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Sustainable power food for you skin

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Abstract

A healthy lifestyle paired with an increasing awareness of the need for sustainable and responsible consumption of resources form part of the outlook of today's consumers. They are in the know about health and ecological aspects of their nutrition and they place their trust in cosmetics that recalibrate skin processes in a way that the skin can help itself.

The microalga *C. vulgaris* and the white lupin, two super foods, are combined together in Cellactive®, the carbon-neutral essential cell boost factor. The active ingredient promotes the integrity of the integumentary system at two levels: in the epidermis, it stimulates the production of adhesion proteins in order to improve cellular cohesion. In addition, it triggers the production of multiple extracellular matrix components in the dermis and helps skin to quickly regain its firmness and elasticity.

Cellactive® belongs to the new generation of climate-neutral cosmetic active ingredients. The transparency provided across its whole supply chain, offsetting of unavoidable CO_2 emissions and support of UN-sustainable development goals comply with the concept of a genuinely "green" cosmetic active.

Chronological ageing on its own, ignoring for the moment extrinsic factors, brings with it a wide range of consequences for our skin (Fig 1).[1,2] For example, the quality of differentiation of keratinocytes in the epidermis decreases, meaning that the thickness of the epidermis and the quality of the skin barrier are also diminished. In the stratum corneum, this can lead to changes in the desquamation process. A destabilised skin barrier cannot protect us properly and malignant cells may invade deeper tissue. The skin initiates inflammatory responses as a protective reaction. These inflammatory processes can have a significant impact on skin ageing. This imbalance in favour of inflammatory messengers tends to be an underlying, on-going process, which permanently stresses the skin, causing damage and accelerating the ageing processes.

In ageing skin, the basement membrane, the dermoepidermal junction, atrophies. The basement membrane grows thinner, is damaged and becomes permeable leading to destabilisation of the skin. There is reduced expression of types IV and VII collagen and consequently the organisation of the papillary dermis is modified. As a result, important water-retaining molecules such as hyaluronic acid, versican and other proteoglycans disappear leading to less hydrated skin.

Chronological ageing not only impacts on the upper layers of the skin but also on much deeper skin layers. The thickness of our reticular dermis roughly doubles until we reach the age of 50 years after which it begins to deteriorate. Furthermore, fibroblasts as long-lived cells undergo age-associated accumulated damage leading to cellular senescence and thus the collagen and elastin density of the dermis continuously decreases, which is one of the hallmarks of skin ageing.[3]

A balanced and healthy diet and enough physical activity will support the needs of the skin from within. Consequently, the application of a topical skin super food can complement healthy dietary strategies and give the strength and necessary boost to the weakest links in the skin, ensuring the resilience and protective power that skin needs during everyday activities.

Cellactive® (INCI: Water, Chlorella Vulgaris/Lupinus Albus Protein Ferment, Sodium Benzoate, Potassium Sorbate) combines the valuable ingredients of two 'kings of foods'; the green alga *Chlorella vulgaris* and the protein rich lupin, which help to maintain the skin's dynamic state of equilibrium and at the same time actively promote a better environment as we ensure a CO_2-neutral carbon footprint across the whole supply chain.

Materials and methods

In vitro

Improvement of cell cohesion

Primary human keratinocytes were seeded in six well plates. Half of the wells were supplemented with 1% Chlorella Vulgaris/Lupinus Albus Protein Ferment for 72 hours. Subsequently calcium was added to the cultures triggering stabilisation and expression of adhesion structures at the site of cell-cell contacts. After 0 hours, 3 hours and 6 hours post calcium switch, the tissue-like cell layer was detached from the well floor and disrupted by means of carefully defined mechanical stress (10 pipette

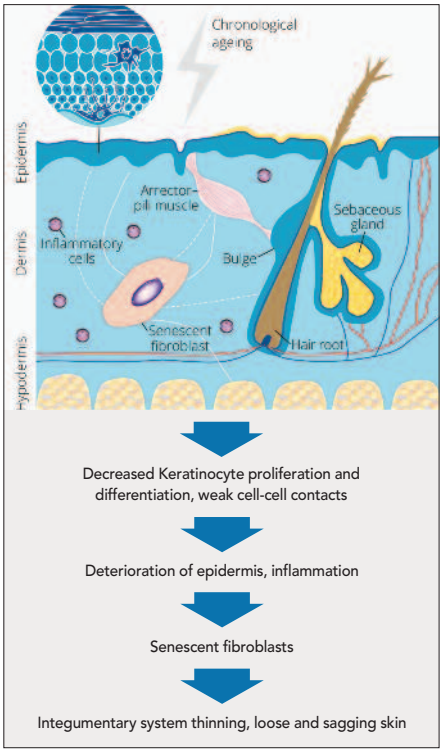

Figure 1: Diagrammatic representation of the integumentary system and consequences of chronological ageing. The enlarged section in the image depicts weakened cell–cell contacts and decreased quality of keratinocyte differentiation.

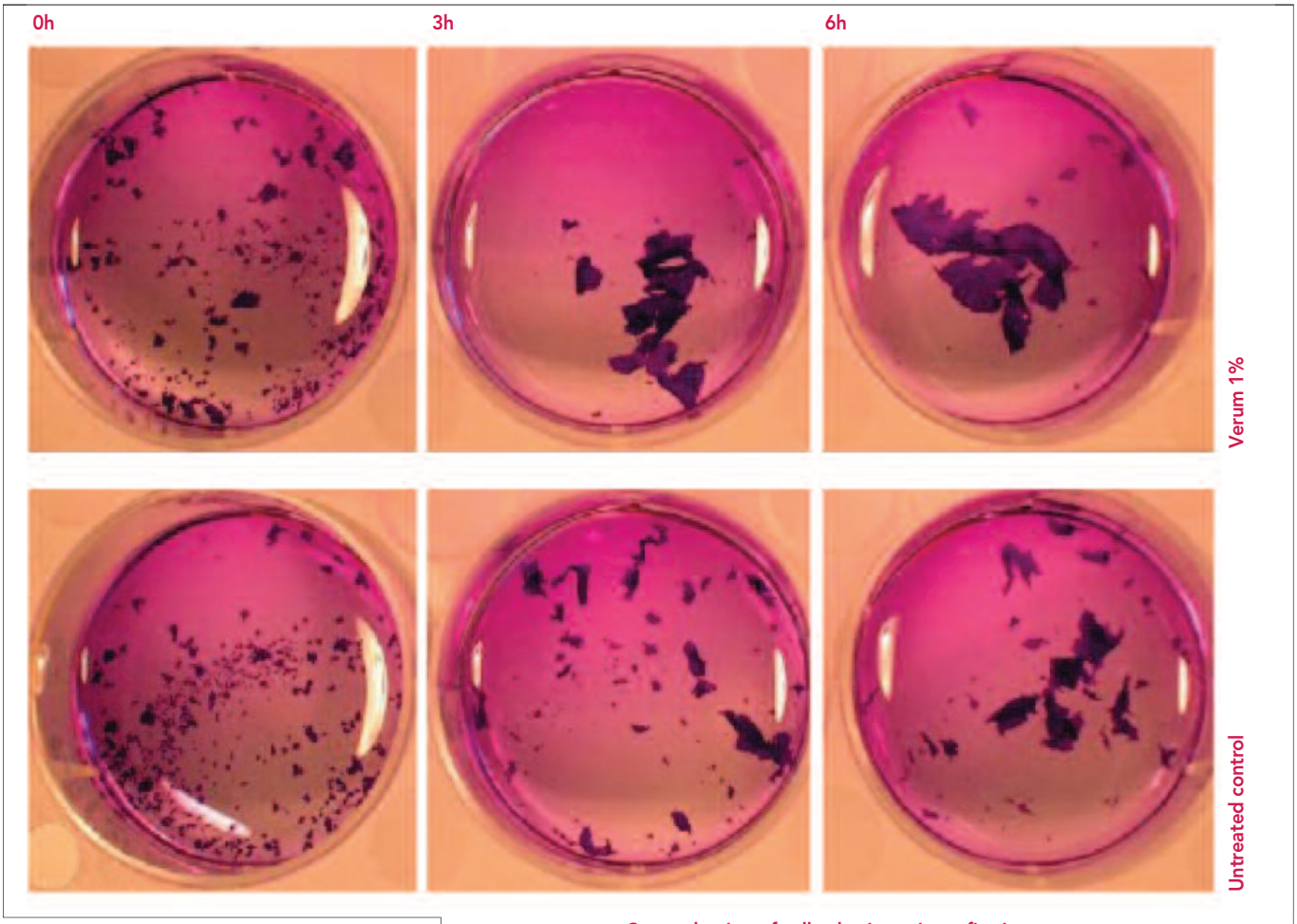

Figure 2: Chlorella Vulgaris/Lupinus Albus Protein Ferment enhances the resistance to mechanical stress of keratinocyte sheets. The cell-cell contacts were significantly strengthened following incubation with 1 % Chlorella Vulgaris/Lupinus Albus Protein Ferment. An unpaired Student's t-test was performed. The statistical values in black are the results of comparison with baseline.

strokes in the case of the 0- and 3-hour samples and 30 strokes in the case of the 6-hour samples). Crystal violet was used for staining and the result was documented by photographic images.

In a parallel experiment, cells were immunofluorescently stained with various antibodies to analyse adhesion molecules present at desmosomes, such as E-cadherin, α- and β-catenin. The microscopic analysis was performed after 0 and 3 hours.

Promotion of ECM integrity and control of inflammatory response genes

Gene expression in the case of inflammatory response and ECM integrity were analysed using two different 3D skin models (EpiDermFT and MatTek full thickness skin) following topical application of 0.1 % Chlorella Vulgaris/Lupinus Albus Protein Ferment or vehicle control. The promotion of ECM integrity and control of inflammatory processes are potential ways of counteracting chronological skin ageing.

Quantitative reverse transcriptase polymerase chain reaction (TaqMan qPCR) was used to measure changes in gene expression. In brief, the amount of an expressed gene in a cell can be determined by means of fluorescent dye detection of the number of the RNA copies of that gene. The greater the initial number of copies of the nucleic acid target, the sooner a significant increase in fluorescence is observed.

In vivo

Rapid effects with regard to skin elasticity and firmness

The *in vivo* study was performed in accordance with the principles of good laboratory practice (GLP), good clinical practice (GCP) and in compliance with quality assurance system requirements. The study also conformed to the provisions of the Declaration of Helsinki of the World Medical Association. All study participants signed a written informed consent form at the beginning of the study. In a double-blind, placebo-controlled study, 20 female subjects, aged 35 to 65 years, with healthy, Caucasian skin were tested. The volunteers applied either placebo or verum emulsion

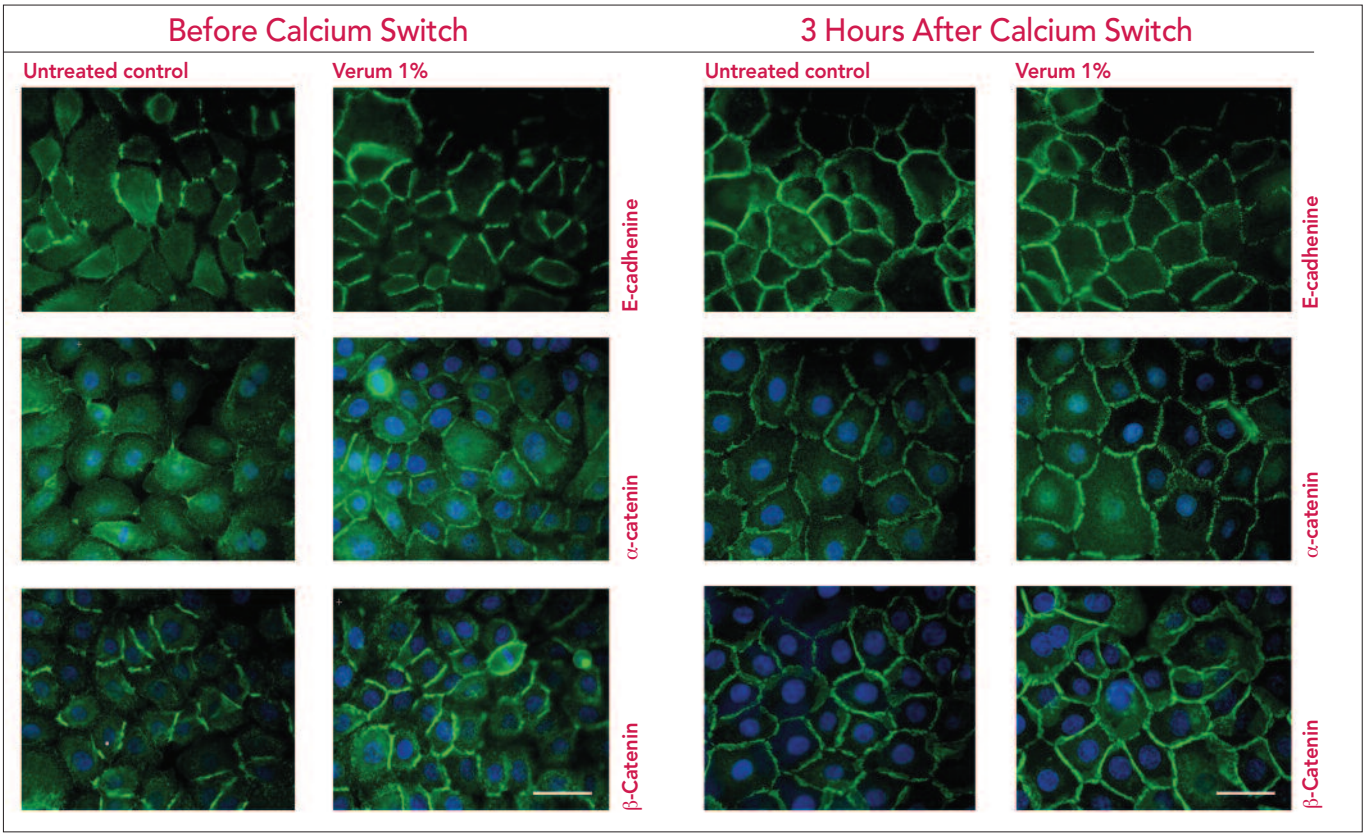

Figure 3: Chlorella Vulgaris/Lupinus Albus Protein Ferment increases stabilisation of various adhesion proteins. Cell incubation with 1% of Chlorella Vulgaris/Lupinus Albus Protein Ferment for 72 hours led to an increased expression of the cell adhesion molecules E-cadherin and α- and β-catenin compared to the untreated control (left panels). Three hours after calcium switch, the expression of the proteins increased in the untreated control as well as in the case of Chlorella Vulgaris/Lupinus Albus Protein Ferment-treated cells; however, this was more pronounced in the latter case. Scalebar: 50 µm.

or gel containing 0% or1 % active twice daily to the inner side of forearm for 14 days. Skin elasticity and firmness were determined using a conventional cutometry technique.

Results

Improvement of cell cohesion

There were clear differences in the resistance of keratinocyte cell sheets to disruption by mechanical stress between the control and cells exposed to Chlorella Vulgaris/Lupinus Albus Protein Ferment. The difference was most apparent in the case of the samples 3 hours post calcium switch (Fig 2, top). The difference in the ratio of cell sheet fragments in the case of control- and Chlorella Vulgaris/Lupinus Albus Protein Ferment-exposed cells was significant: 15% initially, 57% after 3 hours and 52% 6 hours post calcium switch (Fig 2, bottom).

In the epidermis, calcium levels continuously increase from the basal layer to the stratum corneum, reaching their maximum in the stratum granulosum, where constricted junctions function as a barrier to ions. It can thus be assumed that stabilisation of adherens junctions (desmosomes) is likely to be most marked in this area. The results of our cell culture experiment corresponded to the observed physiological effects.

The immunofluorescence imaging showed that the exposure of human keratinocytes to 1% Chlorella Vulgaris/Lupinus Albus Protein Ferment for 72 hours resulted in increased stabilisation of adhesion proteins compared to untreated control (Fig 3, left). Three hours after elevation of calcium levels, the extent of staining of the adhesion molecules E-cadherin, α- and β-catenin was increased and encompassed the entire perimeters of the cells. As expected, levels of adhesion molecules were also elevated in the untreated control but to a much lesser extent. (Fig 3, right).

Promotion of ECM integrity and reduction of inflammatory response genes

The changes to gene expression following topical application of 0.1% Chlorella Vulgaris/Lupinus Albus Protein Ferment with regard to inflammatory response (Fig 4, top) and ECM-integrity genes (Fig 4, bottom) took the form of differing effects in the epidermal and full thickness 3D skin models. While the epidermal layer skin culture exhibited almost no changes to the expression of the selected genes - except in the case of elastin (ELN) and the tissue inhibitors of metalloproteinases (TIMP 1 and 2 genes) - in the full thickness model there was in general decreased gene expression with regard to inflammatory response and enhanced ECM-integrity gene expression. This experiment demonstrates the limited expressiveness of the epidermal skin model. While elastin and TIMP regulation were responsive, the model failed to reflect the situation in full thickness skin as shown by the corresponding model. The difference in the gene regulation effects demonstrates the importance of intact tissue composition for skin homeostasis in terms of keratinocytes and fibroblasts.[4] Furthermore, levels of the most important building blocks of ECM – the collagen/elastin network (types I and IV collagen and elastin), collagen and elastin interconnections (decorin, dermatopontin, fibrilin and fibronectin), and water retention molecules (versican) - were enhanced, showing that Chlorella Vulgaris/Lupinus Albus Protein Ferment had a reconstructive effect across the whole integumentary system. In addition, levels of inhibitors of ECM degradation (TIMPs) increased while concentrations of inflammatory genes associated with activation of matrix-degrading enzymes were reduced.

In vivo

Rapid beneficial effects on skin elasticity and firmness

A significant improvement to skin firmness after just 14 days of application was observed for both formulations, a gel and emulsion containing 1% Chlorella Vulgaris/Lupinus Albus Protein Ferment (Fig 5). In the case of elasticity there was a significant effect of the gel formulation and visible improvement with regard to the

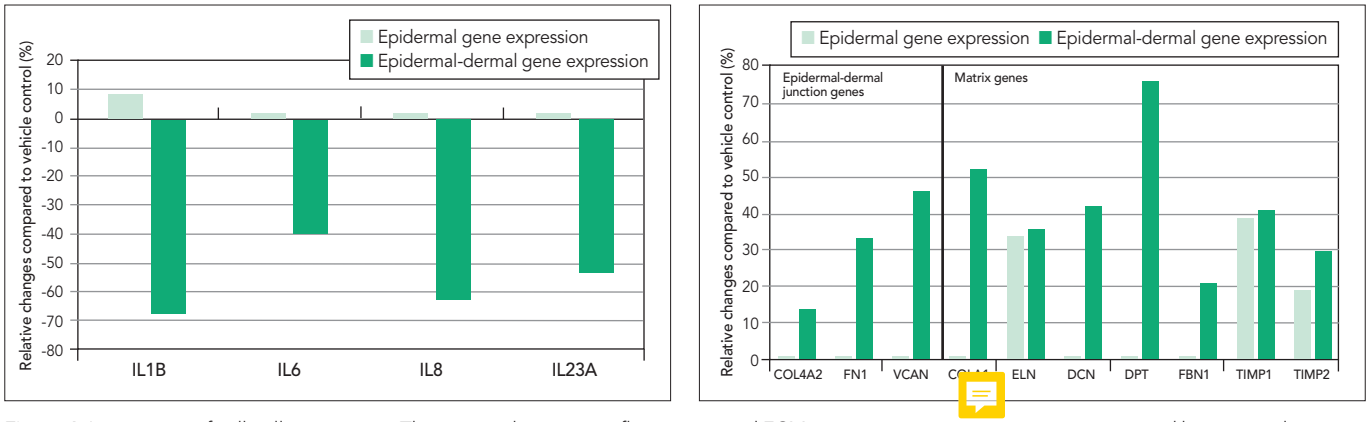

Figure 4: Importance of cell-cell interactions. There were changes to inflammatory and ECM-integrity gene expression in reconstituted human epidermis and the full thickness skin model. Chlorella Vulgaris/Lupinus Albus Protein Ferment improved bilateral interaction between epidermis and dermis and revitalised the whole integumentary system.

emulsion formulation. Overall, we can say that our test products containing 1% Chlorella Vulgaris/Lupinus Albus Protein Ferment induced a change in the biomechanical properties of the skin, improving both its firmness and elasticity.

Discussion

During chronological ageing our skin is constantly modified and re-organised. This dynamic process used to maintain equilibrium entails many challenges and cells have to cope not only with their daily problems but also master environmental impacts. Keratinocytes and fibroblasts accumulate over time a wealth of the hallmarks of ageing. This cellular fatigue becomes visible to us in the form of loose and sagging skin, deep wrinkles, dull hair and a generally overall unhealthy appearance of skin and hair (Fig 1). With increasing age our skin needs more valuable nourishing ingredients from inside and outside.[5]

Chlorella Vulgaris/Lupinus Albus Protein Ferment contains water-soluble ingredients of two "kings of superfoods", supplying skin with precious essential and non-essential amino acids, proteins, various carbohydrates, minerals and vitamins (essential cell boost factor). Amino acids are important for the production of epidermal and dermal structures such as collagens; they are part of the natural moisturising factor class and may work as anti-oxidants.[6] The primary fuel of skin cells is glucose, which is freely transported across

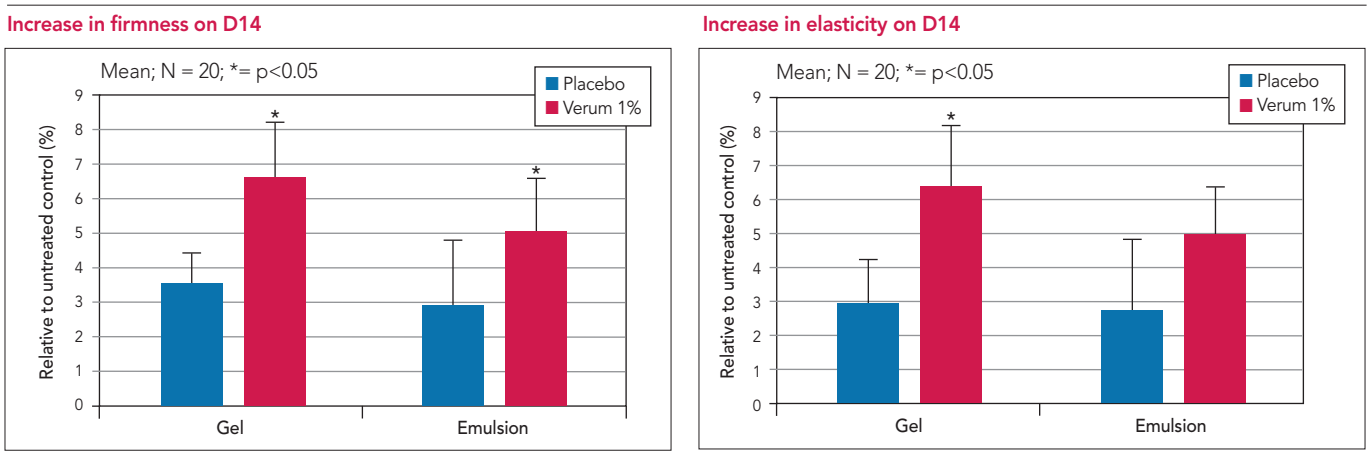

Figure 5: Improvement of biomechanical skin properties. Results for firmness and elasticity after 14 days of use of 1 % Chlorella Vulgaris/Lupinus Albus Protein Ferment incorporated in different cosmetic formulations. For statistical analysis an ANOVA, Tukey HSD test was performed. The statistical values in black are the results of comparison with baseline.

the membranes with water and many other small molecules.[7] The consumption rates are similar to those observed in resting skeletal muscles. The uptake of topically applied skin nutrition optimises cellular nourishment and thus enhances cell vitality, leading to a stronger and healthier integumentary system.

All in all, Chlorella Vulgaris/Lupinus Albus Protein Ferment as a power nutrient boosts and vitalises cells in the skin (Fig 6). Increased keratinocyte proliferation (data not shown) leads inevitably to more robust cell-cell contacts visible in the form of enhanced expression of adherens junction proteins (E-cadherins and α- and β-catenins) (Fig 3). This in turn ensures keratinocyte differentiation,[8] which is essential for the epidermal barrier function.[9] A healthy epidermal skin layer with enhanced cell-cell contacts guarantees improved skin cohesion and resistance to mechanical stress (Fig 2). Increased production of basement membrane building blocks such as types IV and VII collagen ensures more resilient binding to the dermis. Robust binding of epidermal keratinocytes also promotes the gate-keeping function of the basement membrane. Higher levels of dermal fibrous elements such as elastin, also the collagen- and elastin-connecting elements decorin, dermatopontin, fibronectin, fibrilin and water-retaining proteoglycans such as versican (Fig 4) are evidence of enhanced fibroblast and keratinocyte vitality,
which improves skin firmness and elasticity (Fig 5). The Chlorella Vulgaris/Lupinus Albus Protein Ferment carbohydrate stachyose, a tetrasaccharide related to raffinose, is known to stimulate fibroblasts.[10] Moreover, it has been reported that fibroblast senescence was significantly reduced following application of an aqueous extract of *C. vulgaris*, which resulted in a significant decrease in the biomarkers of ageing.[10] In addition, enhanced production of tissue inhibitors of metalloproteinases (TIMPs), an effect that results in a decreased inflammatory response,[11] further promotes the integrity of the ECM.

Conclusion

Cellactive® supplies the ideal combination of nutrition to the skin, safeguarding reliable and maximum maintenance of skin homeostasis and ensuring cells remain energised and vital, thus counteracting chronological ageing and the impact of external factors. It gives skin a vibrant and healthy-looking appearance. Furthermore, as the lupin employed is farmed in conformity with the key principles of sustainability while a cutting-edge technology – cultivation in sealed photobioreactors – is used to obtain *C. vulgaris*, this product may represent a paradigm for future cosmetic developments. PC

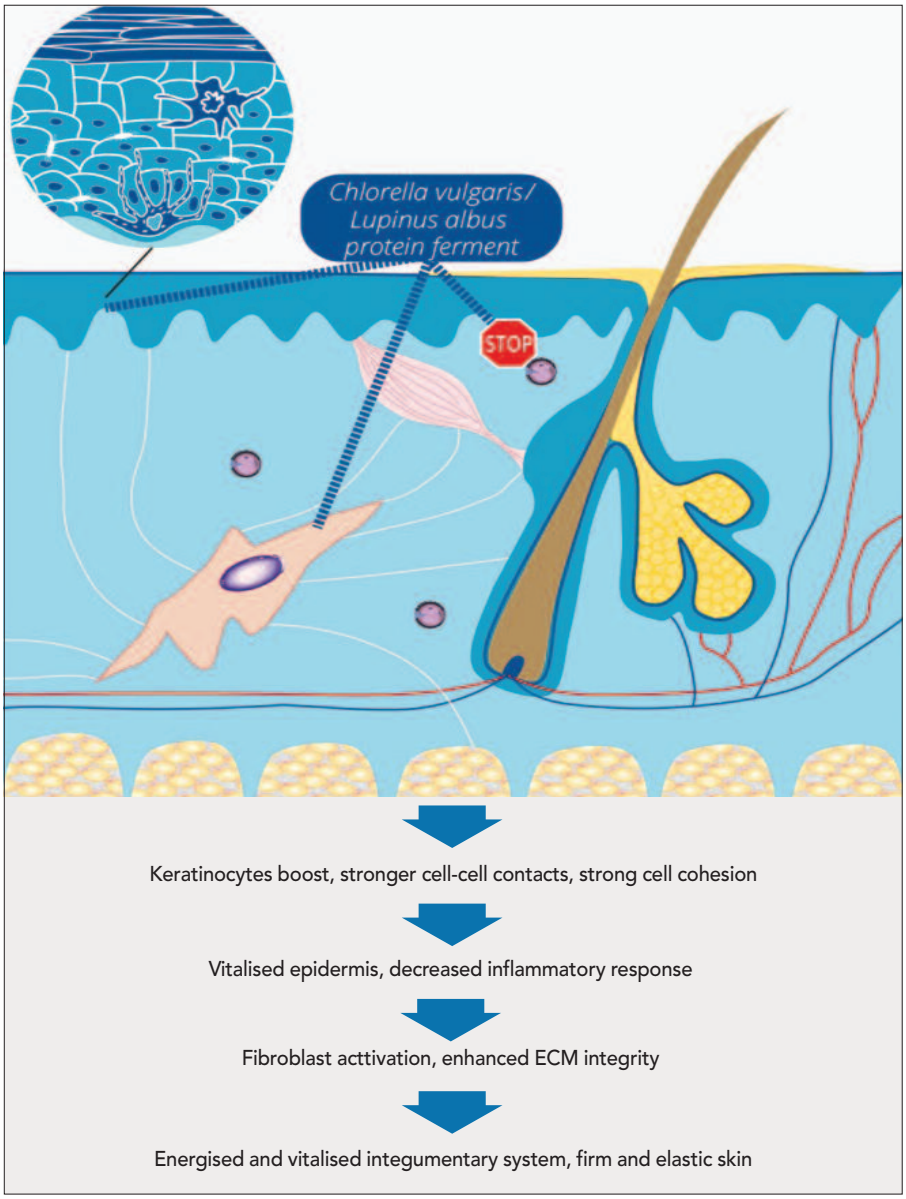

Figure 6: Diagrammatic representation of the mode of action of Cellactive®.

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