ProRenew Complex CLR™

Postbiotic approach to skin renewal and resurfacing

for fine cosmetics · since 1926

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Postbiotic approach to skin renewal and resurfacing

CONTENTS

ProRenew Complex CLR™

Dosage: 3.0%
pH range: 4.0–7.0
INCI Name: Lactococcus Ferment Lysate

ProRenew Complex CLR™ is preserved with sodium benzoate.

Also available as **ProRenew Complex CLR™ NP**, stabilized with phenylpropanol and levulinic acid/sodium levulinate.

For information on formulations or natural certifications, such as COSMOS and NATRUE scan or click QR code

Application

- ■ skin regeneration
- ■ barrier maintenance and repair
- ■ skin protection
- ■ scalp care

■ tested ❑ recommended

Summary

ProRenew Complex CLR™ acts on essential features in the aging process of the skin, its ability to successfully adapt to a constantly changing environment and to effectively renew itself.

ProRenew Complex CLR™ positively influences the speed and quality of epidermal growth. The production of essential proteins and enzymes in skin quality is clearly increased, barrier function and cell cohesion are improved, and skin renewal is accelerated, promoting skin health. Additionally, ProRenew Complex CLR™ has shown to effectively promote desquamation, the shedding of dead cells.

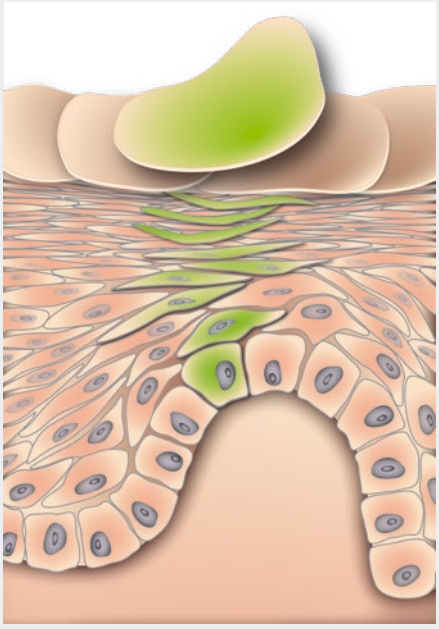

Fig. 1: Epidermis is the most dynamic part of our body

Epidermal turnover: approx. 4 weeks

- 2 weeks from basal layer to SC
- 2 weeks in the SC until surface is reached and dead skin cells are shed

Per day

- 1 sheet = 2 m^2 = 1.5 g of dead skin cells is shed

During an average lifetime

- Epidermis renews itself approx. 1000 times
- Approx. 30 kg of dead skin cells are shed

Fig. 2: Successful skin renewal results in healthy-looking skin

Fig. 3: Epidermal skin aging has profound consequences for the skin's quality and appearance

ProRenew Complex CLR™

INTRODUCTION

Skin aging is a highly multifaceted phenomenon – scientifically and from the consumer's point of view. 50+ year-old consumers are reported to be confused by the tremendous amount of choices they are confronted with in the increasingly saturated facial skincare market. Their confusion is further increased by the many incoherent and overlapping claims that are communicated on the packaging and in advertisements. Modern-day 50+ year-old consumers are perfectly comfortable with their age. They are not obsessed with looking younger but want to look the best they can. And that means looking as healthy as possible.

Healthy-looking skin is closely related to the actual main purpose of our skin: to function as a physical barrier between our body and the harmful outside world. Our skin's top layer, the epidermis, is responsible for maintaining this function.

Epidermis, the most dynamic part of our body

The renewal process of the epidermis is a product of a careful and sensitive balance between proliferation of the living keratinocytes in the basal layer and the differentiation process which takes place above the basal layer, one in which the differentiating keratinocytes move slowly upwards. In healthily functioning skin, approximately 14% of the basal keratinocytes are in the process of cell division at any given time. They go through 3–5 cell divisions before they detach from the so-called basement membrane, after which they enter the extremely well-organized and delicate process of differentiation and move upward. The total turnover time of epidermis is reported to be between 3–5 weeks. During half of this time, the cells are part of the stratum corneum, where in the process of moving upwards they continue to differentiate until they are fully transformed into corneocytes, reach the skin surface, and are shed.

Although the stratum corneum constitutes a strong and effective physical barrier, it is constantly challenged by fluctuating environmental humidity and temperatures, UV, microbes, daily hygiene (water, surfactants), foreign molecules, etc. In order to maintain its vital function as physical barrier, the epidermis is constantly renewing itself, a process which makes it the most dynamic part of our body. Every day an average of one sheet of dead skin cells is shed from the outer surface of our body. During a lifetime, up to 30 kg of dead skin cells are "produced" by our epidermis, and the epidermis renews itself in its entirety approximately 1,000 times. If the epidermal renewal process takes place successfully, skin shows optimum barrier function, hydration level, and esthetic properties (Fig. 2).

Aged epidermis: "stretched" and "slow"

Aging has a significant impact on the renewal processes in the epidermis. The proliferation rate of the keratinocytes in the basal layer diminishes progressively and is reported to be up to 50% slower in people 80+ years old. The effectiveness of the differentiation process is reduced, which also means that the production of key elements in the skin's barrier function is impaired. Additionally and probably most importantly, aging leads to a loss of immunocompetence in the epidermis.

As mentioned above, the reason why skin is renewed so dynamically is because our stratum corneum is constantly challenged and cannot maintain its barrier function without constant renewal. To safeguard the skin's ability to uphold its barrier function, the epidermis can and must act as a "biosensor." The differentiating keratinocytes are able to instantly react and compensate for the negative influence of an outside challenge on the

skin's barrier function. Although the keratinocytes which are in the process of differentiation are no longer formally alive, they are still biochemically and immunologically active. Where the physical barrier properties of our skin can be considered to be the first line of defense, the keratinocytes' ability to react to insults immunologically would be the second line of defense against negative impacts. Their immunocompetence is therefore of eminent importance.

Reduced keratinocyte proliferation, less effective differentiation, and loss of immunocompetence are essential features in both the esthetics and sensitivity of aged skin. Because of these impairments, upholding a healthy barrier function becomes difficult, and skin dries out quickly and easily. Xerosis, for instance, is a common problem for aged skin, especially occurring in the wintertime. Additionally, the excessive use of soaps and harsh cleansers, but also showering too frequently and thereby washing away essential elements of stratum corneum quality, the Natural Moisturizing Factors, are known to induce xerosis. Furthermore, aged skin, which is dry, is wrinklier than healthily moisturized skin. It seems to have lost its healthy rosy appearance and looks grayish (Fig. 3).

Desquamation in aged skin

The process of desquamation is a key constituent of successful epidermal differentiation, ensuring sufficient hydration levels at the surface of the skin, skin smoothness and elasticity, and a beneficial skin surface pH. In the lower part of the stratum corneum, the stratum compactum, corneocytes are anchored to each other with corneodesmosomes. In the upper part of the stratum corneum, the stratum disjunctum, during the upward movement of the corneocytes, the corneodesmosomes are gradually broken down, until the corneocytes reach the surface of the skin. Here the corneocytes are sloughed off one by one.

Aged skin often shows a dull complexion, at the core of which lies an impairment in the desquamation processes in the stratum corneum. The shedding of dead skin cells in aged skin is disturbed which has significant implications for the healthy and appealing appearance of skin.

A well-known approach toward compensating for above problems is the use of alpha- and/or beta-hydroxy acids or enzymes, such as papain or bromelain. These ingredients essentially lead to the degradation of the corneodesmosomes ensuring "peeling" and "resurfacing" of the skin. The desiccated part of the stratum corneum is removed, revealing healthy and moisturized skin. Whereas these artificial ways of peeling are widely used in the cosmetic industry, a smarter and more sustainable approach would be to help skin to reactivate its own endogenous resurfacing qualities from within.

Air pollution and its impact on aged epidermis

Air pollution is a growing concern for the consumer, especially for those who live in big cities. In many big cities the level of air pollution exceeds the maximum values determined by the World Health Organization. Epidemiological studies have clearly shown that people who live in a polluted environment have worse skin barrier function, lower moisture level in the stratum corneum and skin which is more prone to become irritated. Production of filaggrin and KLK7 has been reported to be significantly lower in skin which is exposed to pollution. Moreover, strong indications have been found that skin in a polluted environment shows a lower level of immunocompetence. Interestingly, corneocyte cohesion and the overall desquamation process were also reported to be disturbed in skin in a polluted environment.

ACTIVITY

CLR has developed ProRenew Complex CLR™ (INCI: Lactococcus Ferment Lysate), based on a lysate of *Lactococcus lactis*, to stimulate the skin's self-renewal effectively. This probiotic lactic acid–producing bacterium is grown under specific conditions, after which the obtained cells are lyzed, a process involving the killing and destruction of the bacterial cells.

Probiotic bacteria are well-established in the food industry, and their benefits for the human body are described in many scientific, peer-reviewed papers. In the gut they beneficially influence the composition and metabolic activity of the endogenous bacteria, and some probiotic strains are even able to inhibit the growth of pathogenic bacteria. Probiotic bacteria are also reported to be able to modulate the immune system, essentially improving immunocompetence and, therefore, our body's ability to adapt to negative influences. Interestingly, it is not the whole living probiotic bacterial cell which is needed for the latter activity, it is the constituents and metabolites of these bacteria which are essential. A product obtained from a lysate of *Lactococcus lactis*, as ProRenew Complex CLR™, which essentially contains the cell debris of this bacterium – such as cell fragments, like DNA, metabolites, cytoplasmic compounds, and cell wall materials – should show and, in the studies performed, has shown that it is able to improve both the skin renewal processes and the processes involved in the skin's adaptation to negative influences.

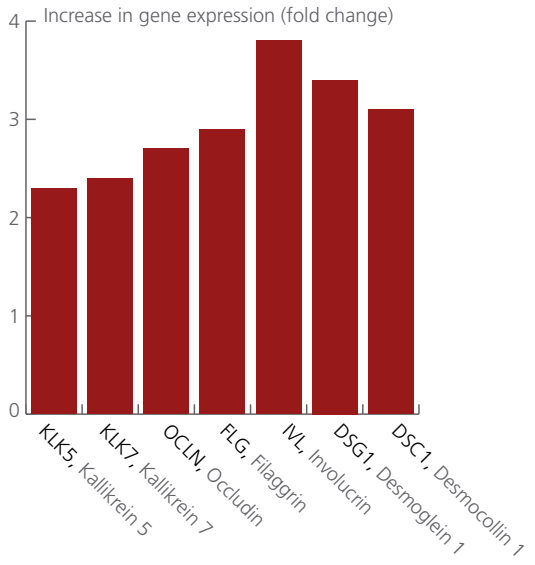

Fig. 4: Activation of gene expression of differentiation-relevant markers

Regulation of gene expression on differentiation-relevant markers

A study was performed where it was assessed whether ProRenew Complex CLR™ was able to activate the expression of differentiation-relevant genes. In the process of successful differentiation of keratinocytes, a variety of different proteins and enzymes is produced. The production of these molecules starts with the expression of a gene, after which the resulting RNA is used in a so-called translation process, where the RNA is "decoded" to produce proteins or enzymes. In this study the RNA was collected and a comparison was made between the gene expression in control cells and those cells treated with ProRenew Complex CLR™.

Normal human keratinocytes from a female, Caucasian volunteer (arm) were treated for 96 h with 1% ProRenew Complex CLR™ in a pre-confluence stage. RNA was extracted by RNeasy protect Midi Kit (Qiagen) and quantified with a Biophotometer (Eppendorf).
The Genearray was performed by an Agilent Whole Human Genome Oligo Microarrays chip.
The expression values are related to non-treated control cells (Fig. 4).

EFFICACY STUDIES – *in vitro assays*

As a consequence of the incubation with ProRenew Complex CLR™, the keratinocytes show a clear activation of gene expression for several essential proteins and enzymes involved in the differentiation process. This indicates that ProRenew Complex CLR™ is able to support the quality of the epidermal renewal processes, a key constituent of healthy skin with a strong barrier function.

Histological evaluation of markers which are essential for successful skin development

Epidermal skin models (epiCS, CellSystems GmbH, Germany) were grown during a period of 14 days. The daily application of ProRenew Complex CLR™ (3% in medium) started on day 4, and it was continued until day 12. On day 4, the three-dimensional growth of the epidermal skin models was still in an early stage. Therefore this experiment allowed the assessment whether experimental manipulation had an influence on their growth. On day 6 no application of ProRenew Complex CLR™ took place. Alternatively to the application of ProRenew Complex CLR™, in order to obtain a suitable control, the epidermal skin models were treated with vehicle (cell culture medium). The epidermal skin models were grown at 37 °C, 5% CO_2, and 95% relative humidity.

This study was performed with the aim to assess whether ProRenew Complex CLR™ would be able to positively influence the quality of the epidermal skin models, histologically determining the presence of different proteins and enzymes (transglutaminase-1, (pro)filaggrin, and caspase-14), which play a crucial role in the successful growth and renewal of epidermis. Additionally, by assessing the thickness of the stratum corneum after 10 days, conclusions could be drawn concerning the speed with which the epidermal skin models had grown. Finally, on day 14, the quality of the barrier function of the epidermal skin models was determined by assessing the live keratinocytes' viability after the topical application of 0.4% SDS. The viability of the live keratinocytes showing healthy mitochondrial activity will only have suffered if sufficient SDS have been able to penetrate through the stratum corneum of the epidermal skin models. Therefore, the better the barrier function of the stratum corneum, the lower the amount of SDS is able to penetrate through and the less the live keratinocytes suffer, and therefore the higher their viability.

Assessment of transglutaminase 1, loricrin, (pro)filaggrin and caspase-14

Stainings were conducted, and one epidermis was used to check the histological structure of the tissues at the time indicated. The epidermal skin models were completely immersed in 4% formaldehyde for fixation. The fixed sample was then embedded in paraffin, de-paraffinized, sliced, mounted, and stained for antibodies (i.e. 'first antibodies', see below) against transglutaminase 1, (pro)filaggrin and caspase-14 according to standardized methods.

Staining and photography

The second antibody directed against the host of the first antibody was coupled to the substrate-converting enzyme peroxidase (HRP) by the biotin-streptavidin complex. The AEC substrate chromogen, which yields a red reaction end product at the site of the target antigen, was used to visualize the specific antibody-mediated staining according to standard procedures.

Transglutaminase 1

The cornified envelope (CE) is a complex assembly of different structural proteins, including involucrin and loricrin. These proteins are cross-linked, and involucrin is covalently attached to extracellular ω-hydroxyceramides. These structural features ensure the CE's mechanical strength, make it insoluble and, in cooperation with corneodesmosomes, ensure the cohesion of the stratum corneum. Transglutaminase 1 is the enzyme which is primarily responsible for building up this vast network of molecules. It is considered one of nature's most important "glues," without which the CE cannot be formed. Dry skin and xerosis, two typical phenomena seen in aged skin, are associated with a significant decrease in maturation of the CE, illustrating the importance of transglutaminase 1. Moreover, it is reported that there is a reduced presence of transglutaminase 1 in photoaged skin.

Microphotographs were taken with a Zeiss Axiovert microscope equipped with a Zeiss Axiocam camera and Zeiss Axiovision image analysis software.

Antibody

IgG isotype antibody (Abnova, H00007051-B02P). Retrieval of the antigen was done by trypsin pre-treatment (37 °C; 20 min).

Results

Treatment of the growing epidermal skin model with ProRenew Complex CLR™ led to an activation in the production of transglutaminase 1, as seen in the clear difference in red color compared with the control, the epidermal skin model which was not treated with ProRenew Complex CLR™. Histological pictures were made (magnification 400x, Fig. 5).

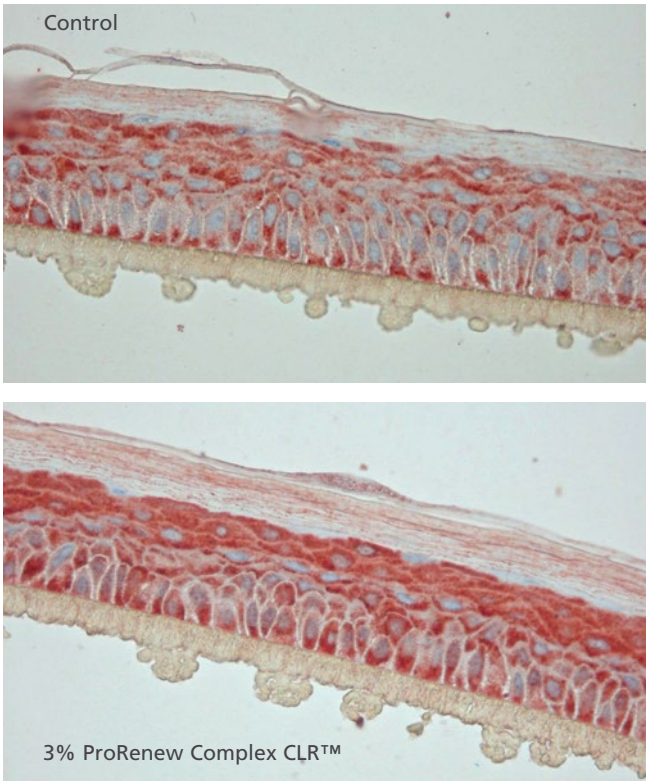

Fig. 5: Expression of transglutaminase 1 with ProRenew Complex CLR™ is clearly higher than in the control

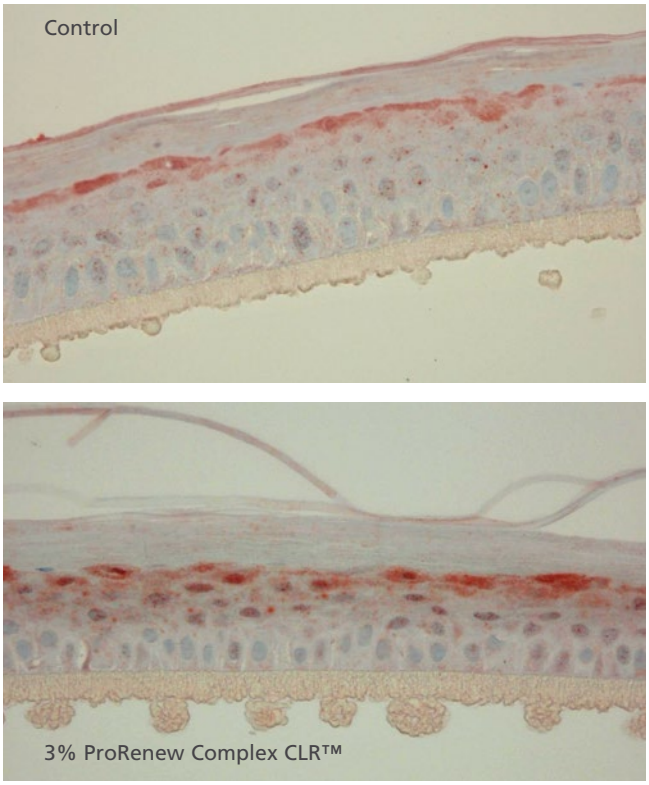

Fig. 6: Expression of loricrin with ProRenew Complex CLR™ is clearly higher than in the control

Loricrin

At >80% of the CE's protein mass, loricrin is the CE's main component in young skin, strongly contributing to its physical strength and mechanical properties. This changes during the aging process, where the expression of loricrin decreases almost 3-fold. A process called 'inflammaging' might well lie at the basis of these changes. TNFα is a pro-inflammatory cytokine described to play a role in micro-inflammation-induced aging, so-called 'inflammaging'. TNFα reduces the expression of loricrin during the differentiation process. Loricrin is insoluble, but mechanically flexible and the aging-related change in the mechanical properties of the skin is thought to at least partly originate from the loss of loricrin from the CE. A reduced loricrin content in the CE is also associated with a higher susceptibility to mechanical stress, possibly contributing to skin tending to become more vulnerable to mechanical stress with age. Loss of loricrin from the CE during aging also contributes to loss of skin barrier function and barrier homeostasis.

Antibody

IgG isotype antibody, GeneTex, Cat.No. GTX116013. Retrieval of the antigen was done by boiling in citrate buffer (pH 6; 8 min).

Results

Treatment of the growing epidermal skin model with ProRenew Complex CLR™ led to an activation in the production of loricrin, as seen in the clear difference in red color compared with the control, the epidermal skin model which was not treated with ProRenew Complex CLR™. Histological pictures were made (magnification 400x, Fig. 6).

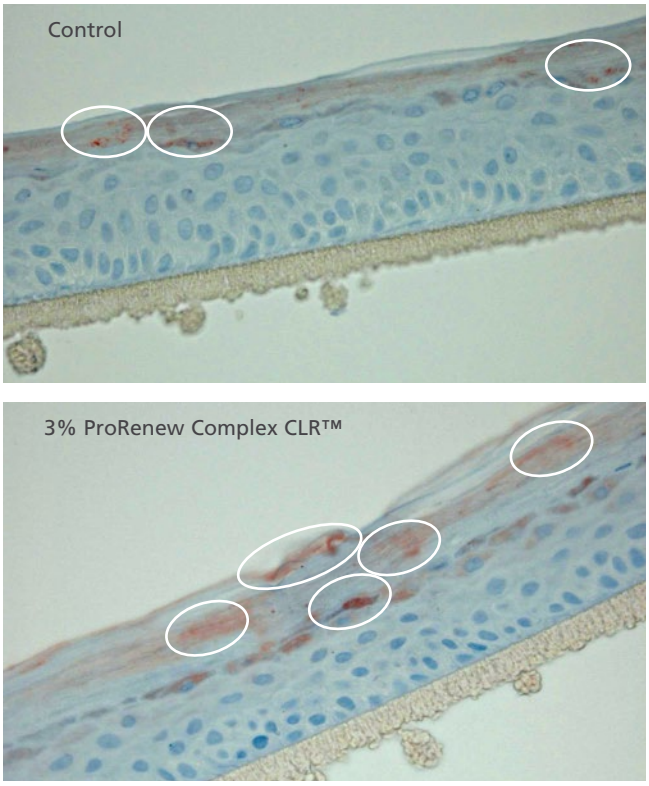

Fig. 7: Expression of (pro)filaggrin with ProRenew Complex CLR™ is higher than in the control

Profilaggrin and Filaggrin

(Pro)Filaggrin can be considered to be one of the most important molecules in our skin. It plays a crucial and multifaceted role in many different aspects of our skin's quality and is the second-most abundant protein, after keratin, in the epidermis. Filaggrin interacts with keratins, causing their aggregation into macrofibrils, in which the intermediate filaments are aligned in tightly packed parallel arrays. This permits extensive cross-linking of the keratin filaments and is a critical process in both cell flattening and "cornification". Filaggrin-keratin structures are reported to be part of the cornified envelope. Filaggrin is obtained through the proteolytic cleavage of profilaggrin, and is further proteolytically cleaved to obtain amino acids and amino acid derivatives (e.g., PCA and UCA), which are the core ingredients of Natural Moisturizing Factors (NMF). The NMF are solely responsible for binding water inside the corneocytes, which is crucial for making the surface of the skin soft and supple and avoiding cracking. For this reason, dry skin feels rough and is mechanically vulnerable. In elderly skin a reduced production of (pro)filaggrin is described, illustrating the relevance of supporting its production in order to obtain an effective anti-aging effect and healthily functioning skin.

Antibody

IgG1 isotype antibody (Genetex, GTX23137). Retrieval of the antigen was done by boiling in citrate buffer (pH 6; 8 min). The antibody interacts with both profilaggrin and filaggrin.

Results

Treatment of the growing epidermal skin model with ProRenew Complex CLR™ led to an elevated presence of (pro)filaggrin, as seen in the difference in red color compared with the control, the epidermal skin model which was not treated with ProRenew Complex CLR™. Histological pictures were made (magnification 400x, Fig. 7).

Caspase-14

Reduced expression of caspase-14 is associated with increased Transepidermal Water Loss (TEWL), indicating that caspase-14 is vital for the quality of the skin's barrier function. In this context, caspase-14 has been described to be of vital importance in the maintenance of normal and healthy keratinocyte differentiation, cornification, the architecture of the SC, and even the formation of the cornified envelope, especially under skin barrier–challenging conditions. As described above, elderly skin shows a lack of ability to properly react to external challenges, making caspase-14 an extraordinarily important element of anti-aging skincare.

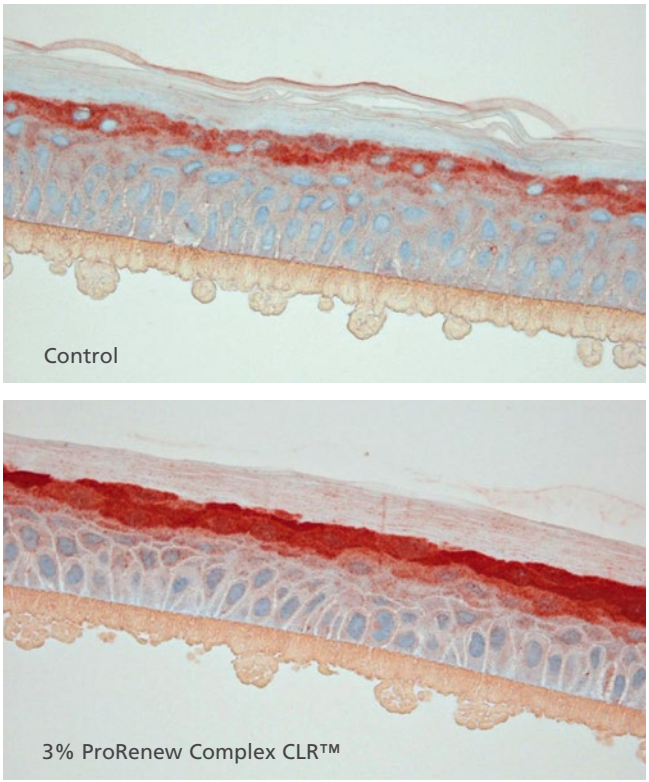

Fig. 8: Expression of caspase-14 with ProRenew Complex CLR™ is clearly higher than in the control

Antibody

IgG isotype antibody (Genetex, GTX85087). Retrieval of the antigen was done by trypsin pre-treatment (37 °C; 20 min).

Results

Treatment of the growing epidermal skin model with ProRenew Complex CLR™ led to an activation in the production of caspase-14, as seen in the clear difference in red color compared with the control, the epidermal skin model which was not treated with ProRenew Complex CLR™. Histological pictures were made (magnification 400x, Fig. 8).

Evaluation of the speed of epidermal growth

The epidermal skin models were grown under the influence of ProRenew Complex CLR™. The speed with which the epidermal skin models grew can be determined from assessing the thickness, i.e. number of cell layers of the respective strata corneum, at a time point when the epidermal skin models are not fully grown yet (day 10).

Method

Staining was conducted, and one epidermis was used to check the histological structure of the tissues at the times indicated. The epidermal skin models were completely immersed in 4% formaldehyde for fixation. The fixed sample was then embedded in paraffin, de-paraffinized, sliced, mounted, and stained for Haematoxylin-Eosin (H&E). Histological pictures were made (magnification 400x).

Results

The epidermal skin models grown under the influence of ProRenew Complex CLR™ showed a distinctly thicker stratum corneum on day 10 (Fig. 9). This clearly indicates that ProRenew Complex CLR™ is able to accelerate the epidermis' renewal processes.

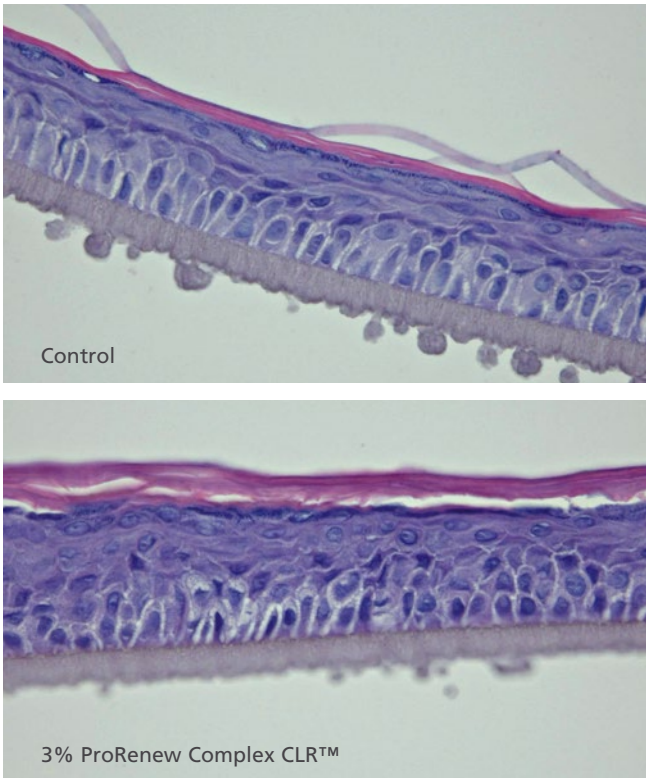

Fig. 9: Treatment with ProRenew Complex CLR™ leads to acceleration of epidermal growth

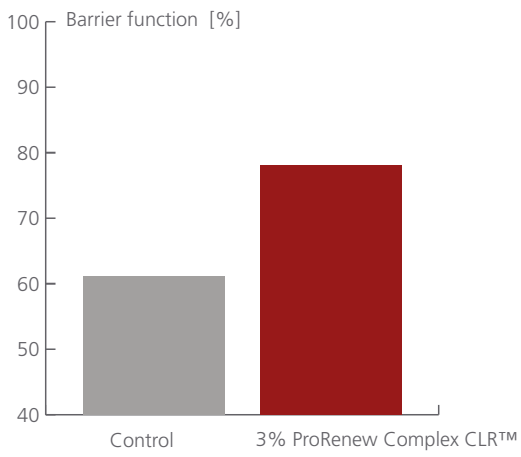

Fig. 10: Treatment with ProRenew Complex CLR™ clearly leads to a better barrier function

Determination of the barrier function of the epidermal skin models

On day 14, the quality of the barrier function of the epidermal skin models was determined by assessing the live keratinocytes' viability after the topical application of 0.4% SDS. The viability of the live keratinocytes showing healthy mitochondrial activity will have only suffered if sufficient SDS has been able to penetrate through the stratum corneum of the epidermal skin models. Therefore, the better the barrier function of the stratum corneum, the lower the amount of SDS is able to penetrate through and the less the live keratinocytes suffer, and therefore the higher their viability.

Method: MTT assay

The epidermal skin models were transferred to MTT assay medium to determine the remaining barrier and viability of the tissues.

The cell viability was measured by dehydrogenase conversion of MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) into a formazan salt. MTT assay was performed by transferring the tissues to well plates containing 300 µl MTT assay medium (1 mg MTT/ml) and further incubation in a humidified atmosphere (37 °C, 5% CO_2, 95% relative humidity). After a 3 h MTT incubation the tissues were transferred to a new well plate, and the blue formazan salt formed by cellular dehydrogenases was extracted with 2 ml isopropanol per tissue for 2 h at room temperature or overnight at 2–8 °C. A total of 200 µl of the isopropanol extract was transferred to a 96-well plate (in duplicate per model) and the optical density (OD) of the extracted formazan was determined in a spectrophotometer at 550 nm with isopropanol as a blank. The ODs of controls were used to calculate the vitality within each group, and the ODs of SDS-treated tissues were used to calculate the remaining barrier, i.e., the relative cell viability as percent of the negative control tissues.

Results

Values obtained with untreated and undamaged epidermal skin models were set at 100%. Compared to control, the epidermal skin models grown under the influence of ProRenew Complex CLR™ clearly showed higher viability and, therefore, a better barrier function (Fig. 10).

CONCLUSIONS

ProRenew Complex CLR™ was shown to be able to accelerate the growth of the epidermal skin models, indicating that it is able to support the renewal processes in the epidermis. Additionally it was shown that, by elevating the production of transglutaminase 1, (pro)filaggrin, and caspase-14, ProRenew Complex CLR™ has a significant influence on the quality of the skin renewal process, as these molecules are essential for the quality of the skin. This observation was further supported by the assessment of the quality of the barrier function by topically applying SDS on the epidermal skin models, where the models which were treated with ProRenew Complex CLR™ clearly showed a better barrier function.

Antimicrobial defense

Apart from producing stratum corneum, obtaining a strong and effective physical barrier function and its ability to act as a "biosensor," the epidermis actively manages the composition of the resident microflora on top of the skin. In doing so it prevents exogenous pathogenic bacteria from colonizing and invading the skin. Differentiating keratinocytes actively produce antimicrobial peptides (AMPs), which contribute to the skin's antimicrobial defense. The production of AMPs is considered to be a constituent of healthy and effective keratinocyte differentiation and, as such, is co-regulated with all other events in this process. Skin barrier disruption, a constant phenomenon as skin is permanently challenged by external factors (sunlight, fluctuating environmental humidity, temperature, etc.), has been shown to lead to a rapid loss of AMPs from the stratum corneum. The failure of elderly skin to properly react to these external challenges means that it has difficulties in re-obtaining a healthy AMP pool and, therefore, maintaining a healthy microbiome on top of the skin. Additionally, it has been shown that skin aging leads to a reduced production of AMPs, particularly cathelicidin. Conversely, it was reported that the support of barrier repair leads to an elevation of the production of AMPs.

β Defensin-1 (BD-1)

BD-1 is constitutively produced and released from differentiating keratinocytes and corneocytes, and shows strong antimicrobial effects against Gram-negative bacteria (e.g., *E. coli, P. aeruginosa*) as well as yeast (e.g., *C. albicans*). Interestingly, apart from its role as an antimicrobial, BD-1 has been shown to be an active component in the differentiation process in the epidermis.

Cathelicidin (LL-37)

Like β Defensin-1, LL-37 is constitutively expressed by keratinocytes. This expression is dependent on the status of the differentiation process. It is worthy of note that LL-37 production is up-regulated upon wounding or a bacterial challenge. This is a process which has been described to lose its functionality in aged skin. LL-37 shows strong antimicrobial activity against many different Gram-positive and Gram-negative bacteria, such as *Streptococci* and *S. aureus*, but is also able to effectively fight *C. albicans*. In its antimicrobial role, LL-37 is known to synergistically cooperate with β Defensins. LL-37 has even been reported to have antiviral properties. It is described to function as a so-called "alarmin," a substance which is responsible for "communicating damage" in order to activate healing processes. This is one of the reasons why it is important to induce LL-37 production in elderly skin, as elderly skin shows reduced immunocompetence and, therefore, a reduced ability to adapt to external stresses and to act as a "biosensor." LL-37 even promotes cell proliferation, another important topic in the skin renewal process in aging skin.

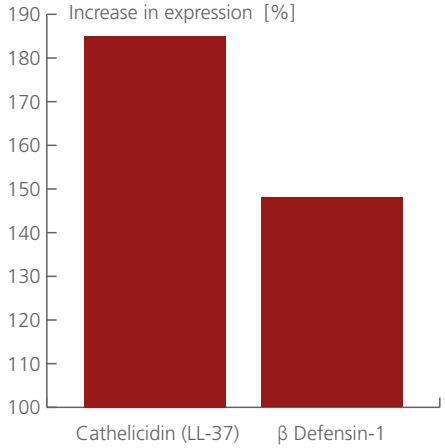

Fig. 11: Stimulation of Cathelicidin (LL-37) and β Defensin-1 expression

Method

Values were determined after 48 (Cathelicidin, LL-37) and 72 h (β Defensin-1, BD-1) pre-incubation in the presence of 1% ProRenew Complex CLR™.

The keratinocytes were lyzed before an ELISA assay was performed (HyCult Biotech). Control was set at 100%.

Results

ProRenew Complex CLR™ was able to significantly boost the production of both cathelicidin and β Defensin-1 (Fig. 11). This attribute strongly indicates that ProRenew Complex CLR™ can support the epidermis in maintaining a healthy, nonpathogenic skin microbiome, another key feature to be addressed in aging skin.

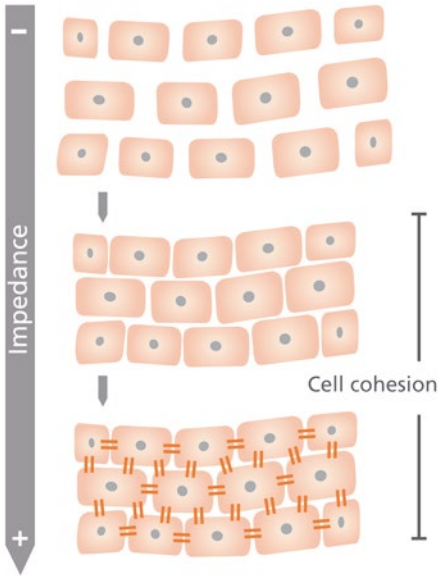

Fig. 12: Increase in impedance during establishing cell cohesion as measured with ECIS

Keratinocyte cohesion

Reduced keratinocyte cohesion, i.e. "attachment," is a well-described phenomenon in elderly skin. This can be analyzed with Electric Cell-substrate Impedance Sensing (ECIS). ECIS is an automated, noninvasive method to monitor cell behavior, which allows the observation of cell cohesion. The principle behind ECIS measurement in this study with ProRenew Complex CLR™ is that in the process of reaching confluence (a well-established cellular network of keratinocytes, where they are anchored to each other, resembling the bottom left part of Fig. 12) the impedance – the electrical resistance of the keratinocyte-system – increases until it reaches a plateau. In the process of reaching confluence, the keratinocytes first proliferate and then build a cellular network, during which process cell cohesion is increased.

Method

Human keratinocytes were seeded in an electrode array chip for 24 h, after which, at t = 0, 0.1% ProRenew Complex CLR™ and control were applied. The impedance was recorded as a function of time. Impedance measured after application of control was set at 0%.

Results

ProRenew Complex CLR™ and control were applied before the keratinocytes had reached confluence. Within the first hour after applying the substances, the impedance measured on the cells treated with ProRenew Complex CLR™ showed a steep increase compared to the impedance measured on the cells treated with control (Fig. 13). This indicates that ProRenew Complex CLR™ was able to positively influence cell cohesion. Interestingly, the plateau in the difference in impedance with control, which was reached after approximately 3 hours, was stable over the following hours, demonstrating that, compared to control, the positive influence of ProRenew Complex CLR™ leads to a sustainable improvement in cell cohesion. This demonstrates that ProRenew Complex CLR™ potentially has a significant impact on the quality of skin.

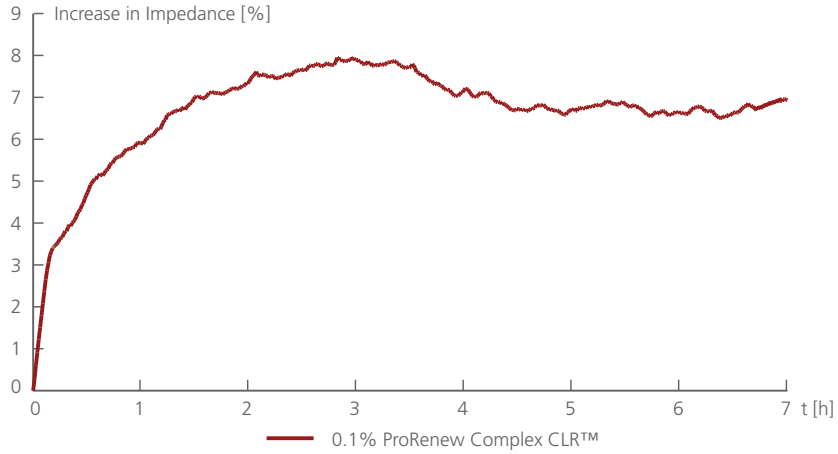

Fig. 13: Increase in keratinocyte cohesion and proliferation

EFFICACY STUDIES – *in vivo assays*

In order to further prove that ProRenew Complex CLR™ is able to support aging skin in its renewal processes, *in vivo* studies were conducted, where both the speed and the quality of the skin's renewal processes were assessed and the influence of ProRenew Complex CLR™ on corneocyte cohesion and the desquamation process in the stratum corneum was determined.

Influence on the quality and speed of skin's renewal process

In this study, test products were applied on designated skin areas on the inner forearm of 5 female volunteers (47–63 years old) for 2 weeks, twice daily. After that, tape stripping was performed to remove stratum corneum and induce skin damage, and the application was continued twice daily for another 4 days. Skin renewal and barrier recovery where determined by measuring stratum corneum thickness and TEWL (transepidermal water loss) at different time points after tape stripping.

Skin renewal

A Vivascope® 1500 (Lucid Inc., Rochester, NY, USA) was used in the determination of the thickness of the stratum corneum at different time points after tape stripping, thus providing information on the speed with which the stratum corneum was rebuilt. This is vital information from which conclusions can be drawn about the actual skin renewal process, as stratum corneum and its corneocytes are a "product" of successful and effective processes of growth in the layers below the stratum corneum. Moreover, this provides information on the skin's ability to adapt, i.e., to act as a "biosensor," as skin which is able to adapt quickly will show faster recovery from barrier disruption by tape stripping.

With the Vivascope® 1500 a laser beam is scanned into the skin, from where it is then reflected by the skin cells' different components. As a result, different cell structures can be detected. The stratum granulosum – the layer below the stratum corneum – shows different cell structures than the corneocytes in the stratum corneum. By precisely focusing at different depths in the stratum corneum until the cells of the stratum granulosum can be recognized, the thickness of the stratum corneum can be determined.

Results

By measuring the thickness of the stratum corneum 1 hour, 2 and 4 days after tape stripping it was clearly shown that the skin treated with ProRenew Complex CLR™, as compared to the skin treated with placebo, showed a significantly accelerated growth of the stratum corneum (Fig. 14). Additionally, with ProRenew Complex CLR™, a pronounced positive trend could be seen in the kinetics of the speed with which the growth of the stratum corneum commenced, as compared to placebo. The distinct indications which were obtained in the *in vitro* studies were thus confirmed in this *in vivo* study. The positive influence of ProRenew Complex CLR™ on the skin's renewal processes could be clearly demonstrated.

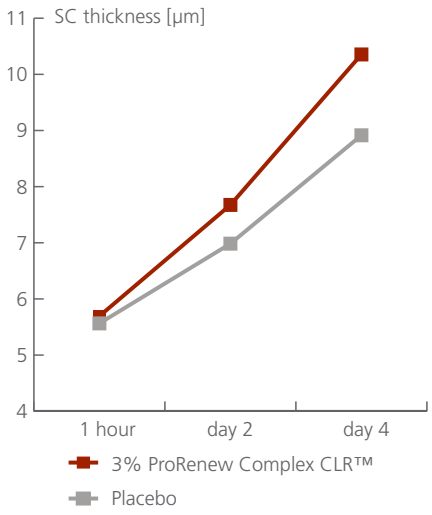

Fig. 14: Acceleration of skin renewal

Skin barrier recovery

TEWL is a well-established unit for measuring the quality of the barrier function of skin. With a Tewameter TM 210 (Courage & Khazaka, Germany), measuring the TEWL after tape stripping and comparing it to skin which was not tape-stripped and not treated (positive control), the recovery of the barrier function was determined. Clearly illustrating the effectiveness of the tape stripping, the TEWL directly after tape stripping (t = 0) was measured to be in the range of 90–95 g/hm², whereas the skin which was not tape-stripped showed a TEWL of approximately 10 g/hm². TEWL was further measured 1 hour, as well as 2 and 4 days after tape stripping.

Results

The skin treated with ProRenew Complex CLR™ clearly showed an increased rate of recovery in a healthy barrier function of skin (Fig. 15). Additionally, a pronounced positive trend could be seen in the kinetics of the speed with which barrier recovery took place.

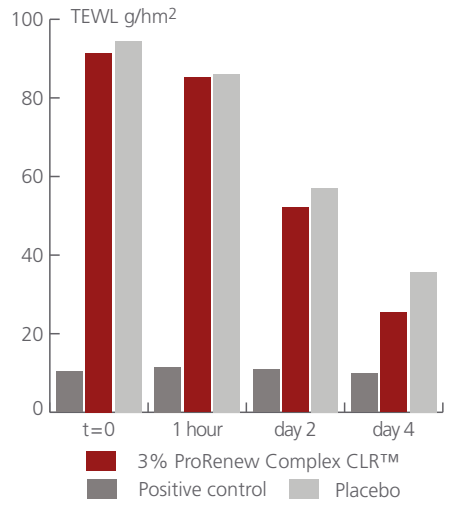

Fig. 15: Stimulation of barrier recovery

Influence on desquamation and corneocyte cohesion

In the stratum corneum, where efficient desquamation processes take place, a clear gradient in cell cohesion can be detected: strong at the bottom and increasingly weaker toward the surface of the skin, where the corneodesmosomes are gradually degraded toward the skin's surface (Fig. 16). The stratum corneum of aged skin, however, shows a different gradient in cell cohesion. Corneodesmosome degradation toward the surface is disturbed and cell cohesion is stronger at the surface of the skin and tends to be lower in the lower layers of the stratum corneum (Fig. 17).

The influence on the production of, among others, KLK5, KLK7, procaspase-14, caspase-14 and filaggrin in the stratum corneum, which are required to maintain a sufficient level of hydration and effective proteolytic degradation of the corneodesmosomes at the surface of the skin, is essential for the skin's endogenous abilities to regain and maintain its natural desquamation properties.

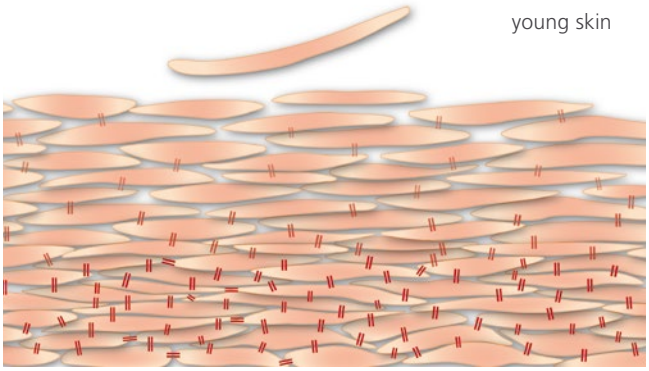

Fig. 16: Healthy desquamation as seen in young skin

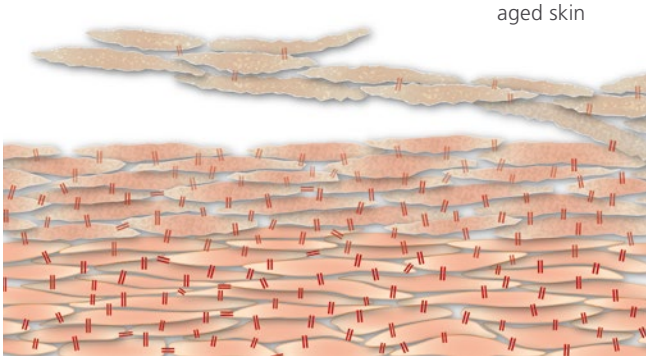

Fig. 17: Desquamation in aged skin is disturbed

In order to assess whether ProRenew Complex CLR™ contributes to activating the skin's natural desquamation, i.e. resurfacing processes, an *in vivo* study was performed where the gradient in cell cohesion in the stratum corneum was determined.

Eighteen volunteers (52–70 years old) applied a formulation containing 3% of ProRenew Complex CLR™ and corresponding placebo on designated areas on the outer forearms, showing mild photoaging, twice daily for 42 days. On day 43 of the study, the treated skin areas were tape-stripped, totaling 15 strips per area. The total mass of proteins on the strips was determined on strips 1–5, 6–10 and 11–15. Strips were extracted in 1N NaOH, protein concentration was measured photometrically (595 nm) and protein mass on the strips determined from a calibration line.

Keratin is the main constituent of the corneocytes, and is by far the most abundant protein in the stratum corneum. The amount of protein on the strips, therefore, is a measure for the amount of corneocyte material present on the strips, where the amount of corneocyte material inversely correlates with intercorneal cohesion, hence desquamation.

Results

Treatment with ProRenew Complex CLR™ clearly leads to a more pronounced gradient in protein mass on the different strips as compared to the skin treated with placebo (Fig. 18). In the skin treated with ProRenew Complex CLR™, on the first 5 strips, taken from the top layers of the stratum corneum, more corneocyte material was found, and on the last 5 strips (11–15) less corneocyte material was found.

The amount of proteins found on the strips inversely correlates with cell cohesion. Taking the above results obtained on strips 1–5, 6–10 and 11–15, reveals a clear difference in the gradient of cell cohesion determined in skin treated with ProRenew Complex CLR™ as compared to placebo (Fig. 19). The graphs show corresponding trend lines, deduced from the results obtained on protein mass.

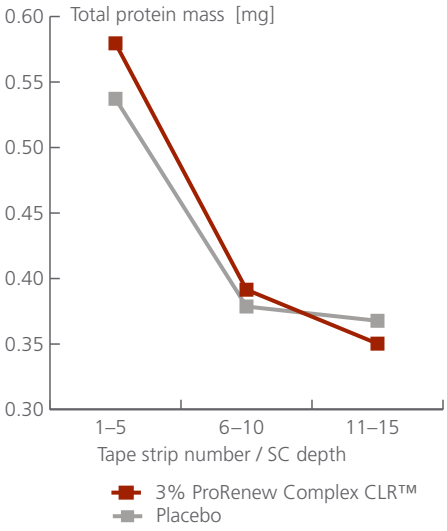

Fig. 18: Influence on protein mass on tape strips vs. tape strip number, i.e. stratum corneum depth

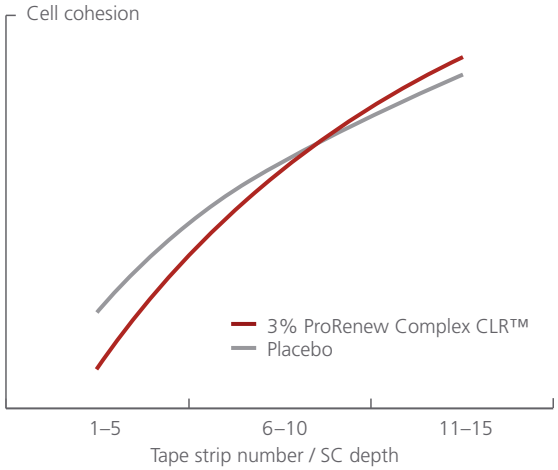

Fig. 19: Promotion of a beneficial gradient of cell cohesion in stratum corneum, i.e. effective desquamation

CONCLUSIONS

The interest of the modern-day anti-aging skincare user mainly lies in slowing down the skin's aging process and maintaining healthily functioning skin. Nowadays the fact that skin ages – as does the rest of the body – is something which is considered to be a fact of life, but also something to which you can take at least a preventative approach.

It was shown that ProRenew Complex CLR™, which is based on a lysate of probiotic bacteria, can significantly support the most important feature of skin, its ability to quickly and effectively renew itself and shed dead skin cells. This is a feature which is not only relevant for aged skin, but increasing evidence shows that skin in a polluted environment has similar problems as aged skin, when it comes to these essential characteristics.

Probiotics are renowned for their ability to support our body's immune system. With ProRenew Complex CLR™, this characteristic was successfully translated to cosmetic skincare. The lyzed probiotic cells in ProRenew Complex CLR™ are able to give a strong boost to the skin's renewal processes, both accelerating it and improving its quality. This was shown both in *in vitro* and *in vivo* studies, from the gene level to the protein and enzyme level, and by making use of ECIS to monitor cell proliferation and cell cohesion. In the *in vivo* studies the results obtained in the *in vitro* studies were demonstrated with extraordinary clarity. After removing the stratum corneum by tape stripping, renewal of the skin and the recovery of its barrier function were shown to be accelerated by the use of ProRenew Complex CLR™. ProRenew Complex CLR™ additionally promotes the natural desquamation processes in the stratum corneum. It thus improves the healthy functioning and appearance of our skin and satisfies the main demands of the modern-day anti-aging skincare user.

BIBLIOGRAPHY

- MA Lefebvre, DM Pham, B Boussouira, D Bernard, C Camus, QL Nguyen: Int. J. Cosmetic Sci., 37 (2015), 329–338. Evaluation of the impact of urban pollution on the quality of skin: a multicentre study in Mexico.
- M Rinnerthaler, J Duschl, P Steinbacher, M Salzmann, J Bischof, M Schuller, H Wimmer, T Peer, JW Bauer, K Richter: Exp. Dermatol., 22 (2013), 329–335. Age-related changes in the composition of the cornified envelope in human skin.
- SJ Brown, WH Irwin McLean: J. Invest. Dermatol., 132 (2012), 751–762. One remarkable molecule: filaggrin.
- E Hoste, P Kemperman, M Devos, G Denecker, S Kezic, N Yau, B Gilbert, S Lippens, P De Groote, R Roelandt, P Van Damme, K Gevaert, RB Presland, H Takahara, G Puppels, P Caspers, P Vandenabeele, W Declercq: J. Invest. Dermatol., 131 (2011), 2233–2241. Caspase-14 is required for filaggrin degradation to natural moisturizing factors in the skin.
- A Alikhan, HI Maibach: Textbook of aging skin, Edited by MA Farage et al., Springer Verlag, Berlin, Heidelberg, Germany (2010), Chapter 40. Biology of Stratum Corneum: Tape Stripping and Protein Quantification.
- KM Aberg, MQ Man, RL Gallo, T Ganz, D Crumrine, BE Brown, EH Choi, DK Kim, JM Schröder, KR Feingold, PM Elias: J. Invest. Dermatol., 128 (2008), 917–925. Co-regulation and interdependence of the mammalian epidermal permeability and antimicrobial barriers.
- R Ghadially, BE Brown, SM Sequeira-Martin, KR Feingold, PM Elias: J. Clin. Invest., 95 (1995), 2281–2290. The aged epidermal permeability barrier.

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