

MultiMoist CLR™

mapping the moisturization puzzle



for fine cosmetics · since 1926



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APPLICATION

- Moisturizing
- Hair conditioning and protection

■ tested □ recommended

MultiMoist CLR™

Dosage: Skin: 3.0% Hair: 1.0%

pH range: 3.0–8.0

INCI Name: Fructooligosaccharides, Beta Vulgaris (Beet) Root Extract, Water

MultiMoist CLR™ is unpreserved.

For information on natural certifications, such as NATRUE and COSMOS, see QR code or: <https://www.clr-berlin.com/products/multimoist-clr/>



SUMMARY

MultiMoist CLR™ activates production of the vitamin D receptor (VDR) and promotes the VDR's activation. MultiMoist CLR™ also potentiates the effect of vitamin D. As a consequence the skin becomes more moisturized. By making use of 5 different objective instrumental approaches, the immediate and long term moisturizing properties of MultiMoist CLR™ were convincingly proven. Additionally, the activity of MultiMoist CLR™ was proven in a consumer study, where the subjective improvement of skin moisture content, skin smoothness and the look of skin were confirmed.

MultiMoist CLR™ consists of a powerful synergistic blend of fructooligosaccharides and *beta vulgaris* (beet) root extract.

MultiMoist CLR™ also has strong properties for hair and scalp care. Please ask for the MultiMoist CLR™ hair care brochure.

"WHAT IS A MOISTURIZER?"

CONSUMER PERCEPTION

Most people use a moisturizer every day of their life. Moisturizers are so familiar we hardly think to ask, "What is a moisturizer?" The skin is the most complex organ of the human body and the one with most need for everyday care and attention, so moisturization should be a key goal. Our skin plays a vital role in maintaining our physical and mental health. Keeping it in best condition is essential.

Consumers will choose what they think works best. It is what the consumer believes about performance that drives purchase. Therefore perception of performance is incredibly important. Smooth skin is perceived as young skin. Most people want smooth skin because it makes them look and feel younger. This is where moisturizing skincare products play a pivotal role. Hydration helps create a smooth, soft, and elastic skin. The appearance of the skin is another essential and vital factor in a person's self-esteem. Visible indicators of dry skin, are a lackluster surface, dry white patches, flakes, cracks and even fissures. Skin moisturizers do not just make the skin feel soft, they make it look attractive. These are important characteristics to consider when developing moisturizing cosmetics and proving their efficacy.

The science behind skin dryness vs. moisturization

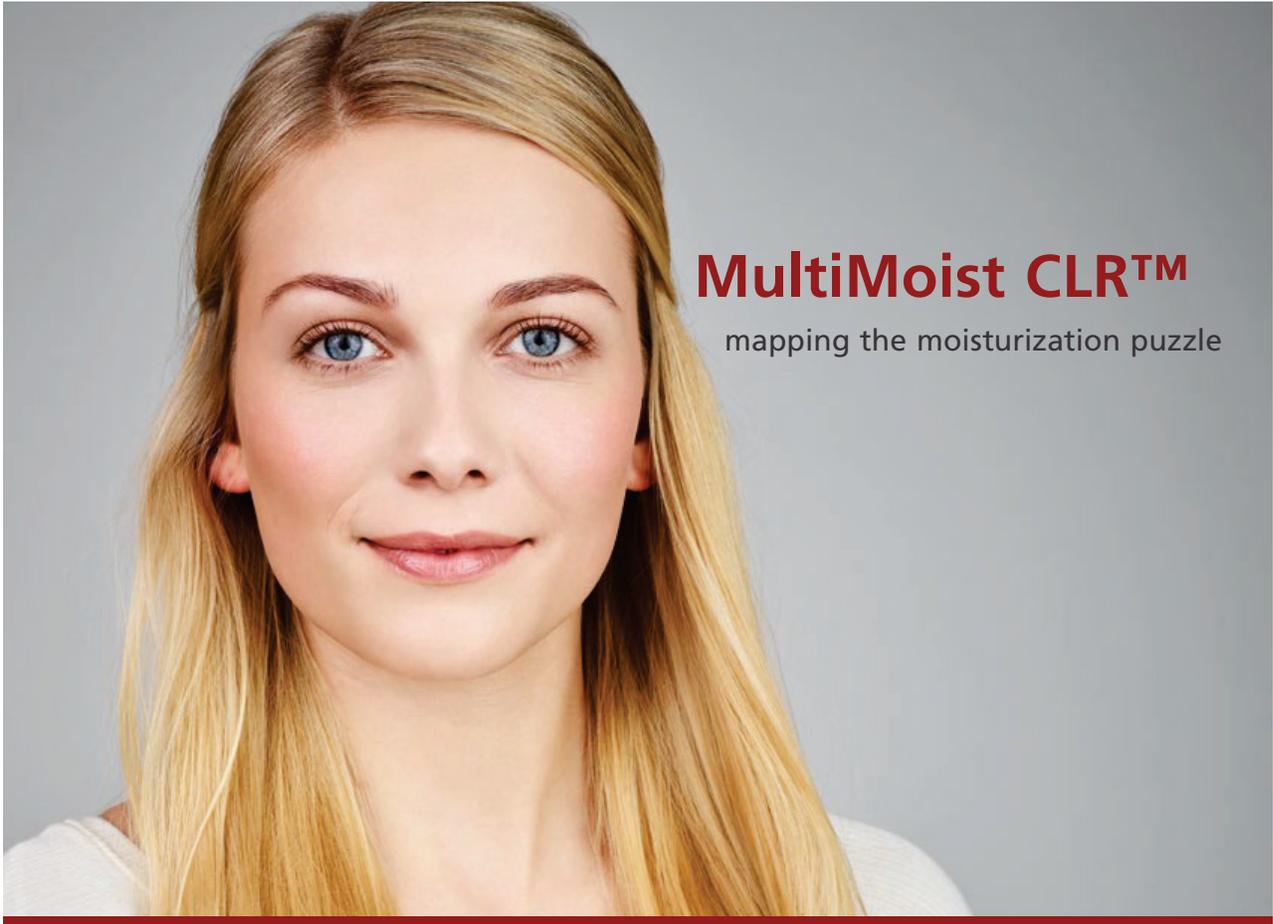
In 1978, the world-renowned dermatologist Dr. Albert Kligman introduced the following definition: "A moisturizer is a topically applied substance that overcomes the signs and symptoms of dry skin."

Millions of people suffer from skin dryness. Dry skin problems are heavily influenced by weather conditions. Dry skin is far worse in the winter months, when low relative humidity is often accompanied by low temperatures and dry overheated homes. A dry, hot climate is also apt to lead to dry skin. The critical factor is the absolute moisture content of the air. Any drop in the relative humidity of the environment will lead to a corresponding decrease in skin surface moisture levels.

The stratum corneum (SC) provides protection and a controlled permeable barrier to the external environment while being subject to highly variable conditions including changing temperature, humidity, mechanical and abrasive contact. In addition, the SC must withstand daily application of topical cleaning agents and potentially damaging acute and chronic chemical exposure.

The suppleness and overall functionality of the SC is closely related to its water content. This is reflected in the signs and symptoms of dry skin. These may manifest as scaling with an associated reduction of mechanical flexibility and an uncomfortable feeling of tightness, in addition to pruritus, flaking, chapping, erythema, pain, and stinging. Additionally, dry skin is dull and lifeless in appearance.

Physiologically, the top of the SC is one of the tissues in the human body with the lowest water content. Naturally, as water is being constantly lost from the skin surface, a water gradient is established within the different layers of the SC. The natural hydration levels in the SC are between 15% at the skin surface and 40–45% at its innermost layer compared with 70–80% within the granular layer (stratum granulosum). A selective retention of water in the SC is required and this function is largely dependent on the presence of the so-called Natural Moisturizing Factors (NMF). Dry skin is described to contain less than 10% of water, which relates strongly to a lack of the presence of NMF.



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The NMF comprises hygroscopic molecules, produced during the epidermal differentiation process, which are able to bind water at the surface of skin. The NMF may represent up to 15–30% of the corneocyte mass. The NMF is, for the bigger part, produced by the enzymatic breakdown of filaggrin. This process can only take place successfully with optimal water levels in the SC as the activity of these enzymes is strongly dependent on the water level in the SC. This implies that the SC of dry skin not only lacks water but also contains reduced levels of NMF, which is required to bind water in the SC. In this way, a vicious circle is created. The dry skin cycle caused by the impaired NMF production can be disrupted by increasing water levels in the SC by the application of moisturizers, i.e., substances whose application results in increased water levels in the SC.

The ever-changing world, prevalence of dry skin, and vitamin D

Over the past decennia the world, in the broadest sense of the word, has gone through tremendous changes. Many of these changes are relevant to our health and our skin in particular. Improved economic situations and urbanization have led to a significant increase in the number of people working and living indoors, mostly in an extremely dry (air-conditioned or heated) environment. Personal hygiene practices have also considerably changed. A growing part of the world population follows stricter and more frequent personal hygiene practices. Sun protection has undergone an increase in acceptance in the world population as well, where significantly more consumers now use sun protective products than a few decades ago. In short, on average our skin is increasingly exposed to influences which promote skin

dryness (dry environment, personal hygiene) and decreasingly exposed to sunlight.

Whereas sunlight has many deleterious influences on skin, it has one important positive outcome: it provides our skin with the ability to produce vitamin D. Apart from the increase in the use of sunscreen protection products, there are other challenges related to vitamin D. The world population is becoming more obese. In some countries obesity has even quadrupled. An increase of body mass is correlated with a lower level of vitamin D. Yet another important fact is that the capability to produce Vitamin D is inversely correlated to people's age. As we know, demographically, the world population is aging.

The prevalence of so-called hypovitaminodosis D is now indicated as a global major health problem. Interestingly, a chronic deficiency insystemic vitamin D is described to be linked to specific hallmarks of aging. A lack of vitamin D is associated with an increase in oxidative stress and inflammation and a decreased ability for cell repair. These are all vital factors in organismal aging and skin aging in particular. Vitamin D protects the skin against the hazardous effects of many skin aging-inducing agents, including ultraviolet (UV) radiation and is described to help in treating sunburn.

The role of vitamin D in those processes in the skin which are vital for its ability to bind water have been described in scientific literature. Vitamin D deficiency is common in people suffering from atopic dermatitis, a skin disease which is associated with reduced skin barrier function and skin dryness. Additionally, vitamin D is

an important initiator of filaggrin production, which is of particular note in this context, filaggrin being the most important raw material for the production of the NMF.

In more detail, the so-called vitamin D receptor (VDR) is essential for epidermal differentiation. The VDR is the receptor to which vitamin D binds to elicit its biological effects. Interestingly, the presence of vitamin D leads to an increase in the expression of the VDR. In this sense, vitamin D is able to potentiate its own biological effects, as with more VDR, beneficial biological processes which lie downstream of the activation of the VDR are activated more effectively. An increase in expression of the VDR leads to an improvement of epidermal differentiation, which, as suggested above, includes an increase in filaggrin production and, therefore, more moisturized skin.

Development of quality moisturizing skincare formulations

A quality moisturizer formulation should not only reduce skin dryness, but also prevent its return. Today, the amelioration of dry skin symptoms is still the primary benefit of skincare products to the consumer.

Many ordinary humectants (glycerin, sodium pyrrolidone carboxylate, urea, and sodium lactate) are used in cosmetic skincare formulations, but application of aqueous solutions of ordinary humectants has not shown to increase the hygroscopicity of the skin. Moisturization of skin is, therefore, not simple and should not be taken lightly.

It is of eminent importance to consider the core problem of skin dryness. Whereas demographic and sociological changes are impossible to influence, focus can and should be placed on the main downstream physiological consequence of these changes: hypovitaminodosis D, i.e., a lack of the activation of the VDR.

THE COMPLEXITY OF CLAIM SUBSTANTIATION

Regulatory bodies and competent authorities are increasingly demanding over cosmetic claim support and the substantiation of cosmetic claims. The consumer too wants proven results. The times of “hope in a jar” are behind us.

Proving the moisturizing effect of a cosmetic product sounds easy enough, but the fact of the matter is that it is quite difficult. The efficacy of skin moisturizers should be proven both objectively, using high-end methodology, and subjectively, in relation to the average consumer’s perception of dry skin vs. moisturized skin.

Proving the efficacy of MultiMoist CLR™ to be an efficacious moisturizer demands intelligent study designs which provide information from all relevant angles:

- quantification of water in the skin
- quantification of water-binding properties
- quantification of visual skin dryness
- consumer perception
- short-term and long-term effects

As described above, cosmetic skin moisturization is all about supporting the upper layers of the SC in holding on to water. Objective measurement of the amount of water is, therefore, paramount in obtaining proof about the moisturizing effect of cosmetic formulations. Here, it is important to distinguish between short-term moisturizing effects and long-term moisturizing effects. Short-term effects are not predictive of the ability of moisturizing formulations to be effective after long-term use of the products. A good moisturizing formulation should obviously work both directly, and the effect should be long-lasting. Both short-term and long-term measurements should therefore be undertaken.

In vivo approaches

Measurement of capacitance

Capacitance methods are commonly used mainly because of their simplicity. Alterations in epidermal hydration will result in increased SC capacitance values. A drawback of water measurement using capacitance methods is that substances other than water may influence the results. For example, glycerin will influence the readings directly while urea is known to alter the dipole moment of keratin, also influencing the results. The use of glycerin or urea in cosmetic moisturizers will lead to false positive results when capacitance of the skin is measured with the aim of proving a moisturizing effect, i.e., an increase in water level due to a cosmetic formulation containing these ingredients.

Another potential pitfall with capacitance methods is that they are also impacted by skin morphology. Differences in their readings can be seen as a result of the inclusion of air gaps in the measurement substrate due to the skin's texture. Additionally, depending on the measurement frequency and the geometry of the electrodes, the hydration measured corresponds to a varying degree of skin depth which is almost impossible to determine with precision.

It is evident that this type of measurement can only provide arbitrary values which would be risky to interpret in terms of absolute water content in a given skin compartment. Therefore, in the quest to prove the efficacy of moisturizing formulations, other methods have to be taken into account.

In two *in vivo* studies to obtain proof of the moisturizing activity of MultiMoist CLR™ the capacitance instruments Corneometer CM825™ and MPA 5 CPU (Courage & Khazaka electronics) were used. The studies were performed at two different external laboratories and on different types of skin.

MoistureMap: "capacitance imaging"

Skin capacitance measurement as described above gives the average capacitance of the whole contact area covered by the probe, while MoistureMap displays a more detailed distribution histogram of the capacitance values.

MoistureMap is a two-dimensional image analysis technique. MoistureMap images give graphical information on the near surface hydration distribution and the micro topography of the skin. On the 1.8 x 1.28 mm silicon chip of the sensor, over 90,000 (360x256) capacitors are located every 50 µm. A MoistureMap image corresponds precisely to what the sensor of a device measuring the capacitance of the skin actually "sees."

The MoistureMap device measures the penetration of an electromagnetic field. As a conductive material, moisturized skin reflects the signal, making the resulting pixel darker, while less conductive material, such as dry skin, will make the signal go further inside so that the resulting pixel will be lighter. Increasing the skin moisture level increases conductivity, which means darker pixels. MoistureMap additionally indicates the distribution of hydration on a given surface, but the image's mean gray level, i.e. mean hydration level, is another useful parameter which can be deduced from the results obtained with technology.

Moisture Accumulation Test (MAT)

Both single-probe capacitance measurements and the Moisture-Map technology only detect superficial hydration and are limited in that they provide only instantaneous data. These are drawbacks which need to be compensated for when objectively assessing the moisturizing capacities of a cosmetic skincare formulation.

The moisture accumulation test (MAT) gives information about the quantity of moisture the SC may accumulate during a given time. This can be performed with the Novameter, which can measure continuously after bringing the probe in contact with the skin surface. The probe remains on the skin for three minutes, thereby creating occlusive conditions. The MAT measures the accumulation of water under the probe every 0.5 minutes. Water accumulation is evaluated by calculating the area under the time curve until the three-minute mark.

It is well-described in literature that decreased NMF content (i.e. a decreased ability to bind water) correlates with lower water accumulation during the MAT. Dry and flaky skin shows low water accumulation, for instance. Inversely, a cosmetic moisturizing skincare formulation, which is designed to increase the water-binding capacity of the SC, should lead to higher water accumulation (i.e., a higher area under the curve) as measured with MAT. Performing a MAT is therefore a useful tool in obtaining a relevant body of evidence when proving the efficacy of a cosmetic moisturizer.

Squametry: "skin dryness evaluation"

One of the prime characteristics of dry skin is certainly its visual appearance: a whitish surface appearance and scaliness. This provides another important parameter in obtaining proof of the efficacy of a moisturizer. The use of adhesive tape strippings to facilitate observations of the skin surface was first reported by Wolf in 1940. When tape is pressed against the skin, the outermost, loosely adherent portion of the SC will stick to the tacky adhesive. Upon removal the tape provides a specimen which retains the topographical relationships of the skin surface. This technique is called squametry.

Direct objective methods for assessments are preferable over clinical assessments as these are difficult to standardize and can show a lack of objectivity as well as discrimination in borderline cases. Objective methods are based on sampling the superficial portion of the SC with tape strips and quantifying the amount of scales or squames using imaging techniques where scales appear white due to their air content. These scales can be automatically sized and counted to give a measure of the degree of skin dryness, correlating to skin appearance.

Skin smoothness

Another important parameter for the evaluation of moisturizing properties for cosmetics is skin smoothness. Dry skin is rough and increasing the smoothness of the skin is particularly relevant, as consumers associate skin dryness with rough skin. Skin smoothness can be assessed using Primos technology. With this optical technology, the precise three-dimensional topography of skin can reliably be determined. From the information obtained the smoothness of skin can be deduced.

Subjective aspects of skin moisturization

Objective measurements of skin capacitance, MoistureMap and the MAT give evidence based on electrical characteristics of the skin, and squametry, including image analysis, offers objective information on the severity of the appearance of skin dryness. These methods are all part of the total body of evidence concerning whether or not a skin moisturizer actually works. Another important factor should be taken into account as well, though: as suggested in the first section of this article, the perception of the consumer should also be taken into account. The consumer perceives dry skin as rough, whereas moisturized skin feels smooth and pleasant. A moisturizing skincare formulation should therefore not just objectively increase the water content in the skin but should also to a significant degree positively affect important subjective skin parameters that are related to skin dryness, such as skin feel and look.

EFFICACY STUDIES – *in vitro* assays

Influence on VDR expression and activation

The presence of vitamin D leads to the activation of VDR production by keratinocytes. A reduced presence of vitamin D, which is a relevant problem, leads to a reduced production of the VDR, as discussed above. The VDR as such is only active when the serine residues are phosphorylated. When assessing the relevance of an active ingredient in inducing the VDR and potentiating its activity, the activation of the VDR should therefore also be taken into account.

As a first step in the *in vitro* studies seocalcitol, a synthetic analogue of Vitamin D, was assessed for its ability to increase the expression of the VDR and the activation of the VDR. Here the focus was on the analysis of VDR-SerP.

Normal human epidermal keratinocytes were incubated for 72 hours in and without the presence of seocalcitol. Results of this study are presented in figure 1. Expression of VDR and VDR-SerP without the influence of seocalcitol is set at 100%.

As could be shown in this experiment, seocalcitol was able to clearly increase the production of the VDR and its activation. A dependence of concentration of seocalcitol could also be observed.

In a following study the effect of 0.5% MultiMoist CLR™ on the expression of the VDR and its activation was determined. Here too normal human epidermal keratinocytes were utilized and were incubated with and without MultiMoist CLR™ for 72 hours. Results are shown in figure 2.

The treatment with MultiMoist CLR™ led to somewhat more pronounced results as compared to the treatment with seocalcitol, illustrating the potency of MultiMoist CLR™ to activate so-called VDR signaling, which should lead to the improvement of epidermal differentiation and, hence, the hydration state of the top layer of the skin, the SC.

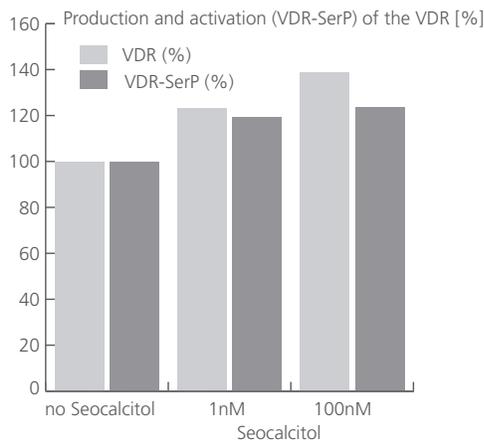


Fig. 1: Influence of seocalcitol on the expression of the VDR and its activation (VDR-SerP) by normal human epidermal keratinocytes after 72 hours of incubation.

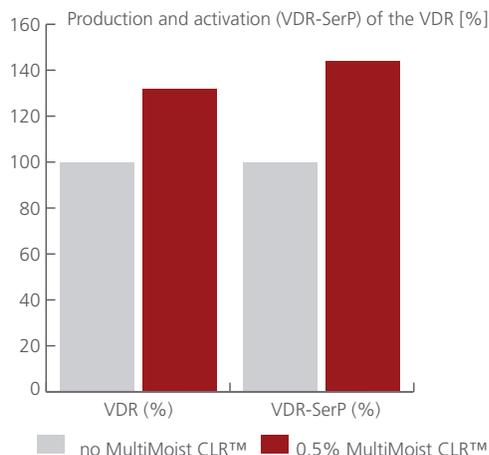


Fig. 2: Influence of MultiMoist CLR™ on the expression and activation (VDR-SerP) by normal human epidermal keratinocytes after 72 hours of incubation.

In another experiment it was assessed whether MultiMoist CLR™ was able to potentiate vitamin D–induced effects on expression of the VDR and its activation. Here normal human epidermal keratinocytes were incubated for 72 hours with and without the presence of seocalcitol (a vitamin D analogue at 1nM) and MultiMoist CLR™ at 1%.

Results obtained on VDR expression are presented in figure 3, and corresponding results relevant to the activation of the VDR (VDR-SerP) are presented in figure 4.

As described in the first *in vitro* experiment (figure 1), here too seocalcitol was able to increase and activate the VDR. The addition of MultiMoist CLR™ clearly showed its potentiating effects. This leads to the conclusion that MultiMoist CLR™ can amplify the effects of vitamin D, which is an important realization, as a lack of vitamin D in the skin is a common phenomenon.

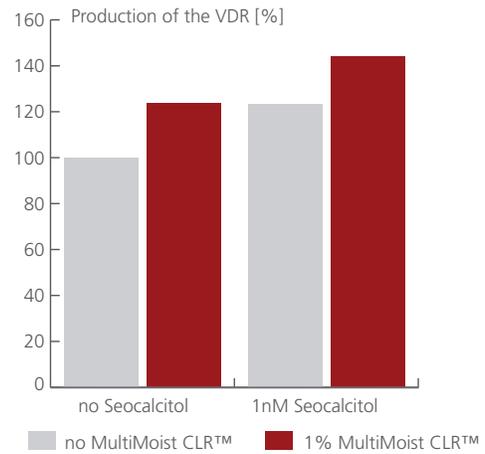


Fig. 3: Influence on expression of the VDR after 72 hours of incubation of normal human epidermal keratinocytes with and without the presence of seocalcitol and MultiMoist CLR™.

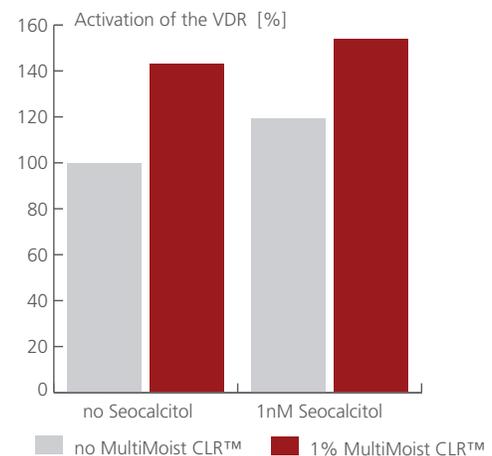


Fig. 4: Influence on the activation of the VDR (VDR-SerP) after 72 hours of incubation of normal human epidermal keratinocytes with and without the presence of seocalcitol and MultiMoist CLR™.

Influence on parameters relevant to epidermal differentiation

In the above experiments it was clearly shown that MultiMoist CLR™ is able to increase the expression of the VDR and its activation as well. This should lead to the improvement of epidermal differentiation and, ultimately, better barrier function and more moisturized skin.

In order to prove this, a monolayer of normal human epidermal keratinocytes was analyzed for relevant signs of epidermal differentiation. In obtaining a two-dimensional monolayer, keratinocytes proliferate until they reach confluence. In this situation the monolayer is fully developed, and keratinocytes then can initiate the differentiation process, the process of building the epidermis.

After reaching confluence, two important parameters were analyzed. First, the proliferation rate was determined through BrdU analysis. BrdU is a thymine analogue which, when provided to proliferating keratinocytes, is incorporated into the DNA structure of daughter cells. The more BrdU is incorporated in cells, the more proliferation has taken place. Inversely, as with reaching confluence the proliferation rate should go down, a reduction of BrdU indicates that the cells have reached confluence and should therefore start to enter the differentiation process. This is something which should be expected from the presence of vitamin D and its corresponding receptor (VDR, VDR-SerP).

Normal human epidermal keratinocytes were incubated with and without the presence of seocalcitol (0.001 nM) and MultiMoist CLR™ (0.5%) for 72 hours. Then the proliferation rate of the cells was determined. Results are presented in figure 5. Proliferation rate without the presence of seocalcitol or MultiMoist CLR™ is set at 100%.

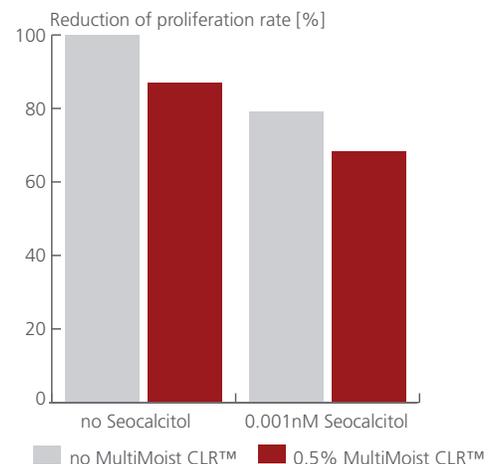


Fig. 5: Influence on proliferation rate of normal human keratinocytes by seocalcitol and MultiMoist CLR™ after 72 hours.

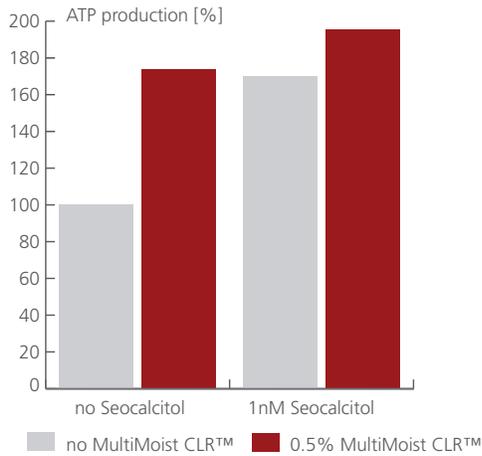


Fig. 6: Influence on ATP production by normal human keratinocytes of seocalcitol and MultiMoist CLR™ after 72 hours.

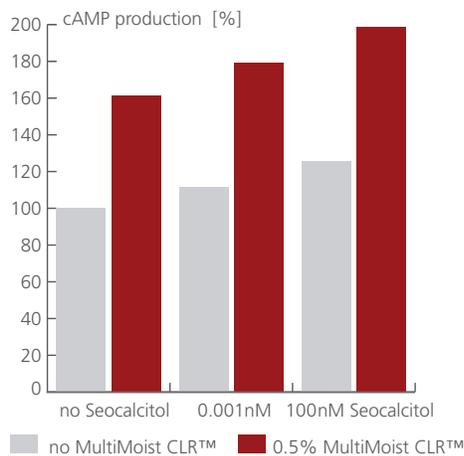


Fig. 7: Influence on cAMP production by normal human keratinocytes of seocalcitol and MultiMoist CLR™ after 96 hours.

The results of these experiments are unambiguous: the incubation with seocalcitol alone led to a clear reduction of proliferation, i.e., the initiation of the epidermal differentiation process. MultiMoist CLR™ also showed that it was able to have similar effects. As with the above-described experiments, MultiMoist CLR™ was able to potentiate the influence of seocalcitol, i.e. vitamin D, on this important parameter.

The initiation of the epidermal differentiation process is extremely energy-intensive. One of the major relevancies of vitamin D is that it initiates cellular processes within the keratinocytes, enabling them to produce more energy, which can be measured through the expression of ATP. In a following experiment the influence of seocalcitol (1nM) and MultiMoist CLR™ (0.5%) on ATP production by normal human epidermal keratinocytes was determined. The cells were incubated with and without the presence of seocalcitol and MultiMoist CLR™ for 72 hours, after which ATP was determined. The results are summarized in figure 6. ATP production without the presence of seocalcitol or MultiMoist CLR™ is set at 100%.

As with the results described above, seocalcitol increased the production of ATP by normal human epidermal keratinocytes. Interestingly, MultiMoist CLR™ alone had similar effects. The combined effects of seocalcitol and MultiMoist CLR™ were approx. 20% higher than those of MultiMoist CLR™ or seocalcitol alone. These results also underline the potency of MultiMoist CLR™ in initiating epidermal processes which are important in ultimately obtaining well moisturized skin.

A last important parameter was assessed by analyzing the ability to increase the production of cyclic adenosine monophosphate (cAMP). cAMP is an important inducer of terminal differentiation in the epidermis. Whereas the above parameters (VDR, VDR-SerP, BrdU and ATP) were assessed after 72 hours of incubation with test substances, this parameter was analyzed after 96 hours of incubation. In our *in vitro* studies, after 72 hours confluence was reached and differentiation processes were initiated. After an additional 24 hours the keratinocytes became even more engaged in the differentiation process, and this is where cAMP becomes important.

Normal human keratinocytes were incubated with different concentrations of seocalcitol and MultiMoist CLR™ for 96 hours. Subsequently the cells were analyzed for the presence of cAMP. The results are shown in figure 7. cAMP production without the presence of seocalcitol or MultiMoist CLR™ is set at 100%.

Conclusions from *in vitro* studies

A lack of vitamin D in the skin is a relevant problem in the modern world. Skin dryness is another common problem, and both should be seen in the same light. MultiMoist CLR™ activates production of the VDR and promotes VDR activation. MultiMoist CLR™ also potentiates the effect of vitamin D, as was shown when MultiMoist CLR™ was combined with seocalcitol.

MultiMoist CLR™ has an important positive influence on parameters which are relevant for epidermal differentiation, and in these experiments MultiMoist CLR™ was also able to potentiate the effect of vitamin D.

These results clearly show that MultiMoist CLR™ has an important influence on cellular processes in the skin which are relevant for the quality of the epidermis and, hence, the moisture level of the SC. MultiMoist CLR™ can substantially compensate for the lack of vitamin D in the skin.

EFFICACY STUDIES – *in vivo* assays

Skin capacitance: Corneometer CM825™ and MPA 5 CPU (Courage & Khazaka electronics)

The moisturizing effect of MultiMoist CLR™ at 3% in a cosmetic formulation was compared to a control formulation not containing MultiMoist CLR™ (placebo) in two different studies. First of all, using a Corneometer MPA 5 CPU, the eight-hour moisturizing effect of MultiMoist CLR™ was measured on normal skin after a single application on 20 volunteers. The results are presented in figure 8.

After 2 hours the formulation containing MultiMoist CLR™ performed 78% better than the corresponding placebo formulation. After 4 hours this difference was 65%, and after 8 hours 173%.

In a second study, the direct moisturizing activity of MultiMoist CLR™ was assessed on dry skin on 22 volunteers with the Corneometer CM825™. After 2 hours measurements were performed, and the results are presented in figure 9.

Although the relative difference between the formulation containing MultiMoist CLR™ and its corresponding placebo were already remarkable in the study performed on normal skin, the results obtained with the study on dry skin further confirm the potency of MultiMoist CLR™ in directly moisturizing skin.

“Capacitance imaging” with MoistureMap® MM 100 (Courage & Khazaka electronics)

To further underline the direct moisturizing effects of MultiMoist CLR™, the study including 22 volunteers with dry skin was continued with assessing the skin moisture level using the MoistureMap technology. A remarkable difference between the formulation containing MultiMoist CLR™ and its corresponding placebo could be detected in this way as well. See figure 10 for the results obtained.

The formulation containing MultiMoist CLR™ outperformed the placebo formulation by more than three times.

Individual results are shown in figure 11. The direct moisturizing activity of MultiMoist CLR™ and the fact that it clearly performs better than the corresponding placebo formulation can easily be seen from the MoistureMap pictures.

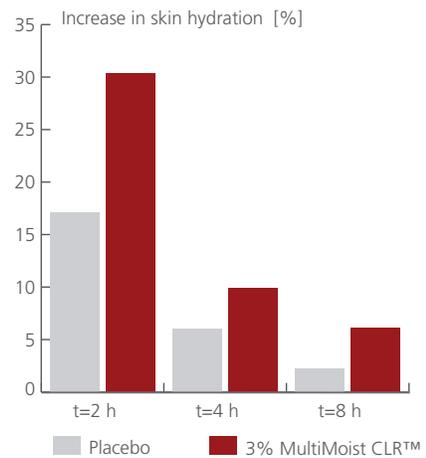


Fig. 8: Relative improvement of skin moisture after 1 application on normal skin (%). Measured with Corneometer MPA 5 CPU.

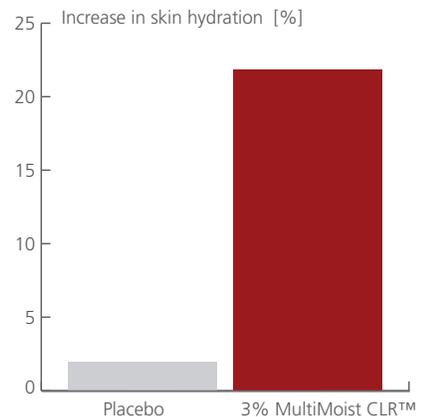


Fig. 9: Relative improvement of skin moisture after 1 application on dry skin (%). Measured with Corneometer CM825™ 2 hours after application.

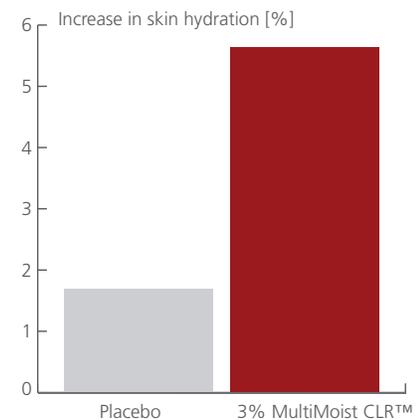


Fig. 10: Relative improvement of skin moisture after 1 application on dry skin (%). Measured with MoistureMap® MM 100 2 hours after application.

INDIVIDUAL RESULTS

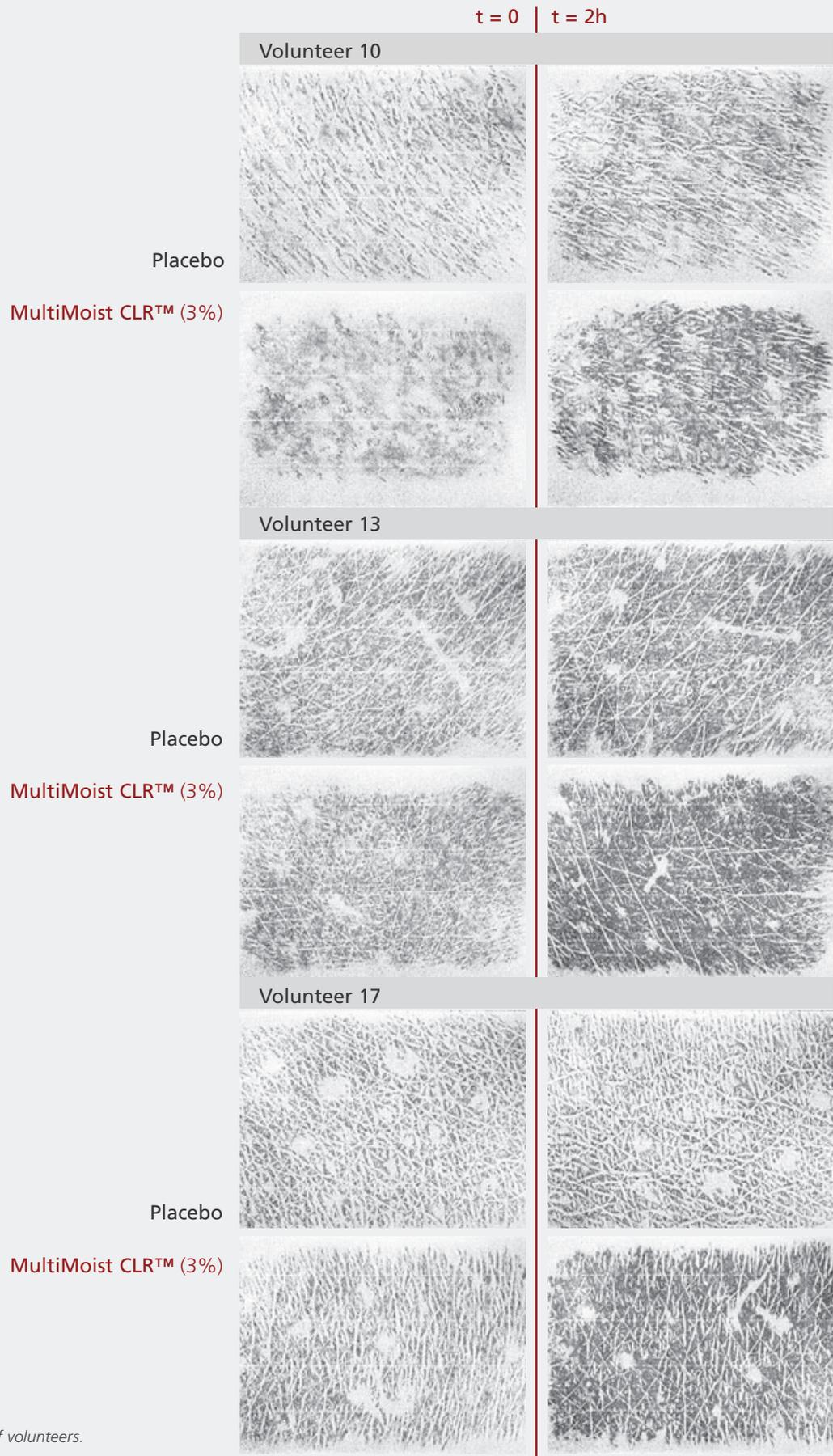


Fig. 11: Individual results of volunteers.

Moisture Accumulation Test (MAT): Novameter® DPM 9003 (NOVA Technology Corp)

Both short-term and long-term moisturizing efficacy was determined with a Novameter. This study was also performed on 22 volunteers with dry skin. Here the direct moisturizing effects were determined 2 hours after a single application. The long-term moisturizing effects were determined after 21 days of twice daily application, with the last application of the test formulations taking place 12 to 16 hours before the measurements.

Apart from placebo and the corresponding formulation with 3% MultiMoist CLR™, a third formulation was included in this study. The base formulation was identical to the base formulations of placebo and that with MultiMoist CLR™, but contained 3% of a benchmark moisturizing active ingredient which can be assumed to be one of the most successful high-end moisturizers in the cosmetic industry. The results are shown in figure 12. Untreated control is set at 0%.

The direct moisturizing activity of MultiMoist CLR™ was already convincingly proven using the corneometer and MoistureMap. It was further affirmed by performing the moisture accumulation test. Comparable to the results obtained with MoistureMap, the formulation containing MultiMoist CLR™ clearly outperformed the corresponding placebo formulation by more than five times.

MultiMoist CLR™'s ability to directly hydrate the skin is particularly strongly revealed in this study when a comparison is made with the benchmark moisturizer: MultiMoist CLR™'s moisturizing effect two hours after the application was almost twice (98%) as strong as that of the benchmark ingredient.

The long-term moisturizing properties were proven with MAT as well. Even 12 to 16 hours after the last application of the test formulations, after having applied these formulation two times per day for a total of 21 days, the formulation containing MultiMoist CLR™ convincingly showed a long-lasting moisturizing effect as well, 35% better than its corresponding placebo.

Here too, in the comparison with the benchmark moisturizing ingredient, MultiMoist CLR™ clearly showed superior results. After 21 days the moisturizing effect of MultiMoist CLR™ was 10% better than that of the benchmark.

Where the direct moisturizing properties of MultiMoist CLR™ might be accounted for by its inherent ability to support the skin in effectively binding water, the long-term moisturizing properties, measured up to 16 hours after the last application of the test formulation, clearly indicates that MultiMoist CLR™ is able to support the skin in producing its own water-binding ingredients (e.g. NMF) too.

Squametry: Diagnoskin System® (Laboratoire BIO-EC)

The assessment of the moisturizing efficacy of MultiMoist CLR™, squametry was performed on 22 volunteers with dry skin. With this approach the visual dryness of skin was determined using a technology which allows for the analysis of images of squames and scales on tape strips. This was performed after 21 days of twice daily application of test formulation and, as with the MAT study, 12 to 16 hours after the last application of the test formulations. The results are presented in figure 13.

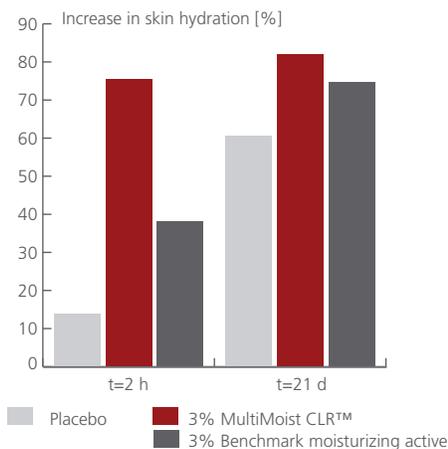


Fig. 12: Relative improvement of skin moisture after 1 application on normal skin (%), measured with Novameter® DPM 9003 in a moisture accumulation test 2 hours after application and after 21 days of twice daily application.

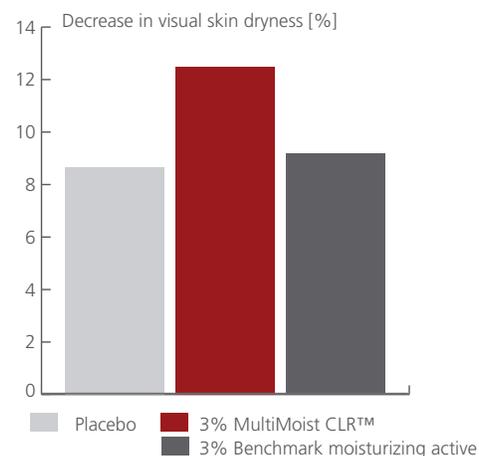
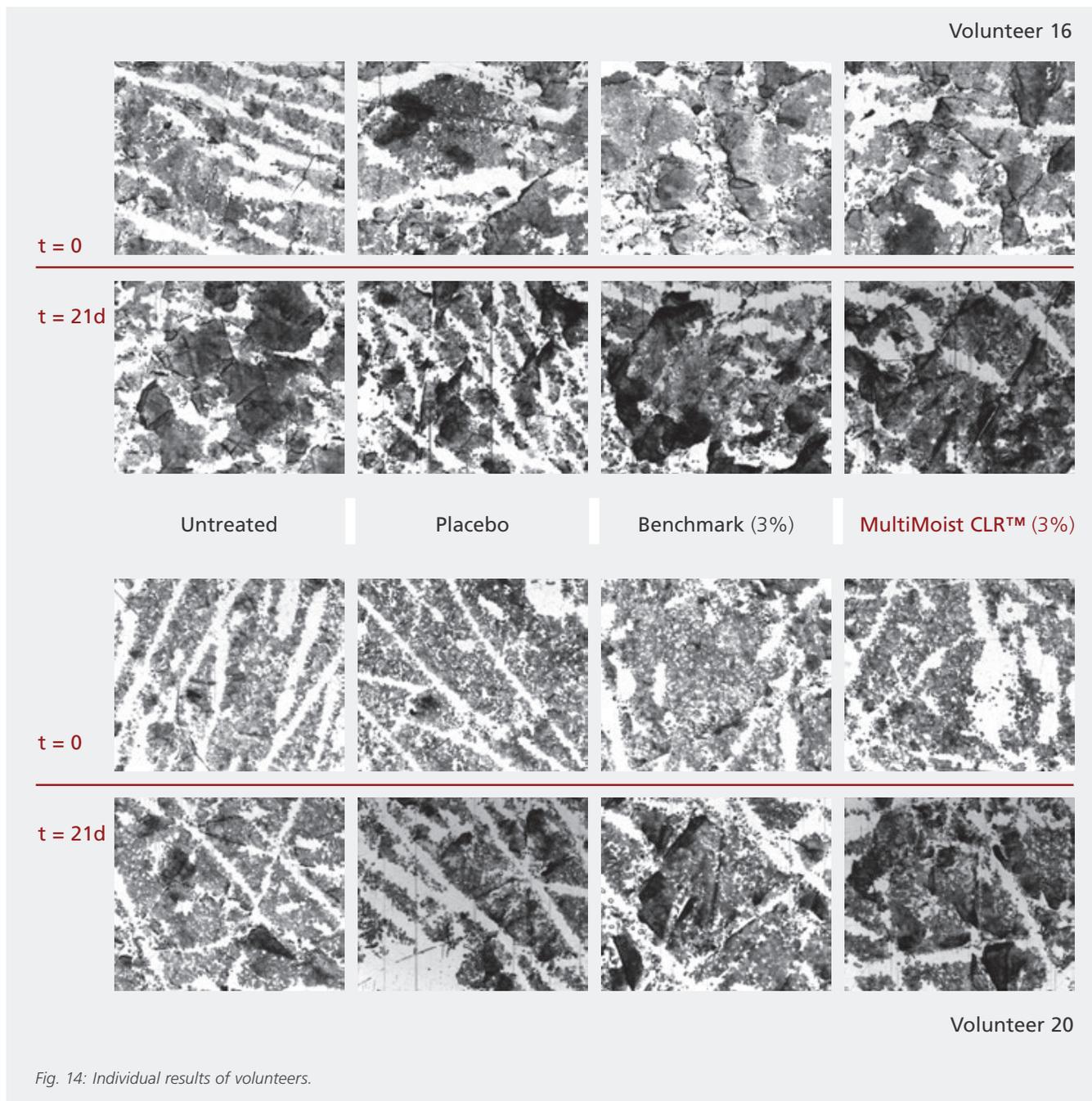


Fig. 13: Relative decrease in visual skin dryness as determined with the Diagnoskin System® (%) after 21 days of twice daily application.

As in all other parameters, the formulation containing MultiMoist CLR™ clearly outperformed its corresponding placebo on this parameter too. The formulation containing MultiMoist CLR™ showed a 44% improvement as compared to the placebo formulation.

The benchmark moisturizing active ingredient was included in this study as well. As with the above-described MAT, here too it was clearly shown that MultiMoist CLR™ has a more pronounced effect than the benchmark. With MultiMoist CLR™ the visual aspects of dry skin were reduced 36% more effectively than with the corresponding formulation containing 3% of the benchmark ingredient.

Individual results are shown in figure 14. These pictures clearly illustrate MultiMoist CLR™'s superiority compared to corresponding placebo and formulation containing the benchmark active ingredient.



Skin smoothness

As mentioned above, one of the main expectations the consumer has from skin moisturizers is that they should make skin soft and smooth immediately. The immediate skin smoothing properties of MultiMoist CLR™ were determined on normal skin after a single application on 20 volunteers. Skin smoothness was assessed after 1 and 2 hours after applying test formulations by making use of a Primos® 5.7 high res (GFMeßtechnik GmbH, Teltow, Germany). The results are presented in figure 15. Untreated control is set at 0%.

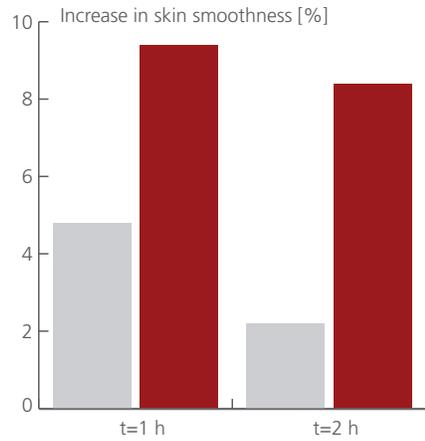


Fig. 15: Relative increase in skin smoothness after 1 application on dry skin (%). Measured with Primos® 5.7 high res one and two hours after application.

Consumer study

As a last piece of the puzzle providing proof of MultiMoist CLR™ being a strong moisturizer, relevant to consumer perception of skin moisturization, a consumer study was performed. 20 volunteers with dry skin applied the test formulations once, after which they assessed different skin parameters, relevant for skin moisturization, by filling out a questionnaire. The parameters were assessed 30 minutes, 1, 4 and 24 hours after the application of the test formulations. Results are shown in figures 16, 17 and 18.

The results from this consumer study were clearly in line with the results obtained with the instrumental assessments of the moisturizing properties of MultiMoist CLR™. For a period of at least 24 hours after one single application, skin felt more moisturized, felt smoother and looked better than the skin which was treated with placebo.

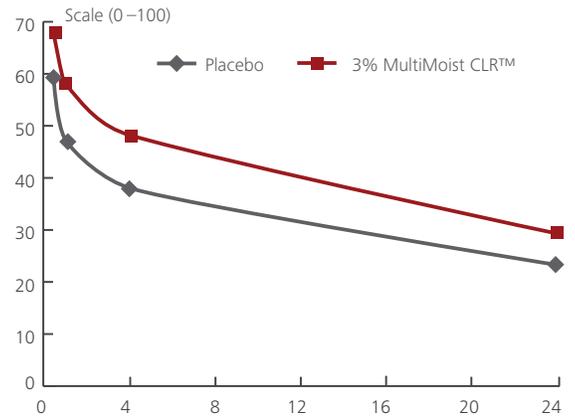


Fig. 17: How far do you judge the formulation to make your skin feel smoother?

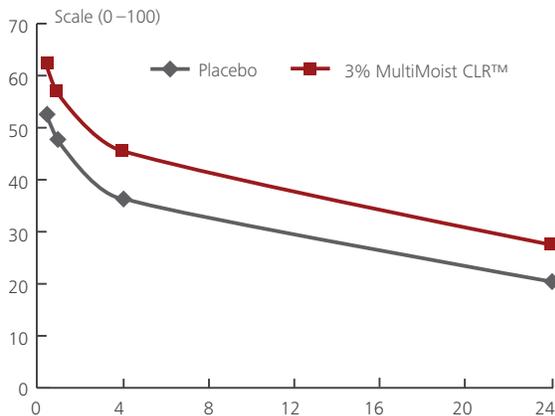


Fig. 16: How do you judge the moisturizing effect of the formulation?

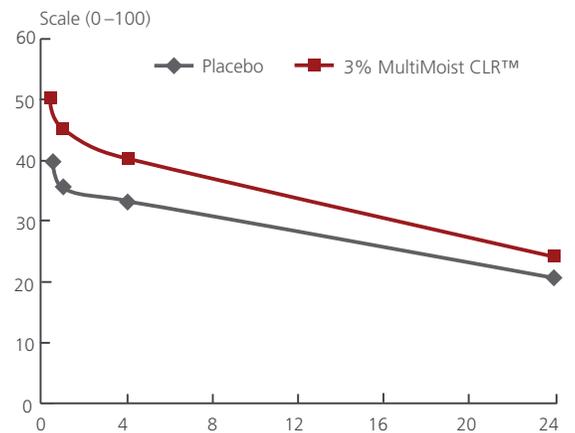


Fig. 18: How far do you judge the formulation to improve the look of your skin?

CONCLUSIONS

Only when a well thought-out combination of methods is utilized to prove the *in vivo* moisturizing efficacy of an active ingredient can clear and objective conclusions be drawn. The various study methods used in finding proof for MultiMoist CLR™'s moisturizing potency complement each other and, without exception, showed clear and strong moisturizing effects from MultiMoist CLR™.

The direct moisturizing properties of MultiMoist CLR™ are remarkable and proven with separate approaches – normal skin versus dry skin and corneometer vs. MoistureMap vs. moisture accumulation test. After one single application, both quantification of skin smoothness and consumer perception of skin smoothness showed that MultiMoist CLR™ works on this relevant parameter of skin moisturization. 24 hour-effects were proven on the perception the moisturized feel of skin and the improved look of skin.

Short-term effects measured relatively quickly, after 21 days of twice daily application of the test formulations, might lead to positive results, however this does not guarantee relevant information on long-term moisturizing properties. Proving long-term moisturizing effects should be performed a significant amount of time after the last application of the test formulations.

12 to 16 hours after the last application it was clearly shown that MultiMoist CLR™ has potent moisturizing properties. This was proven both with the moisture accumulation test and with squametry. Both methodologies provide two totally different points of view on the topic of skin moisturization. The moisture accumulation test provides proof in the objective quantification of water in the skin. Consumers perceive dry skin to be rough and unattractive and do not necessarily see the link between water and these skin features, although they realize that a good moisturizer works for them. Squametry supplies important objective information on these skin features.

MultiMoist CLR™'s potent moisturizing properties were not only proven against placebo, they were also clearly underlined in the direct comparison with a benchmark moisturizing ingredient. This ingredient is extremely successful and has proven itself in the market. Cosmetic chemists trust this product to perform when aiming for moisturizing activities in a cosmetic formulation. It was therefore important to put MultiMoist CLR™'s efficacy in the context of the efficacy of the benchmark ingredient.

The results obtained with MAT and squametry, particularly those obtained with MultiMoist CLR™ and the benchmark ingredient, illustrate the complexity of proving moisturizing properties *in vivo*. Whereas the difference in results obtained with MAT after 21 days are relevant, they might be interpreted as "just 10%." On visual appearance of dryness the difference between the two is 36% after 21 days.

MultiMoist CLR™ has been tested with different complimentary and relevant technologies. It was shown that MultiMoist CLR™ moisturizes immediately and exceptionally strongly, and MultiMoist CLR™ has proven long-term moisturizing effects.

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