Topical Growth Factors for Skin Rejuvenation

Frank Dreher

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Abstract

Growth factors play a key role in the regulation of numerous cell processes including wound healing. More recently, they have been recognized also for use in skin rejuvenation. Aged skin reveals a similarly altered growth factor response as a chronic wound. Growth factors may reduce signs of skin aging due to their capacity to promote dermal fibroblast proliferation and to stimulate extracellular matrix formation. Growth factor products for skin rejuvenation can contain either recombinant growth factors, growth factors as part of conditioned cell culture media, or growth factors as part of cell lysates. Numerous randomized controlled clinical trials demonstrated the good tolerability and efficacy of those products. Today, there is evidence that the signs of aging skin may be best improved with a balanced mixture of growth factors.

Introduction

Growth factors are polypeptides or proteins that play a key role in the regulation of numerous cell processes [1]. Together with cytokines, they help regulate division, differentiation, chemotaxis and adhesion, trafficking, activation, apoptosis, survival, and transformation of cells. Growth factors are able to mediate activities within a specific

F. Dreher (✉)
MERZ North America, Inc., San Mateo, CA, USA
e-mail: frank.dreher@merz.com

M.A. Farage et al. (eds.), *Textbook of Aging Skin*,
DOI 10.1007/978-3-642-27814-3_100-3

tissue microenvironment at extremely low concentrations. The growth factor binds to its corresponding receptor, located on the outer surface of the target cell, and ultimately, through a complex cascade of events, elicits a response.

Growth factors are key regulators of the wound healing process [2], and their topical use for cutaneous wound healing has been extensively described [3–5]. Clinical trials have indicated that the topical administration of certain growth factors reduces healing time and improves the rate of wound closure. These particular growth factors include epidermal growth factor (EGF), fibroblast growth factor-2 (FGF-2), transforming growth factor-beta (TGF-β), and platelet-derived growth factor (PDGF).

While for the most part, adult cutaneous wounds heal with scar formation to restore tissue integrity after wounding, in utero, rapid and perfect skin repair occurs [6]. Although complex and not fully understood, current research indicates that at specific ratios, several growth factors and cytokines participate in the scarless wound repair that is observed in fetal skin.

Richard E. Fitzpatrick first demonstrated that topical growth factors help improve such signs of skin aging as wrinkles [7]. In fact, it is recognized that aged and photodamaged skin, related to an altered growth factor response, reveals attributes similar to a chronic wound [8]. Growth factors may reduce signs of skin aging due to their capacity to promote dermal fibroblast proliferation and to stimulate extracellular matrix formation, including that of collagen and hyaluronic acid.

A study on the role of growth factors in human skin aging revealed that TGF-β1 and its downstream target, connective tissue growth factor (CTGF), are significantly reduced in aged human skin in vivo [9]. Moreover, it was shown that in a serum, a higher ratio of insulin-like growth factor-1 (IGF-1) to its binding protein (insulin-like growth factor binding protein-3 or IGFBP-3) was associated with a lower perceived age. This association was established because of its connection with reduced skin wrinkling [10]. However, whether or not high levels of IGF-1 delay the onset of skin wrinkles remains to be investigated.

This chapter provides a brief overview of growth factors and wound healing including their role in scarless skin repair and focuses on the review of clinical studies of topical growth factors for skin rejuvenation.

Growth Factors in Wound Healing

Wound healing is a regulated process consisting of distinct phases: hemostasis, inflammation, granulation tissue formation, and remodeling [3]. It is well recognized that growth factors are one of the key regulators of the wound healing process [2]. After skin injury occurs, platelets arrive and release several growth factors and cytokines. Once released, these proteins initiate the wound healing process by attracting neutrophils and monocytes. The result is an acute inflammatory phase that lasts between 1 and 2 days in normal wounds. After that, the growth factors (which include FGF-2, PDGF, and TGF-β) promote cell proliferation and new tissue formation that also includes fibroblast-initiated extracellular matrix production. During this time, the number of inflammatory cells decreases and the number of fibroblasts in the wound area increases. Stimulated by EGF, epithelial cells from the wound margins and dermal appendages migrate under the scab for re-epithelialization. During the final phase of wound healing, the extracellular matrix is reorganized. Over time, collagen type III, which was initially produced by fibroblasts, is gradually replaced with collagen type I. This type I collagen has a higher mechanical strength than its type III counterpart because of a higher degree of cross-linking. Several growth factors participate in the remodeling phase, which can take up to 2 years to complete, and often results in scarring. High levels of TGF-β1 and TGF-β2 are associated with scar formation [11].

As in the case of chronic wounds, sometimes healing can be significantly impaired. In contrast to acute wounds, pressure and non-healing dermal ulcers produce reduced levels of growth factors EGF, FGF-2, TGF-β, PDGF, and VEGF (vascular endothelial growth factor) [4]. It would seem evident, therefore, that topical applications of a

mixture of growth factors would help patients with chronic wounds: for example, those growth factors that have been well studied in chronic wound healing such as EGF, FGF-2, TGF-β, PDGF-BB, VEGF, or KGF-2 (keratinocyte growth factor) [3]. In fact, recombinant human PDGF-BB (at a concentration of 0.01 % in a hydrogel vehicle) was the first topical growth factor formulation to be approved in the United States for the treatment of deep neuropathic diabetic foot ulcers.

Wounds heal slower with age [12]. In the elderly, re-epithelialization is significantly delayed as the rate of collagen formation decreases. Wound healing becomes impaired as dermal fibroblasts decrease and as the proliferative capacity of keratinocytes, dermal fibroblasts, and vascular endothelial cells is reduced. Animal experiments reveal that reduced productions of growth factors and their receptors may be responsible for impaired wound repair in aged mice [13, 14].

While cutaneous wound healing may result in scar formation in adults, wound healing in utero (fetal wound healing) is scarless [6]. Early gestation fetuses in both animals and humans have the remarkable ability to heal skin wounds without forming a scar. It has been established that scarless wound repair is independent of the obvious environmental differences (i.e., amniotic fluid) between fetus and adult and can progress outside the uterus [6]. In fact, the fetal fibroblast was found to be the main effector cell for scarless wound healing [15]. Fetal fibroblasts have the ability to deposit collagen in a fine, highly organized pattern during fetal skin wound repair that is indistinguishable from the surrounding uninjured dermal collagen. This is in contrast to the coarse pattern of collagen deposition observed during wound healing in adults. Skin is primarily composed of collagen types III and I. The former is smaller and finer than the latter, and although type I is the predominant form in both fetal and adult skin, collagen type III is more abundant in fetal skin. In humans, collagen type III comprises 30–60 % of the total fetal skin. In adults, it comprises only 10–20 % [6]. As the fetus develops, the ratio of skin collagen types I to type III approaches that of adults, correlating with the transition from scarless wound repair to scar formation. TGF-β is believed to be one of the most implicated growth factors in regulating scar formation and for this purpose has been widely studied [6, 14]. Three highly homologous isoforms are known in humans: β1, β2, and β3. In fetal wounds, low levels of TGF-β1 and TGF-β2 and high levels of TGF-β3 are associated with scarless repair. Conversely, in the wounds of adults, predominantly TGF-β1 and TGF-β2 are present, which is linked to fibrosis and ultimately scarring. As TGF-β1 and β2 neutralizing antibodies, or exogenous TGF-β3, have in part been shown to prevent scarring [16, 17], so too should growth factors, and cytokines other than TGF-β participate in scarless wound repair. For example, PDGF, VEGF, and FGF isoforms were implicated in the transition from scarless to scar-forming wound repair [6]. Despite an increase over the past decades in our knowledge of the mechanisms of wound healing, that of fetal wound healing remains only partially understood.

Skin Aging

Skin's mechanistic, protective, and restorative properties are impaired with age. Skin aging occurs through two biologically distinct processes: intrinsic and extrinsic aging [18]. Intrinsic skin aging is a naturally occurring process caused by an accumulation of age-associated degenerative changes, such as progressive telomere shortening and oxidative damage as a result of aerobic cellular metabolism [19]. In human skin, intrinsic aging is characterized by dermal atrophy (thinning), flattening of the rete ridges, and a reduction in dermal fibroblasts. A loss of vascular network or a weakening of blood vessels also causes intrinsic aging. Intrinsic aging has a significant effect on the dermal collagen fiber network. Collagen biosynthesis steadily declines toward the third and fourth decades of life. Subsequently, it remains at levels too low to permit mature skin to repair and replace the collagen it loses as part of the degenerative, age-associated process [18]. Collagen depletion is the age-related consequence of a

decrease in collagen formation and an increase in collagen-degrading enzymes (such as matrix-metalloproteases-1 or MMP-1). Elastin provides skin with elasticity and resilience. Until around 50 years of age, elastin biosynthesis remains constant. After that, it declines sharply [18, 20]. Although it is established that both elastase and also MMP-2 play a role in elastin degradation, the underlying etiology of age-related changes in elastin is not well understood.

Extrinsic or premature skin aging is interconnected with the intrinsic components of skin aging. Premature skin aging is primarily caused by photodamage – the exposure of unprotected skin to ultraviolet irradiation from the sun. Sun exposure is thought to account for up to 80 % of facial aging [19]. Smoking, air pollution, and poor nutrition are other causes of premature skin aging. Chronic sun exposure promotes degradation of collagen and elastin, resulting in the accumulation of abnormal amorphous elastic material in the dermis (solar elastosis). The increased degradation of extracellular matrix proteins caused by sun exposure, combined with the decreased regenerative capacity of dermal fibroblasts caused by aging, lead to the characteristic signs of photoaged skin (deep wrinkling, furrowing, and the loss of skin elasticity).

Skin Rejuvenation with Growth Factors

A large variety of antiaging ingredients and sunscreens are used today in an attempt to slow or reverse skin aging [19, 21]. One such ingredient that has recently emerged is the growth factor. The following paragraphs review growth factors and their role in skin rejuvenation.

There are three distinguishable categories of skincare products that contain growth factors:

- Products that contain single or multiple recombinant growth factors
- Products that contain a combination of growth factors as part of conditioned cell culture media
- Products containing a mixture of growth factors as part of cell lysates

Skincare Products with Recombinant Growth Factors

Several products available today are marketed as containing one or more recombinant growth factors – usually TGF-ß1 or EGF. Regrettably, limited studies exist that report on the efficacy of such products for skin rejuvenation. These particular recombinant growth factors are often obtained from yeasts or plants that are altered to produce the desired growth factor proteins. This process, which involves genetic engineering, is known as "recombinant DNA technology." In wound healing, TGF-ß1 and EGF are two of the more widely studied growth factors. So predictably, several antiaging products are marketed as containing those growth factors.

A study investigated the efficacy of an antiaging formulation containing TGF-ß1 and ascorbic acid in a silicon base [22]. In the study, 7 of the 12 (58 %) female participants indicated that they saw a visible improvement in their wrinkles and skin texture after 3 months of twice-daily use. This perception was confirmed by a panel of four dermatologists who used a five-point facial wrinkle visual scale to assess the high-quality digital photographs taken before and after the treatments. A later study [23] reported that for reducing wrinkles, the same TGF-ß1-containing product was superior to a similar cream that did not contain TGF-ß1. In the study, 4 of the 12 (33 %) participants noticed an improvement when using the product free of TGF-ß1. Both products were well tolerated.

More recently, a study in 29 female subjects demonstrated that serum containing barley-bioengineered EGF [24] improved visible signs of aging in the facial skin when used twice a day for 3 months. Clinical evaluations showed statistically significant improvements within the first month of use in the appearance of pore size, skin texture, fine lines, rhytids, and various dyschromatic conditions. These trends continued to improvement for the duration of the study. The serum was well tolerated with minimal treatment-related complications.

A study of a preparation comprising recombinant EGF, bFGF, KGF-2, IGF-1, and hyaluronic

acid [25] demonstrated that the formula significantly improved periorbital wrinkles after 4 and 8 weeks of twice-daily use. An investigator assessed the subjects using a skin roughness analysis.

Because the particular skin functions that are affected by aging are responsive to growth factors, it would make sense that a skincare product containing more than one growth factor would be efficacious in reducing the visual signs of skin aging. However, limited clinical data exist that report on skincare products comprising more than one recombinant growth factor. The majority of skincare products that contain multiple growth factors obtain these proteins using technologies other than recombinant DNA technology. These product types are further described in the following paragraph.

Skincare Products with Growth Factors as Part of Conditioned Cell Culture Media

Currently, there are several lines of skincare products that contain a mixture of natural (nonrecombinant) growth factors as a part of the conditioned media. These growth factor ingredients are manufactured in a biotechnology process that involves collecting the cell culture media after the cells have been cultured. The cell culture media, also known as conditioned media, consists of growth factors and cytokines, as well as remnants of the original medium that was used to culture the cells. It does not, however, contain cells.

The first growth factor ingredient used for skin aging that was obtained from conditioned media (Tissue Nutrient Solution; TNS®) is collected after culturing neonatal human dermal fibroblasts [7]. It is reported to contain over 110 growth factors, cytokines, and soluble matrix proteins [22]. Since its introduction, several scientific articles have reported on the efficacy of TNS for skin rejuvenation.

One journal article described a study involving 14 subjects that used TNS in a hydrogel formulation [7]. The study reported that after 2 months of twice-daily application, an improvement of 12.2 % ($p \leq 0.05$) was observed in the periorbital area and 8.5 % ($p = 0.09$) in the perioral area. Photodamage was clinically graded using a nine-point visual wrinkle scale. At the beginning and end of each treatment session, photodamage and skin roughness of the lateral cheek area were assessed using silicon replica technology and punch biopsy.

In a 3-month split-face study of the hydrogel, 14 of the 19 (74 %) subjects completing the study showed improved or no worsening of their averaged wrinkle scores [21]. The severity of wrinkles was assessed by four dermatologists using a five-point visual scale and high-quality digital photographs taken before and after each treatment. The same study included a comparison of a TGF-β formulation and the TNS hydrogel. Twenty-seven of the 31 (87 %) participants showed improved or no worsening of their average wrinkle scores after treatment with the single growth factor formulation. On average, the wrinkle scores of all 31 subjects improved by 12 % [23]. Using a selected statistical model, the investigators concluded that there was no statistically significant difference between the hydrogel formulation with the growth factor mixture and the formulation with the single growth factor. Both products were well tolerated.

The antiaging benefit of TNS hydrogel was confirmed in a double-blind, placebo-controlled study of 60 subjects [26]. For at least 4 weeks after enrollment, each subject used a basic skincare regimen consisting of a gentle skin cleanser and a daily facial moisturizer, which provided a sun protection factor (SPF 15). After that, subjects were randomized into two groups and instructed to apply to their skin either the active hydrogel or the vehicle twice a day for 6 months. They were instructed to apply the respective hydrogel formulation after skin cleansing and before application of the SPF15 moisturizer. The investigator clinically assessed treatment efficacy by using a five-point visual scale and silicon replica technology. An independent panel of three dermatologists reviewed photodamage from photographs using an 11-point comparative scale. Assessments were conducted at the baseline visit at 3 months and

again at the 6-month visit. As assessed by the investigator after 6 months, in the active group (27 subjects), photodamage parameters, namely fine wrinkling (0.33 ± 0.55), mottled pigmentation (0.48 ± 0.58), and tactile roughness (0.52 ± 0.85) score units significantly decreased from baseline. In the vehicle group (26 subjects), mottled pigmentation (0.65 ± 0.69) and tactile roughness (0.58 ± 0.81) also decreased. Fine wrinkling, however, did not significantly decrease (0.15 ± 0.46). In the vehicle group, photographic evaluation showed a worsening of photodamage indicated by a negative change in the photodamage scores. The active group showed either a slight improvement or a substantial prevention of worsening in photodamage as indicated by a smaller change from baseline at 6 months. Whereas the difference between active and vehicle group was not statistically significant ($p = 0.323$) after 3 months, there was a leaning toward a statistically significant difference ($p = 0.083$) after 6 months. When analyzing the data from only those with severe photodamage, the five subjects who used the active hydrogel for 6 months showed a statistically significant improvement (by 0.4 points). The six subjects in the vehicle group, however, showed a statistically significant worsening (by 1 point) in photodamage scores. The study showed that after 3 and 6 months of treatment, the active hydrogel reduced fine lines and wrinkles better than the vehicle, when a silicon replica technology was used to assess roughness and shadow parameters of the periorbital area. After 3 months of treatment, no significant differences between the active and vehicle groups were noticed in either the reduction of deep lines or in skin roughness ($p = 0.290$). In the reduction of fine line shadows ($p = 0.045$), the differences were either significant or tended toward statistical significance. After 6 months, the differences were either significant or tended toward statistical significance in the reduction of deep lines and roughness ($p = 0.06$) and in the reduction of fine line shadows ($p = 0.072$). Although one subject treated with active gel discontinued because of dislike of the cosmetic attributes of the product, overall the active hydrogel was well tolerated.

In a 3-month study of 37 subjects, a serum containing TNS hydrogel, combined in situ with collagen-building peptides, antioxidants, and depigmenting agents, was evaluated for the improvement of the visible signs of facial photodamage [27]. After 1 month of treatment, the clinical evaluations showed statistically significant reductions in coarse and fine wrinkles and improvements in skin tone, texture, and radiance with continued improvements after 2 and 3 months. Decreased skin extensibility and increased resiliency were further measured. The serum was well tolerated with no treatment-related adverse events reported during the study period.

Recently, another company introduced a product containing growth factors derived from human cell-conditioned media. The growth factors were obtained from neonatal cells cultured under conditions of low oxygen tension. Although the so-obtained conditioned media contain a variety of growth factors and cytokines (including VEGF, KGF, and IL-8), it is devoid of TGF-β proteins [28]. In a proof of concept study, two products with this conditioned media were tested over 3 months in 21 female subjects with mild to moderate photodamage, fine lines, and wrinkles [29]. The study results showed that after 2 weeks, tactile roughness, texture, and wrinkles significantly improved ($p < 0.05$) from baseline and continued to improve. At 12 weeks, an improvement over baseline reached 44.9 %, 46.2 %, and 29.9 %, respectively. No treatment-related adverse events were recorded during the study.

Skincare Products with Growth Factors as Part of Cell Lysates

Inspired by the scarless wound healing properties of fetal skin cells, another company developed a skincare product with the cell lysate of fibroblasts (Processed Skin Cell Proteins; PSP®). The cells are obtained from a dedicated cell bank of cultured human fetal fibroblasts. The cell bank, which originated from a single biopsy of donated skin tissue, was established for the purpose of

developing wound healing products. PSP is produced from cultured fibroblasts that are harvested by centrifugation. The conditioned culture liquid (or spent culture media) produced during the process is removed and discarded as it consists of cellular waste, red dye, and remnants of unwanted culture media. The collected fibroblasts are washed with a physiological buffer to help remove any lingering traces of culture media, and the cell walls are freeze-thawed. This freeze-thaw process ruptures the cell membranes, leading to cell lysis, and allows the fetal fibroblast cell lysate to be obtained.

PSP is made up of a combination of proteins and other cytokines that include human growth factors. Proteomic analysis of the formula detected more than 100 growth factors and cytokines, including those growth factors most studied in the regulation of cutaneous wound healing [2]. Additionally, PSP was shown to contain several anti-inflammatory cytokines, including the interleukin-1 receptor antagonist (IL-1RA). In their study, Kupper and Groves demonstrated that IL-1RA exerts an anti-inflammatory response by competitively binding the IL-1 receptors without triggering a signal [30]. Other cytokines such as tumor necrosis factor α (TNF-α), interferon γ (INF-γ), and the tissue inhibitors of metalloproteinases 1 and 2 (TIMP-1, TIMP-2) were also detected in PSP.

In an 8-week study to evaluate the antiaging benefits of the first cream with PSP, doctors Gold and Goldman assessed 20 female subjects between the ages of 35 and 65 years with demonstrable facial wrinkles [31]. Investigators determined improvements using a five-point visual score of both clinical assessments and photographs of perioral and periorbital wrinkles. Subjects determined improvements using a questionnaire. After 1 month of treatment, a statistically significant ($p \leq 0.05$) reduction in periorbital and perioral wrinkles was observed. After 2 months, the periorbital wrinkles decreased by an additional 17 % and the perioral wrinkles by 13 %. After the full 8-week study period, of all the subjects who completed the study, 83 % showed an improved average wrinkle score of at least 0.5 units around the eye area and 50 % around the mouth.

PRIMOS, or Phase shift Rapid In-vivo Measurement of Skin (PRIMOS, GFM, Tetlow, Germany), is an optical three-dimensional (3D) in vivo skin-measuring device that allows contact-free, direct, and fast measurement of skin surface topography at high resolution. PRIMOS measurements are based on a digital parallel stripe pattern-imaging technique that is projected onto the surface of the skin and depicted in the charge-coupled device (CCD) chip of a high-resolution camera. The light patterns are created by a digital micromirror projector (Texas Instruments, Irving, Texas) that is valuable when applied to optical 3D in vivo skin measurements because exposure times are short, and the high light intensity can be controlled point- and pixel-wise, or both. Because the device is contact-free, it is less prone to artifact buildup and, therefore, more reliable than the silicon replica technology commonly used for topography purposes [33, 34]. Complex mathematical algorithms embedded in the analytical software reconstruct the data into highly precise 3D skin surface images, which allow accurate measurements of the depth of fine lines and wrinkles. Additionally, the short exposure time guarantees that the involuntary movements of the subject do not influence the captured data. Finally, PRIMOS allows the recorded data (i.e., the precisely located skin wrinkles) to be matched and thus accurately compared from the before- and after-treatment images (See Fig. 1a, b).

In a study by Gold and colleagues, subjects treated with PSP cream were assessed after quantitatively measuring skin surface topography using the PRIMOS device [32]. Eighteen female subjects completed a double-blind, placebo-controlled split-face study aimed at assessing skin topography after 2 months of twice-daily Bio-Cream application. The study revealed significant improvements in periorbital skin topography. When the after-treatment was compared to the before-treatment images, skin surface roughness was shown to have decreased between 10 % and 18 % ($p \leq 0.05$), depending on the roughness parameters. The roughness parameters for maximum depths R_{zmax}, R_{3zmax}, and R_{zISO}, in particular, decreased by 15 % or more after the

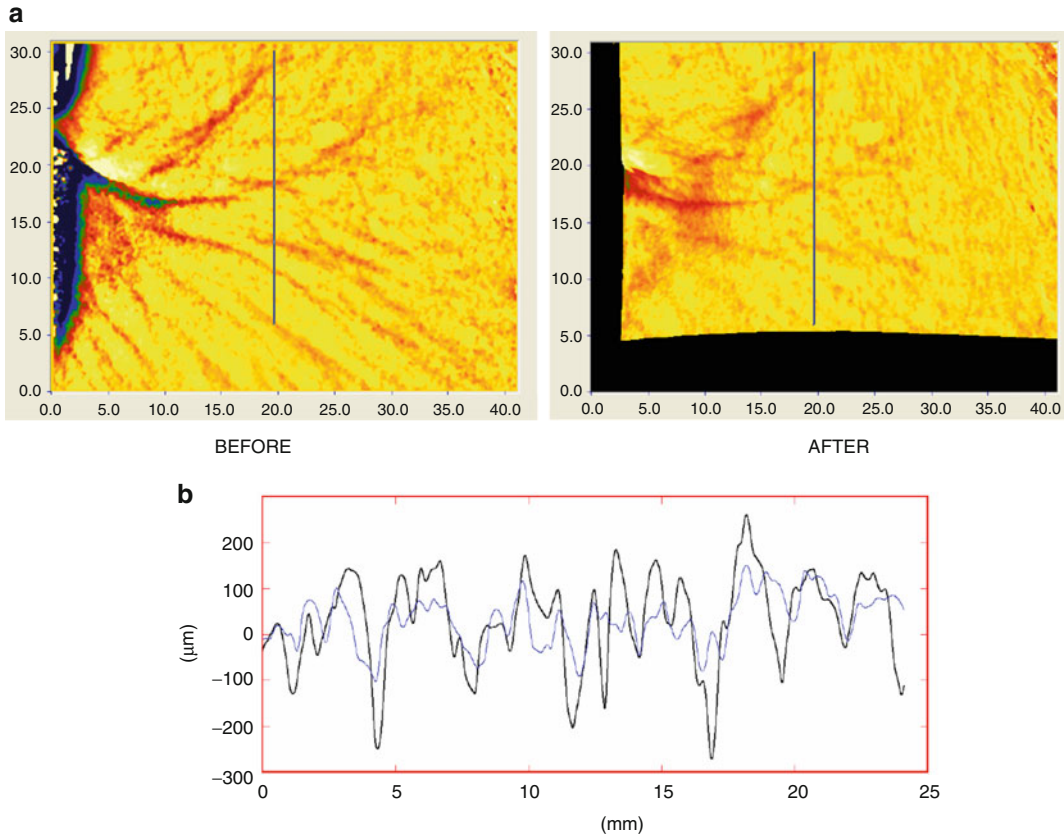

Fig. 1 Example of PRIMOS-3D color-coded skin topography picture of 40 × 30 mm periorbital skin measurement area before (**a**, *left*) and after (**b**, *right*) 2 months of twice-daily use of the human growth factor and cytokine skin cream. The color-coded scale for skin depth is in millimeters (mm). The pictures were matched in order to overlap the periorbital surfaces of the before and the after measurement. This allows analysis of structural changes caused by the treatment along cut lines. The skin profile along the vertical cut lines of major periorbital wrinkles including the crow's feet is shown in (**b**). As compared to the baseline profile (**b**, *black profile*), a significantly smoother profile representative for decreased skin roughness or a reduction in the depth of fine lines and moderate and deep wrinkles was obtained after the treatment (**b**, *blue profile*)

study period. The "extreme" roughness parameters described such pronounced signs of periorbital facial skin aging as moderate and deep wrinkles, including those of crow's feet. In contrast to the cream, twice-daily treatments for 2 months with the placebo formula (which was identical to the PSP cream) did not result in significant changes to the roughness parameters R_{zmax}, R_{3z}, R_{3zmax}, and R_{zISO}. Only R_a and R_z significantly decreased (by about 10 %). After the study period, the differences between active and placebo groups for Rz_{max}, R_{3z}, and R_{zISO} were statistically significant ($p \leq 0.05$). The differences between active and placebo for R_a (Remark: Although the average roughness R_a is one of the most frequently used parameters in surface measurement, this parameter does not inform about all aspects of the surface. Surfaces with the same Ra may differ significantly in shape. In order to distinguish surfaces, other parameters about peaks and valleys and profile shapes and spacing are commonly used.), R_z (Remark: R_z is a mean parameter; here, it is the average of five single roughness depths over the entire sample length. This parameter has some advantages in finding extremes in the roughness, but it is not sensitive to single unusual features of the surface. R_{zmax}, which corresponds to the maximal R_z within the

five measuring length, seems a better descriptor for unusual features of the surface. In the present case, deep wrinkles such as crow's feet can be regarded as such an unusual feature. R_{zISO} is another parameter which helps to further describe rather extreme and unusual skin surface patterns), and R_{3zmax} did not reach statistical significance. The difference, however, for R_{3zmax} was close to significant ($p = 0.06$) and may have reached statistical significance if more subjects had participated in the study. The baseline values for the roughness parameters did not differ significantly between the active and placebo groups. The cream was well tolerated.

The antiaging benefits of skin cream with PSP were confirmed in an additional study investigating improvements of signs of facial skin aging when used for a prolonged period (6 months) [35]. The 11 female subjects who completed the study all underwent a punch biopsy for light and electron microscopy evaluation at the beginning and end of the treatment periods. Further evaluations included photographic and clinical assessment of skin for signs of facial wrinkles using the same five-point visual wrinkle scale as was used in the 2-month IL-1RA study by Kupper and Groves [30]. After the 6-month treatment period, signs of periorbital wrinkles were reduced by 33 % and perioral wrinkles by 25 %. Furthermore, chin texture improved by 21 % and cheek by 39 %. All subjects tolerated the cream well without the occurrence of adverse events.

An eye cream with PSP was evaluated in a multicenter study investigating periorbital skin rejuvenation [36]. Thirty-seven female subjects between the ages of 36 and 65 years completed the study. Results revealed that on average, signs of wrinkles – lower eyelid bags, sagging, and dark circles – and skin texture were significantly reduced ($p \leq 0.05$) by between 14 % and 28 % after 6 weeks of twice-daily application. A subject questionnaire further confirmed the clinical improvements. The subjects reported that their "tired look" improved on average by 32 %. The eye cream was well tolerated and all subjects "liked the way it felt." The formulation's efficacy, excellent tolerability in the delicate periorbital skin area, and pleasant sensory properties explain why a large majority (78 %) of the subjects indicated that they would continue regular use with the eye cream. In addition to PSP®, Lumière Bio-restorative Eye Cream contains caffeine, bisabolol, and glycyrrhetinic acid, which may contribute to the cream's observed efficacy.

Discussion

Even though the crucial role of growth factors in cutaneous wound healing is extensively studied and well recognized, the beneficial use of growth factors for skin rejuvenation has only been confirmed in the last few years. About a dozen clinical studies and articles describing the safety and efficacy of topical growth factor products for skin rejuvenation in humans exist in professional medical and scientific journals today. While a few articles report on recombinant growth factor products, the majority of articles describe antiaging studies with skincare products that contain natural mixtures of growth factors and other cytokines that are obtained by either collecting the conditioned cell culture medium or by cell lysis. All of the articles, however, demonstrate that growth factor products are effective at improving the signs of facial skin aging. Some studies indicate that efficacy is a function of time, with signs of significant wrinkle reduction seen only after prolonged product use (i.e., after 6 months or more). These observations seem comparable to those of some retinoic acid studies [37], where the severity of photodamage improves only after prolonged topical treatments. Placebo-controlled studies on skincare products that contain growth factors, such as TGF-β, TNS, and PSP, report that when compared, the signs of skin aging of the active groups are significantly more reduced than those of their vehicle counterparts. It is worth noting that vehicles are not completely "inactive," since they do contain moisturizers, and the effects of moisturizing can produce some improvements in fine lines and wrinkles. Even with that being the case, placebo-controlled studies clearly demonstrate that topical growth factors are superior in reducing the signs of skin aging than moisturizers alone.

In theory, growth factors can participate in skin rejuvenation at various levels due to their multifunctional activities. However, to be effective, growth factors must penetrate the skin to reach their respective cell surface receptors (keratinocytes, fibroblasts, or endothelial cells). Despite their relatively large size (>5 kDa), growth factors are able to penetrate intact skin. In this, they are not alone, as other large proteins have the same ability, for example, latex protein allergens (3–26 kDa) and botulinum toxin type A (900 kDa) – although to a lesser extent [38, 39]. Growth factors are also effective at very low concentrations (10^{-9}–10^{-12} of a mole). Thus, a low level of penetration is more than sufficient to induce a response. Proteins such as growth factors predominantly penetrate the skin through a vertical pathway, also known as the skin's "natural imperfections." These imperfections include the follicular apparatus of hair follicles, sweat glands, and microlesions in the interfollicular stratum corneum [40].

Those cutaneous functions that are impaired by the aging process seem to respond well to growth factors. Thus, the signs of aging skin may be improved with the topical use of an appropriate mixture of growth factors. However, due to the complexity and enormous expense associated with the development and manufacture of growth factor-containing products, only a limited number of skincare products are currently available that incorporate a mixture of growth factors. The question, therefore, of what combinations of growth factors and cytokines are most effective for skin rejuvenation remains to be further investigated.

Most recently, a novel peptide mixture consisting of matrikine and matrikine-like peptides was shown to provide statistically non-distinguishable anti-wrinkle benefits as compared to PSP and TNS [41]. A matrikine is a matrix-originating peptide with cytokine activity that is generated from the degradation of connective tissue proteins. The mixture (Micro-Protein Complex; MPC™), which comprises capryloyl carnosine (a dipeptide), palmitoyl tripetide-1 acetate, and tetrapeptide-21, demonstrated an ability to stimulate in vitro the formation of collagens I, III, and VII, elastin, and hyaluronic acid – all of which deplete considerably as skin ages. This indicates that matrikines, in particular MPC, may be valuable growth factor alternatives for skin rejuvenation.

Conclusion

In the last decade, topical growth factor products have become well accepted for use in skin rejuvenation. Numerous randomized controlled clinical trials demonstrated their good tolerability and efficacy. Today, there is evidence that the signs of aging skin may be best improved with a mixture of growth factors. Yet, what combinations of growth factors are most optimal for skin rejuvenation by topical application remains to be further investigated.

Cross-References

- ▶ Aging and Anti-aging Strategies
- ▶ Cosmetics and Aging Skin
- ▶ Cosmetic Anti-aging Ingredients
- ▶ Topical Peptides and Proteins for Aging Skin

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