Crocus Bulb Extract to Communicate Reorganization in Deep Skin for Visible Improvement of Stretch Marks

Daniel Schmid*, Esther Belser, Stefanie Kröni and Fred Zülli

*Corresponding author

Mibelle Group Biochemistry, Switzerland

August 2014

Abstract

The altered skin relief found in stretch marks reflects structural modifications in the skin. These alterations occur in the dermis, a skin tissue that cannot be easily reached by topically applied cosmetic actives. A strategy for the treatment of stretch marks could be to utilize the communication processes between the cells of the outer skin layer, the epidermis, and the cells of the subjacent dermis. Communication is normally mediated by growth factors and cytokines released by one sort of cells that reach other cell types by diffusion. In a series of cell culture assays, an extract of crocus bulbs was shown to stimulate epidermal keratinocyte cells to release growth factors into the medium. The cell-free medium incubated with dermal fibroblast cells was found to enhance the expression of elastin, of the elastin-processing enzyme lysyl oxidase-like 2 and of the connective tissue growth factor (CTGF). The crocus bulb extract applied directly to fibroblast cells was without effect. The results clearly demonstrated that the crocus bulb extract induced secretion of messenger compounds in the epidermis that could enhance the synthesis of matrix proteins in the dermis. Using the non-invasive two-photon microscopy, a cream with the crocus bulb ingredient was found in a clinical trial to activate the synthesis of collagen and elastin in the upper dermis. In a study with 18 volunteers with stretch marks, a cream with the crocus bulb ingredient significantly reduced redness and roughness in the analyzed stretch marks.

Introduction

Striae distensae, commonly known as stretch marks, arise from progressive stretching of the skin during pregnancy or obesity. The marks appear perpendicular to the direction of the greatest tension indicating rupture of the connective tissue. Stretch marks undergo an evolution from an initial inflammatory phase (striae rubra) to a later chronic phase (striae alba). Compared to the normal surrounding skin, in stretch mark areas the skin is characterized by flattening and thinning of the epidermis due to a loss of collagen and flattening of rete ridges in the underlying papillary dermis (1,2). A number of studies suggest that fibroblasts play a key role in the pathogenesis of stretch marks (3). Elastin was shown to be reduced in the papillary dermis and expression of fibronectin and type I and III procollagen was found to be significantly lower in fibroblast from striae. A cosmetic ingredient to treat stretch marks should reactivate the synthesis of the above-mentioned structural matrix components. The metabolism of fibroblasts is controlled by growth factors. Enhancement of extracellular matrix production is a major effect of TGF-β and its downstream mediator CTGF. A decreased expression of TGF-beta and CTGF by aged skin fibroblast cells is known to be responsible for the age-dependent loss of regeneration capacity in the skin (4). Thus stretch marked skin and aged skin in general should be treated with growth factors. The use of human growth factors in cosmetic products is forbidden. There are cosmeceutical products on the market based on biotechnologically produced growth factors. Theoretically they could be used for cosmetic products but their efficacy is very limited because of instability and lack of penetration. Growth factors are proteins with a defined spatial conformation and too big to penetrate into the skin. They are not stable in cosmetic emulsions and are normally quickly degraded by protease enzymes after topical application. They most probably just adhere to the outermost skin layers (stratum corneum), because these molecules are too big to penetrate deeper into the skin. Topically applied growth factors cannot reach fibroblast cells of the dermis, where they should bind to membrane receptors to induce the synthesis of matrix proteins. This paper describes an approach to stimulate the growth factor-dependent synthesis of matrix proteins in the dermis by using epidermis / dermis communication processes. A screening assay based on human primary keratinocyte and fibroblast cells was developed to find an ingredient that encourages keratinocytes to produce messenger compounds such as growth factors that reactivate in fibroblasts the synthesis of matrix proteins. Keratinocytes were incubated with a series of extracts of different plants. After incubation, the keratinocyte cells were separated and the culture supernatants (conditioned medium) were used to culture fibroblast cells in them. At the end of the incubation, the expression of

growth factors and matrix proteins in the fibroblast cells was analyzed. Clearly the best results were found for a Crocus chrysanthus bulb extract.

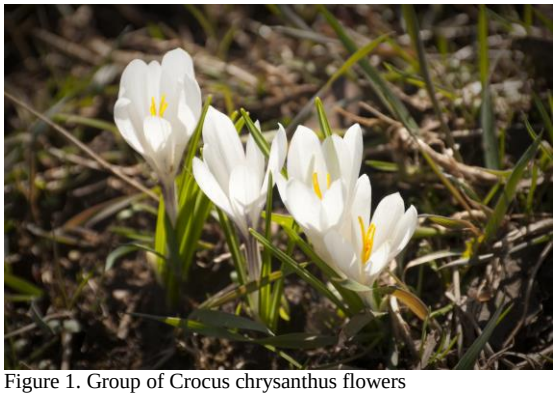


Figure 1. Group of Crocus chrysanthus flowers

A cosmetic ingredient containing the crocus bulb extract (DermCom; INCI: Crocus Chrysanthus Bulb Extract, Acacia Senegal Gum and Water) was then analyzed in clinical studies for densification of the skin and improvement of stretch marks.

Material and Methods

Preparation of the Crocus Bulb Extract

Plant bulbs at 12% w/w were homogenized in a 15% ethanol solution and incubated at 50°C for 4 hours. The extraction was done in a DIG-MAZ 50 system (samtech Extraktionstechnik GmbH). The extraction process was controlled by chromatographic fingerprint analysis (Waters, ACQUITY UPLC system, C18 column).

Screening Assay with Conditioned Media

Primary human keratinocytes from a 50 year old donor were cultured in culture medium for 24 hours. The medium was then replaced with the assay medium (Epilife medium) containing or not (control) the plant extracts at non-toxic concentrations based on a preliminary cytotoxicity assay (MTT). The keratinocytes were then incubated for 72 hours. At the end of the incubation, the cell cultures were centrifuged to prepare cell pellets and culture supernatants (conditioned media). The cells were used to analyze the expression of 30 genes of growth factors and cytokines typically expressed in keratinocytes. The expression analysis was done by quantitative PCR (LightCycler® system; Roche Molecular System Inc.). The conditioned media were used to treat normal human dermal fibroblasts (pool of donors over 50 years old). Beforehand, the fibroblasts were cultured for 24 hours in a normal culture medium and then for 72 hours in an assay medium (Epilife medium). The medium was then removed and replaced by either the conditioned media, the assay medium alone or the assay medium containing the plant extracts at non-toxic concentrations based on a preliminary cytotoxicity assay (MTT). The fibroblasts were then incubated for 24 or 72 hours. At the end of the incubation, the fibroblasts were isolated and used for expression analysis of 61 genes known for their central role in fibroblast biology and cutaneous aging. All experimental conditions were performed in triplicate.

Clinical Pilot Study Using Two-Photon Microscopy

A cream with 0.4% DermCom was tested in a study over 4 weeks on a 53 year old Caucasian woman. The cream was applied twice a day to the inner forearm skin. The other forearm was treated with the placebo cream. Two-photon microscopy pictures were taken at baseline conditions, after two and after four weeks. Image stacks were acquired using Olympus Fluoview 1000 MP two-photon microscopy setup customized for human skin imaging in vivo. The volunteer's forearm was placed under the microscope objective (25x water immersion) and the skin was gently adhered to the proprietary holder using double-sided adhesive tape. For repeated imaging sessions, the same skin spot was targeted every time. Two-photon images were obtained simultaneously in two modes: autofluorescence (AF) and second harmonic generation (SHG).
Image acquisition setup
Illumination: 800 nm
Emission filters:
Channel 1 – band pass 397-412 nm (SHG, color-coded red)
Channel 2 – band pass 455-490 nm (AF, color-coded green)

Clinical Anti-Stretch Mark Study

A cream with 2% DermCom was tested in a study over 56 days on 18 women aged between 18 and 50. The volunteers presented striae alba either on external or internal thighs, on the stomach or on the breasts with a right and left symmetric distribution. It was a placebo-controlled half side study with twice daily product application. Before the first product application and on days 28 and 56, on stretch mark skin lightness (L*) and redness (a*) were measured with the Chromameter® CR300 (Konica Minolta, L*a*b chromatic system) and skin roughness was analyzed with PRIMOSpico® (GFMesstechnik).

Results and Discussion

Normal human fibroblast cells were incubated in a conditioned medium derived from keratinocyte cultures that had been treated with plant extracts. Gene expression analysis in the fibroblast cells showed that the conditioned medium of keratinocytes treated with the crocus bulb extract strongly stimulated the expression of genes involved in the synthesis of matrix proteins.

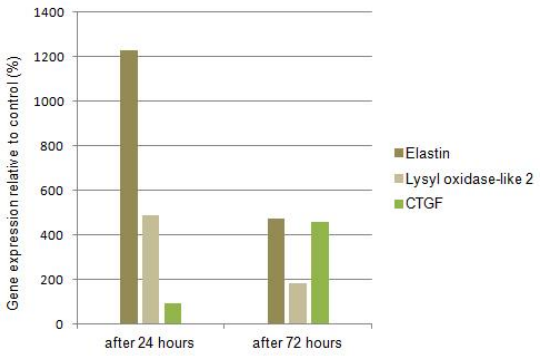


Figure 2. Effect on gene expression in fibroblast cells after incubation for 24 and 72 hours with a keratinocyte conditioned medium. The keratinocytes were treated with the crocus bulb extract. The values are compared to gene expression values of fibroblasts after incubation with the conditioned medium of untreated keratinocytes (100%).

Compared to the conditioned medium of untreated keratinocytes, the expression of elastin was enhanced 12-fold and of lysyl oxidase-like 2, 5-fold after 24 hours' incubation. After 72 hours' incubation, the expression of CTGF was also enhanced 5-fold. The lysyl-oxidase-like enzymes are important for elastic fiber formation by cross-linking elastin. The expression of lysyl-oxidase-like enzymes is reported to be reduced in aged skin (5). Incubation of fibroblast cells with the crocus bulb extract did not affect gene expression, indicating that the effect in fact depends on compounds secreted by keratinocytes. Treatment of keratinocytes with the crocus bulb extract was found to up regulate the expression of several growth factors known to play important roles in epidermis / dermis communication.

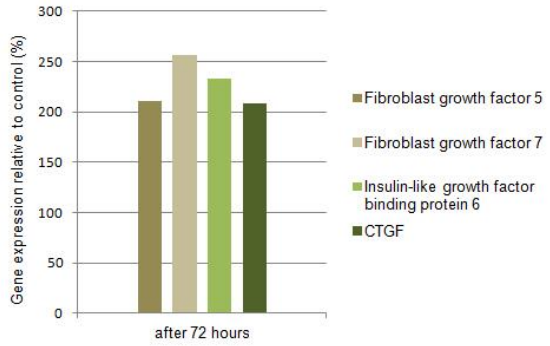


Figure 3. Effect of the crocus bulb extract on gene expression in keratinocytes

Members of the fibroblast growth factor family are involved in a variety of biological processes including cell growth, morphogenesis and tissue repair. Insulin-like growth factors promote the accumulation of the extracellular matrix in various cell types, including fibroblasts (6). Insulin-like growth factor binding proteins modulate the activity of the growth factors. The cell culture assays with all the relevant control conditions showed in fact that the stimulatory effect of the crocus bulb extract on the synthesis of matrix proteins is mediated by growth factor messenger compounds released by keratinocytes. Thus, there is a realistic chance for in vivo efficacy because keratinocytes can be reached by cosmetic actives.

A first clinical pilot study was performed using two-photon microscopy for non-invasive analysis of the skin tissue. Compared to confocal microscopy, multiphoton microscopy such as two-photon microscopy makes skin imaging possible at greater depths. Near infrared wavelengths are used to build up a tissue contrast based either on autofluorescence generated for example by elastin and NADH or based on second harmonic generation induced by collagen proteins. Already after two weeks' application, there was a clear improvement seen in the skin zone treated with the cream containing the crocus bulb ingredient (DermCom) at 2% (verum) compared to the placebo cream treated skin.

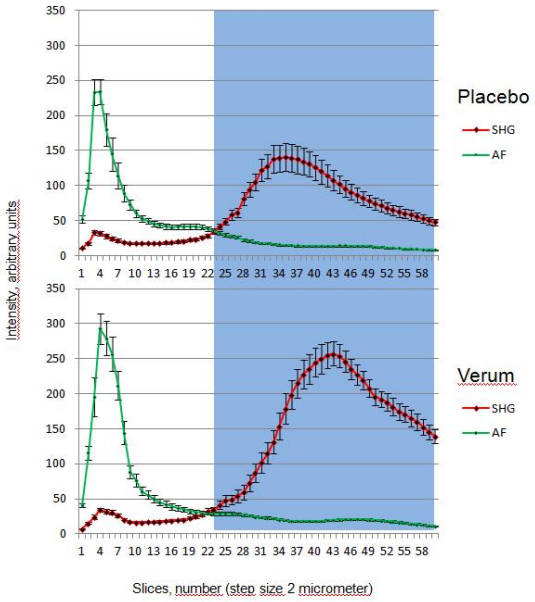


Figure 4. Autofluorescence (AF) and second harmonic generation (SHG) signal of the skin after two weeks' application

The area in blue, corresponding to the papillary and reticular layers of the upper dermis, was integrated to define collagen and elastin content. The amount of collagen was significantly increased over the baseline by as much as 115% for the verum.

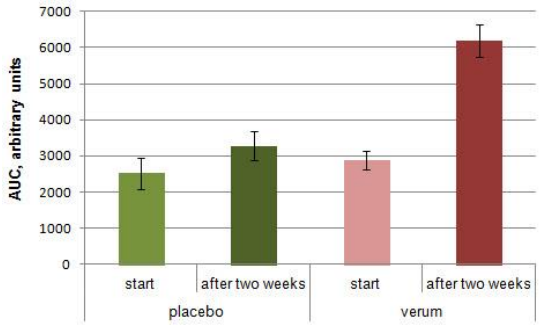


Figure 5. Increase of collagen amount in the upper dermis after two weeks' application

For the placebo, an increase of 30% was found. The difference between the two forearms was highly significant ($p<0.001$). The amount of elastin was increased by 25% for the verum and slightly decreased by 7% for the placebo.

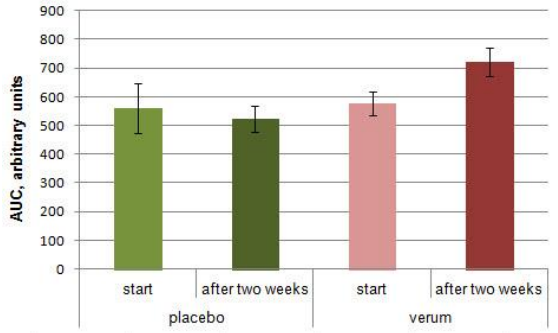


Figure 6. Increase of elastin amount in the upper dermis after two weeks' application

The difference between the two forearms was again significant ($p<0.01$). In addition to these quantitative changes in collagen and elastin amounts, the two-photon microscopy pictures showed also qualitative differences between verum and placebo. The verum was found to clearly reduce the size of wrinkles. The wrinkles in the placebo treated skin zone are visible as dark, crack-like openings that are wide at the depth of 12 microns and become narrower at 24 and 48 micron depths.

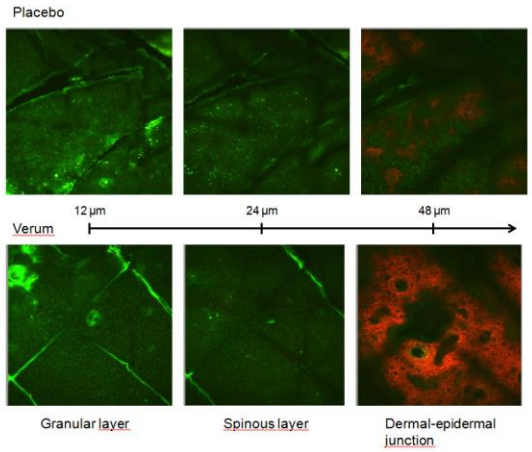


Figure 7. Two-photon microscopy images of the epidermis after two weeks'

By contrast, there was a complete absence of such dark openings, even in the superficial layers, in the verum treated skin. The tendency to increase the amount of collagen and elastin observed at week 2 persisted also at week 4. However, the difference between the two forearms was less pronounced after 4 weeks.

The efficacy of the crocus bulb ingredient (DermCom) in stretch mark treatment was assessed in a placebo-controlled clinical study. A cream with 2% DermCom was applied on stretch marks of one half of the body and the placebo cream on marks of the other half. The redness in the stretch marks treated with DermCom was significantly reduced after 56 days.

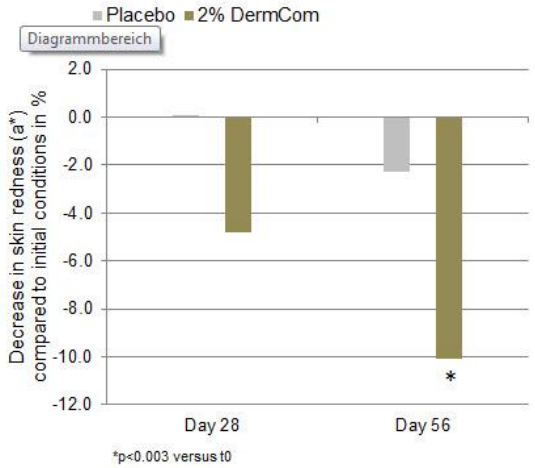


Figure 8. Decease in the redness of stretch mark skin after 28 and 56 days of treatment with a cream containing 2% DermCom

There was only a minor effect of the placebo cream. Already after 28 days, lightness was increased significantly in marks treated with DermCom.

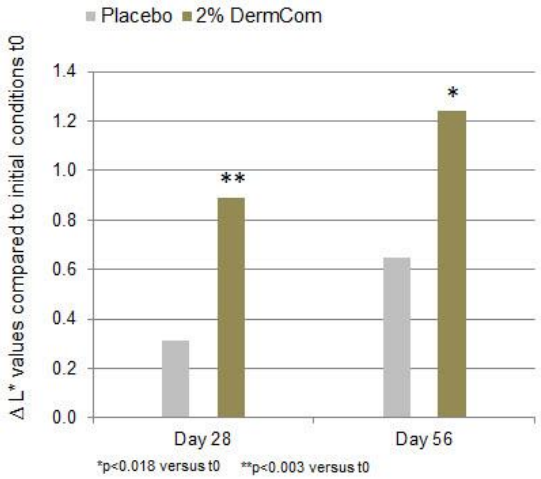


Figure 9. Increase in lightness in stretch mark skin after 28 and 56 days of treatment with a cream containing 2% DermCom. A Δ L* of 0.3 units is a visible difference.

The placebo cream was only slightly active. Also roughness was already significantly reduced already after 28 days application of the cream with DermCom.

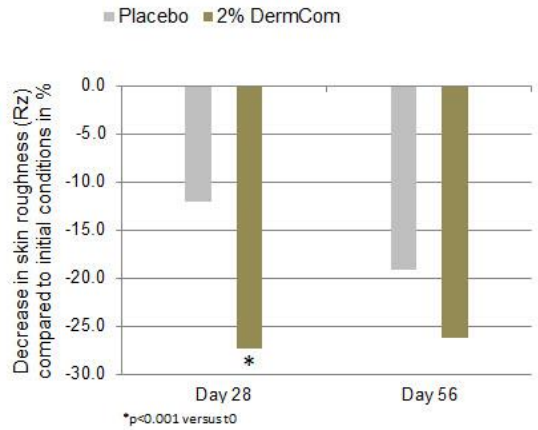
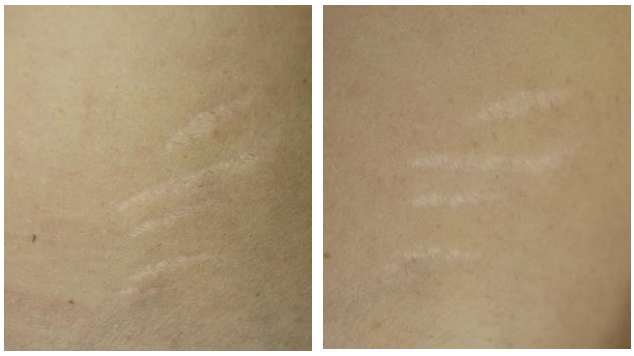


Figure 10. Decrease in the roughness of stretch mark skin after 28 and 56 days of treatment with a cream containing 2% DermCom

The visible effect of the treatment with DermCom is demonstrated by before/after pictures.



Before After

Figure 11. Digital photo of stretch marks before treatment and after 56 days of treatment with a cream containing 2% DermCom

CONCLUSIONS

Successful treatment of stretch marks needs a reorganization of the dermis. But the dermis is a skin layer that cannot be easily reached by cosmetic actives. Using cellular communication between epidermis and dermis could solve the problem. An extract of crocus bulbs was found to stimulate the release of keratinocyte-derived growth factor messengers that induced the neo-synthesis of matrix proteins in the dermis. Clinical studies demonstrated the efficacy of DermCom in the stretch mark treatment. But the dermis is also the origin of wrinkle formation because of an age-related imbalance in the synthesis and degradation of extracellular matrix components. Thus, using cellular communication between epidermis and dermis is a general anti-aging strategy.

References

1. D.H. McDaniel, Dermatologic Clinics, 20, pp. 67-76 (2002)

2. R.E.B. Watson, E.J. Parry et al., British Journal of Dermatology, 138, pp. 931-937 (1998)

3. K.S. Lee, Y.J. Rho et al., Clin. Exp. Dermatol., 19, pp. 285-288 (1994)

4. T. Quan, Y. Shao et al., J Invest Dermatol, 130, pp. 415-424 (2010).

5. G. Pascual, C. Mendieta et al., Histol Histopathol, 23, pp. 179-186 (2008).

6. B.D. Schreiber, M.I. Hughes et al., Clin Nephrol, 43, pp. 368-374 (1995).

Bolimattstrasse 1, 5033 Buchs / Switzerland
Phone: +41 62 836 17 31 Fax: +41 62 836 14 05
info@mibellebiochemistry.com, www.mibellebiochemistry.com

mibelle GROUP biochemistry