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Corneocytes: relationship between structural and biomechanical properties

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Key Message: This review paper comprises a comprehensive insight of the morphological and topographical features of corneocytes forming the outer layer of the stratum corneum (SC) and relates these characteristics to the mechanical resilience of the SC.

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Abstract

Skin is the interface between an organism and the external environment and hence the Stratum Corneum (SC) is the first to withstand mechanical insults that, in certain conditions, may lead to integrity loss and the development of pressure ulcers. The SC comprises corneocytes, which are vital elements to its barrier function. These cells are differentiated dead keratinocytes, without organelles, composed of a cornified envelope and a keratin-filled interior, and connected by corneodesmosomes. The study of their morphology has revealed two maturation states (fragile/immature and resilient/mature cells) in normal skin. An increased number of immature cells has been observed in dry skin and inflammatory diseases such as Psoriasis Vulgaris. Furthermore, the presence of villous-like structures on the surface of corneocytes has been suggested as a biomarker for Atopic Dermatitis

and has been associated with aging. However, the function of corneocytes as building blocks of the SC and their role in skin integrity maintenance and the development of pressure ulcers has yet to be fully resolved. The current review will focus on the relationship between the morphological, structural, and topographical features of corneocytes and their mechanical properties, to understand how corneocytes assist the SC in maintaining skin integrity and in responding to mechanical insults.

Introduction

Human skin is composed of three main dynamic layers, namely the hypodermis, dermis, and epidermis [1]. The latter is the outermost layer (Fig. 1), being a waterproof barrier protecting the body against the entry of foreign bodies and physical and mechanical insults [2]. It is a stratified and squamous epithelium that can be subdivided into four discrete layers, in which keratinocytes, the main cell type, undergo a differentiation process, from the inner layer, at the epidermal-dermal interface (stratum germinativum or basal cell layer), to the SC. [3, 4] The SC is usually described by a "brick and mortar" structural model [5] (Fig. 2) where the corneocytes are completely flattened and their keratin filled-interior is surrounded by protein and lipid envelopes (cornified envelopes and cornified lipid envelopes, CE and CLE, respectively) [6]. Adjacent corneocytes are connected by corneodesmosomes (CDs) for which localization and density depends on the depth of localization of the cells in the SC [7]. Intercellular lamellae of lipids, such as ceramides, cholesterol, and fatty acids, are first synthesized in the stratum granulosum [6] and compose the "mortar" of the SC.

The SC functions as a barrier between the organism and the external environment, being responsible for the control of trans-epithelial water loss (TEWL) and the selective absorption of compounds [8]. The thickness of the SC varies depending on body site - that of the cheek and back of the hand being 17 and 30 μm, respectively, while that from the palm being much thicker (173 μm) [9] - although this will vary somewhat for different individuals. The complex hierarchical structure of the SC has two main functions: while corneocytes and CDs mainly contribute to the mechanical resistance of the layer, the intercellular lipids are mainly involved in the barrier function [10], preventing water loss and the entry of foreign bodies. The mechanical resistance of the skin to environmental insults is a critical function of the structural cohesion of the SC and the mechanical resilience of the corneocytes, which depends on the differentiation process of the keratinocytes in the epidermis.

There are several reviews on the composition and biochemistry of the stratum corneum (SC) that mainly reveal the mechanisms underlying its barrier function in healthy skin and several disorders [11-13]. However, the mechanical function of SC has been addressed in less detail. Many studies have examined the pathways leading to the loss of skin integrity under the action of applied stresses, but there is still not a consensus on the underlying mechanisms. The current review aims to consider the relationship between the structure and morphology of corneocytes and their biomechanical properties since these dead cells are the first elements to sense load and friction.

Corneocytes

Keratinocytes proliferate in the basal layer of the epidermis and migrate upwards, undergoing a sequential differentiation that culminates with death by cornification [14]. Cornification is usually described by three main events: formation of an intracellular keratin network (with the loss of organelles); assembly of the CE with the CLE covalently attached and selective degradation of CDs. The maturation of the corneocytes of the SC and desquamation of superficial cells are well-controlled processes that depend on several different elements such as proteases and their inhibitors, a controlled pH gradient, and regulated hydration of the SC [7]. The different steps of corneocyte maturation are reflected by their morphology and surface topography as discussed below.

Morphology and Topography

The morphology of corneocytes (Table 1, Fig. 3) was first studied in 1939 [15], which reported a diameter of approximately 25-35 μm and a single-sided surface area of 700-900 μm^2 and it is of great interest in dermatology and cosmetic science since the appearance of corneocytes is increasingly associated with several skin conditions. Later, cells from the forehead and hand were observed to have the smallest surface area of 700-800 μm^2, and those from the upper arm, thigh, and axilla had the greatest surface area of 1000-1200 μm^2 [16-18], which indicated that corneocytes from high proliferative regions are usually smaller [19]. An increase in surface area of corneocytes with age has also been reported [17, 20, 21], e.g. corneocytes from the axilla have a surface area of ≅ 750 μm^2 for babies, ≅ 1000 μm^2 for children, ≅ 1200 μm^2 for adults, and, finally, ≅ 1400 μm^2 for the elderly. Gorzelanny et al. [22] using Atomic Force Microscopy (AFM) showed that corneocytes from aged SC (Fig. 3(b)) presented a surface area of 753 ± 120 μm^2, and those from young subjects skin (Fig. 3(a)), a smaller area of 555 ± 80 μm^2. Moreover, the thickness of corneocytes has been reported to be inversely related to the cross-sectional area of the cells: the largest cells are usually the thinnest [23]. Furthermore, it was reported that there is a 40% decrease in thickness for corneocytes sampled from the upper SC (first tape strip), compared to those in the inner SC (20^{th} tape strip) of about 40% [20], and a corresponding increase in the surface area [24].

In the context of representing the 3D morphology of a corneocyte, a flattened Kelvin tetrakaidecahedron geometry has both a hexagonal cross-section as well as hexagonal upper and lower faces that are consistent with a simple 2D top-view model of the SC [25, 26]. Close-packing of SG keratinocytes of this shape allows them to maintain an effective tight junction barrier as cells suffer cornification and move upward to the SC. This geometry represents an optimal shape for space-filling

and those authors propose that it also represents the basis for the barrier function and physical strength of the horny layer.

Mechanical properties of corneocytes

The mechanical properties of corneocytes are important since the dead envelope of cells that constituting the SC must have physical properties that enable them to experience deformation in response to body movement and physical insults [27]. The skin regularly endures a range of physical challenges e.g. walking and wearing tight shoes, wearing belts and tight clothes, and when in contact with skincare and grooming products such as shavers. The stretched or compressed SC must be able to return to its original state without structural changes. Hence, understanding the mechanical properties of single cells assist us in delineating the phenomena underlying the mechanical resistance of the skin, the maintenance of integrity, and damage processes. Corneocytes were observed to fold readily but to strongly resist elongating forces [27], and the Young's modulus was calculated to be ≅ 0.45 GPa. Later, using a micromanipulation technique, two populations of corneocytes were identified in normal skin based on the force required to compress the cornified envelope: resilient (833 ± 431 μN) and fragile CEs (135 ± 32 μN) [28] and a greater ratio of fragile to resilient CEs was observed in dry skin.

More recently, the mechanical properties of corneocytes have been mainly investigated using AFM to measure indentation loading curves and applying contact mechanics models, e.g. Hertz-Sneddon, DMT or JKR [29], to obtain the Young's modulus (a representative loading curve is shown in Fig. 4). There is a large variability in the Young's modulus determined for corneocytes: from 1.34 MPa up to 0.4 GPa [30-32] (Table 2). The force spectroscopy mode of AFM requires great control of the method and a rigorous analysis of the data such that the contact mechanics model and the geometry of the probe greatly influence the results. Pyramidal and conical probes, for example, can be regarded as spheres or paraboloids of revolution for small indentations, in which a nominal radius is usually assigned. However, for larger indentations, the probe can be treated as either a cone or pyramid with half-angles dependent on the indentation depth. Another important point to consider is the stiffness of the cantilever: if it is softer than the sample, the measured stiffness of the sample will be mainly that of the probe. We speculate that some of the probes used in the literature were too soft in this respect and resulted in Young's moduli > 1 GPa.

The influence of the different components of the cells was assessed using nanoneedles mounted on cantilever with a spring constant of 35 N/m [33] and the Oliver-Pharr model [34] to fit the data. The reduced Young's modulus was calculated to be ≅ 100 MPa for indentations < 50 nm and 500 MPa at greater depths. This work can be related to that by Milani et al. [35], in which the authors calculated

the apparent Young's modulus for different depths by applying the Hertz model to intervals of force as a function of indentation. Using pyramidal probes and 2.5 N/m stiffness cantilevers, the stiffness of cells from 3 different SC depths (1st, 7th, 14th tape strips) was measured. A decrease in the Young's modulus was established as a function of the depth of indentation. For the 1st strip, an apparent modulus of ≅ 250 MPa for the CE and 300-520 MPa for the keratin bundles, while for cells deeper in the SC, a modulus of about 150 MPa for the CE and 250-300 MPa for the keratin-matrix were calculated. The increase in stiffness with proximity to the skin surface correlates with the enlargement and the thinning of cells along that axis.

However, the protocol for the collection of corneocytes might influence the results obtained. While most authors use the tape stripping method to collect cells, the influence of the tape on the compliance of the AFM force curves is not described. Moreover, Richter et al. [36] studied the swelling of corneocytes by AFM Tapping Mode (TM), using a silanization technique to firmly attach the cells to a silicon substrate. In their TM images, they did not find phase contrast differences between cells in air and the silicon substrate. However, cells in water showed a considerable phase contrast when compared to the substrate. The authors attributed this to the viscoelasticity of the swollen cells and concluded that the absence of phase contrast in the case of the cells in air was consistent with the description of corneocytes as being brittle and hard [36]. More rigorous viscoelasticity experiments, such as stress-relaxation using AFM, are nonetheless needed to confirm this assumption. Additionally, it should be noted that this procedure includes an overnight drying step of the sample followed by a washing step. Because the cells are allowed to dry overnight, it is difficult to state that their mechanical properties are close to those measured when the tape stripping method is employed to collect corneocytes.

Moreover, a divergence of the mechanical properties of single cells from those obtained when cells are integrated in the SC is expected. When integrated in the tissue, corneocytes are subjected to different forces, such as interplanar and peripheral adhesion forces (explored in the Section below regarding the CE), which may influence their mechanical properties.

Keratins: role in cell mechanical strength

Keratins have long been recognized as the main component contributing to the mechanical strength of keratinocytes [37, 14]. Their expression is selective and matches the degree of differentiation of cells across the epidermis. Basal keratinocytes are mitotically active and express keratins 5 and K14, with a minor amount of K15 [38]. As keratinocytes migrate upwards and suffer differentiation, they start expressing keratin 1 and keratin 10, with a loss of K5 and K14 expression [39]. Additionally, there is expression of K2 in the upper spinous and granular layers [40]. Furthermore,

in the palmar-plantar epidermis K9 is prominently expressed in the suprabasal differentiating layers [41] and K6, K16, K17, and K19 are also expressed in specific spatial patterns and their expression can be rapidly induced upon various challenges, such as wounding and infection [42]. Since corneocytes are dead cells composed of a strong protein-lipid envelope surrounding these keratin bundles, studies of the isolated influence of keratin filaments on their mechanical properties are difficult to perform. Nevertheless, as seen in the previous section, it is possible to make a distinction between the contribution of the cornified envelope and the cell interior using AFM force spectroscopy. Furthermore, by studying the phenomena occurring with the viable cells of the epidermis, it is possible to extrapolate knowledge to the product of their differentiation i.e. corneocytes. For example, it has been observed in multiple studies that mutations in keratin genes, such as in the disruption of filament formation or alteration of properties and dynamics of bundle assembly, reduce the strength of cells [43, 44].

Living human keratinocytes were studied under loading and unloading cycles of single cells by AFM and it was observed that a typical force of 0.4 ± 0.1 μN was required to reach 30% compression and 6.0 ± 2.0 μN for an 80% compression of the original height [43]. The Young's moduli of the keratinocyte membrane and cytoplasm were calculated to be 23 – 38 MPa and 120-340 kPa, respectively, which is a factor of 25-30 greater than that of the cytoskeleton of T cells. This shows that keratins are responsible for the high stiffness of keratinocytes. A correlation between molecular integrity of keratin filaments (K5 and K14) and mechanical toughness of epithelial cells was also found [45]. The modulus of mutant cells (not expressing K14) was 343 ± 18 and 412 ± 74 Pa for the nucleus and cytoplasm, respectively, while for controls, a greater cell stiffness was observed of 459 ± 31 Pa for the nucleus and 752 ± 100 Pa for cytoplasm.

In a different study, Akinshina et al. [46], attempted to verify the results obtained by Jokura et al. [47]. The latter studied the effects of the Natural Moisturising Factor (NMF) components on the SC using nuclear magnetic resonance (NMR), rheology, and Scanning Electron Microscopy (SEM). They observed that treating excised SC with water would release the NMF component, leading to a reduction in the mobility of keratin intermediate filaments (IFs) and a smaller value of the elastic modulus. The electron micrographs suggested that, in the absence of NMF, the keratin filaments tend to associate more tightly. They concluded that the former results indicated that the increase in corneocyte rigidity is due to increased intermolecular attractive forces between keratin filaments. They modelled the interactions between keratin IFs suspended in different media and found that the N-tails of the keratin filaments act as the 'glue', while the C-tails are responsible for maintaining a certain distance between the IFs. The small charged species comprising the NMF then act in the prevention of the attractive forces between the protruding terminal domains of IF helical cores. When

they are eliminated, the attractive forces are sufficiently large to 'glue' the filaments together and thus increase the stiffness of the corneocyte. This denotes the complex relationships existing in SC cells in order to maintain mechanical integrity.

The stretching of cultured keratinocytes also provides an insight into the response of cells to certain types of mechanical stimuli. When normal human keratinocytes and HaCat cells are stretched, the expression of K10 was completely suppressed after 24 h, and the K6 expression was induced; that is, the mechanical stimulus suppressed the differentiation of the cells [48]. The response of cultured cells to mechanical stimuli raises the question of what happens to keratin expression when the skin is subjected to clinically relevant pressures, such as those of a patient lying in bed or seated in a wheelchair: are the keratinocytes able to adapt in the presence of mechanical loading? Using a device to periodically stimulate keratinocytes (over 80 h), it was observed that the keratin distribution of the cells was amorphous and partly granular, as described for corneocytes [49]. The expression of a 67 kDa keratin was found in mechanically stimulated HaCat cells, which is not normally expressed by cultured keratinocytes. Furthermore, Yamaguchi et al. [50] found that non-palmoplantar keratinocytes gained the ability to express K9 when co-cultured with palmoplantar fibroblasts.

The influence of the morphology and composition of plantar skin on the tolerance to load has been studied [51]. Plantar SC was found to be a factor of 16 thicker than the SC from nonplantar skin, with greater interdigitation between the epidermis and dermis and with a 2.1 increase in fluorescence intensity of desmoglein 1, a key component of CDs. Moreover, plantar skin was observed to deform less with loading (factors of 1.6 and 3.4 less under compression and shear, respectively). Moreover, the SC showed a factor of 3 increase in the Young's modulus, and factors of 4.8 and 7.2 increase for viable epidermis and dermis respectively. Using a pyramidal probe, the change of the Young's modulus with depth proved to be more gradual for plantar skin (18% decrease per 100 μm) than for nonplantar skin (84% decrease per 100 μm). The authors suggested this may assist in mitigating stress concentrations by eliminating "hotspot" areas of stress. Plantar skin seems to mitigate the effect of mechanical insults by diverse structures and mechanisms: thicker SC is associated with greater stiffness and more cohesion since there is a greater number of desmosomes. To study the mechanical properties of individual corneocytes would be a logical next step to complement the knowledge about this type of SC.

Cornified Envelope (CE)

Apart from keratin bundles that fill the interior of corneocytes, two other components contributing to the mechanical strength of these cells and the mechanical resistance of SC must be considered. These are the CD and the CE. Extensive reviews on the formation and composition of both structures

have been presented elsewhere [52, 14, 7, 8]. Briefly, the cornification process begins with the synthesis of an immature CE beneath the plasma membrane of the keratinocytes. This envelope undergoes maturation by the covalent attachment of certain precursor proteins (such as involucrin, loricrin, and SPRs - small proline-rich proteins) to produce a quite rigid structure [52]. Transglutaminase activity allows the cross-linking of proteins via γ-gamma-glutamyl-ϵ-lysine isopeptide bonds, which reinforces the envelope [53]. Some authors have observed that the composition of CEs is variable and dependent on the type of substrate available to the transglutaminases [54], with some considering the possibility of the incorporation of CD proteins later in the CE maturation [55]. Additionally, the maturation of CEs culminates with the covalent attachment of ceramides, fatty acids, and others to involucrin and loricrin, creating a hydrophobic coating [56, 57]. These bound lipids are called the cornified lipid envelope and are thought to be a scaffold for the intercellular lipids of SC [58-60], further stabilizing and promoting the plasticity of this top layer.

The maturation of the CE follows the movement of corneocytes in the SC and many authors have attempted to discriminate mature from immature CEs using microscopy and immunostaining techniques. CEs from different SC depths were first observed by Normarski contrast microscopy, which allowed the identification of two different populations of CEs: 'fragile' (CEf) and 'resilient' (CEr) [54]. These two states of maturation can be found in the most superficial layer of SC, although the amount of CEf is small in normal skin and elevated in dry skin [28]. Moreover, the ratio between resilient and fragile CEs in the case of inflammatory skin disorders was found to be decreased in Psoriasis and Atopic Dermatitis [61].

Most recently, the standard technique for assessing CE maturity is the double staining of lipids with Nile red and immunostaining of involucrin. Nile red is used to stain lipids covalently bound to the CE, the last step of corneocyte maturation, while immunostaining of involucrin is performed to distinguish non-matured CEs [62]. It is suggested that the loss of involucrin staining during corneocyte maturation must be a result of the covalent attachment of lipids to CE proteins, in which case the epitope of involucrin might be hidden for recognition by the antibody upon immunostaining. Corneocytes were reported to become less and less mature with increasing SC depth [63, 64]. This morphological feature is related to the mechanical strength of corneocytes as discussed previously: a decrease in Young's modulus of corneocytes with depth from the first strip (250 MPa) to the seventh strip (150 MPa) for the cornified envelope [35] and a similar trend reported when measuring the force necessary to compress both CE types [28].

Guneri et al. [57] applied conventional methods and a novel one to evaluate the maturity of CEs. They found that the anti-involucrin and Nile red approach was limited in discriminating the maturity

of CEs in the deeper SC layers of photo exposed cheek (PE) and photoprotected post-auricular (PP). With this method, both deep layers (PP9 and PE9, ninth tape strip) would be considered immature. Although, when relying on the mechanical integrity of CEs, by analysing the morphology after sonication, differences were found in the proportion of immature CEs in the deeper layers of PE cheek (43 ± 6.5 % less maturity), but PP9 seemed to be less perturbed by this mechanical stress. This means that, although the traditional method would regard PP9 CEs to be immature, they cannot be considered immature in their response to mechanical stress, suggesting that the maturation of corneocytes depends both on the formation of a mechanical resistant cornified envelope and on the establishment of a lipid envelope [60] surrounding the rigid CE. In summary, the maturation of CEs is intimately related to the stiffness of corneocytes: maturated corneocytes are stiffer not only because of the type of keratin bundles composing their matrix but also because of the cross-linking occurring in the cornified envelope.

Corneodesmosomes

In addition to the maturation of the cornified envelope, another important step in cornification is related to the formation and degradation of CDs. These structures are responsible for the cohesion of the deeper layers of SC that bind the corneocytes together. The importance of these components was demonstrated by the absence of corneodesmosin expression, which results in the premature detachment of corneocytes from SC in mice and humans [65]. The precise distribution of desmoglein-1 (DSG1) in corneocytes as they transit in SC has been studied (Fig. 5) [66]. The outer layer or mature SC is characterized by a honeycomb-like structure, in which the cornified cells are attached only laterally in the same cell layer [67]. This feature is only absent in palmar and plantar regions, where CDs are found to be dispersed over the whole surface of the cells. These junctional structures are modified desmosomes [68, 69] involved in desquamation, a complex process regulated by several proteases and inhibitors, first expressed in the stratum granulosum and secreted to the extracellular space. Peripheral CDs are thought to persist in the outer SC, being protected from enzymatic degradation by tight junctions that surround them [70]. A correlation between TEWL readings and the localization of DSG1 has also been established, with the presence of DSG1 on the periphery of cells being associated with a healthy SC (lower values of TEWL), and if dispersed on the whole surface, corresponding to a reduced barrier function (higher TEWL) [66]. Moreover, the honeycomb feature of mature SC seems to result in improved flexibility of the whole layer by attenuating mechanical insults, such as bending or flexing, allowing the shape of corneocytes to adapt and the outer SC to perform minimal “slide” over the inner SC [71].

Furthermore, the digestion of CDs increases with increasing relative humidity (r.h.) [72]. It was also found that at high (80% r.h.), but not at low (44% r.h.) humidity, glycerol further enhances CD digestion. When the glycerol treated SC was extended at 80% r.h., the extensibility of the SC increased dramatically, indicating a reduction of intercorneocyte cohesion. This behaviour is suggested to be caused by the influence of humidity on the activity of desquamatory enzymes. When an inhibitor of serine proteases was introduced, the moisture-dependent increase in tissue extensibility was prevented. Finally, the stiffness of the SC was largely unchanged even at high doses of UV radiation, while the fracture strength and fracture strain of the SC decreased with increasing UV exposure [73, 74], indicating that UV influences cellular cohesion, dominated by both intercellular lipids and CDs, while not interfering with the mechanical resistance of the tissue, which is mainly controlled by the keratin matrix and the rigid cornified envelope of corneocytes. A dispersion in the localization of DSG1 in the tissue, indicating degradation of CDs after UV radiation treatment was also observed [74].

Corneodesmosomes seem to play a major role in the cohesion of SC, which can be divided into two main regions: the deeper-immature SC, which is much more cohesive, and the outer SC, for which flexibility and plasticity may depend on intercellular lipids and the honeycomb localization of CDs. It is important to note that the honeycomb structure of the outer SC seems to be preferred in most body regions, but not for the palmar and plantar zones [75, 74]. In these regions, there prevails a strong cohesion between corneocytes, with CDs dispersed on the whole surface. This cohesiveness in combination with greater Young's modulus (greater stiffness) may be an evolutionary advantage since they are subjected to greater pressures and other forces even if it is associated with decreased suppleness and barrier function.

Maturation of corneocytes and surface texture

When analysing the surface of corneocytes using SEM and AFM, small circular protrusions of a few hundreds of nanometres (termed circular nano-objects - CNOs) are observed on the basal side of the cells (Fig. 6 (c) [76]). Cells from the palms and soles were observed to have an irregular shape, with excessive wrinkling on their surfaces and sometimes villi-like structures, while those from the axilla, umbilical region, and the back were presented a hexagonal shape, with trabeculae that were parallel to the cell border, forming a regular network [77]. Similar differences were found using AFM TM to analyse the topography of cells from the forearm (smooth topography) and the palm of the hand (with the presence of numerous small protrusions) [78].

The distribution of these structures is not homogenous: some cells are covered with CNOs, while some cells are smooth with a subcellular preference of CNOs at the periphery. Such protrusions have been associated with cell-to-cell adhesion [79] and assigned to aging [22] and some skin states and

disorders [16, 22, 80-82], as reviewed in detail by Riethmüller [83]. Using a computer vision algorithm, Franz et al. [84] were able to determine the mean number densities of CNOs over cell surfaces; they termed this parameter the Dermal Texture Index (DTI). It was found to be greatest on lesion areas of AD skin (DTI - 200-800), but also to be greater in non-lesion areas, when compared to healthy skin controls (DTI ≅ 100). An increase in DTI was also found in cases of loss of function of filaggrin [85]. The same authors observed corneodesmosin (a key component of CDs) to be present in CNOs by immunogold electron microscopy. Although the literature does not define a conclusive connection between CNOs and CDs, it is reasonable to explore such relations. It is known that the degradation of filaggrin leads to an increase in NMF concentration that is responsible for water retention, which is an important factor for the activation of proteases involved in the degradation of CDs, as mentioned above. Consequently, it is reasonable to relate a mutation of the filaggrin loss-of-function gene with a greater number of CNOs by the impaired degradation of CDs when the water content is decreased. If CD degradation is impaired, corneocyte maturation might be compromised, which would explain the defect in barrier function attributed to the intercellular lipids that might not have the space to rearrange viz., a greater number of CDs implies a greater cohesion of the SC, but a loss in barrier function.

Additionally, corneodesmosin was detected on the surface of SC using simultaneous topographic and recognition imaging (TREC) technique based on AFM [86]. The authors found the density of corneodesmosin to be about 1000 proteins/μm^2 on the surface of corneocytes from superficial SC and about 1200 in deeper layers of SC. Additionally, the size of the recognition spots was larger in the deeper SC, which is in agreement with the described progressive degradation of corneodesmosin during the desquamation process [87]. The topographic and recognition images showed that the protein is located mainly on 'bead-like' structures observed by the authors, although not every structure presented recognition sites [86]. Using the same technique (TREC), Danzberger et al. studied the location of glycans on the corneocyte surface [88]. Glycan moieties were demonstrated to protect CDs from proteolysis, and so to be involved in the desquamation process [89]. In this study, almost all recognition sites for glycans were on top of 'bead-like' topographical elevations (height between 1.1 – 4 nm) [88]. The authors suggested that these structures could be remnants of CDs [13].

An unequivocal correlation between morphology and function of these structures is still lacking, and work to relate the above topographical feature with the mechanical characteristics and maturation states of corneocytes should be done. A study by Guo et al. carried out relevant research by exploring the adhesion of single corneocytes in different layers of the SC. Both peripheral and interplanar adhesion increased with depth. Moreover, cells from the most superficial layers showed a weaker interplanar adhesion compared to the peripheral one, which is consistent with the localization

of CDs in the superficial SC [90]. This anisotropy of adhesion forces agrees with the protective barrier role of the SC, allowing for a resistance to stretching and shearing forces, while not compromising the integrity of the layer.

Conclusions

Corneocytes are the "bricks" of the SC (Fig. 7) primarily responsible for the mechanical strength and stiffness and contributing to the barrier function. Being the product of differentiation of keratinocytes, these cells create distinct regions in the SC: the inner SC characterized by immature cells with a fragile cornified envelope and a uniform distribution of CDs; and the upper SC with resilient CEs and a honeycomb distribution of CDs.

Regarding their morphology, it is important to note: 1) the regional differences in the surface area and thickness (200 nm to ≤ 1 μm). Corneocytes from body sites usually more protected from chemical, physical and mechanical insults (ventral region of arms and legs and abdomen, for example) generally have a greater surface area (1000-1200 μm^2) and are thinner than those exposed to insults, such as the forehead, cheek, palms, and soles (700-800 μm^2) [91]. 2) The increase in the surface area of corneocytes with age, and finally, 3) the correlation between the surface area, thickness, and location of these cells in the SC, with upper cells presenting a greater surface area and smaller thickness than deeper cells [92, 20, 93], which depends upon the sequential maturation of corneocytes along the SC. This morphological maturation is ultimately related to the mechanical properties of corneocytes [35], with immature cells from the deeper regions of the SC being less stiff than those at the surface [28, 35]. Corneocytes are more rigid compared to other cell types (the Young's modulus of other cell types such as muscle [94] and fibroblasts [95] are typically a few kPa), and presenting two distinct mechanical regions: the cornified envelope (100-250 MPa) and the keratin matrix (250 – 500 MPa).

The SC is usually described as having two different regions, the stratum compactum and the stratum disjunctum that relate to the maturation of corneocytes, particularly in the distribution of CDs over the cell surface, as represented in Fig. 5. There is a progressive degradation of CDs from the inner to the outer SC, the latter being characterized by a honeycomb structure. The presence of CNOs seems to be related to CDs, since these structures have been observed to contain corneodesmosin. However, a clear relation between their presence in normal skin and their function is still lacking. Moreover, the SC from different body sites possesses different characteristics, particularly that from palmar and plantar regions, which is much stiffer and cohesive with a uniform distribution of CDs over the surface of the cell [75]. Although the stiffness of plantar corneocytes seems to arise from the expression of K9, further mechanical studies are needed to comprehend the maturation of the cornified envelope in such regions, particularly how it is affected by the persistence of CDs in the upper SC.

To understand the morphological and mechanical properties of corneocytes is a crucial step in revealing the mechanisms underpinning skin barrier function and integrity loss. Intercellular lipids are normally considered to be the barrier function elements of SC; however, the optimal arrangement of this "mortar" seems to depend on the correct formation of a cornified protein and lipid envelope that serves as a scaffold. The literature reviewed indicates that the relationship between the maturation of corneocytes and the mechanical resistance is one of the most critical steps in the formation of an effective SC barrier. When the morphology of the corneocytes is perturbed, an impaired barrier function is observed, and some skin disorders may arise. Furthermore, in the presence of water, there is increased degradation of CDs, which may compromise the response of the SC towards loading.

However, there are still gaps in the knowledge about these cells, particularly in their response to mechanical loading and frictional forces and on the role of the structural lipids attached to the CE on the desquamation process, as well as the implications of their presence on the skin microbiome. It has been suggested that the CLE may, on one hand, function as a scaffold for the organization of the extracellular bilayers and, on the other, to contribute to the cohesion of the SC [60]. Moreover, it is thought that the CLE may function as a selective body of permeation; allowing free transmembrane passage of water, while restricting the loss of larger hygroscopic molecules out of the cell, such as filaggrin breakdown products [60].

It is also relevant to mention the overlooked relationship that seems to exist between the accelerated degradation of CLE and atopic dermatitis [96]. AD is frequently aggravated by the colonization of bacteria secreting ceramidase or the activation of endogenous ceramidase activity [97], which, by contributing to the degradation of the CLE, may induce the release of hygroscopic products from the cell interior, resulting in the appearance of xerosis [98]. A recent study using AFM showed that the adhesion of Staphylococcus aureus to corneocytes from AD patients is controlled by NMF [99]. Reduced filaggrin expression, and consequently low levels of NMF, has been shown previously to facilitate the colonization by these bacteria [100]. The authors postulated that the strong adhesion of Staphylococcus aureus to the cells originates from an increased exposure or expression of ligand proteins on the SC surface. Topographic images show that the surface morphology of corneocytes from low-NMF skin samples present a high density of villous protrusions, which were the regions where strong adhesion bonds were concentrated [99]. Finally, the authors also point out that bacterium-skin adhesion is greatly enhanced by physical stress, as shown by the considerable increase in strength of adhesion bonds subjected to tensile forces [99].

Finally, it would be relevant to study the modifications imposed by pressure on the expression of keratins as well as on the maturation of the cornified envelope, both *in vitro* and *in vivo* and to

consolidate the knowledge related to mechanical properties of corneocytes of different body sites and site-dependent cell maturation.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Author Contributions

Ana Évora contribution wrote the original draft of the paper. It was edited by Simon Johnson, Zhibing Zhang and Michael Adams.

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Figure Legends

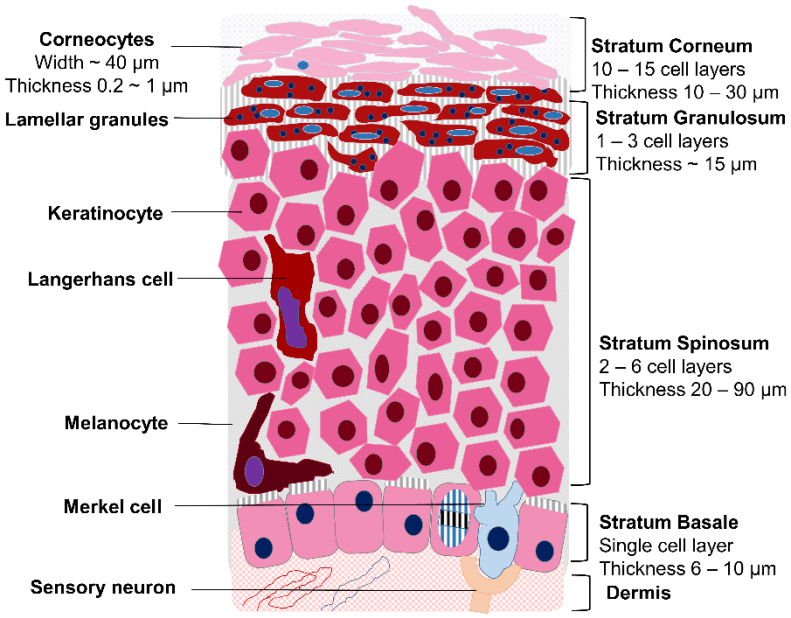

Fig. 1. Schematic diagram of the epidermis: keratinocytes proliferate in the basal cell layer and move upwards in the stratum spinosum where they start to suffer a differentiation process while migrating upwards in the epidermis. This process involves a synthesizing step in the stratum granulosum and culminates with death by cornification and maturation of corneocytes in the outermost layer of the stratum corneum.

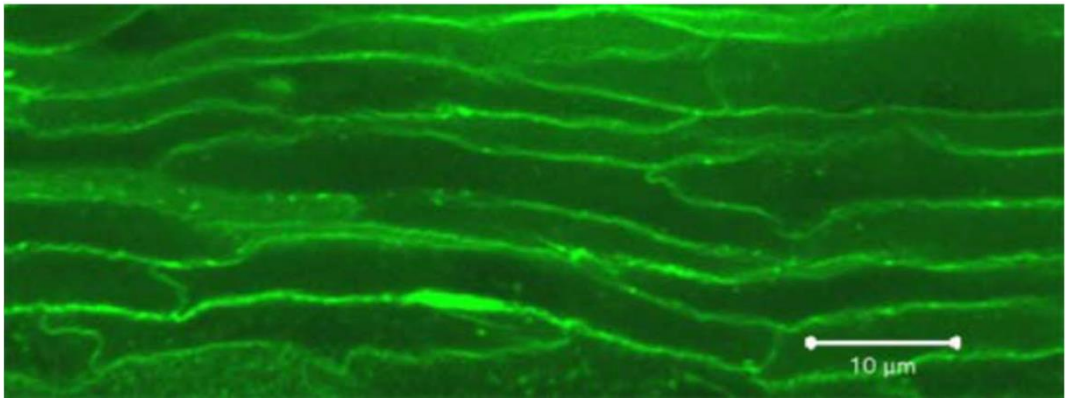

Fig. 2. Confocal fluorescence images after staining with Nile red in alkali solution to swell the corneocytes in order to render them visible in an optical microscope (cells are expanded by a factor of 5 in the thickness dimension). The specimen was mechanically and optically cross-sectioned in the same apical-to-basal plane, dorsum skin. The image illustrates the 'bricks and mortar' structure of the SC. Reproduced, with permission, from Talreja et al. [5]

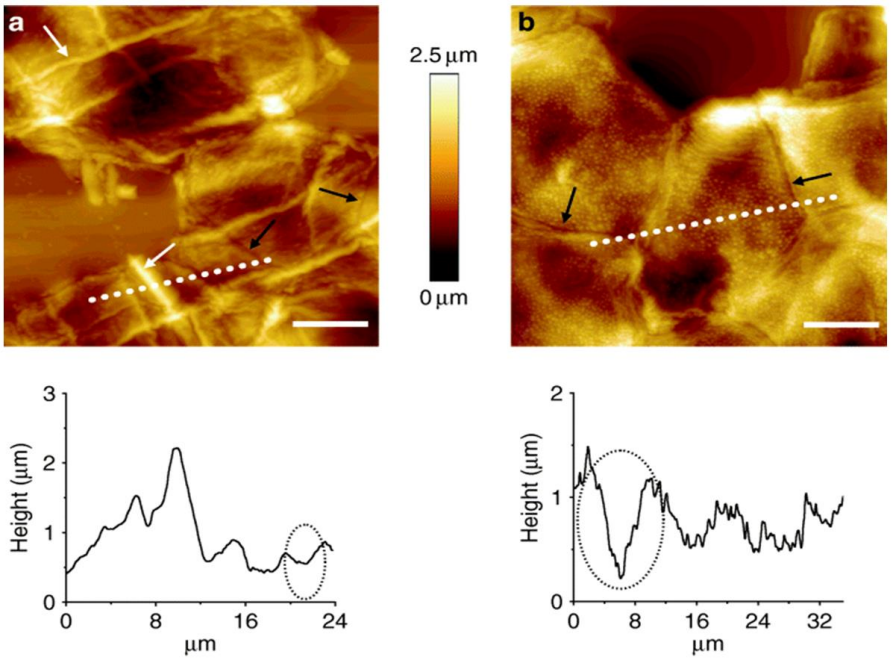

Fig. 3. 3D image of corneocytes from a young donor a) and from an aged donor b). Height is given in colour brightness – lateral dimension by scale bar (10 µm). The white arrows point to filamentous structures so far unidentified, while the black arrow indicates cell–cell contacts between two corneocytes. The dotted line indicates a cross-section that is displayed below. The dotted circle indicates the intercellular gap. Reproduced with permission from Gorzelanny et al. [22]

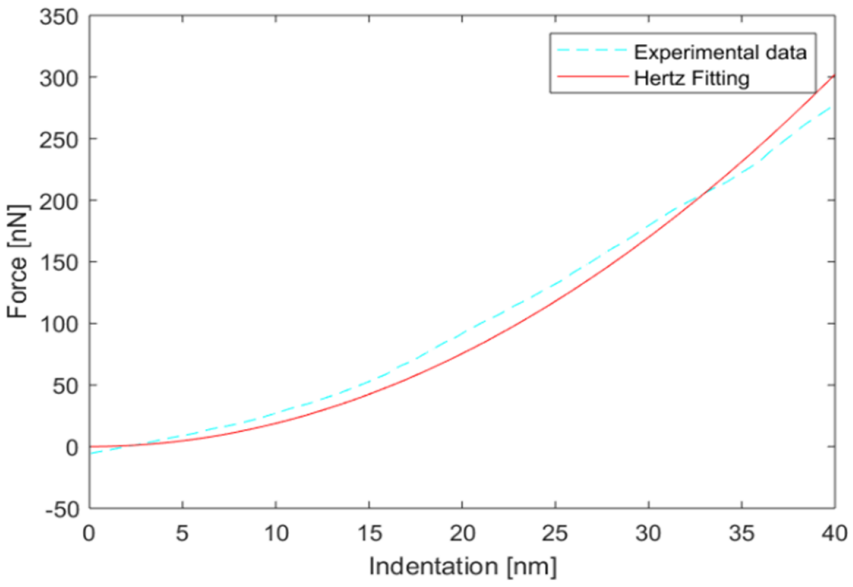

Fig. 4. Representative force as a function of indentation depth and Hertz-Sneddon fitting obtained on inner forearm superficial corneocyte (2nd tape strip) at room temperature using a JPK Nanowizard instrument with a 26 N/m stiffness cantilever and a pyramidal tip. [76]

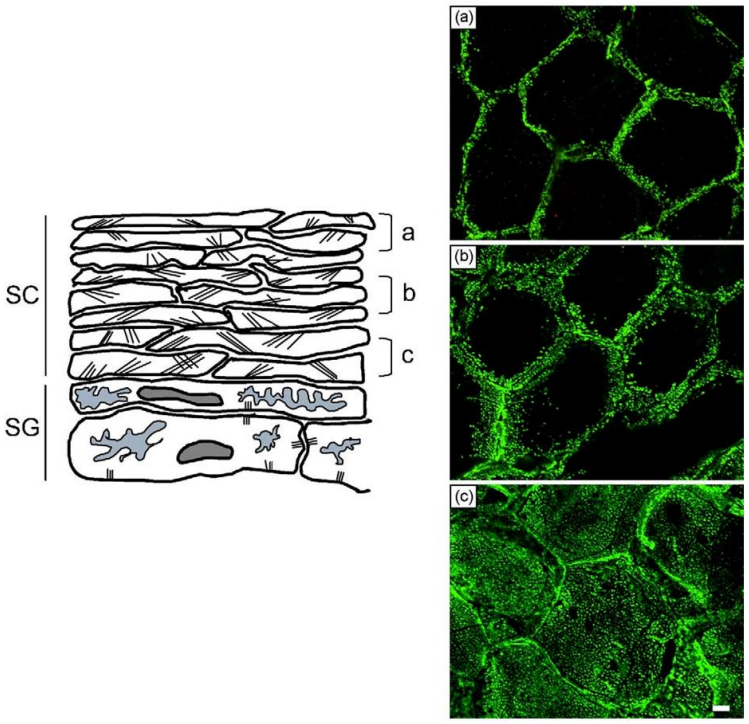

Fig. 5. Desmoglein 1 immunostaining in the stratum corneum from the inner to outer SC. 11 tape strips were performed on the inner upper arm. In the inner SC (c), desmoglein 1 was localized over the entire surface of the corneocytes, while in the middle layer (b) it was localized mainly in the periphery, persisting in this region in the outermost layer (a). Bar = 5 µm. Reproduced with permission from Naoe et al. [66]

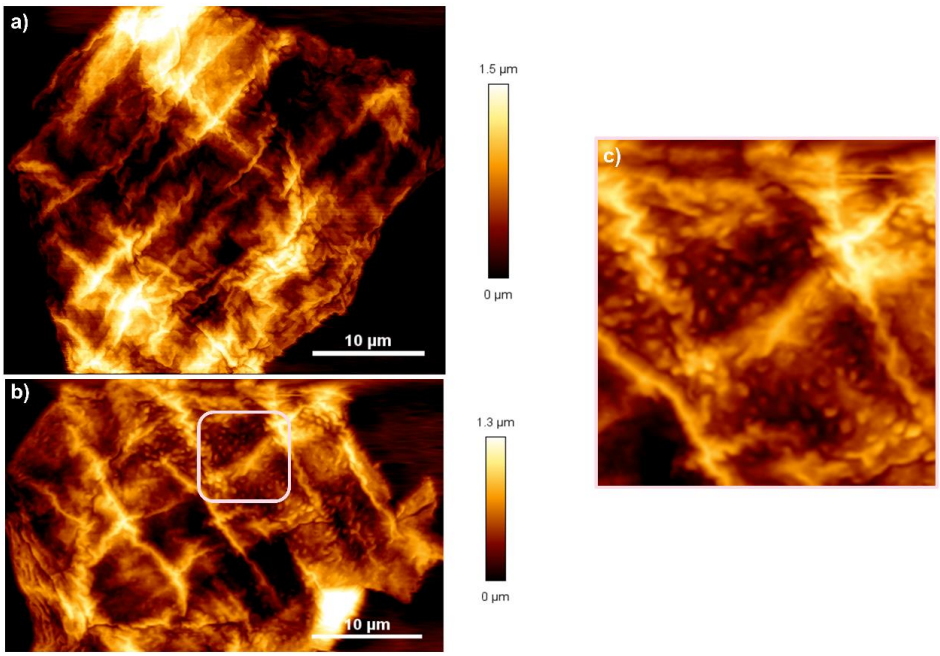

Fig. 6. AFM reveals surface topographical features on superficial corneocytes (2nd strip) from the inner forearm of one volunteer. Height is given in colour brightness – lateral dimension by scale bar (10µm).

The images were obtained in the contact mode (cantilever stiffness of 1 N/m and a pyramidal tip). (a) A smooth corneocyte presenting ridges on its surface. (b) A corneocyte presenting CNOs on its surface, highlighted by the white box. (c) Zoom image of (b) of CNOs present on the cell surface. [76]

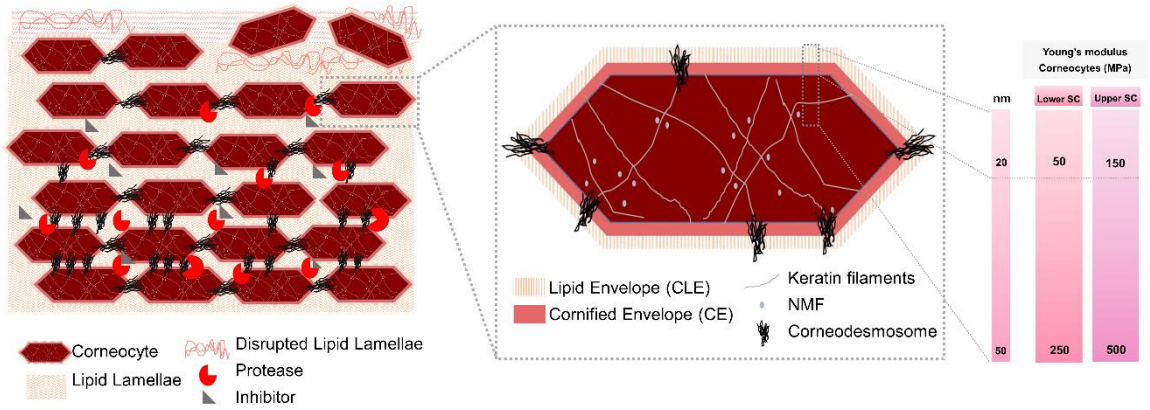

Fig. 7. "Brick and mortar" illustration of SC (stratum corneum), which can be theoretically classified into two main zones. Deeper SC (stratum compactum) that is close to the stratum granulosum, which represents the compact SC with CDs present over the whole surface of the corneocytes. Outer SC (stratum disjunctum) with the transition to desquamating surface, which is characterized by looser corneocytes (CDs create a honeycomb pattern). The lipid lamellae is well formed and imparts some plasticity compared to the cohesive deeper SC. Moreover, the morphology and strength of the corneocytes is consistent with this classification since the inner corneocytes are softer than those at the surface. The maturation of the cornified envelope may assist in compensating for the loss of cohesion of the outer SC with a greater mechanical strength of the individual cells.

Table 1 Surface area of corneocytes from different body sites, ages and genders.

Surface area (μm²)					
Body region	**Babies**	**Children**		**Adults**	**Aged**
Scalp	~ 700 [16]	~ 800 [16]	**Male**	~ 900 [16,17]	1000 [16]
			Female	~ 1100 [16,17]	
Forehead	~ 750 [16]	~ 750 [16]	**Male**	~ 750 [16,17]	900 [16]
			Female	~ 900 [16,17]	
Axilla	~ 800 [16]	~ 1000 [16]	**Male**	~ 1200 [16,17]	1400 [16]
				~ 1148 ± 11 [19]	
			Female	~ 1250 [16,17]	
				1176 ± 11 [19]	
Upper arm	~ 800 [16]	~ 1000 [16]	**Male**	~ 1000 [16,17]	1200 [16]
				1098 ± 12 [19]	
			Female	~ 1050 [16,17]	
				1145 ± 14 [19]	
			-	~ 1000 [20]	
Forearm	~ 700 [16]	~ 900 [16]	**Male**	~ 850 [16,17]	1100 [16] 753 ± 120 [22] 1000-1300 [21]
				~ 1004 ± 12 [19]	
			Female	~ 1100 [16,17]	
				~ 1018 ± 21 [19]	
			-	~ 840 [18]	
			-	555 ± 80 [22]	
			-	600 – 1000 [21]	
			-	1000 ± 200 [24]	
Hand	~ 650 [16]	~ 700 [16]	**Male**	~ 800 [16,17]	~ 1000 [16]
			Female	~ 1000 [16,17]	800 – 1050 [21]
				600-850 [21]	
Abdomen	~ 800 [16]	~ 800 [16]	**Male**	~ 1000 [16,17]	~ 1300 [16]
				1130 ± 8 [19]	
			Female	~ 1400 [16,17]	
				1180 ± 18 [19]	
				~ 850 [18]	
Thigh	~ 750 [16]	~ 800 [16]	**Male**	~ 1100 [16,17]	~ 1300 [16]
				1184 ± 17 [19]	
			Female	~ 1300 [16,17]	
				1188 ± 14 [19]	
Heel	-	-	**Male**	~ 800 [16,17]	-
				1104 ± 12 [19]	
			Female	~ 1000 [16,17]	
				1101 ± 12 [19]	
Cheek	-	-	-	~ 680 [20]	-
			-	750 ± 150 [24]	
			-	800 – 1000 [20]	

Table 2 Mechanical properties of corneocytes measured by different techniques as defined by the value of the Young's modulus.

Methods	Young's Modulus
Fontbrune's double micromanipulation system **Corneocytes dispersed in water**	450 MPa [27]
Scanning Probe Microscopy **Indentation: < 10 nm** **Cantilever spring constant: -** **Probe: spherical** **Contact mechanics model: Hertz**	0.4 GPa [30]
Treatment of skin with moisturizer for 10 days **Scanning Probe Microscopy** **Indentation: < 10 nm** **Cantilever spring constant: 0.1 N/m** **Probe: pyramidal** **Contact mechanics model: JKR**	Before treatment: 50 ± 20 MPa After treatment: 25 ±15 MPa [31]
Treatment of skin with moisturizer for 5 days **Scanning Probe Microscopy** **Indentation: < 10 nm** **Cantilever spring constant: 0.6 N/m** **Probe: spherical** **Contact mechanics model: JKR**	Before treatment: 1.34 ± 0.46 MPa After treatment: 1.87 ± 0.85 MPa [32]
Scanning Probe Microscopy **Indentation: < 50 nm (cornified envelope) and > 50 nm (cell interior)** **Cantilever spring constant: 35 N/m** **Probe: nanoneedles (diameters of 40 and 75 nm)** **Contact mechanics model: Oliver-Pharr**	For < 50 nm: 100 MPa For > 50 nm: 500 MPa [33]
Scanning Probe Microscopy **Indentation: < 50 nm (cornified envelope) and > 50 nm (cell interior)** **Cantilever spring constant: 2.5 N/m** **Probe: pyramidal** **Contact mechanics model: Hertz**	**Upper SC (1st Tape strip):** For < 50 nm: 250 MPa For > 50 nm: 300 – 520 MPa **Inner SC (14th Tape strip):** For < 50 nm: 150 MPa For > 50 nm: 250 – 300 MPa [35]