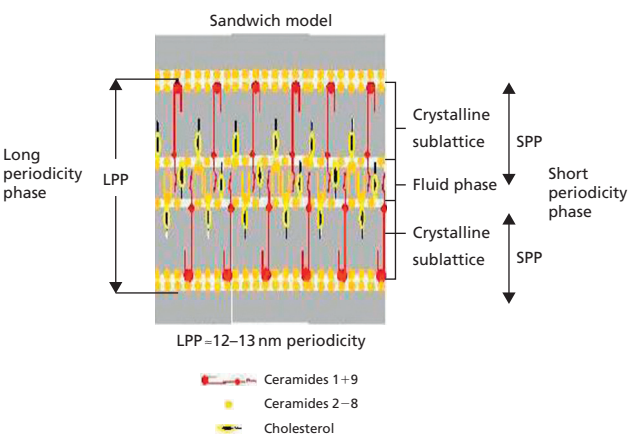

Figure 3 Diagrammatic representation of the sandwich model. Modified from Bouwstra *et al*[4]

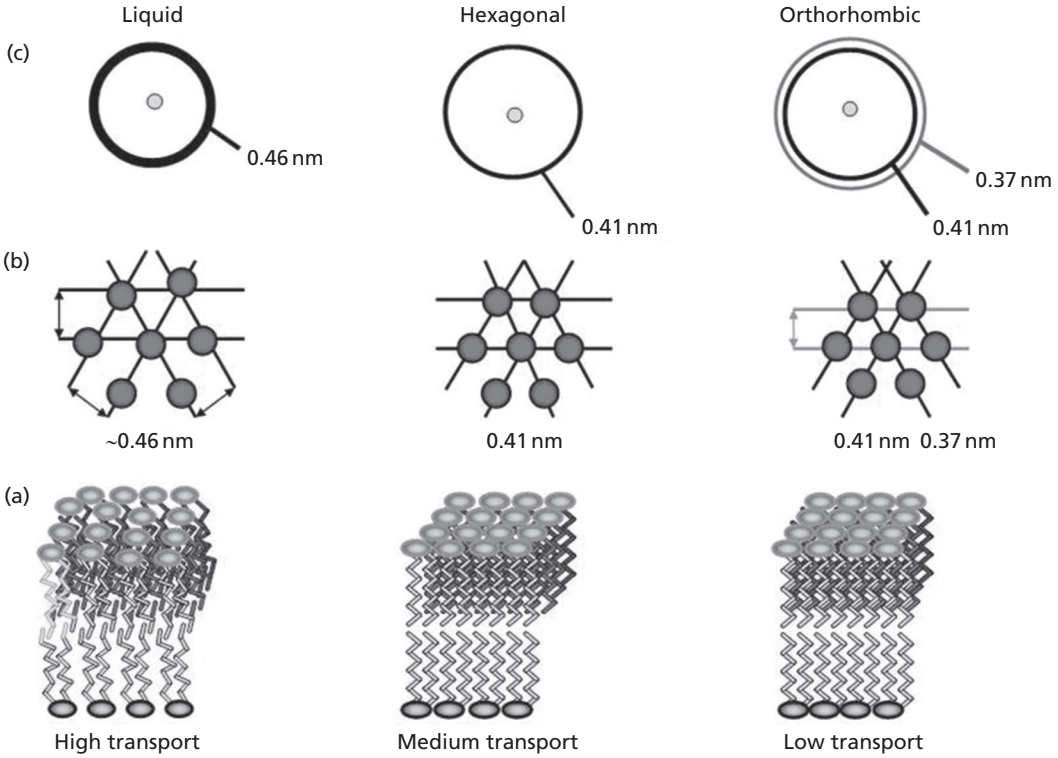

Figure 4 Wide angle X-ray diffraction: a schematic representation of the positions of the alkyl chains in the liquid, hexagonal and orthorhombic arrangement in the plane of the lipid lamellae. (a) Lipid arrangement in the lipid lamellae. (b) Schematic representation of the distances between the lipid tails in the plane parallel to the lamellae, together with the corresponding lattice planes and the distances between the lattice planes, referred to as spacings. (c) The corresponding diffraction patterns of a liquid, hexagonal or orthorhombic lateral packing. In a liquid phase (high permeability) arrangement of the hydrocarbon chains is not very well defined, resulting in a broad reflection at a spacing of 0.46 nm. In a hexagonal packing (medium permeability) the hydrocarbon chains of the lipids are equally distributed in the structure. The distance between the lattice planes is approximately 0.41 nm. This results in one strong reflection in the diffraction plane. The orthorhombic phase (low permeability) is a very dense structure in which the hydrocarbon chains are not equally distributed in the lattice, resulting in two different distances between lattice planes, namely 0.37 and 0.41 nm. This corresponds to two high intensity diffraction planes in the diffraction plane. Adapted from Bouwstra *et al*[39]

A greater proportion of hexagonally packed lipids are found in subjects with atopic dermatitis compared with healthy skin.[42] Animal skin may also be different, for instance pig stratum corneum appears to only have hexagonally-packed lipids in the stratum corneum *in vivo*.[43]

Stratum corneum proteases and desquamation

The transition of the CE_f to CE_r has been discussed above but the processing of the corneodesmosomes has not. This is a water dependent enzymatically-mediated process (which occurs naturally if the stratum corneum is not stored adequately) and facilitates a necessary imperceptible loss of corneocytes called desquamation.[44,45] Stratum corneum chymotrypsin-like (KLK7) and trypsin-like (KLK5) kallikrein activity was reported several years ago, but recently many others have been identified (KLK5, KLK6, KLK7, KLK8, KlK10, KLK11, KLK13 and KLK14).[46–50] Others such as cathepsins are also found in the stratum corneum. These are all believed in some way to play a role in corneodesmolysis and a cascade of activation from inactive pro-forms has been proposed. In that respect the stratum corneum trypsin-like activity is important. Increased and decreased activity of these enzymes has been reported depending on the skin condition of interest. For example, reduced activity of KLK 5 and 7 has been reported in dry skin conditions, and KLK5 in non-eczematous atopic dermatitis skin i.e. atopic xerosis.[15,51] However, increases in stratum corneum serine protease activity (KLK5, urokinase, plasmin and a newly-identified stratum corneum tryptase-like protease) have been associated with elevated transepidermal water loss, especially on facial body sites.[52,53] All enzymes, except chymotrypsin-like kallikreins, were found to positively correlate with increasing values for transepidermal water loss and negatively correlate with skin hydration. However, a 4-bp AACC insertion in the 3′ untranslated region of the *KLK7* gene has been reported especially in subjects who did not have elevated levels of immunoglobulin E (extrinsic atopic dermatitis). It is thought that this insertion could increase the half-life of KLK7 mRNA leading to increased levels of KLK7 in affected individuals.[54] Other inflammatory proteases have also been found in the stratum corneum, such as plasmin and furin.[55] Nevertheless, the most compelling evidence for the role of excess serine protease activity in the pathogenesis of atopic dermatitis in humans comes from Netherton syndrome.[56] Netherton syndrome includes atopic dermatitis as one of its

manifestations. Mutations in the serine protease inhibitor Kazal-type 5 (*SPINK5*) gene, which encodes the lympho-epithelial Kazal-type 5 serine protease inhibitor (LEKTI), have been linked to Netherton syndrome. Mutations in the *SPINK5* gene have also been associated with atopic dermatitis. Reduced levels of LEKTI are thought to lead to a thinner stratum corneum because of uncontrolled serine protease degradation of the corneodesmosomes. Indeed, recently elevated stratum corneum protease activity was reported in subjects with acute eczematous atopic skin conditions who also had a thinner stratum corneum.[57] Recently another LEKTI inhibitor (LEKTI-2 a specific KLK5 inhibitor) has been identified, but its impact on the desquamatory process has not been established yet.[58–60] Nevertheless, it is highly likely that this elevated protease activity was the cause of the compromised stratum corneum condition. Current therapy for the treatment of atopic dermatitis has been largely directed towards ameliorating TH2-mediated inflammation and pruritis.[61] Pseudoceramide-dominant and ceramide creams have been shown to aid barrier repair, but these findings of increased protease activity provide compelling evidence to warrant the use of serine protease inhibitors.[57,62–65]

Stratum corneum tortuosity

One should not forget the importance of stratum corneum tortuosity in controlling stratum corneum barrier function; the total architecture of the stratum corneum is important for its barrier function.[66,67] Subjects with eczematous atopic dermatitis have not only abnormalities in stratum corneum lipid composition and lipid organisation, but also problems in their filaggrin biology, and probably just as importantly their stratum corneum is thinner and the corneocytes are considerably smaller of the CE_f-like type, leading to a very much reduced path length for penetration of ingredients into the living epidermis.[14,16,42,57]

Stratum corneum antimicrobial barrier

The antimicrobial barrier of the skin, other than just a lower skin surface pH, has been of great interest over the last 10 years. Sebaceous and eccrine innate molecules residing on the surface of the stratum corneum constitute the first 'chemical barrier' of the skin. Sebum contains many antimicrobial lipids including sapienic acid (*cis*-6-hexadecenoic acid).[68] Eccrine sweat contains antimicrobial proteins such as dermacidin and antimicrobial enzymes such as lysozyme.[69] Within the conventional stratum corneum lipids are other antimicrobial activities such as defensins, cathelicidins, psoriasin, catestatins and RNAses as well as antimicrobial sphingoid bases.[70] One can even assume that the plethora of proteases and protease inhibitors may also be involved in the stratum corneum innate immune system to dampen exogenous protease activity or to degrade any potential protein allergen.[71] Reduced levels of antimicrobial molecules are known to occur in several disease states.[72] It may be pertinent here that further KLK-induced proteolytic fragments of LL-37 exacerbate the inflammatory response in rosacea.[73] It is quite possible that these are generated in other barrier compromised conditions. Antimicrobial agents from commensal bacteria may also provide further antimicrobial shields. Phenol-soluble modulins (PSMs) produced by *Staphylococcus epidermidis* have recently been shown to exert selective antimicrobial action against skin pathogens such as *Staphylococcus aureus*.[74] Additionally these PSMs functionally cooperated with each other and LL-37 to enhance antimicrobial action.

Conclusions

21st Century research on the stratum corneum has progressed at great pace (Table 1). Our knowledge on the complexity of stratum corneum biology, chemistry and biophysics is ever growing, but we are still far from understanding the intricate control mechanisms in the stratum corneum that bring about

Table 1 Key outcomes of last decade of research

Key outcome
1 The original 'bricks and mortar' model is now recognised as a continuous poly-proteinaceous structure of varying thickness (the bricks are tightly interconnected by corneodesmosomes in all layers of the stratum corneum; but the bricks are actually more like natural moisturising factor-containing keratin sponges as they hydrate extensively) interspersed between a continuous highly-ordered lamellar and largely orthorhombically-packed lipid phase.
2 The functional humectancy/corneocyte swelling role of natural moisturising factor has been shown by electron microscopy and the enzymes involved in its formation from filaggrin have been further deiminated, with peptidylarginine deiminase, caspase 14, calpains and bleomycin hydrolase being key enzymes involved.
3 The single gel phase lipid model and the sandwich model of lipid organisation have been developed of the last few years but the importance of the lateral as well as lamellar lipid packing states has become recognised. Increased hexagonal lipid phases can be observed in barrier perturbed conditions.
4 Several different types of corneocytes have been identified in the stratum corneum depending on the quality of the stratum corneum. The two most predominant are the 'fragile' corneocyte (CE_f) and 'rigid' phenotype (CE_r), of which the former occurs in higher quantities in dry skin due to reduced transglutaminase activity. These corneocyte phenotypes together with the thickness of the stratum corneum contribute to the tortuosity of the stratum corneum, which is important for barrier function.
5 The ever growing family of desquamatory proteases and their inhibitors are being documented. However, an excess quantity of stratum corneum trypsin-like activity together with stratum corneum tryptase-like, plasmin and urokinase have been observed in barrier compromised conditions and especially in stratum corneum from patients with eczematous atopic dermatitis, which is believed to contribute to a thinner stratum corneum.
6 The role of the stratum corneum as a chemical and biological antimicrobial shield has taken centre stage in the last few years, in which skin pH still plays a role.

its full and proper structural maturity. Anyone involved in cosmetic or dermatological development needs to consider this plethora of research that has only been developed thanks to the tenacity of both industrial and academic scientists alike.

Declarations

Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

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