

Analysing vitamin D and skin hydration

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Over the past decade the world has gone through tremendous changes. The world's population increasingly follows stricter and more frequent personal hygiene practices. Significantly more consumers now use high SPF sun protective products than a few decades ago. Urbanisation has led to a significant increase in the number of people working and living indoors, often in a heated or air-conditioned, environment. The COVID-19 crisis has had an additional dramatic impact on the fact that people remain indoors.

In short, on average our skin is increasingly exposed to influences which promote skin dryness (dry environment, personal hygiene) and decreasingly exposed to sunlight. Sunlight provides our skin with the ability to produce vitamin D and decreased exposure to UV light leads to a deficiency in vitamin D in the human body.

In addition to the above, the capability to produce vitamin D is inversely correlated to people's age. As we know, demographically, the world population is ageing. Interestingly, a chronic lack in systemic vitamin D ('hypovitaminodosis D') is described to be linked to specific hallmarks of ageing.

Vitamin D and the skin

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 Natural Moisturizing Factors (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

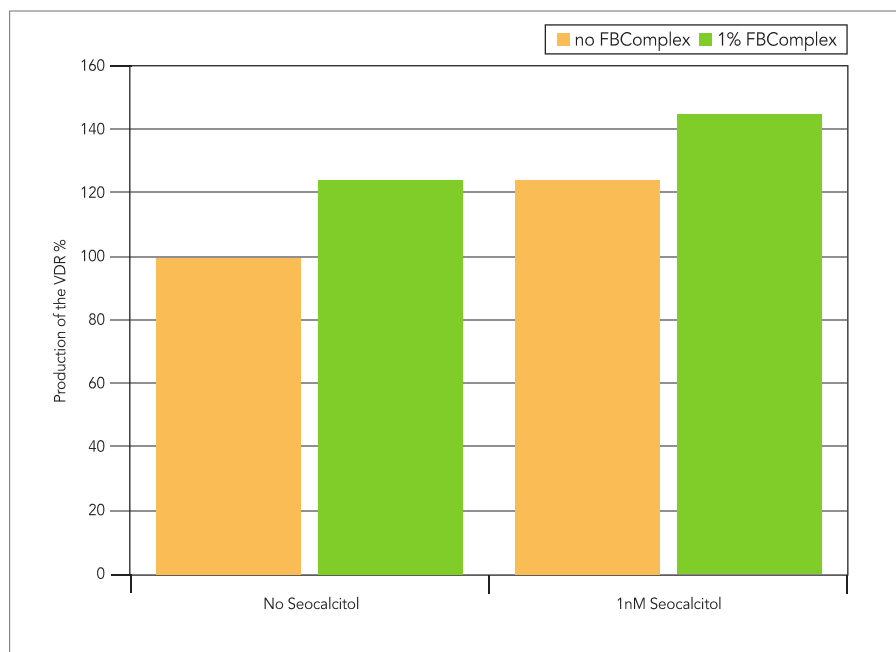


Figure 1: Influence on expression of the VDR after 72 hours of incubation of normal human epidermal keratinocytes with and without the presence of seocalcitol and FBComplex.

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 moisturised skin.

Making the most of our vitamin D

A lack of vitamin D can possibly, at least partly, be compensated for by the use of oral vitamin D supplements. Another approach, supporting the skin, is to allow the epidermal keratinocytes to make better use of the little vitamin D which is present. Increasing the production and activation of the VDR leads to a higher probability for vitamin D to elicit its beneficial effects for the skin. CLR Berlin developed MultiMoist CLR™ (FBComplex, INCI: Fructooligosaccharides, Beta Vulgaris (Beet

Root Extract, Water). *In vitro* and *in vivo* studies were performed with FBComplex with the purpose to prove its beneficial effects on production and activation of the vitamin D receptor and its properties as a moisturising active ingredient.

In vitro results, Influence on VDR expression and activation

It was assessed whether FBComplex 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 calcitriol analogue, calcitriol is the biologically active form of vitamin D) and FBComplex.

Results obtained on VDR expression are presented in Figure 1. VDR expression without the presence of seocalcitol or FBComplex is set at 100%. Corresponding results relevant to the activation of the VDR (VDR-SerP) are presented in Figure 2.

The treatment with FBComplex led to

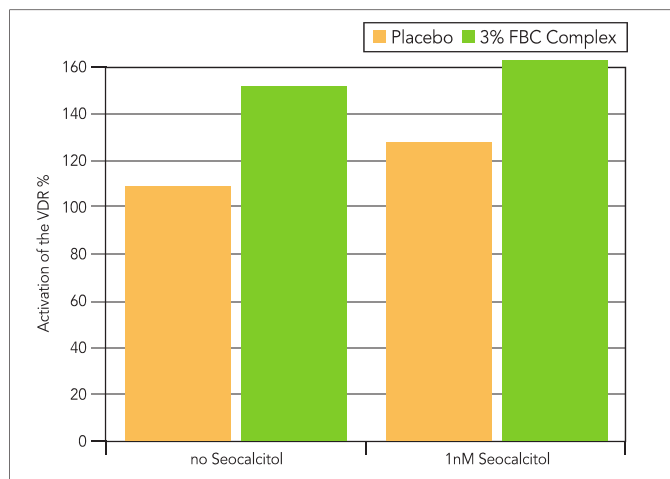


Figure 2: Influence on the activation of the VDR (VDR-SerP) after 72 hours of incubation of normal human epidermal keratinocytes with and without the presence of seocalcitol and FBComplex.

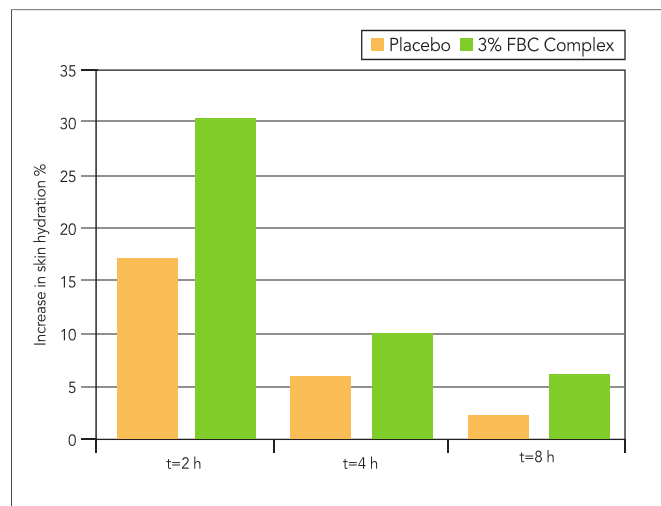


Figure 3: Relative improvement of skin moisture after 1 application on normal skin (%). Measured with Corneometer MPA 5 CPU.

pronounced results as compared to the treatment with seocalcitol alone. The addition of FBComplex to seocalcitol clearly showed the potentiating effects of FBComplex. This leads to the conclusion that FBComplex can amplify the effects of vitamin D.

Additional *in vitro* studies showed results which were in line with the above. Through its action on the VDR, FBComplex was shown to support epidermal growth.

In vivo approaches to proving skin hydrations

Regulatory bodies and competent authorities are increasingly demanding cosmetic claim support and the substantiation of cosmetic claims. The consumer too wants proven results. The times of 'hope in a jar' are largely gone.

Proving the moisturising effect of a cosmetic product sounds easy enough, but the fact of the matter is that it is a complex endeavour. The efficacy of skin moisturisers is best proven both objectively, using high-end methodology, and subjectively, in

relation to the average consumer's perception of dry skin vs. moisturised skin.

The known measuring techniques quantifying the hydration level in the skin are not flawless. To be able to draw 'watertight' conclusions from the results obtained, a combination of techniques is best used. Additionally, the 'hydration' level as measured with these techniques, do not necessarily correlate with consumer perception of hydrated vs. dry skin. Dry skin feels rough and looks dull to the consumer. These parameters must therefore be quantified as well. Lastly, a consumer study should be performed in order to validate the results obtained with the instrumental methods.

In our studies we first used skin capacitance as a parameter of skin hydration. For this, 2 types of the Corneometer were used (CM825 and MPA 5 CPU, Courage & Khazaka electronics). Additionally, the MoistureMap technology (MoistureMap® MM 100 (Courage & Khazaka electronics) was used. This is a 'capacitance imaging' technology giving

graphical information on the near surface hydration distribution over a larger skin area. After that, a Moisture Accumulation Test (MAT) was performed with a Novameter DPM 9003 (NOVA Technology Corp). The MAT gives information about the quantity of moisture the SC may accumulate during a given time. 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.

Where the above technologies only give quantitative information on 'water in the stratum corneum', two other technologies were used to address the more subjective aspects of skin hydration. Squametry ("skin dryness evaluation") was performed with Diagnoskin System® (Laboratoire BIO-EC). By tape stripping the surface of the skin, the loosely adherent portion of the SC sticks to the adhesive. The scales can be automatically sized and counted to give a measure of the degree of skin dryness, correlating to skin appearance. Dry skin is perceived to be rough. Skin roughness was

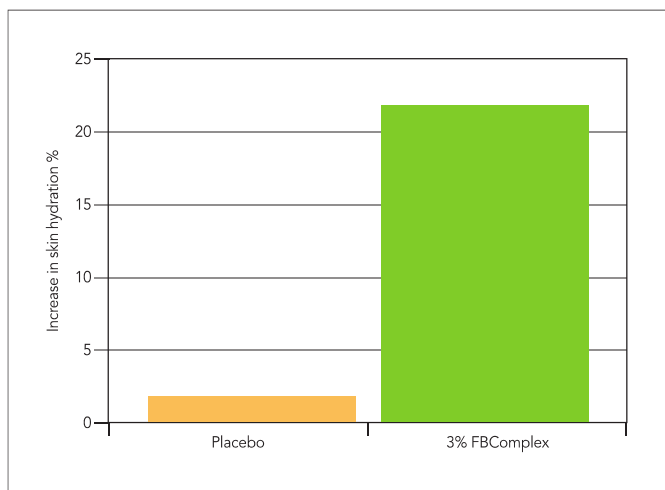


Figure 4: Relative improvement of skin moisture after 1 application on dry skin (%). Measured with Corneometer CM825™ 2 hours after application.

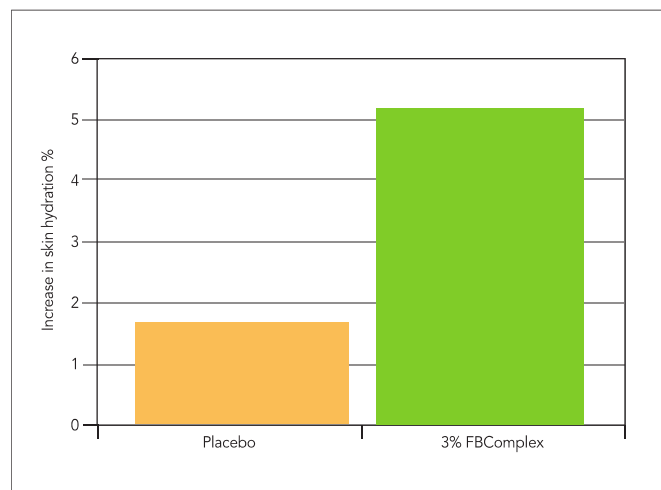


Figure 5: Relative improvement skin moisture after 1 application on dry skin (%). Measured with MoistureMap® MM 100 2 hours after application.

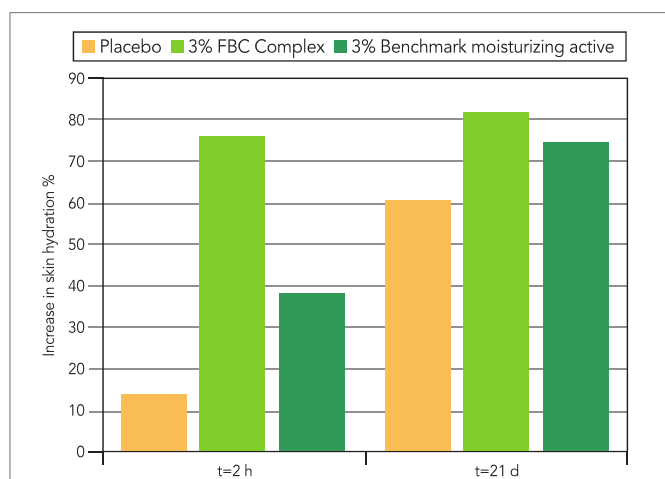


Figure 6: Relative improvement of skin moisture after 1 application on normal skin (%), measured with Novameter® DPM 9003 in a moisture accumulation test 2 hours after application and after 21 days of twice daily application.

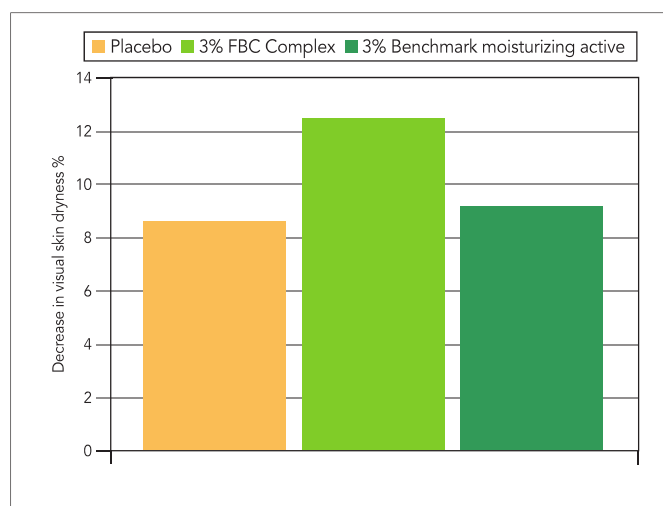


Figure 7: Relative decrease in visual skin dryness as determined with the Diagnostics System® (%) after 21 days of twice daily application.

assessed using a Primos 5.7 high res (GF Messtechnik). With this optical technology, the precise 3-dimensional topography of skin can reliably be determined. A consumer study was performed as well and the combined results of all *in vivo* studies were clear.

Skin capacitance

The moisturising effect of FBCComplex at 3% in a cosmetic formulation was compared to a control formulation not containing FBCComplex (placebo) in two different studies. First of all, using a Corneometer MPA 5 CPU, the eight-hour moisturising effect of FBCComplex was measured on normal skin after a single application on 20 volunteers. The results are presented in Figure 3. After 2 hours the formulation containing FBCComplex 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 moisturising activity of FBCComplex 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 4. Although the relative difference between the formulation containing FBCComplex 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 FBCComplex in directly moisturising skin.

Capacitance imaging

To further underline the direct moisturising effects of FBCComplex, 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 FBCComplex and its corresponding placebo could be detected in this way as well. See Figure 5 for the results obtained. The formulation containing FBCComplex outperformed the placebo formulation by more than three times.

Moisture accumulation test

Both short-term and long-term moisturising efficacy was determined with a Novameter. This study was also performed on 22 volunteers with dry skin. Here the direct moisturising effects were determined 2 hours after a single application. The long-term moisturising 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% FBCComplex, a third formulation was included in this study. The base formulation was identical to the base formulations of placebo and that with FBCComplex, but contained 3% of a benchmark moisturising active ingredient which can be assumed to be one of the most successful high-end moisturisers in the cosmetic industry. The results are shown in Figure 6. Untreated control is set at 0%. The direct moisturising activity of FBCComplex 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 FBCComplex clearly outperformed the corresponding placebo formulation by more than five times. FBCComplex's ability to directly hydrate the skin is particularly strongly revealed in this study when a comparison is made with the benchmark moisturiser: FBCComplex's moisturising effect two hours after the application was almost twice (98%) as strong as that of the benchmark ingredient.

The long-term moisturising 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 FBCComplex convincingly showed a long-

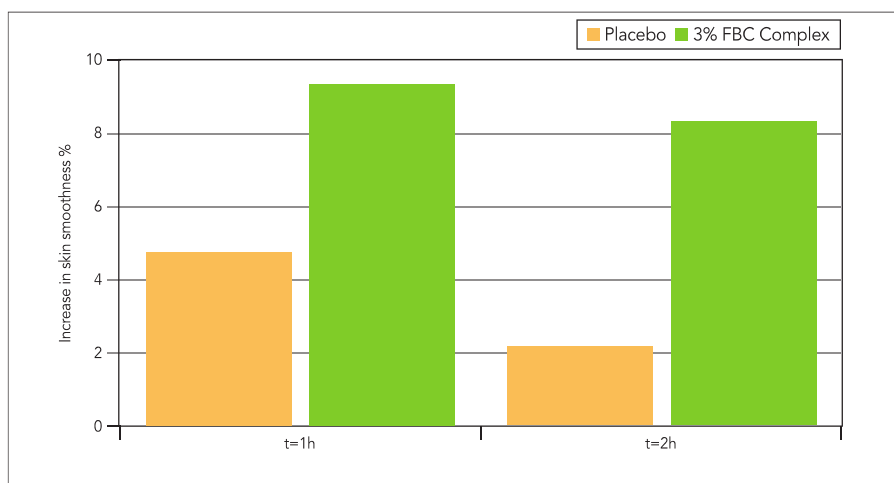


Figure 8: Relative increase in skin smoothness after 1 application on dry skin (%). Measured with Primos® 5.7 high res one and two hours after application.

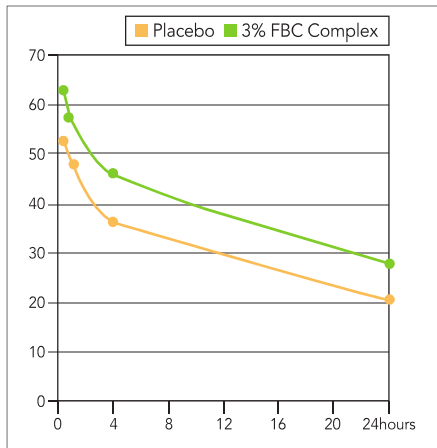


Figure 9: How do you judge the moisturising effect of the formulation?

lasting moisturising effect as well, 35% better than its corresponding placebo. Here too, in the comparison with the benchmark moisturising ingredient, FBCComplex clearly showed superior results. After 21 days the moisturising effect of FBCComplex was 10% better than that of the benchmark.

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

Squamestry

The assessment of the moisturising efficacy of FBCComplex, squamestry 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 7. As in all other parameters, the formulation containing FBCComplex clearly outperformed its corresponding placebo on this parameter too. The formulation containing FBCComplex showed a 44% improvement as compared to the placebo formulation. The benchmark moisturising active ingredient was included in this study as well. As with the above-described MAT, here too it was clearly shown that FBCComplex has a more pronounced effect than the benchmark.

With FBCComplex the visual aspects of dry skin were reduced 36% more effectively than with the corresponding formulation containing 3% of the benchmark ingredient. These results

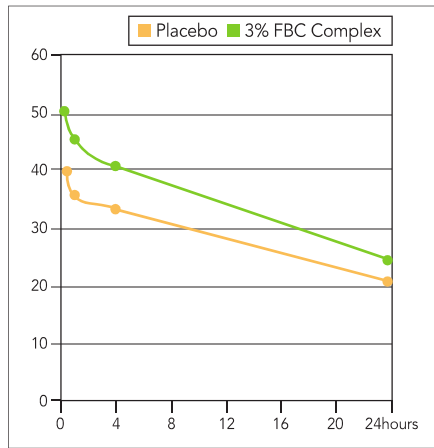


Figure 10: How far do you judge the formulation to make your skin feel smoother?

clearly illustrate FBCComplex's superiority compared to corresponding placebo and formulation containing the benchmark active ingredient.

Skin roughness

As mentioned above, one of the main expectations the consumer has from skin moisturisers is that they should make skin soft and smooth immediately. The immediate skin smoothing properties of FBCComplex 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 8. Untreated control is set at 0%.

Consumer study

As a last piece of the puzzle providing proof of FBCComplex being a strong moisturiser, relevant to consumer perception of skin moisturisation, 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 moisturisation, 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 10, 11 and 12. The results from this consumer study were clearly in line with the results obtained with the instrumental assessments of the moisturising properties of FBCComplex. For a period of at least 24 hours after one single application, skin felt more moisturised, felt smoother and looked better than the skin which was treated with placebo.

Conclusions

Lack of vitamin D is a common problem and has significant impact on the quality of skin, for instance its hydration level.

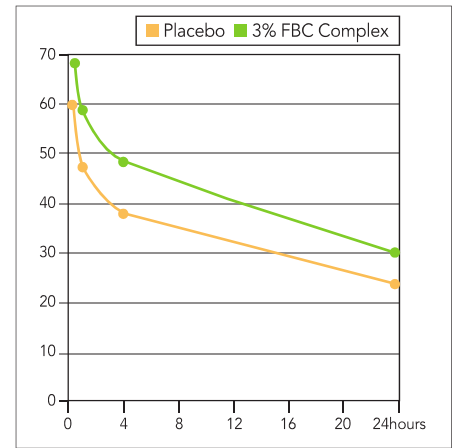


Figure 11: How far do you judge the formulation to improve the look of your skin?

FBCComplex is able to induce the production and the activation of the VDR. Mimicking the *in vivo* situation, where FBCComplex was combined with a vitamin D analogue showed that FBCComplex could potentiate the effect of vitamin D as well.

The various study methods used in finding proof for FBCComplex's moisturising potency complement each other and, without exception, showed clear and strong moisturising effects from FBCComplex. The direct moisturising properties of FBCComplex 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 FBCComplex works on this relevant parameter of skin moisturisation. 24 hour-effects were proven on the perception the moisturised feel of skin and the improved look of skin.

12 to 16 hours after the last application, after a 21-day skin care regime, it was clearly shown that FBCComplex has potent moisturising properties. This was proven both with the moisture accumulation test and with squamestry. FBCComplex's properties were not only proven against placebo, they were also clearly underlined in the direct comparison with a benchmark moisturising ingredient. The results obtained with MAT and squamestry, particularly those obtained with FBCComplex and the benchmark ingredient, illustrate the complexity of proving moisturising properties *in vivo*. On visual appearance of dryness, the difference between the two is 36% after 21 days. FBCComplex has been tested with different complimentary and relevant technologies. It was shown that FBCComplex moisturises immediately and exceptionally strongly and has proven long-term moisturising effects. PC