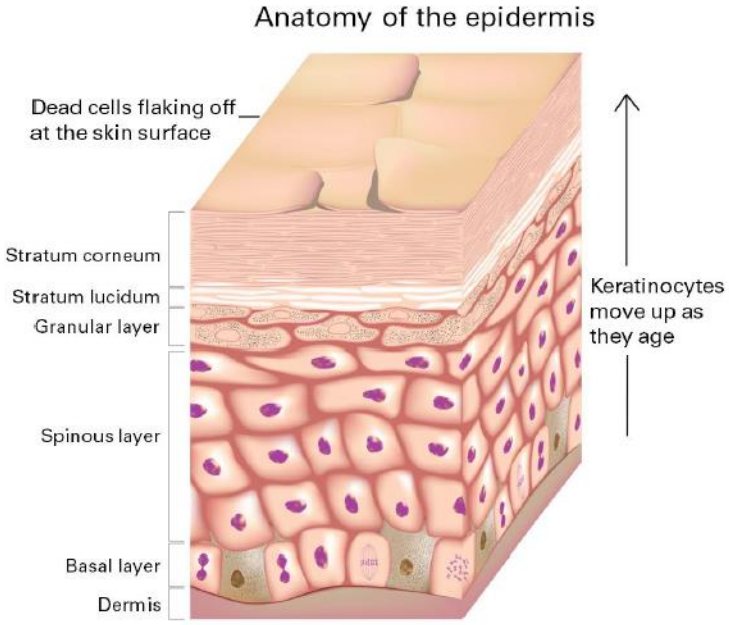
SYNERGIE SKIN
CLEAN SCIENCE

CLINICAL DATA – PAPAIN MOLECULAR FILM

An altered desquamation leads to dry skin

The normal desquamation of corneocytes in the stratum corneum (SC) is essential, not only to retain the healthy appearance of skin, but also to provide protection against physical, chemical and biological aggressions.

The epidermis is the outermost layer of the skin and it is comprised of four layers: basal, spinous, granular and the stratum corneum. The basal layer is composed of keratinocytes that migrate to the SC undergoing biochemical changes to evolve to corneocytes. This maturation process is known as cornification.



Corneocytes are non-viable anucleated cells that form the SC and are held together by corneodesmosomes, which are constituted by protein complexes. These cohesive forces are progressively degraded by specific hydrolytic enzymes to allow cell shedding at the surface of the skin, a process known as desquamation [1].

Desquamation is related to the constant and controlled cell renovation that takes place in the basal layer. The rhythm of mitosis of keratinocytes allows a regeneration of the SC, replacing the desquamated corneocytes for new ones [2, 3].

In healthy skin, the complex process of complete cell turnover takes around 30 days [4]. With ageing, this turnover rate of the SC decreases, and the skin becomes rough and dry because the interaction between corneocytes is higher than in young skin [5]. The solution would be able to help break the linkages between corneocytes to remove old cells and stimulate the creation of new ones.

The water path in the SC is key in hydration

In dry, flaky skin conditions, corneodesmosomal linkages are not degraded efficiently and corneocytes accumulate on the skin's surface layer [6] leading to the formation of visible powdery flakes. The inability to degrade these structures reflects a decreased activity of the enzymes, either through a reduced synthesis, inherent loss of activity, leaching from the surface layers of the SC or changes in the surrounding lipid-rich microenvironment, which may indirectly reduce enzyme functionality.

There is a connection between dry skin and altered desquamation: improve one factor and the other one also improves [7]. An impaired desquamation leads to a disturbance of the water barrier function and hence to a dehydration tendency of the SC. On the other hand, skin dryness is able to alter the desquamation process [8].

Water is essential for the normal function of skin, especially the stratum corneum [9]. The physical packing of the corneocytes creates a tortuous path for molecules to traverse, effectively increasing the diffusion length and thereby improving the SC barrier function. However, the reduction of water flux and loss through the tissue is not the sole cause of the apparent discontinuity in hydration between different corneocytes layers, elective retention of water is required as well. The health of the SC depends on the maintenance of the SC water content at an optimal level, a function predominantly handled by the natural moisturising factor (NMF) [10].

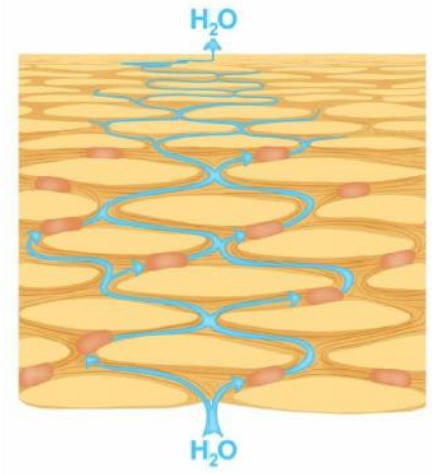


Fig. 1. Water path in the stratum corneum.

An impaired degradation of corneodesmosomes may disturb the water barrier function and lead to dehydration; and, conversely dryness is able to alter desquamation.

The NMF provides a natural shield to the skin

Found exclusively in the SC, the NMF is a complex mixture of low-molecular-weight, water-soluble compounds which are present in high concentrations within corneocytes (as much as 10% of their dry weight) and represent up to 20-30% of the overall dry weight of the SC [8]. The NMF contains amino acids, sugars, lactate, urea, pyrrolidone carboxylic acid (PCA) and inorganic ions such as chloride, sodium, potassium, calcium and magnesium.

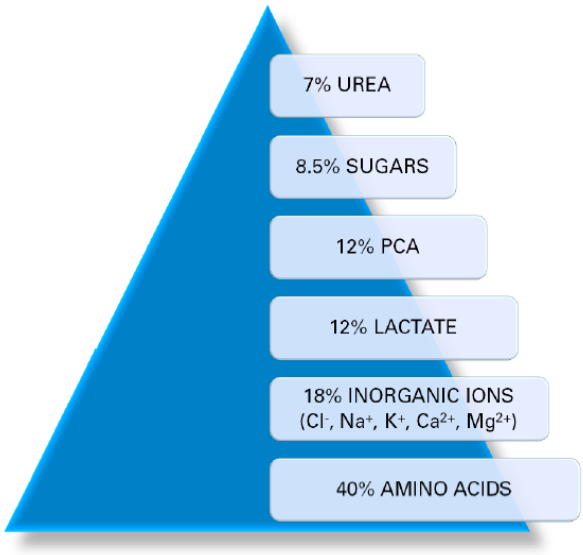


Fig. 2. Composition of the NMF.

The NMF is a natural protection shield against dehydration which also plays an important role in the physical properties of the SC [8]. Hygroscopic NMF components absorb atmospheric water and dissolve it in their own water, acting as very efficient humectants. This humectancy allows the outermost layers of the SC to remain hydrated despite the desiccating action of the environment. The water retained by the NMF acts as a plasticizer on the corneocytes proteins providing elasticity to the cells: if deprived of water the SC is prone to snapping on mechanical stress, it becomes less extensible and the forces required to stretch the skin are greater [9].

Furthermore, the NMF also contributes to the essential regulation of the pH in the SC and plays a role in the maintenance of the aqueous or semi-aqueous environments needed by hydrolytic enzymes involved in the normal desquamation process [1].

The NMF components provide a natural shield that protects the skin against dehydration and help in the physical properties of the skin such as elasticity.

Exfoliators help with desquamation

There are different ways to prevent skin from becoming rough and dry due to ageing. Physical exfoliators erode the surface of the skin cells; as rubbing is continued and an additional force is exerted, skin cells will continue to forcibly desquamate in a non-specific manner. Common ingredients of manual exfoliation include pumice, loofahs and buff puffs. Microdermabrasion is also a common exfoliating method that uses a combination of fine abrasive tip of crystal and vacuum suction applied to the skin.

Chemical exfoliation includes ingredients such as alpha-hydroxy acids (AHAs), salicylic acid or N-acetylglucosamine [11]. They cause superficial skin cell removal as a result of their ability to disrupt intercellular bonding between cells.

Enzymatic peeling uses specific proteases to break the cell-cell interactions and accelerate desquamation. Papain is an enzymatic exfoliator which is naturally present in papaya (*Carica papaya)*. This cysteine protease hydrolase enzyme is a single peptide

with 212 amino acids and has a molecular weight of 23KDa stabilised with 3 disulfide bonds [12].

The active site of papain is formed by two amino acids: Cys-25 and His-159. This latter amino acid interacts with Cys-25 and allows its deprotonation, which then performs a nucleophilic attack on the carbonyl of carbon of a peptide sequence, forming an acyl-papain intermediate. The enzyme is finally deacylated by water and the enzyme recovers its original structure releasing the carboxy terminal portion of the peptide.

Recent studies have shown that papain promotes the proteolytic digestion of the first layers of the SC and decreases cross-linking in corneodesmosomes region. A large amount of the intercellular material is lost in the first layer of the stratum corneum, corneodesmosomes are devoid of their plug material, most of the extracellular components are digested and the corneocytes are separated [13].

Papain stimulates exfoliation by digesting inter-corneocyte cohesion enzymatically; this proteolytic enzyme decomposes proteins into smaller fragments causing a softening effect to the skin and a sloughing of corneocytes [13].

There are several types of exfoliators; physical, chemical and enzymatic. The papain enzyme which induces the removal of the superficial skin cells thereby helping with desquamation.

Papain molecular film, peeling and moisturisation, a powerful combination

Molecular films are very thin monolayers with interesting cosmetic benefits, conferring a soft, light, and pleasant sensation for the skin. Due to their particular rheology, they show good spreadability which facilitates the absorption of functional ingredients. Thanks to a better compactness and distribution of molecules, the film is more homogenous and thinner, hence having a greater contact area with the skin.

Papain molecular film was specially designed to target both desquamation problems and dryness. It contains papain which helps to increase the SC turnover by degrading corneodesmosomes and allowing the removal of corneocytes in a correct way helping to prevent rough and dry skin. This molecular film provided better desquamation properties on human epidermal keratinocyte cell cultures than papain alone *in vitro.*

Moreover, Papain molecular film also contains ingredients of the NMF, both ions (magnesium, potassium…) and amino acids (serine, alanine, proline) which help to restore the levels of moisture and preserve the natural barrier function of the epidermis, by providing a refill of the NMF ingredients. Furthermore, it was demonstrated to provide an immediate increase of skin moisturisation in a panel of volunteers after only 24h, and also a long-lasting effect after 20 days of treatment.

Papain molecular film helps to regulate desquamation and refills the NMF ingredients thereby preventing dryness and enhancing hydration.

In vitro efficacy
Peeling assay

The activity of the Papain molecular film in detaching human epidermal keratinocytes was determined and compared with that of the papain alone *in vitro.*

The cells were treated with Papain molecular film or a solution with 0.75% papain (the same papain concentration as in the molecular film).

The cells were incubated at room temperature for different times (10, 20, 30 and 45 min), and then the well medium was collected and the desquamated cells were counted with a particle counter.

The molecular film proved to increase its desquamation efficacy with time while a solution with the same concentration of papain presented a decreased peeling activity.

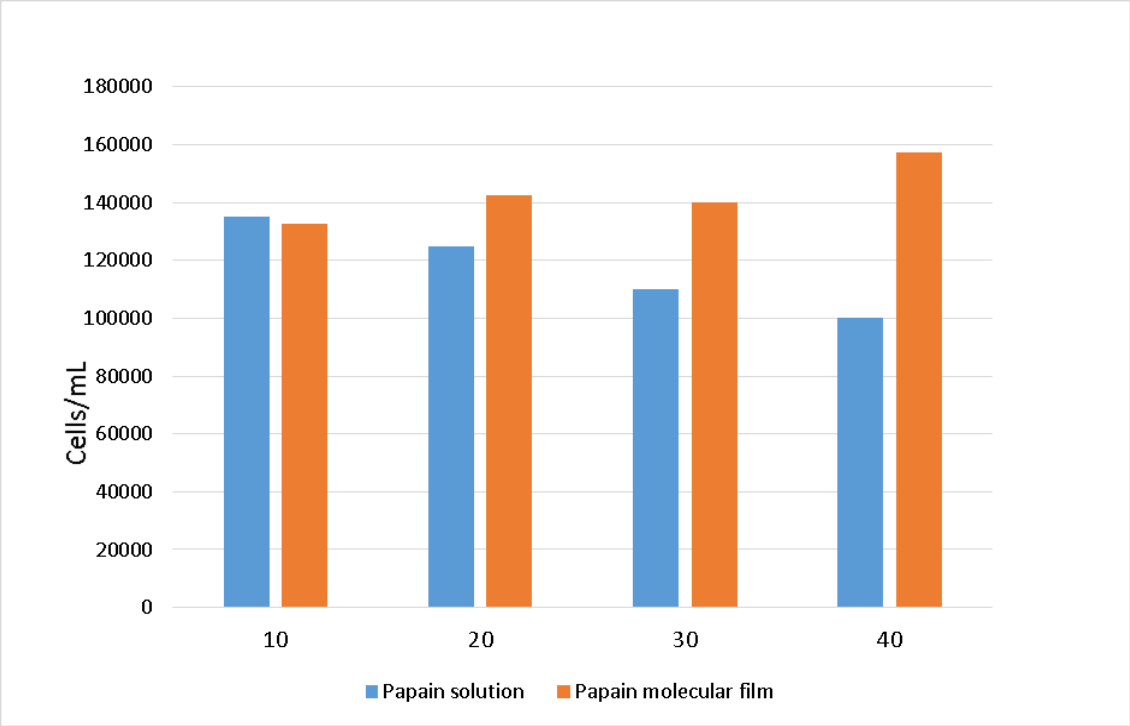


Figure 3 – Cells per millilitre obtained at different incubation times with Papain molecular film or with a papain solution.

In vivo efficacy
Moisturisation increase

The moisturising profile of Papain molecular film was determined by corneometry. With the corneometer principle, the moisture of the outer layer of the skin was determined by means of a capacity measurement.

A panel of 20 female volunteers between the ages of 20 and 73 applied a gel with 7% Papain molecular film on one side of the fact, twice daily for 20 days. A placebo gel was used to treat the other side of the face. The volunteers were acclimatised for 45 minutes at 22°C and 60% relative humidity.

Skin moisture measurements were taken after 12h, 24h and 20 days. Readings of three different spots of the treated area were recorded and the values were standardised.

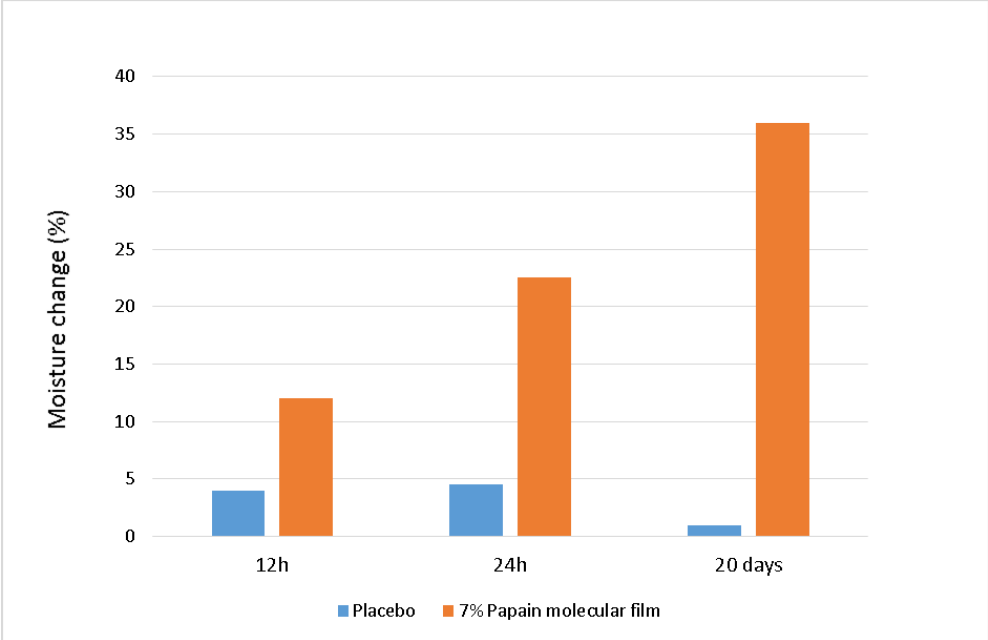


Figure 4 – Moisturisation profile of a gel with Papain molecular film versus a placebo gel.

The treatment with the active gel showed an improvement in the cutaneous hydration by 12.3% only after 12h, and provided a long-term effect increasing hydration by 23.1% and 35.4%, after 24h and 20days, respectively. The placebo gel did not produce any significant effect on skin moisturisation.

Cosmetic properties

Papain molecular film:

- Increases SC turnover by degrading corneodesmosomes and allowing the removal of corneocytes in an efficient way.
- Improves desquamation of human epidermal keratinocytes cell cultures versus papain alone.
- Contains ions and amino acids present in the NMF of human skin and restores the levels of moisture and natural barrier function of the epidermis, by providing a refill of NMF ingredients.
- Provides an immediate improvement in moisturisation after only 12 h (12.3%), increasing after 24h (23.1%) and 20 days (35.4%) presenting a long-lasting effect on cutaneous hydration levels, as proven *in vivo* (7% Papain molecular film).

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