INTRODUCTION

Skin hydration is defined as the water content of the epidermis and the dermis (1). The inner milieu of our body consists of about 70% water (2). Approximately 20% of water present in the body is accumulated in the skin, with 60–70% of this amount being accumulated in the dermis (3). Water is important for the structure and mechanical properties of many proteins and their mutual interactions (4). Functionally, the amount of water in the skin can be divided into free and bound water. In healthy skin, most of the water is bound to macromolecules. The ability of the skin to hold water is primarily related to the stratum corneum (SC), which plays the role of barrier to water loss (5). The retention of water in the SC is dependent mainly on the presence of natural hygroscopic agents within the corneocytes and the SC intercellular lipids ordely arranged to form a barrier to transepidermal water loss (5). The glycosaminoglycan polymer hyaluronic acid (HA, hyaluronic acid) provides a scaffold on which sulfated proteoglycans and matrix proteins are organized. These supramolecular structures are able to entrap water and ions to provide skin with hydration and turgor. HA occurs in both dermis and epidermis, with dermis containing the greater proportion (6, 7). HA present in the epidermis may play a role in an epidermal barrier function and SC hydration (5).

Unbound water molecules bind to each other in tetrahedron form (8). The lack of interaction between water and surrounding molecules contributes to dry appearance of skin. Water content can vary depending on vary factors as skin site, skin depth, body mass index, age, sex, diurnal hour (9-15), seasons and climates (16).

On the other hand, Hydration is the most basic consumer expectation toward a skin care product and is therefore, the most common and functional benefit promised by the cosmetics industry; at the same time, skin moisturizing formulations represent a major category of products in the skin care business (17). Proper hydration is absolutely critical to obtaining healthy skin (18). Similarly, the occurrence of healthy skin is linked to a suitable water content and flow through all layers. Exists a plethora of endogenous and exogenous molecules that contribute to the maintenance of water in the skin, covering various mechanisms and interactions that influence skin functionality and structure (19). The challenge of formulations and researchers is to adapt the formulas to the needs of skin hydration and compliance with user expectations, who expects a high efficacy regardless the technology used.

In this review will describe some aspects of hydration at different skin depths, molecules and formulations involved in the modulation of skin water content, and present methodologies used in the evaluation and the information that they provide about the complex process of the skin hydration and influence for moisturizers.

EPIDERMIS

The epidermis thickness is variable. It had been reported between 40 - 240 µm thick, depending on the measuring area and method used (20-22). Water originates in the deeper epidermal layers and moves upward to hydrate cells in the outermost skin layer, the stratum corneum (SC). Aquaporins...
content expressed in g/cm³. From this water profile across the epidermis the border between SC and SG can be estimated, due to the steep drop in water concentration from the inner to the outer side of the SC [2]. Water content drops from approximately 70% at the inner SC to only 30% at the skin surface, and this slope of the curve becomes flatter in the SG [2].

**STRATUM CORNEUM (SC)**

Graphic:

![Figure 1. Skin water content profiles.](image)

**a.** Water profile across human skin with percent water expressed as grams of water per total grams (water plus dry mass) of tissue. SC: stratum corneum; G: stratum granulosum; P: stratum spinosum; B: stratum basale.

**b.** Water concentration curve with water content expressed in grams/cm³. Graphics modified from references 26 and 2, respectively.

The inner milieu of our body consists of about 70% water, while the surrounding air at ambient conditions contains less than 1% water, so at ambient humidity the air is far from saturated [2]. Water originates in the deeper epidermal layers and moves upward to hydrate cells in the outermost skin layer, the stratum corneum (SC), eventually being lost to evaporation. Then, an evaporation barrier is needed to maintain body water homeostasis. The SC, with its normally minute thickness between 10 - 20 µm in most areas of the body, functions as the main evaporation barrier [2, 20, 31]. Its architecture is the most important factor in water flux and retention in the skin, and in overall level of moisturization. SC damage affects the capacity to retain water, which leads to drying of the skin impairing the barrier function. In the SC, free water is able to diffuse from the skin to the outer environment, while bound water is associated with many molecules, such as filaggrin and other NMF such as amino acids, pyrrolidone carboxylic acid (PCA), lactic acid, urea, glucose and mineral ions [32], throughout the epidermal layers [1]. Furthermore, water is inhomogeneously distributed in the SC [7]. Bouwstra and colleagues [2003] observed hydration level in the central part of SC is higher than in the superficial and deeper cell layers, and water domains are mainly present within the corneocytes and not in the intercellular regions. At a very high hydration level (300% wet/wet), the corneocytes are strongly swollen (except for deepest cell layers adjacent to the viable epidermis), and cell thickness increases linearly with the hydration level: swelling of cells mainly occurs in the direction perpendicular to the skin surface. At an increased hydration level, the corneocyte envelope more efficiently surrounds the cell content compensating for the increased cell volume [7]. SC thickness can increase up to two-fold by hydration for...
few hours (31). It has been observed changes in the SC water levels during different seasons. The water content in face skin decreased in autumn, especially near the eyes and upper-cheek (33). Dry skin in the winter involves scaling, defects in water holding and barrier functions, and decreased ceramide (CER) levels in the SC (34).

The SC intercellular space is enriched in lipids organized into lamellar bilayers. It has been proposed that the organization of SC lipid bilayers could be stabilized by a partial interdigitation between the two leaflets (16, 35, 36). In human SC the major lipid classes are ceramides (CER), cholesterol (CHOL) and saturated long chain free fatty acids (FFAs); the ratio between these lipid classes is approximately equimolar (37), and they are responsible for the permeability barrier and water holding functions of the epidermis (34).

The CHOL molecules play an important role in the permeability of SC, since partly immobilize the hydrocarbon chains of the lipids, making the lipid bilayer less deformable and thereby decreases the permeability of the bilayer to small hydrophilic molecules (16). The relative rigid lipid bilayers of the SC also are characterized by a high content of unusually long chain CER (38). Each long chain ceramide species has unique properties that contribute to SC organization and thereby provide the SC with its barrier function (39). Levels of total CER and the specific CER species with sphingoids 6-hydroxy sphingoamine (CERNP) and phytosphingosine (CERNH), are indicators of normal keratinization, show significant positive correlations with conductance values and play important roles in maintaining lamellar stability in the membrane assembly (34, 37, 40).

In dry skin, the level of long chain CER decreases (41) and there are changes in composition of CER subtypes (42). The increase of CER levels plays an important role in improving and/or preventing dry skin symptoms (34). Some studies show active molecules (vg. niacinamide, Eucalyptus extract) could increase the levels of enzymes related to the anabolism of CERNP and CERNH in keratinocytes, thus improving SC function (34, 43, 44). Fatty acids, essential components of natural lipids, determine the physiological structure and function of the skin. (45).

Depletion of lipids in SC can occur with ageing, cleansing, environmental conditions or dry skin disorders (33, 34, 46). The lipid bilayers are targets for influence of moisturizing products (43). Fourier transform infrared spectroscopy (FTIR) studies using lipid mixtures containing isolated CER, CHOL and FFAs, mimicking the lipid composition and organization in SC, show that some lipophilic moisturizers can interact with SC lipids, rendering the more densely packed (47) In addition to the thickness of the bilayer, the packing of the lipids within a bilayer can be expected to control the overall barrier properties. Proposed forms of bilayer organization include orthorhombic, hexagonal and fluid or less rigid bilayers (48). Among these three forms, orthorhombic represents the most compact packing with the highest barrier properties and the fluid form represents the least. Proportions of these may vary depending upon the relative levels of various lipids and their chain length in the bilayer (49). SC lipid simulation studies suggest distinct roles for each lipid species within the bilayer (16).

In experiments conducted by Das et al. (2009), CER compose a dense bilayer phase, whereas the smaller, more rigid CHOL molecules serve to secure and condense the bilayer, thus increasing the lipid density within. In contrast, FFAs function to relieve the stresses induced by the dense and rigid CHOL/CER bilayer (16). It is possible that the shorter chain fatty acids (such as C18) associated with the fluid phase of the bilayer provide flexibility, whereas the longer chain fatty acids (>C20) are associated with the more rigid crystalline phase (50). The stabilization of the orthorhombic lateral packing of the lipids might reduce the water loss from the skin, and contribute to the moisturizing effect of the lipophilic moisturizers (47). On the other hand, some evaluations of product ranking show increase in efficacy when lipids are added to the formulations (51), and recordings of Near Infrared Spectroscopy (NIR) had showed differences in the regions belonging to CH and NH groups, rather than purely on the water bands, when skin is treated with a lipid moisturizer indicating the interaction and moisturizing effect on the skin (52).

Keratin is the major non-aqueous component of SC which accounts for 80% of its dry weight (53). Keratin also is a major factor (together with filaggrin derived free amino acids) determining SC hydration level and water holding capacity. The water holding capacity depends on the structural organization of the corneocyte keratin-associated membrane network in a cubic-like rod-packing symmetry, with a lipid bilayer between apposed keratin intermediate filaments. This structure demonstrates the close lipid association of keratin and the insolubility of keratin and promotes the reduction in cell volume and hydration level between SG and SC layers (54). Lipids and keratins play very important role in formation of permeability barrier. It is important in the hydration process of the skin, the SC extracellular matrix, which is metabolically active, as it changes both structure and function as it transits to the surface, and contains not only lipids but also enzymes, other structural proteins, and antimicrobial peptides that could impact barrier function and water holding capacity (55).

Hyaluronic acid (HA) present in the epidermis, regulates keratinocyte differentiation and SC-extracellular lipid formation required for normal SC structure and epidermal barrier function, by interacting with its receptor CD44, on keratinocyte cell surface (56). Xylose, component of glycosaminoglycans (GAGs), is a keratinocyte HA synthesis-stimulating and skin-hydrating agent (57).

DERMIS

The inner layer of the skin, the dermis, is between 1 and 4 mm thick (20), and it consists mainly of connective tissue. Dermis thickens as it binds more water (58, 59). In the dermis, the collagen fibers, the interstitial space GAGs and the proteoglycans, can absorb large quantities of water (60), determining the intrinsic turgor of youthful skin. With aging of the skin, the collagen fiber network is stretched, reduces its absorption capacity and water retention. Dermal hydration is highly related to the content and distribution of GAGs. GAGs are widely distributed throughout the skin, GAGs most often present in human skin are hyaluronic acid (not attached to a protein core) and the proteoglycan family of chondroitin sulfates (GAGs attached to a protein core). GAGs bind up to 1000 times their volume in water (4). Some conditions influence on GAGs behavior. As was shown by confocal laser scanning.
microscopy. GAGs in photodamaged skin are abnormally deposited on elastic material clumped in the papillary dermis, rather than diffusely scattered as in young skin (8). This aberrant localization interferes with normal water binding by GAGs, despite their increased number (4). Age also leads to increased hydrophobicity and folding of proteins (v.g. collagen, elastin), which in turn, leads to a decreased interaction of dermis proteins with water. Hence, in aged skin, water is found in the tetrahedron form, bound to itself rather than other molecules (4).

**INGREDIENTS AND MOISTURIZING FORMULATIONS**

Evidence from several clinical and *in vitro* studies shows increase in the SC water content due to application of moisturizing formulations. The use of effective and safe moisturizers is beneficial in the treatment of dry skin and skin barrier disorders. Cornneocyte morphology and keratinocyte biology also are targets for moisturizing products (43). The use of some moisturizers during a week can cause increase in the area of cornneocytes up to 3% (61). When samples of excised skin treated with moisturizers are visualized by cryo-scanning electron microscopy, the SC appears as a region of swollen cornneocytes trapped between two layers of relatively dry cornneocytes (62). Centrally located cornneocytes are more sensitive to moisturizer application than cornneocytes in non-swelling regions, and the change in SC thickness is most influenced by the change in the thickness of this central swelling region, where cells swell because much NMF is available for water retention, while in upper non-swelling region, for example, NMF is easily lost due to the skin surface’s frequent contact with water (63). This water distribution correlates with the NMF distribution in SC (62). Changes after the application of topical moisturizer product involve the skin surface by reducing the micro-scales and epidermal irregularity, as was revealed by reflectance confocal microscopy (1). Alike, depending of their composition, moisturizers are able to change the mRNA expression of certain epidermal genes essential for keratinocyte cycle (v.g. involucrin, transglutaminase, kallikreins) (64).

Likewise, formulations that contribute to the improvement of the skin hydration at different depths, interact in a specific way with structure of the skin, and the diversity of ingredients can provide efficacy through different mechanisms (65). Vary authors have found that profile of hydration can change depending on type of moisturizing formulation (15, 53, 66, 67). And, when water content averages are compared, the effect of distinct formulations on the SC could be different at the time (15). In the same way, it was observed that emollients can present different mechanisms of hydration (68), and a good moisturizing performance depends mainly on the choice of the emollients and can enhanced by choosing the right emulsifier (69). Moisturizers can improve skin hydration but only some formulations improve SC thickness, water gradients and hydration (70), and this is due to compositional differences between the products. For example, niacinamide [nicotinamide, vitamin B3] could contribute to this effect (70).

With respect to the dermis, there are very few studies that report a relationship between the change in water content and application of moisturizing products. Miosek et al (2013) observed that after the use of a moisturizing cream, they were obtained statistically significant differences between the echogenicity of the superior layer of the dermis on the chin and cheek, suggesting dermis thickening due to formulation application (71). Likewise, in our recent study, we further note temporal significant changes in the dielectric constant of the dermis, to effective skin depths of 500 µm and 1500 µm, after moisturizing formulations were applied (15), which could indicate an increase in the dermal water content. We have not found other studies that show this same. Future studies are necessary to reinforce these results, trying with other different available measurement methodologies and/or biochemical approaches that could see for example some kind of molecular signalling mechanism that could lead to increased dermis water content.

On the other hand, the water content at 2500 µm depth not showed significant changes by any of the formulations used (15). This is consistent with other researches where they did not observe any significant differences in the inferior (reticular) layer of dermis post application of moisturizing cream, while the superior layer bind water, when skin moisturization treatments where monitored by high frequency ultrasound (58, 71). Another study show similar results, where neither moisturizer use, age, body mass index [BMI] nor hair removal had any significant effect on the dielectric constant values at effective skin depth of 2500 µm (13). Moreover, in a long-term study, the daily performance of massage after moisturizer application neither substantially promoted the moisturizer efficacy (72).

Exists a plethora of endogenous and exogenous ingredients that contribute to the maintenance of water in the skin, covering various mechanisms and interactions that influence skin functionality and structure. Moisturizers are based on occlusive substances, such as petrolatum and dimethicone, and humectant substances, such as glycerin and urea (19). According to their chemical nature of the ingredients can be classified in lipophilic and hydrophilic moisturizers, and they can show different actions (62). Some hydrophilic moisturizers could penetrate much more readily than lipophilic moisturizers, and the latter are abundantly present in the upper regions of the SC (73, 74). Lipophilic moisturizers may penetrate into the SC and interact with the SC lipids to provide an increased barrier to water loss (v.g. esters) or remain on the surface of the skin, thereby preventing water evaporation from the SC by their occlusive properties (v.g. petrolatum); and hydrophilic moisturizers can be hygroscopic substances of low molecular weight that could penetrate the SC, and act as humectants, mimicking the role of NMF [v.g. glycerol, hydroxyethyl urea, mon- or di- saccharides] (75). Equally there may be differences between moisturizers of the same type or changes in efficacy when they mixed with other. For example, glycerol can induce swelling of cornneocytes and formation of intercellular water domains, whereas urea formulations can result only in the formation of intercellular water domains (76).

The efficacy of a moisturizer is influenced by factors such as consumer compliance and skin dryness grade (1, 51). This point concern partly to the composition of the formulation and sensorial properties of emollients. Emollient is defined in reference to the plasticizing and smoothing effect on a rough
and dry skin surface and they are multifunctional ingredients supporting multiple formulation claims: skin feel agents, solvents for numerous actives, permeation enhancers, glass or shine control, skin protectants against damaging environment among others [79]. The efficacy of a moisturizer is influenced by factors such as consumer compliance, skin dryness, skin type and the moisturizer composition [1, 51]. There is a direct dependency of skin physiology and product efficacy. The drier the skin, the higher the increase of hydration [51]. On the other hand, it is noted that an improvement in skin hydration does not necessarily imply an improvement in other parameters. For example, an increased moisturization performance does not necessarily result in increased skin elasticity and that therefore a combination of mechanisms had to be influencing what is perceived by consumers as a positive skin response to emollient application [68]. Long-term use of moisturizers can significantly increase skin capacitance, independently of dose, and does not change the mechanical properties or TEWL [77, 78].

CONCLUSIONS

In the course of this review we have detailed some components involved in maintaining water content through the depths of the skin. An alteration or unbalance of a component triggers a skin disorder that leads to the generation of dry skin. Similarly the absence or lack of water retention indicates an impaired barrier function of the skin. Greater interaction between evaluation methods and mechanisms is required to achieve a greater understanding of the regulation and interaction of water within the skin. The correlations between in vivo and molecular results is important for the development new targets and actives for skin care. Moisturizing formulations are widely used to combat dry skin. These formulations contain moisturizing agents that act by different mechanisms and interact in a specific way and change the flow of water at the different skin layers. The necessity of evaluate different moisturizers through their capacity to increase the skin hydration level on different depths arises to obtain objective information in the mechanisms and offers accordance with consumer needs. Is necessary deepen in the interaction of formulations with different skin layers and influence on the water content. In the dermis has not reported study enough for the influence of moisturizers that confirm changes in the water content and biochemical pathways. One single evaluation method is not sufficient in order to fully understand the hydration mechanisms, requiring a combination of in vitro and bioengineering techniques in vivo to evaluate the impact and the mechanisms of ingredients and formulations in the maintenance and recovery of skin hydration. The hydration skin research has come a long way in identifying key issues and findings that have improved moisturizing products; however there is still a longer way, where always expected something incredible that is waiting to be discovered.

REFERENCES

The Anti-Ageing Skin Care conference will focus on new approaches to prevention and treatment. It will discuss the changes from anti-wrinkle and moisturising creams to the effective contemporary formulations of today and the future that have an effect beyond that of the stratum corneum.

Over the two days of the conference expert speakers will present and discuss topics related to skin ageing mechanisms, treatment and prevention. They will explore how regulators view these advances in anti-ageing skin care technologies and associated effectiveness claims will be discussed in an open forum.

Sessions

Tuesday 7 June

Session 1: Skin ageing processes and causes of premature skin ageing

KEYNOTE - Skin ageing beyond UV: basic mechanisms and clinical implications
Prof Jean Krutmann, Leibniz Research Institute for Environmental Medicine, Germany

Oxidative stress and ageing - the effects of environmental pollution, sunlight and diet
Prof Mark Birch-Machin, Newcastle University, UK

The unique microenvironment of the ageing human adult scalp
Prof Des Tobin, Centre for Skin Sciences, UK

Premature skin ageing induced by air pollution: new in vitro insights approaching realistic conditions
Prof Imke Meyer, Symrise, France

Session 2: New technologies and treatments for premature skin ageing

KEYNOTE - Understanding ageing, its limits and possibilities
Prof Suresh Rattan, Aarhus University, Denmark

Benefits of topical Q10 treatment on human skin
Dr Anja Knott, Beiersdorf, Germany

Microarray analysis of skin ageing
Marta Hlobilová, Contipro, Czech Republic

A novel mechanism to prevent solar-light induced oxidative stress
Varun Mathur, The HaliStar Company, USA

A novel active ingredient induces a beneficial dermal remodelling, leading to clinical improvement on photoaged skin
Alain Mavon, Driflame, Sweden

Wednesday 8 June

Session 3: Advertising and claim support for anti-ageing skin care products

Smoothing the way from script to screen - getting your skin care ads to air
Niamh McGuiness, Clearcast, UK

Supporting breakthrough anti-ageing skin care claims
Dr Jack Ferguson, Skinnovation Ltd, UK

Session 4: Assessment and delivery of anti-ageing skin care benefits

Anti-ageing skin care strategies
Prof Paul Matts, Procter & Gamble, UK

The influence of age, ethnicity and anatomical site on skin mechanics and composition
Dr Michael Sherratt, University of Manchester, UK

In vivo methods to evaluate anti-photo ageing and anti-pollution efficacy claims of cosmetic products
Dr Stephan Bielfeldt, proDERM, Germany

Optical Coherence Tomography (OCT) - a new tool for quantification of skin ageing
Jon Holmes, Michelson Diagnostics Ltd, UK

Ex vivo evaluation of efficacy of topical skin formulations
Ardeshir Bayat, Science of Skin, UK

Programme co-ordinator: Dr Jack Ferguson, Skinnovation Ltd, UK

More speakers to be announced shortly

Programme co-ordinator: Dr Jack Ferguson, Skinnovation Ltd, UK

Sessions

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