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CELLACTIVE® – Care for Hair and Climate

E. Besic Gyenge, S. Hettwer, B. Suter, S. Breitenbach, B. Obermayer

abstract

The microalga *Chlorella vulgaris* and the white lupin are well known sources of valuable nutritional building blocks such as amino acids, proteins and carbohydrates. Both superfoods are combined together in CELLACTIVE®, the Carbon Neutral Essential Cell Boost-Factor for skin and hair. The active ingredient promotes the integrity of the integumentary system and it encourages the longevity of hair follicles. Furthermore, it improves hair quality parameters such as the anti-static effect and structure.

Thanks to a dedicated carbon footprint calculation and neutralisation of produced carbon dioxide, CELLACTIVE® paves the way for a new generation of climate-neutral cosmetic active ingredients with excellent transparency across their whole supply chain while assumption of social responsibility compensates for any unavoidable emissions.

Introduction

Hair appearance is not only dependent on genetic predisposition but is also influenced by external factors, chronological ageing, nutrition and the individual care routine. Application of a topical nutrition preparation can complement healthy dietary strategies and impart the needed strength to skin and hair.

CELLACTIVE® (INCI: Water, Chlorella Vulgaris/Lupinus Albus Protein Ferment, Sodium Benzoate, Potassium Sorbate) contains water-soluble ingredients of two "kings of superfood" supplying the whole integumentary system with valuable essential and non-essential amino acids, proteins, various carbohydrates, minerals and vitamins influencing the living and non-living part of the hair.

The "Kings of Superfood"

Chlorella vulgaris – the Power of Microalgae

C. vulgaris is one of the most remarkable fresh water microalga. The ability of *C. vulgaris* to propagate fourfold within 24 hours and to grow under challenging conditions have motivated many biologists to use it as a model organism to study basic biochemical and physiological aspects such as photosynthesis or even to consider it for use as a component of environmental control and life support systems during space missions [1]. 100 g of Chlorella contains 60–70 g protein (dry weight) and can supply the daily protein requirement of an adult (**Fig. 1**). *C. vulgaris* has many benefits and it is one of the "kings of superfoods". It is detoxifying, anti-ageing, immune-enhancing, prebiotic, repair- and growth-promoting. Its cultivation in photobioreactors is one of the most sustainable forms of production.

Lupinus albus Protein – Nutraceutical Seeds

L. albus is one of the best cover crops. The deep roots loosen the lower soil layers enabling optimal soil conditioning and fertility. Farmed *L. albus* does not need additional water or fertilisers. Furthermore, the beautiful lupin flowers create attractive meadows where our endangered and extremely important bees can collect pollen. Cultivation of lupin is possible

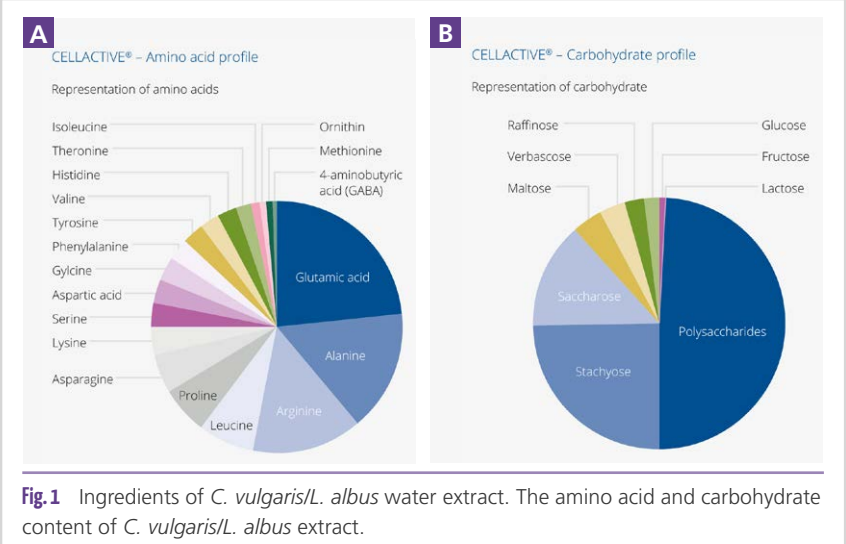

Fig. 1 Ingredients of *C. vulgaris*/*L. albus* water extract. The amino acid and carbohydrate content of *C. vulgaris*/*L. albus* extract.

on almost all continents. The symbiosis of the plant with rhizobia results in the fixation of atmospheric nitrogen for the production of proteins and other nitrogenous substances in seeds while at the same time these contain hardly any starch. Functional foods such as lupin seeds are attracting more interest and are of growing importance in our world. The average protein content ranges from 30 to 40 %. White lupin seeds contain many amino acids and a desirable ratio of ω-6 and ω-3 fatty acids. They are also a valuable source of dietary fibres [2, 3] (**Fig. 1**).

Hair – From Root to Tip

The regulation of periodic hair cycle phases is still not fully understood and it is assumed they involve several control and initiation mechanisms. Nevertheless, some extracellular matrix components such as versican, decorin, type IV collagen, fibronectin, TIMP 1 and 2 play important roles in the cycle process [4-7]. These molecules exhibit specific expression patterns during the hair cycle and have the ability to regulate and influence the duration of the phases. Our gene expression experiment revealed that CELLACTIVE® is able to upregulate the specified genes, thus improving extracellular matrix (ECM) integrity.

Hair growth and the hair cycle need a continuous supply of keratinocytes. The keratinocytes are produced in the matrix region of the hair bulb. At the same time, matrix cells are dependent on a continuous supply of cells from the bulge region and the absence of the bulge follicular stem cells would result in hair loss [8]. Interestingly, these cells are the precursors of both the keratinocytes of the hair follicle and interfollicular epidermis. Furthermore, they are very β-catenin-dependent. The upregulation of β-catenin forces the stem cells to differentiate in favour of hair follicle keratinocytes [9]. Exposure to CELLACTIVE® *in-vitro* resulted in a significant boosting effect on keratinocyte growth. Also observed were increased cell cohesion and elevated expression not only of β-catenin but also E-cadherin (data not shown). E-cadherin is needed to ensure healthy skin differentiation and renewal of hair follicles. The absence of adherens junctions leads to severe epidermal defects, deterioration of hair follicle cycling and subsequent loss of the entire hair follicle [10]. Our *ex-vivo* experiment using hair follicles showed a positive effect on hair shaft growth and prolongation of the life span of the hair follicles.

Topically applied amino acids are known to interact with hair, influencing physical hair properties [11] and these are also essential for the production of epidermal and dermal structures. Topical application of peptides protects and strengthens the hair from outside. Glucose and other carbohydrates are in general very hygroscopic and therefore suitable as anti-static agents. CELLACTIVE® is able to smooth the hair cuticle, providing an effective shield against the external environment and enhancing hair conductivity, leading to an anti-static hair effect. Our *ex-vivo* experiments involving hair tufts have confirmed these assumptions.

Materials and Methods

In-vitro

ECM Gene Expression Pattern (*in-vitro* study)

Quantitative reverse transcriptase polymerase chain reaction (TaqMan qPCR) was used to measure changes in gene expression. Briefly, 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.

Kerationocytes Viability (*in-vitro* study)

NHEK cells (which are very similar to hair follicle keratinocytes [12]), were cultured under standard conditions for 168 hours in the presence of various *C. vulgaris/L. albus* extract (verum) concentrations ranging from 0 % to 10 %. Subsequently performed was an MTT reduction assay to determine the viability of the cells.

Ex-vivo

Hair Follicles Growth (*ex-vivo* study)

The *ex-vivo* method of human hair follicle culture as described by Philpott et al. [13] was used. Briefly, complete hair follicles were prepared and seeded in 24-well plates (1 follicle/well) in a culture medium with and without the verum. Development of the hair shaft was monitored for 14 days with measurement of the length of each follicle. Hair follicle vitality was assessed by means of morphological observation of the hair bulbs. Hair follicle degeneration was observed and documented from day 14 to day 20.

Physical Hair Parameters (*ex-vivo* study)

Anti-static effect: the anti-static effect on hair tufts was investigated using concentrations of 0.5 % and 1 % test compound in water. Virgin and bleached hair tufts were immersed in verum or control solutions (water) for 3 minutes. Each sample was wet-combed, hot air dried for 3 minutes and then dry-combed 20 times at approximately the same combing frequency with a plastic comb. Subsequently taken were digital pictures. This experiment was repeated three times for each test system.

Hair surface: the surface structure of a hair shaft was visualized using scanning electron microscopy (SEM). The hair tufts were bleached for 30 minutes, washed and hot air dried for 3 minutes. Samples were immersed for 3 minutes either in water (control sample) or 0.5 % verum water solution. Subsequently they were hot air dried for 3 minutes and SEM was performed using the standard protocol.

Results and Discussion

ECM Integrity Promotion (*in-vitro* study)

The changes to gene expression following topical application of 0.1 % test substance with regard to ECM integrity genes are shown in **Fig. 2**. The full thickness model exhibited generalised enhanced gene expression. The dynamic changes to ECM during the hair cycle are well documented, with the highest level of expression occurring during the anagen phase [31-34]. The most important building blocks of ECM, such as concentrations of type IV collagen, collagen and elastin interconnections (decorin, dermatopontin, fibrilin and fibronectin) and water retention molecules (versican) were enhanced. These molecules are responsible for ECM integrity but are also able to modulate follicular cycling. Application of *C. vulgaris/L. albus* extract resulted on the one hand in a reconstructive effect across the whole integumentary system but on the other hand may also have a potential influence on the hair cycle. In addition, levels of inhibitors of ECM degradation (TIMPs) were enhanced leading to inhibition of the matrix degrading enzymes, which are mostly upregulated in the catagen and telogen phases.

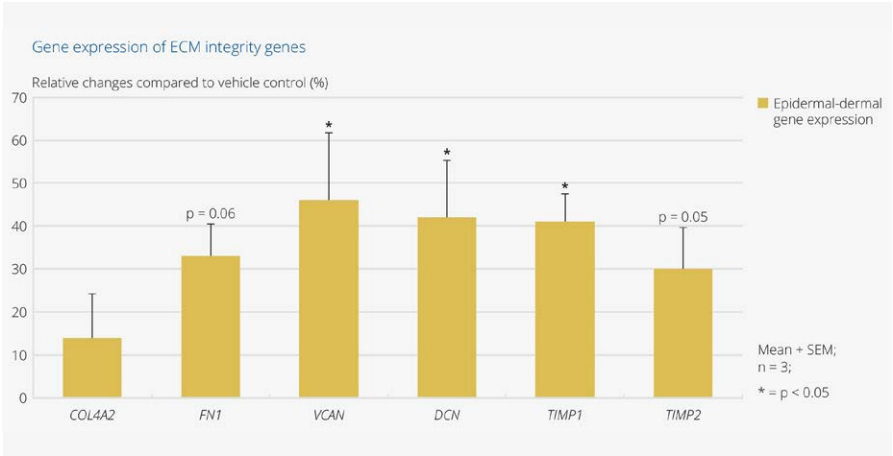

Fig. 2 Importance of a healthy ECM for hair follicles. There were changes to ECM integrity-related gene expression in the full thickness skin model. Verum stimulated the expression of extracellular matrix genes responsible for the maintenance of ECM integrity and hair follicle cycling. An unpaired Student's t-test was performed. The statistical values in black are the results of comparison with vehicle control.

Increase of Keratinocytes Viability (*in-vitro* study)

The experiment demonstrated a significant boosting effect of verum on keratinocyte viability (**Fig. 3**). This effect starting in the presence of a 1 % test compound and greater was significant compared with untreated control samples. At a concentration of 10 %, cell proliferation was increased to such an extent that overpopulation in the well was visible. The combination of ingredients in *C. vulgaris*/*L. albus* extract represents a valuable source of nutrition for cells.

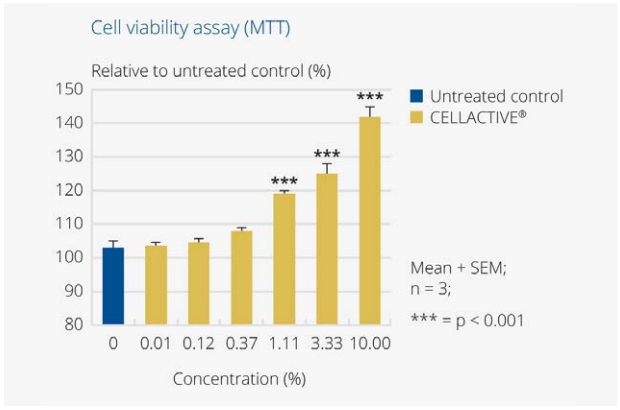

Fig. 3 *C. vulgaris*/*L. albus* extract boosts the growth of keratinocytes. Increasing concentrations of verum lead to significant enhancement of the cells' viability. An unpaired Student's t-test was performed. The statistical values in black are the results of comparison with baseline.

Serum for Hair Follicles (*ex-vivo* study)

Hair growth is dependent on the duration of the anagen phase and also on the hair growth rate or speed. This is influenced by a combination of various factors, such as genetic predisposition and available nutrition. Nutritional deficiencies are not always accompanied by specific signs or symptoms. Nevertheless, cells with higher metabolic rates, such as those in hair, are affected by any such deficiency. Keratin synthesis is impaired leading to alterations to the strength of the hair shaft and a decreased hair growth rate [14].

Exposure to active compound improved the hair shaft progression rate by 4 % on average (**Fig. 4**, upper panel). Additionally, hair follicles treated with verum exhibited a prolonged life span (**Fig. 4**, lower panel). Morphological observations after 20 days revealed that 50 % of hair follicles in the control sample were apoptotic, whereas the verum-exposed samples included only 33 % apoptotic hair bulbs.

In general, it seems that the rich ingredient cocktail in verum is appropriate to the high nutritional requirements of growing hair follicles during the anagen phase.

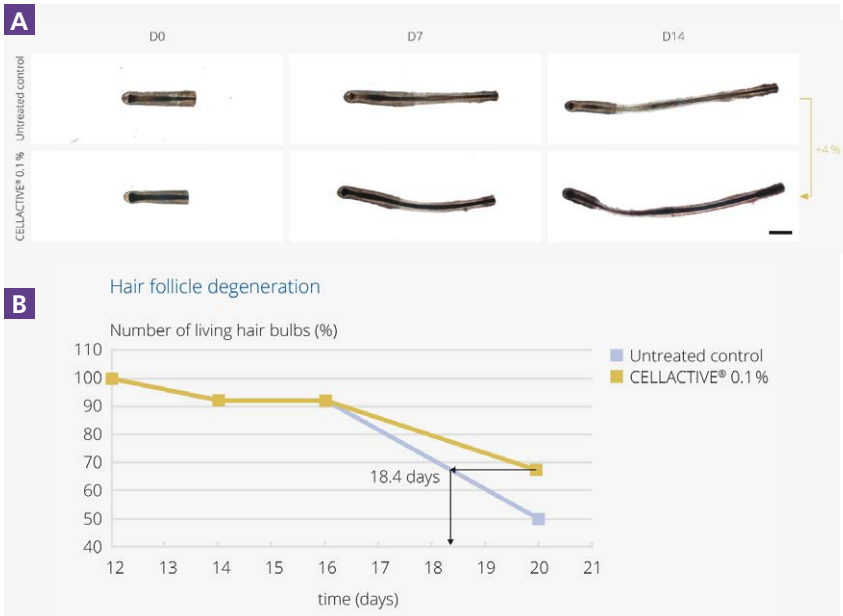

Fig. 4 *C. vulgaris*/*L. albus* extract improves the vitality of hair follicles. The hair follicles were exposed to a concentration of 0.1 % verum. The increase in hair shaft progression was 4 % on average. Furthermore, hair follicles survived for longer in the presence of verum. After 20 days the number of viable follicles in the case of verum treated samples was the same as that for the control samples at day 18.4.

Fig. 5 *C. vulgaris/L. albus* extract causes "discharge" of hair. The conductivity of hair was enhanced following exposure to verum, resulting in less flyaway of the hair (right panel). The effect was dependent on the concentration used. The measurement of HFA revealed a statistically significant difference between verum- and placebo-treated samples (left panel). An unpaired, two-tailed, Student's t-test was performed.

Improvement of the Quality of Damaged Hair (*ex-vivo* study)

Anti-static effect (**Fig. 5**): Glucose and other carbohydrates are known to be very hygroscopic and therefore suitable as anti-static agents. *C. vulgaris/L. albus* extract enhances the conductivity of the hair, resulting in less static loading of the hair. In this experiment two different hair states were investigated, namely virgin and damaged hair. The anti-static effect for all hair states was significantly improved on exposure to verum (**Fig. 5**, right panel). The hair flyaway angle (HFA) was measured for each state (**Fig. 5**, left panel). Exposure to 0.5 % was sufficient to reduce the HFA for all hair states by at least 50 %. Exposure to 1 % resulted in a reduction of HFA by 66 % for virgin hair and almost 80 % for damaged hair. *C. vulgaris/L. albus* extract thus had an anti-static effect on both virgin and on damaged hair. Damaged hair responded significantly better to treatment than virgin hair. Statistical analysis of all verum-exposed samples revealed significant improvements in comparison to placebo-exposed samples.

Hair surface (**Fig. 6**): the damage induced by hair bleaching involves irregular overlay, raising and breakage of the hair cuticle, exposing the hairs' cortex to the environment and thus resulting in rapid moisture loss. The hairs are prone to interlock and tangle easily. Furthermore, it makes hair brittle. The exposure to a concentration of just 0.5 % of verum resulted in a remarkable restoration of the hair cuticle. In the SEM images, the damage to placebo-treated hair is clearly visible in the form of an irregular overlay of the cuticle with cracks and degraded cuticle fragments. The hair cuticle in the case of the sample exposed to 0.5 % showed smoothing, resealing and restoration of the cuticle. The restorative effect to the damaged hair surface following a single exposure to *C. vulgaris/L. albus* extract is marked and clearly visible.

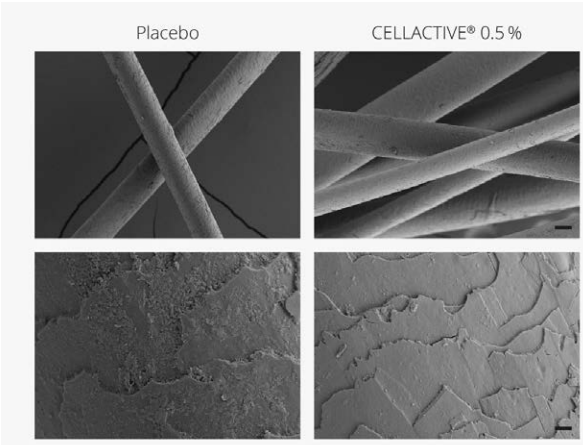

Fig. 6 Images of hair cuticle surface under the microscope. Representative examples of a bleached and placebo-treated (left panel) and 0.5 % (right panel) verum- treated samples. In the case of the *C. vulgaris/L. albus* extract-exposed samples, the hair cuticle was visibly smoothed and resealed. The overall condition of the hair cuticle was thus improved. Scale bar in upper panel 20 µm and 2 µm in the lower panel.

Conclusion

CELLACTIVE® improves interfollicular epidermal cell-cell contacts and serves as a serum for hair follicle and epidermal keratinocytes, leading to a significant enhancement of cellular viability. It has an anti-static effect and a restorative effect on damaged hair cuticles at minimal concentrations. CELLACTIVE®'s major nutritional benefits improve the hair in general – from root to tip. The active ingredient is suitable for hair care formulations, which are efficacious, natural and sustainable at the same time.

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