

EFFICACY OF NOVEL SKIN CREAM CONTAINING MIXTURE OF HUMAN GROWTH FACTORS AND CYTOKINES FOR SKIN REJUVENATION

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Abstract

The crucial role of growth factors and cytokines in cutaneous wound healing is well described, but there has been little investigation into their use for skin rejuvenation. A novel skin cream containing a mixture of human growth factors and cytokines was recently marketed for skin rejuvenation. The mixture was obtained through a biotechnology process using cultured human fetal skin cells, which originate from a dedicated cell bank originally established for the development of products in wound healing. The cream significantly reduced periorbital and perioral wrinkles, as well as improved skin texture of the chin after one month of treatment, which confirms the beneficial use of growth factors and cytokines for skin rejuvenation reported in 2 earlier studies. After 60 days of twice-daily application, 83% of the subject showed an improved average wrinkle score in the eye area, while 50% showed an improved average wrinkle score in the mouth area. In order to exclude a placebo effect, the efficacy of this product should be confirmed with a double-blind, randomized, placebo-controlled study. Also, the difference between mixtures of growth factor and cytokines should be further elaborated.

Introduction

The mechanistic, protective, and restorative properties of skin are impaired with age.¹ Daily exposure to a multitude of environmental stressors including sunlight additionally accentuates cutaneous deterioration related to chronological skin aging.² Clinically, aged skin is characterized by dryness, atrophy, roughness, wrinkling, sagging, as well as alterations in pigmentation.

Histologically, aside from the flattening of the dermal-epidermal junction, intrinsically aged skin displays a loss of extracellular matrix and increased levels of collagen-degrading metalloproteinases, loss of fibroblasts, and vascular network. The main histological change in photodamaged skin is dermal elastosis, or the deposition of abnormal amorphous elastic material.¹

Functional decrements as a result of intrinsic aging include reduced wound healing due to decreased keratinocyte and fibroblast proliferative capacity,³ decreased cutaneous immune responsiveness,⁴ including a reduced cytokine production,⁵ decreased fiber synthesis, and delayed recovery of barrier function after damage.⁶ For instance, the time required to reconstitute a competent skin barrier after damage is more than double in the elderly compared to young adults.⁷ This is further accentuated in photoaged skin.

While the crucial role of growth factors and cytokines in cutaneous wound healing is well described,⁸ their use for skin rejuvenation has only recently been studied.⁹⁻¹¹ Growth factors may be beneficial in reducing signs of skin aging due to their capacity in promoting dermal fibroblast and keratinocyte proliferation and in inducing extracellular matrix formation including collagens.

We report the results of a 2-center study with a novel skin cream that contains a mixture of human growth factors and cytokines (Processed Skin Cell Proteins; PSP[®]) for skin rejuvenation. The mixture was obtained through a biotechnology process using cultured human fetal skin cells originating from a dedicated cell bank established for the development of products in wound healing. Along with some other cytokines, the mixture comprises 16 growth factors known to be involved in the regulation of cutaneous wound healing.¹²

Methods

Study Design

The 2-center study was approved by the Western Institutional Review Board. Twenty Caucasian female volunteers between 35 and 65 years of age, who were not nursing or pregnant, were enrolled. They were of Fitzpatrick skin type I to IV and had demonstrable facial wrinkling in the eye and mouth skin area corresponding to grade 2 or higher in Rao-Goldman's 5-point wrinkle scale (see below). Subjects with any active skin disease affecting the face were excluded from the study. Subjects undergoing any microdermabrasion, light and medium skin peels within one month, any nonablative laser, light or radiofrequency treatments in face within 3 months, any dermabrasion, deep skin peels, ablative laser treatments, Botox[®] or filler injections, or cosmetic surgery in the face within 6 months prior to the start of the study were not allowed to participate. In order to provide sun protection, the use of the sunscreen product Coppertone[®] Shade 45 (Schering-Plough HealthCare Products, Inc., Memphis, TN) was allowed when needed.

Study Product and Treatment

The study product, Bio-Restorative Skin Cream containing a proprietary growth factor and cytokine mixture called PSP was obtained from Neocutis, Inc. (San Francisco, CA). The subjects were instructed to administer the cream to their entire randomly designated half-face in the morning and evening over a period of 60 days.

Clinical Evaluations

The following evaluations were performed:

- a) Photographic assessment at baseline (day 0), day 30, day 60, and after a 2-week period of wash-out (day 74). The half-face was photographed at an angle of 45° with the VISIA-CR Imaging System (Canfield Scientific, Inc., Fairfield, NJ).
- b) Investigator assessment at baseline (day 0), day 30, day 60, and after a 2-week period of wash-out (day 74). The fine lines and wrinkles in the periorbital and perioral skin areas were assessed in each subject by 2 trained investigators using Rao-Goldman's 5-point visual scale: 1) wrinkle absent, 2) shallow but visible, 3) moderately deep, 4) deep with well-defined edges, and 5) very deep with redundant folds.¹⁰ A photographic template providing an example for each wrinkle score for both skin areas was used in order to maximally standardize the assessment. In addition, the investigators also assessed the skin texture of the chin and cheek using the following scale: 1) smooth and soft, 2) slightly coarse and grainy, 3) coarse and grainy, and 4) bumpy and uneven. The data analysis was realized with the average of the scores obtained by the 2 investigators. The differences between the scores after 30, 60 and respectively 74 days and baseline were expressed in percentages of the respective baseline score for each subject.

- c) Subject's self-assessment at baseline (day 0) and day 60. Before and after 60 days of product use, the subjects were asked to fill out a questionnaire on the quality of their facial skin (Table 1). The difference between the scores before and after treatment was expressed in percentages of the respective baseline score for each subject.

Statistical Analysis

The investigator's clinical grading scores after treatment were compared to baseline scores (before treatment) using the Friedman repeated measures analysis of variance on

Table 1. Subject questionnaire for quality of facial skin.

Q1	Do you feel your face's skin is (1) very moist, (2) moist, (3) dry, or (4) very dry?
Q2	Do you feel your face's skin texture is (1) smooth and soft, (2) slightly coarse and grainy, (3) coarse and grainy, or (4) bumpy and uneven?
Q3	Do you feel that your facial lines or wrinkles around the eye area (1) not visible, (2) shallow but visible, (3) moderately deep, (4) deep, or (5) very deep?
Q4	Do you feel that your facial lines or wrinkles around the mouth area (1) not visible, (2) shallow but visible, (3) moderately deep, (4) deep, or (5) very deep?
Q5	Do you feel that your facial skin thickness is (1) thick, (2) normal, (3) thin, or (4) very thin?

Figure 1. Photography of a subject taken before (left) and after (right) 2 consecutive months of twice-daily application of the study cream.



ranks with Student-Newman-Keuls multiple comparison. Statistical significance was accepted when $P \leq 0.05$.

Results

Of the 20 subjects enrolled, 18 subjects averaged 52 ± 8 years of age (between 38 to 65 years) completed the study. One subject left voluntarily due to reasons unrelated to the study product and one was lost to follow-up.

Photographic Assessment

The pictures of one subject taken before and after 2 months of twice-daily application of the study cream are shown in Figure 1. This subject demonstrated improved wrinkles in the periorbital and perioral area as well as tighter and firmer cheek skin.

Investigator Assessment

The averaged periorbital and perioral wrinkle scores at baseline, after 30 and 60 days of twice-daily cream use, and following a 2-week period of wash-out of not using (day 74) are shown in Figure 2.

Starting after 30 days of treatment, a statistically significant reduction in the periorbital and perioral wrinkles was observed. The reduction remained statistically significant after 2 months of treatment as well as after the wash-out. After 30 days, the mean in the periorbital wrinkle score decreased by 9%, whereas the mean in the perioral wrinkle score decreased by 8%. The wrinkle scores further improved

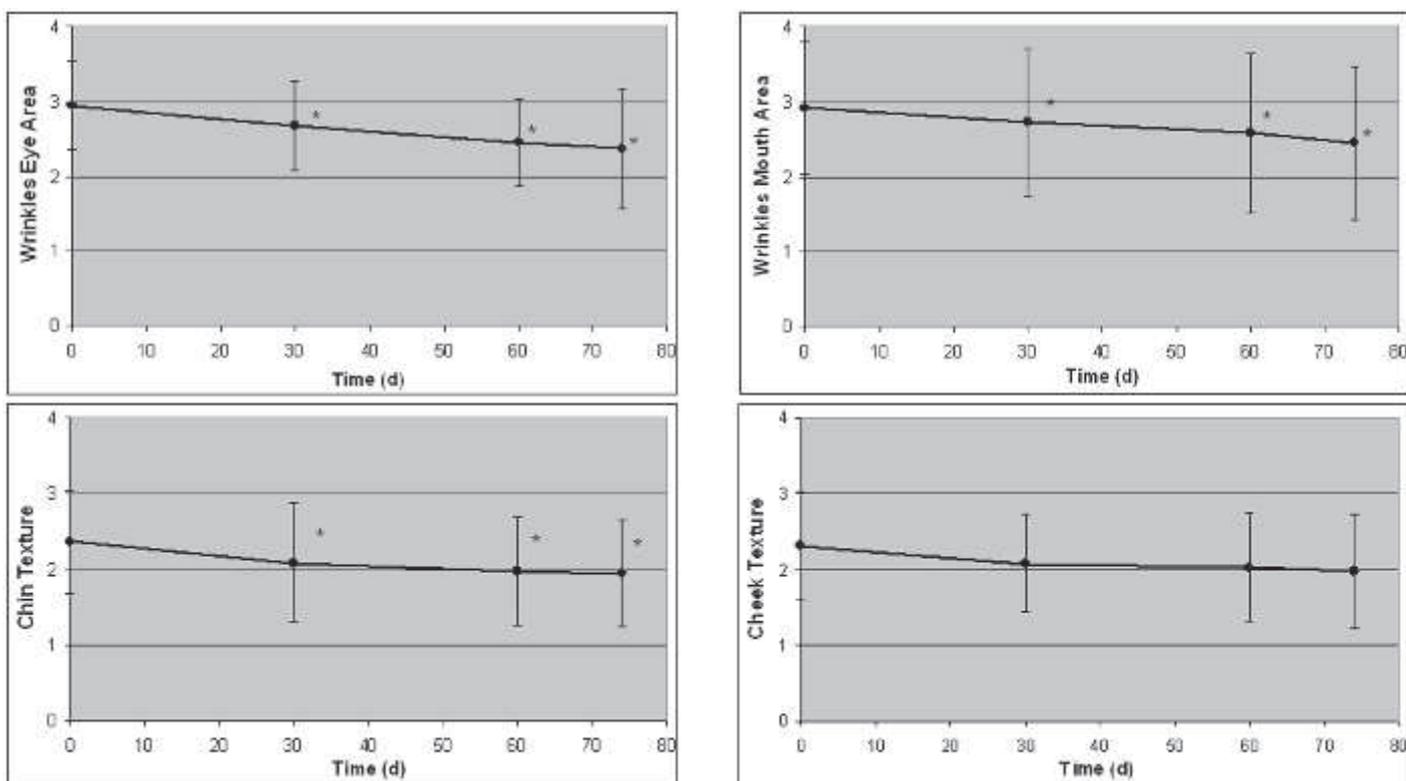
after 60 days; the periorbital wrinkles decreased by 17% and the perioral wrinkles by 13%. After this period, 83% of all subject completing the study showed an improved average wrinkle score (by at least 0.5 units) in the eye area, while 50% of the subjects showed an improved averaged wrinkle score in the mouth area (Figure 3). Interestingly, the wrinkles further improved during the wash-out. After a 2-week period of not using the cream use, a decrease in the periorbital wrinkle score by 20% and in the perioral wrinkle score by 17% was observed.

The chin texture also showed statistically significant improvement after 30 days treatment (Figure 2). The improvement remained statistically significant throughout the entire study period. The mean in the chin texture score decreased by 13% after 30 days, 17% after 60 days, and 18% after the wash-out. The mean in the cheek texture score also decreased by 9% after 30 days, 12% after 60 days, and 14% after the wash-out. However, these differences did not reach statistical significance (Figure 2). After 60 days, 67% of all subjects completing the study showed an improved average skin texture score (by at least 0.5 units) in the chin, while 56% of the subjects showed an improved averaged skin texture score in the cheek (Figure 3).

Subjects' Self-Assessment

The subjects were asked to rate the quality of their facial skin with a questionnaire before and after treatment. At the

Figure 2. Mean of facial wrinkle and texture scores as a function of time as assessed by investigators. The scores at baseline, after 30 days, and after 60 days of twice-daily cream application as well as following a 2-week period of not using cream or wash-out (at day 74) are given (mean \pm standard deviation, $n=18$).



end of the 60-day treatment period, a 9% decrease in the mean periorbital wrinkle score and a 17% decrease in the mean perioral wrinkles score were reported by the subjects. For both areas, 44% of all subject completing the study reported an improved periorbital and perioral wrinkle score by at least one unit. In addition, 44% reported an increased global facial skin texture, 39% an increased thickness, and 33% a more moist skin.

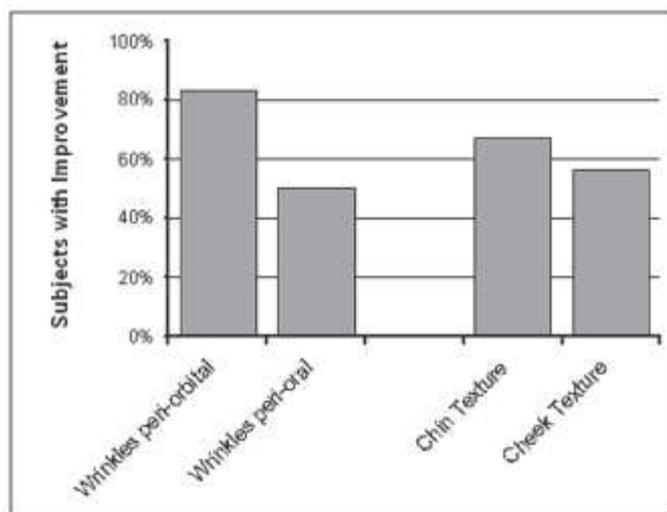
Comment

This study revealed that a cream containing a proprietary mixture of human growth factors and cytokines (called PSP or Processed Skin Cell Proteins) significantly improved facial wrinkles up to 20% after 2 months of twice-daily application.

Together with the earlier studies on other topical growth factor products,⁹⁻¹¹ this study provides further evidence for the beneficial use of growth factors and cytokines in the treatment of skin aging. One study with 14 subjects reported a 12.2% improvement ($P \leq 0.05$) of the periorbital area, while the perioral area improved 8.5% ($P = .09$) after 2 months of twice-daily application of a gel formulation containing a proprietary growth factor solution.⁹ Therefore, a 9-point visual wrinkle scale was used. In another study involving 31 subjects using the same wrinkle scale, a single growth factor formulation comprising recombinant transforming growth factor beta-1 was shown to improve facial wrinkles by 12% after 3 months of twice-daily application.¹¹

In principle, growth factors and cytokines may participate in skin rejuvenation at various levels due to their multifunctional activities. Growth factors and cytokines may promote growth of keratinocytes, dermal fibroblasts, and other cells with decreased proliferative capacity due to aging after topical application, which is similar to observations in cutaneous

Figure 3. Percentage of subjects with improved facial wrinkles and texture as assessed by investigators after 60 days of twice-daily cream application. Improvement in facial wrinkles and texture was defined when the averaged score decreased by at least 0.5 units in a subject.



wound healing.⁸ Furthermore, some growth factors are potent stimulators of the expression of extracellular matrix proteins including collagen.¹³ A reduced capacity to synthesize collagen relative to healthy young skin is characteristic of chronologically and photoaged skin.¹⁴ Collagen degradation is another characteristic feature of photodamaged skin.¹⁵

Cutaneous wound healing is regulated by a large variety of cytokines, growth factors, and their receptors and modulators.⁸ Cytokines and growth factors do not operate in isolation, but form a complex, only partially understood, well-orchestrated network. As a paradigm for ideal tissue repair, scarless wound healing in fetal skin after surgery *in utero* particularly illustrates this orchestration.¹⁶ Based on the fact that a series of cutaneous functions responsive to growth factors are impaired with skin aging, one may speculate that skin aging may also be preferentially treated by an appropriate combination of growth factors and cytokines, similarly to wound healing.¹⁷

Inspired by the unique properties of fetal skin, the growth factor and cytokine mixture contained in the study product was obtained after disrupting cultured fetal skin cells originating from a dedicated cell bank by freeze-thawing. Proteomic analysis of this mixture indicated the presence of most growth factors known to be involved in the regulation of cutaneous wound healing.¹²

To show efficacy for skin rejuvenation, growth factors and cytokines must penetrate skin in order to reach their target (ie, the respective cell surface receptor on keratinocytes or fibroblasts). However, proteins as large as growth factors and cytokines seem unlikely to penetrate skin.¹⁸ On the other hand, various examples of protein contact allergies including latex imply that proteins indeed can penetrate intact skin after topical exposure, at least to a small extent.¹⁹ This level of penetration is sufficient to induce an immediate allergic reaction. Similarly, due to the fact that growth factors and cytokines act at very low concentrations, they may penetrate normal skin in sufficient amounts to reach their respective cell surface receptors thereby inducing a response. Likely, these molecules may penetrate skin predominantly in a vertical pathway through the natural imperfection of skin such as 1) the follicular apparatus of hair follicles, 2) the sweat glands, and 3) microlesions in the interfollicular stratum corneum.²⁰

Placebo data were neither reported for this study nor for the two initial studies on topical growth factor products.^{9,10} However, a recent study with the transforming growth factor beta-1 containing formulation provided evidence that the observed reduction in facial wrinkles can be attributed to the presence of this growth factor.¹²

Conclusion

This study and the previously published studies with products containing a single or a combination of growth factors indicate that topical application of growth factors and cytokines are also beneficial in reducing signs of facial skin aging. In fact, when comparing these study outcomes with

a recently published clinical trial with a 0.1% tretinoin regimen after 12 and 24 weeks,²¹ the reduction in facial wrinkles is rather comparable.

Although there is ample evidence that growth factors and cytokines help reduce signs of skin aging, their beneficial effect for skin rejuvenation as well as differences between mixtures of growth factor and cytokines remain to be further studied.

Disclosure

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