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Role of Physiologically Balanced Growth Factors in Skin Rejuvenation

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Introduction

It is both a pleasure and an honor to introduce this supplement, highlighting some recent research on topically applied skin growth factors, on behalf of my colleagues and co-authors, Drs. Atkin, Cook-Bolden, Fitzpatrick, Kircik, Mehta and Werschler and Ms. Norine.

In recent years, it seems that skin growth factors have become the "new black," billed by the media as the hottest trend in the quest for eternal youth and playing starring roles in a plethora of lotions and potions that seek to populate our office shelves. As clinicians, our challenge is to separate the wheat from the chaff and to offer our patients something more than the promise in a jar that they can obtain from any local drugstore. While I doubt that Albert Einstein had physician-dispensed cosmeceuticals in mind when he remarked that everything should be made as simple as possible but not simpler, his philosophy of judicious simplicity is eminently applicable in this regard. The hypothesis that skin aging is analogous to a chronic wound and that skin rejuvenation is therefore possible via growth factors that stimulate and augment wound healing mechanisms is striking in its simplicity and has some scientific support. However, this apparent simplicity belies the underlying complexity of determining the optimal combination and balance of these growth factors.

In this supplement, my colleagues and I discuss two key concepts that we feel are crucial to furthering understanding of how topical growth factors may mediate skin rejuvenation. The first is that fibroblasts cultured within a three-dimensional matrix may be best poised to produce a physiologically balanced, interactive cocktail of growth factors. The second is that harvesting of these growth factors after they are secreted from their parent fibroblasts is likely to result in optimal biological activity. Further studies are needed to compare the clinical efficacy of cosmeceuticals containing physiologically balanced, naturally secreted growth factors with that of cosmeceuticals containing one or a few synthetic growth factors, growth factors derived from non-physiologically cultured fibroblasts, or growth factors harvested by fibroblast lysis rather than after natural secretion. Are some growth factors *too* "simple" because we cannot fully characterize—and therefore we cannot synthetically engineer—the complex balance, the post-translational modifications or the pre-secretory packaging necessary for optimal activity? If so, then this calls to mind another Einstein aphorism: that not everything that counts can be counted.

I believe you will find the data presented in this supplement to be both scientifically intriguing and of clinical relevance. They provide insight into a new paradigm of skin rejuvenation and a novel strategy for the formulation of therapies to mediate skin repair. They also serves as a reminder of the fact that medicine is, and perhaps always will be, as much art as science and as empiric as it is quantifiable.

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Topically Applied Physiologically Balanced Growth Factors: A New Paradigm of Skin Rejuvenation

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ABSTRACT

Synergistic interaction of multiple growth factors (GF) in skin controls the processes that promote skin repair. GFs have been shown to affect different pathways of skin repair and rejuvenation with many GFs working in close cooperation with one another and with other endogenous agents. Intrinsic and extrinsic aging of skin reduces both the levels of natural GFs and the number and activity of fibroblasts. Supplementing skin's endogenous GFs may enhance natural repair processes and accelerate the reversal of damage caused by intrinsic and extrinsic skin aging. In spite of their large molecular weight, evidence suggests that a small fraction of topically applied GFs penetrating into superficial epidermis can elicit a fibroblast-mediated response in the dermis. GF mixture secreted by human fibroblasts grown in conditions resembling the physiological condition of dermis, and present at high concentrations in a stable formula is most likely to provide an ideal cosmeceutical product. This naturally balanced mixture is also likely to contain other important, but as yet unidentified, substances that affect skin healing. Such a complex mixture cannot be reproduced using synthetic substances. Clinical studies have shown that topical application of products containing high concentrations of a physiologically balanced mixture of GF appears to reverse the signs of skin aging. A synergistic combination of antioxidants, matrix building agents and skin conditioners with physiologically balanced GF provides a novel and comprehensive paradigm of skin rejuvenation.

INTRODUCTION

Overview of Growth Factors and Cytokines in Skin

Growth factors (GF) are regulatory proteins that mediate signaling pathways between and within cells. These naturally occurring proteins are capable of altering cellular growth, proliferation and differentiation under controlled conditions and play an integral part in maintaining healthy skin structure and function.[1] Unlike hormones that work at a site distant from their site of production, GFs work locally on the cells that secrete them and on cells in the vicinity of these secreting cells. Growth factors are produced by all cell types that make up the epidermal and dermal structural components of skin including fibroblasts, keratinocytes and melanocytes. Transitory cells of the hematopoietic system that are associated with skin, such as lymphocytes and macrophages, also produce growth factors that alter skin structure and function. It has been documented that there are extensive communication pathways between epidermal and dermal cells within which growth factors act as messenger molecules.[2] Growth factors that predominantly affect immune functions of the skin are also referred to as cytokines. Cytokines play a critical role in modulation of inflammation following tissue injury and, in close coordination with GFs and other proteins, they stimulate biochemical pathways that produce skin tissue repair and regeneration.[3] For the purpose of this paper, we will use the term GF to refer to both growth factors and cytokines.

Age-Induced Changes to Skin Structure and Function

Skin aging is mediated by the effects of both the natural aging process over time (intrinsic aging) and environmental factors (extrinsic aging) on its cellular and extra-cellular components. It is a complex biological phenomenon consisting of two independent, clinically and biologically distinct processes that simultaneously affect skin structure and function.[4,5] Growing evidence now suggests that the two aging processes have biochemical and molecular pathways that converge around the role of reactive oxygen species (ROS, also known as free radicals).[6,7]

Reactive oxygen species are produced as a result of the absorption of UV radiation by chromophores in the skin.[8] ROS are also generated by excess mitochondrial oxidative energy

as a part of normal cellular metabolism.[9] This free radical overload increases oxidative phosphorylation of cell surface receptors, causing activation of two transcription factors, activator protein 1 (AP-1) and nuclear factor-kappa B (NF-κB), which are components of the MAP kinase signaling pathway. AP-1 activation stimulates transcription of matrix metalloproteinase (MMP) growth factor genes in fibroblasts and keratinocytes.[10] The increased production of MMPs leads to increased degradation of the extracellular matrix. AP-1 mediated reduction in synthesis of procollagen appears to result from two mechanisms: interference of AP-1 with type 1 and type 3 procollagen gene transcription and blocking of the profibrotic effects of TGF-β by impairment of the TGF-β type 2 receptor/Smad pathway.[11,12]

Reactive oxygen species also inactivate tissue inhibitors of metalloproteinase (TIMP), a key regulator suppressing MMP activity in healthy skin.[13] The imbalance in suppression of collagen synthesis and stimulation of collagen degradation eventually results in up to 20% reduction in dermal collagen levels.[11] Activation of NF-κB stimulates transcription of pro-inflammatory cytokine genes including IL-1, TNF-α, IL-6 and IL-8.[14] Inflammation resulting from these cytokines increases secretion of ROS and more cytokines, further augmenting the effect of UV exposure. Ultraviolet exposure and inflammation also cause protease-mediated degradation of elastin and formation of abnormal elastin by fibroblasts. UV light is also an inhibitor of leukocyte elastase, thereby increasing the accumulation of elastotic materials. This accumulation of elastotic materials is accompanied by degeneration of the surrounding collagenous network.

The overall effects of these interlinked biochemical activities is reduction of procollagen synthesis, increase of collagen degradation in the dermal extracellular matrix and irregular elastin deposition. These changes manifest clinically as fine lines and wrinkles and are associated with histopathological features including solar elastosis.

Role of Growth Factors in Skin Repair and Remodeling

Growth factors directly initiate activity that promotes skin repair and also modifies the activities of skin cells and other GFs. They are capable of both stimulating and inhibiting specific actions. The activity of GFs is modulated through other simultaneously synthesized GFs and through various intrinsic factors that interact to maintain homeostatic balance during skin repair. Ongoing research is uncovering more information

TABLE 1.

Partial list of growth factors, cytokines and other proteins identified in active gel and their function in skin (modified from Ref 15)
Fibroblast Growth Factors: bFGF (FGF-2), FGF-4, FGF-6, KGF (FGF-7), FGF-9 Angiogenic and fibroblast mitogen
Hepatocyte Growth Factor: HGF Strong mitogenic activities; three dimensional tissue regeneration and wound healing
Platelet Derived Growth Factors: PDGF AA, PDGF BB, PDGF Rb Chemotactic for macrophages, fibroblasts; macrophage activation; fibroblast mitogen, and matrix production
Insulin Like Growth Factors: IGF1, IGFBP1, IGFBP2, IGFBP3, IGFBP6 Endothelial cell and fibroblast mitogen
Transforming Growth Factor: TGFβ1, TGFβ2, TGFβ3 Keratinocyte migration; chemotactic for macrophages and fibroblasts
Tissue Inhibitor of Metalloproteinases: TIMP1 (MPI1), TIMP2 (MPI2) Prevent enzymatic degradation of collagen and hyaluronic acid
Vascular Endothelial Growth Factor: VEGF Influence vascular permeability and angiogenesis to improve tissue nutrition
Placenta Growth Factor: PLGF Promote endothelial cell growth
Bone Morphogenetic Protein: BMP7 Promote development of nerve cells in developing tissue
Interleukins:
IL1α, IL1β Early activators of growth factor expression in macrophages, keratinocytes and fibroblasts
Interleukin: IL2 Enhance epithelial wound healing
Interleukin: IL6 Mediator of acute phase response to wound and has synergistic effect with IL1
Interleukin: IL10 Inhibits pro-inflammatory cytokines to reduce inflammation prevents scar formation
Interleukin: IL4, IL13 Stimulate production of IL6
Interleukin: IL3, IL4, IL5 Leukocyte maturation and degranulation during inflammatory phase
Interleukins: IL7, IL8, IL15 Leukocyte activation and proliferation during inflammatory phase
Leptin Epidermal keratinocyte proliferation during wound healing
Colony Stimulating Factors:
GCSF, GM-CSF, M-CSF Stimulate the development of neutrophils and macrophages
Proteins of Wnt signaling pathway:
Wnt 4, Wnt 5a, Wnt 7a, Wnt 11 Enhance epithelial cell differentiation, control MMP expression

about the functions of individual GFs during skin repair and, importantly, the synergistic interaction of GFs with each other and with other components of skin repair pathways. It is not yet known whether the presence or absence of a single growth factor is significant. However, current understanding suggests that it is the interaction of many GFs that is significant, with no single growth factor being solely determinant in the outcome of skin repair.

Extensive research into cutaneous wound healing has led to increased understanding of the role of GFs in skin repair and has sparked a great deal of interest regarding their role in skin rejuvenation. ROS-mediated inflammation and matrix degradation resulting from aging can potentially be prevented and reversed by providing a physiologically balanced blend of GFs with proven roles in inducing skin repair processes. Table 1 lists some GF and their skin-related functions.[15] Again, successful skin repair requires an essential balance between the development of inflammation and its rapid resolution; this involves GF such as TGF-β, TNF-α, PDGF, IL-1, IL-6 and IL-10.[3]

After skin injury, GFs act as primary signaling molecules to initiate the repair process by promoting new cell recruitment and growth, production of extracellular matrix and rearrangement of all the components necessary to complete the repair process. TGF-β and PDGF induce migration of fibroblasts to the site of damage.[16,17] Fibroblast growth and activation through action of FGFs, HGH, PDGF, TGF-β , IGFs and PLGF result in synthesis of extracellular matrix components such as collagen, glycosaminoglycans and elastin.[16-21] VEGF facilitates matrix production by providing increased blood supply and tissue nutrition.[22] Long-term remodeling of endothelial tissue is controlled by the action of TGF-β, MMPs and TIMPs.[17,23,24] TGF-β controls fibroblast activation of collagen synthesis. MMPs degrade improperly built or damaged collagen and TIMPs alter MMP activity. The combination of these biochemical processes appears to be essential for remodeling and strengthening of the dermal tissue.

A logical approach to reversing the signs of skin aging is to harness the ability of skin to repair itself and to supplement these healing processes with essential components that have been lost as a result of aging. While the demand for GFs to maintain skin health increases with aging, the levels and activity of endogenous GFs are known to decrease in aged skin.[25,26] Supplementation of GFs that are known to be critical to skin repair in an appropriate medium and in an appropriate combination has significant potential as a strategy for anti-aging skincare.

Topical Application of Growth Factors

It is well documented that hydrophilic molecules larger than 500 Dalton (Da) molecular weight have very low penetration through the stratum corneum.[27,28] Most GF are large hydrophilic molecules greater than 20 kDa molecular weight; thus, they are unlikely to penetrate through the epidermis in measurable quantity to produce pharmacologic effects. However, results of multiple clinical studies show that topical application of these macromolecules may have significant clinical benefits.[15,29-31]

There are several potential routes for penetration of small quantities of large molecules through stratum corneum. The thickness of the epithelium in terminal and vellus hair follicles is significantly lower than the thickness of intra-follicular epidermis[32] and therefore may allow higher penetration of large molecules. The follicular route has been proposed as a delivery mechanism for proteins and liposomes.[33,34] Similar enhancement of delivery may be expected through sweat glands. A small fraction of particles smaller than 10 nm has been shown to penetrate through the skin.[35] Growth factors have a molecular weight in the range of 20 to 150 kDa and may be able to form particles of aggregated proteins in the size range that is known to penetrate skin. For comparison, hemoglobin is a 68 kDa protein with a size of 5.5 nm. Recent studies have shown that vaccines in size over 100 kDa can exert an immunologic response when applied topically, probably due to penetration of a very small amount of protein through intact skin.[36] A similar degree of penetration may suffice for topically applied GFs to produce an effect on epidermal cells. Skin may contain very small defects resulting from dryness, scratching, and use of irritating products that may allow small amounts of large molecular weight materials to penetrate into the viable part of the epidermis.[37] Aged skin is also thinner, more susceptible to perturbations, and takes a longer time to recover from disruption of barrier function.[4,38] As a result of all these circumstances, there may be increased protein penetration into the viable epidermis of aged skin and this may be of particular benefit to the target demographic for cosmeceuticals that are designed to rejuvenate the skin.

Regardless of the mechanism of penetration, the fraction of topically applied GFs penetrating the skin is likely to be very

TABLE 2.

Classes of antiaging actives
Prevent Ongoing Damage
Antioxidants
Sunscreen
Anti-inflammatory
Repair and Regenerate
Growth Factors
Retinoids
Antioxidants
Matrix degradation inhibitors
Protect and Maintain Skin Integrity
Moisturizers
Sunscreen

low. However, natural communication mechanisms between epidermis and dermis may amplify the effect of topically applied GF with minimal penetration. Epidermal–dermal communication pathways play a critical role in mediating the effects of topically applied GFs.[2] Penetration into the uppermost layer of viable epidermal keratinocytes may produce a signaling cascade of GFs that affects cells deeper in the dermis such as fibroblasts.[1,39] Evidence shows the presence of a double paracrine loop whereby epidermal keratinocytes stimulate dermal fibroblasts to synthesize GFs that in turn stimulate keratinocyte proliferation, resulting in amplification of the initial effect of topical GFs.[2,40] Keratinocytes express surface receptors for many GFs including KGF (FGF-7), TGF-β, IL-1, TNF-α, EGF, IFN-γ, and GM-CSF.[1,39,41-43] Penetration of small amounts of these molecules into the viable part of the epidermis after topical application can induce keratinocytes to produce GFs including PDGF, IL-1, TGF-α and TGF-β, which have been shown to exert a paracrine effect on proliferation and activation of dermal fibroblasts, leading to regeneration and remodeling of the dermal extracellular matrix.[1,44]

Optimum Properties of Topical Growth Factors

Skin repair and remodeling is the result of a number of different GFs interacting with each other and with the surrounding cells to improve the quality of the extracellular matrix. Human GFs may be obtained from two principal sources, cultured human cells or genetically engineered micro-organisms. Human cells are capable of producing a wide variety of proteins in response to various stimuli. Fibroblasts or co-cultures of fibroblasts and keratinocytes have been used to produce a mixture of GFs and matrix proteins. Individual GFs are commonly produced in bacteria and yeast and may lack the natural synergistic interactions necessary for optimal skin remodeling and repair.

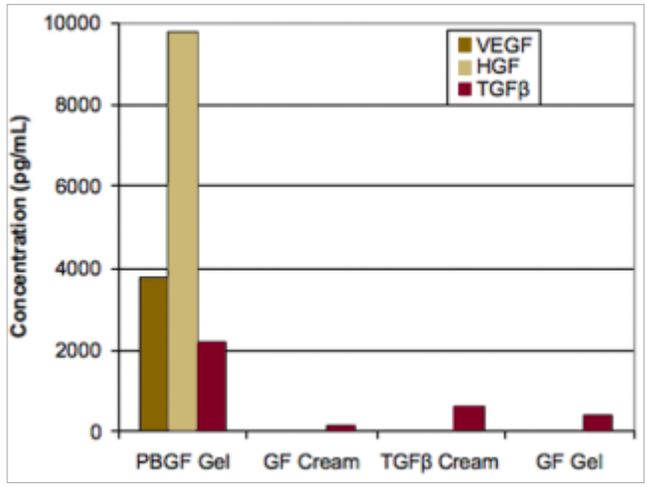
Human cells cultured in a three dimensional network secrete a mixture of many GFs and other proteins capable of promoting wound healing.[45] The composition of this GF mixture varies with cell phenotype and environment. Cells growing under conditions resembling a wound are most likely to produce GFs, cytokines and matrix proteins that assist in wound healing. Another method of collecting a mixture of GFs is to lyse fibroblasts and then collect the intracellular components, including GFs, cytokines and other intracellular materials, that are present at the time of cell lysis.[46] Growth factors secreted naturally from cultured human fibroblasts during the production of extracellular matrix exist as a complex physiologically balanced mixture. The composition of this mixture cannot be duplicated in the laboratory and no single growth factor can produce effects comparable to the effects produced by a physiologically balanced mixture of GFs.

The authors consider the comparison of naturally secreted physiologically balanced GFs with other growth factor mixtures to be analogous to the comparison of breast milk with baby formula. Even after decades of extensive research on the composition of human breast milk, there is still no synthetic product that can adequately replicate the beneficial effects of breast milk on infant development and growth. One problem with attempts to duplicate natural mixtures such as breast milk is that one can only add to the synthetic mixture what can be detected using current techniques for analysis of the natural mixture. Similarly, there are undoubtedly additional proteins and auxiliary substances in the naturally secreted, physiologically balanced GF mixture that play critical roles in development and maintenance of various skin structures.

One such group of proteins with a demonstrated role in skin recovery and repair is the Wnt family of secreted glycoproteins.[47] There is increasing evidence that Wnts are necessary for normal skin development.[48] A physiologically balanced, naturally secreted GF mixture obtained from three-dimensional fibroblast matrix was found to contain at least four different Wnt proteins (Wnt 4, Wnt 5a, Wnt 7a and Wnt 11) when analyzed by standard western-blot methods.[49]

Biological activity of GFs results from binding of an appropriately folded, three-dimensional protein molecule to a cell surface receptor, as a key fits a lock. Natural secretion through the cell membrane—the final step in protein synthesis—is important for the correct folding of many secreted proteins such as GFs.[50] Research indicates that many chaperone molecules assist with the correct folding of proteins in the cytosol and their secretion through cell membranes.[51] Proteins collected from within cells before secretion, as occurs with cell lysis and GF

FIGURE 1. Concentration of selected GF by ELISA in commercial products*



* PBGF Gel – TNS Recovery Complex, SkinMedica; GF Cream – Bio-Restorative Cream, NeoCutis, GF Gel – Bio-Gel, NeoCutis; TGF-β Cream –CRS Cream, Topix

FIGURE 2. Photographs of reduction in periorbital fine-lines and wrinkles after 6 months of treatment with physiologically balanced GF gel

FIGURE 3. Silicone impression of periorbital area at baseline and 6 months after treatment with physiologically balanced GF gel

harvesting, may not have the correct final structure and this may impair biological activity.

In this paper, the authors present data pertaining to a physiologically balanced GF mixture (NouriCel-MD®, SkinMedica, Carlsbad CA), which is harvested after natural secretion from a three-dimensional matrix of dermal fibroblasts induced to produce collagen, the same protein produced during skin repair and remodeling. The combined GFs and other adjunctive substances (Table 1) naturally secreted during the collagen production phase of tissue culture represent the most appropriate mixture to induce skin repair. The authors consider natural secretion to be essential for physiological balance and consequent optimal biological activity of a GF mixture.

Synthetic GFs produced by recombinant bacterial and yeast cultures may not undergo the same post-translational modifications in secondary and tertiary structure as occur in human cell cultures and therefore may have suboptimal biological activity. The activity of any recombinant growth factor must be evaluated using assays capable of showing its biological effects and not merely by immunoassay confirming its presence. Many growth factors of cosmeceutical interest are produced by recombinant bacteria and yeast, including TGF-β, VEGF, EGF, various FGFs and PDGF.[52] While clinical studies have shown that some individual GF have a marginally beneficial effect, more studies must be done to further delineate the role of individual GFs in skin rejuvenation.

The authors believe that a combination of complementary GFs, such as in SkinMedica's Nouricel-MD, is more likely to be clinically effective since multiple GFs are involved in most biochemical processes including wound healing.[3]

In addition to being physiologically balanced, a GF product designed for topical application must contain high levels of proteins that are stable at room temperature, to optimize biological activity. Cosmeceutical products containing mixtures of natural substances such as cellular GFs are generally difficult to analyze for concentrations of active ingredient. Even products manufactured with a single growth factor are not labeled with growth factor content, which makes it difficult to compare product strengths. More sophisticated analysis is needed to assure consumers that the products they are using have reliable quality control. It is important to note that GF and other biologically active peptides are inherently unstable in a non-physiologic environment, unless they are stored frozen at temperatures below -20°C. The presence of surface active additives, alcohols and other protein-denaturing excipients may decrease product stability and compromise its efficacy. In addition, the source of GF affects the total protein concentration and thereby may also affect the product shelf-life.

A study was conducted by Invitrogen Corporation (Carlsbad, CA) to determine the concentrations of VEGF, HGF and TGF-β in four commercially available products; a physiologically balanced GF mixture gel with 50 mg/mL total protein (TNS Recovery Complex, SkinMedica), a GF mixture gel and a GF mixture cream with less than 5 mg/mL total protein and a recombinant TGF-β cream. Extraction methods were developed to optimize the recovery of GF from each product and the extracts were analyzed by enzyme-linked immunosorbent assay (ELISA). Significant differences were found in the levels of GFs (Figure 1) in these products with the physiologically balanced GF gel with high protein concentration showing the highest levels of GF. A separate study confirmed the presence and stability of high levels of GF in this product stored at room temperature throughout its 2 year shelf-life.[15] If quantitative analysis is not possible due to the complexity of the GF formulation, measurement of biological activity should be performed using appropriate techniques, to document product stability throughout the labeled shelf-life.

RESULTS

A number of cosmeceutical products containing either a single human growth factor or a combination of multiple human growth factors and cytokines are currently marketed for skin rejuvenation. Recent clinical studies show that topically applied human growth factors are of benefit in reducing the signs of facial skin aging.[31] A few studies have evaluated the role of single growth factors in controlled wound-healing environments.

These studies demonstrate the importance of growth factors in the repair of damaged tissue,[53] but research into the phases of wound healing has demonstrated that the interaction of multiple growth factors is vital to tissue regeneration.

In an early clinical study with a product containing a fibroblast-derived physiologically balanced GF mixture (TNS Recovery Complex, SkinMedica),[14] patients with Fitzpatrick Class II or greater facial photodamage applied the product twice daily for 60 days. The results demonstrated a statistically significant reduction in fine lines and wrinkles and reduction in periorbital photodamage by clinical grading and by optical profilometry. Measurements of Grenz-zone collagen and epidermal thickness in a 3-mm punch biopsy of the lateral cheek showed a 37% increase in Grenz-zone collagen and a 30% increase in epidermal thickness.[29] It is difficult to conduct vehicle-controlled studies on cosmeceutical products, as emollient topical vehicles cannot truly be classified as "inactive". However, if a product under study contains any new active ingredients, it is imperative that the study includes a reasonably matched vehicle to scientifically validate the effects of the new active ingredient.[54]

In a recent double-blind study, 60 patients were randomly assigned to receive either TNS Recovery Complex or emollient vehicle and to apply it twice daily for 6 months along with a moisturizing cleanser and broad-spectrum SPF15 sunscreen. Treatment with TNS Recovery Complex for 3 months produced greater reduction in fine lines and wrinkles than vehicle treatment, as measured by optical profilometry and evaluator blinded assessment of photographs. The results were either statistically significant ($p \leq 0.05$) or trending towards statistical significance ($p \leq 0.1$). Figure 2 shows improvement in facial photodamage observed in this study and Figure 3 shows a silicone impression of changes to the periorbital rhytides.

The study demonstrates that TNS Recovery Complex, a cosmeceutical product containing a high concentration of physiologically balanced GF, reversed signs and symptoms of skin aging significantly more than an efficacious moisturizer and sunscreen alone.[15] In another multi-center double-blind vehicle-controlled split-face study, subjects applied TNS Recovery Complex to one side of the face and emollient vehicle to the other side for 3 months. Three millimeter punch biopsies of lateral cheek were taken at baseline, at 3 months (end of the treatment) and 6 months (3 months after the end of the treatment). Figure 4 shows biopsy measurements of epidermal and grenz-zone thickness for the treated and control side. The results convincingly demonstrate that topical application of a physiologically balanced GF mixture results in significantly increased production of new collagen, as indicated by increased grenz-zone thickness. The beneficial effects continue for at least 3 months after discontinuation of the treatment.

DISCUSSION

Potential Risks of Growth Factors

Growth factors are key to the control of cellular proliferation and differentiation and, if unregulated, could mediate carcinogenic transformation of cells. The presence of receptors for some GFs on melanoma cells and the expression of certain GFs by cancerous cells[55] have raised concerns about the potential for topically applied GFs to stimulate carcinogenesis. The effects of receptor presence or their increased expression on tumor cells are not yet defined. However, a recent FDA investigation suggests that chronic administration of high concentrations of recombinant PDGF directly into debrided diabetic pressure wounds may result in increased mortality from various types of malignancies that were remote from the site of treatment.[56] The prescription product that was the subject of this FDA investigation contains a more than 1 million-fold higher concentration of PDGF than the highest levels detected in topical cosmeceutical products. In addition, the prescription prod-

FIGURE 4. Increase in epidermis and grenz-zone thickness measured from histopathological analysis of skin biopsy after 3 months of treatment with physiologically balanced GF gel

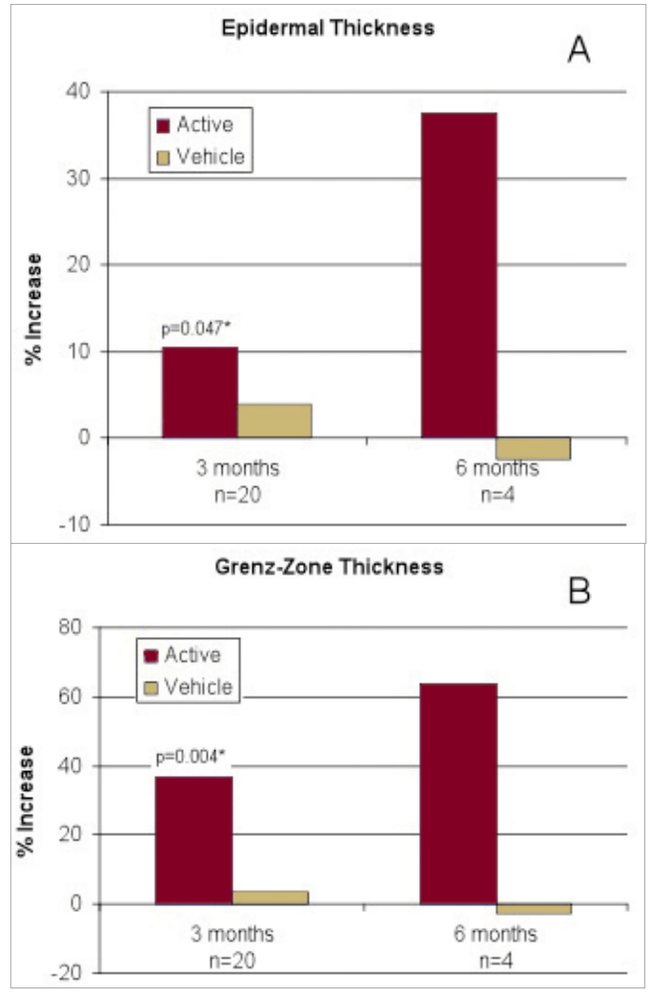
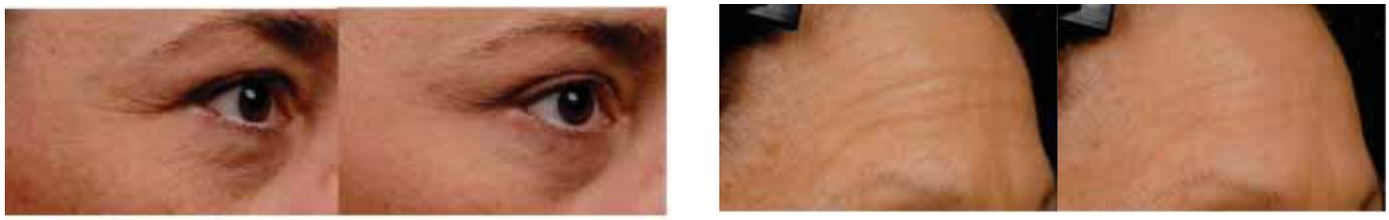


FIGURE 5. Photographs of reduction in periorbital and forehead wrinkle after 1 month of treatment with physiologically balanced GF combination serum (TNS Essential Serum)



uct is applied directly to dermal tissue of a debrided wound, bypassing the epidermal barrier. It appears unlikely that effects observed after applying an extremely high concentration of a single, unbalanced growth factor directly to the dermis can be extrapolated to a topical cosmeceutical product, containing a physiological balance of growth factors applied to essentially intact epidermis. The effects of a physiologically balanced mixture of multiple GF are proposed to be restorative, rather than therapeutic, in nature. One could also propose that the use of a physiologically balanced GF mixture, rather than individual unopposed GFs, might reduce or eliminate the risk of uncontrolled cell growth. We believe that the levels of GFs in skin after application to the epidermis are not significantly higher than are those following pro-inflammatory events such as chemical peels, laser resurfacing or skin infections.

Synergistic Combination of Physiologically Balanced Growth Factors

Physiologically balanced GFs are critical in building extracellular matrix components and form a key component of topical anti-aging skin-care regimens. The synergistic combination of antioxidants, matrix building agents and skin conditioners along with physiologically balanced GFs provide a comprehensive solution to skin rejuvenation. Table 2 lists classes of anti-aging actives and their role in skin rejuvenation.

Antioxidants that are active in aqueous and lipid compartments of the skin help reduce the free radical overload resulting from extrinsic and intrinsic aging processes. Many antioxidants also function as anti-inflammatory agents, protease inhibitors and melanogenesis regulators; therefore it is beneficial to select antioxidants that can provide multiple effects.

Common antioxidants used for skin rejuvenation and repair include vitamin C derivatives,[57] vitamin E derivatives,[58] botanical extracts such as green tea extract[59] and physiological antioxidants such as co-enzyme Q-10.[60] Anti-inflammatory agents prevent generation of excessive free radicals and matrix-degrading enzymes that are produced as a result of intrinsic and extrinsic aging. Matrix degrading enzymes from the matrix metalloproteinase (MMP) family can be suppressed by antioxidants such as ergothionine[61] and botanical extracts such as blackberry leaf[62] and cape jasmine fruit.[63]

The activity of physiologically balanced GFs may be supplemented by peptides and other small molecules designed to activate fibroblasts and increase matrix component production. Small peptides derived from collagen degradation, and modified with hydrophobic side chains such as palmitoyl groups or metal ions, are known to increase fibroblast proliferation and activation, probably via TGF-β mediated pathways.[64] Amino acids critical in collagen synthesis, such as proline and hydroxyproline, are also known to stimulate collagen production in skin.65 Reduction in melanogenesis can be achieved by antioxidants such as vitamin C derivatives[57] and hydroquinone analogs including arbutin.[66] Various forms of retinoids have proven skin rejuvenation and repair benefits by virtue of their action on multiple pathways of skin repair. Topical retinoids remain the mainstay for treating photoaging, given their proven efficacy as measured by both clinical and histologic outcomes.[67] Finally, no skin rejuvenation regimen is complete without inclusion of appropriate sun protection and sun avoidance measures. The best strategy to prevent and reverse the effects of photo-aging is to combine sun avoidance and/or sunscreens with agents to inhibit degradation of extracellular matrix components (MMP inhibitors, antioxidants), to promote synthesis and strengthening of dermal infrastructure (GF, retinoids, vitamin C) and to provide antioxidant protection in hydrophilic and lipophilic regions of the skin (vitamin C, vitamin E, botanical extracts).

A new product, TNS Essential Serum (SkinMedica), containing a combination of physiologically balanced GFs with multiple antioxidants, matrix building peptides and amino acid, skin brighteners, calming and soothing agents and other essential skin nutrients, was recently evaluated in pilot investigations.

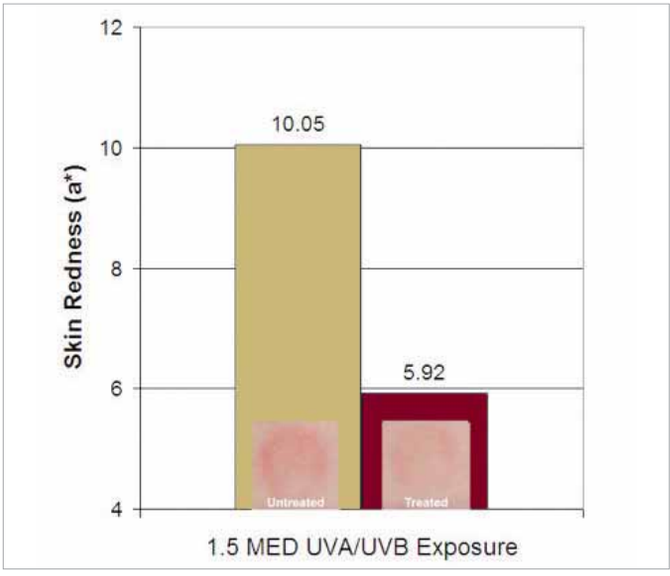
The clinical benefits of this serum combining physiologically balanced GF with other selected ingredients were confirmed in two separate clinical studies. In one of these studies, women with mild to severe wrinkles in the periorbital area used the GF combination serum twice daily for 3 months. They experienced rapid and significant improvement in the signs and symptoms of skin aging, as evaluated by both subjective and objective techniques. Photographs of one subject in Figure 5 show a rapid improvement in fine lines and wrinkles on the forehead. Subject self-assessments conducted monthly throughout the study confirm that the beneficial effects of the GF combination serum

are readily observed by users. Almost all study participants rated their overall satisfaction with the product performance as "excellent" or "good" and over 90% expressed a desire to continue using the product.

The antioxidant benefits of the GF combination serum were evaluated in a second clinical study with 11 participants. Exposure to UV light produces a variety of free radicals in the skin with subsequent tissue damage that manifests as erythema. The presence of adequate antioxidants in the skin neutralizes free radicals and prevents skin damage and resultant erythema.[68] Participants in this study were treated with the GF combination serum twice daily for 10 days on a sun-protected area of the lower back to build an adequate antioxidant reservoir in the skin. The treated areas and adjacent untreated sun-protected control areas of the lower back were then subjected to UVA/UVB exposure using a solar simulator. Resultant erythema sites were photographed and analyzed for the degree of redness using image analysis software. Areas treated with the GF combination serum showed up to 50% reduction in UV-induced erythema in comparison to untreated areas, thus demonstrating the photo-protective effects of the selected antioxidant formulation in the GF combination serum (Figure 6).

Results from these two studies demonstrate that the ingredients in this GF combination serum improve fine lines and wrinkles, hyperpigmentation, skin tone and texture, and skin elasticity, counteract environmental damage, and rapidly improve skin smoothness and tightness. The result is skin with a more even-toned, youthful appearance.

FIGURE 6. Up to 50% reduction in UV induced erythema in skin treated with physiologically balanced GF combination serum (TNS Essential Serum) compared to untreated skin



CONCLUSION

The functions of growth factors in the natural skin repair process are complex and incompletely understood; however, it appears that skin repair and regeneration is dependent on the synergistic interaction of multiple GFs and other proteins. The role of GFs in reducing signs of skin aging is being further explored using novel techniques such as intra-dermal injection of adipose-derived stem cells to increase collagen production.69 Multiple controlled clinical studies have already demonstrated the benefits of physiologically balanced GF in the reversal of skin aging. The observed reduction in fine lines and wrinkles correlates with stimulation of neo-collagenesis, as evinced by increased grenz-zone thickness and increased epidermal thickness.

This evidence suggests that physiologically balanced GFs should be considered as a cornerstone in the treatment of skin aging. The biochemical processes mediating skin repair depend on a number of other agents besides physiologically balanced GFs, including natural proteins, co-factors, vitamins and essential nutrients. This is the rationale for complementing the skin rejuvenation effects of physiologically balanced GFs with a combination of antioxidants, matrix building agents and skin conditioners.

The use of a novel treatment serum containing multiple growth factors, cytokines, peptides and a concentrated blend of antioxidants produced significant improvement in the visible signs of facial photodamage. A balanced combination of these key agents may provide a good first-line treatment for mild-to-moderate photodamaged skin. Additional studies are needed to fully characterize the effects of this combination of ingredients on skin repair and regeneration, using objective techniques that measure changes in the collagen and elastin content of skin.

DISCLOSURES

Dr. Deborah H. Atkin has been a consultant and speaker for SkinMedica, Inc.

Dr. Fran W. Cook-Bolden is a clinical investigator for SkinMedica, Inc., a consultant, speaker and serves on advisory boards.

Dr. Richard E. Fitzpatrick is the founder of SkinMedica, Inc., a stockholder, member of the BOD, investigator and consultant/speaker for SkinMedica, Inc.

Dr. Leon Kircik is a speaker, investigator, consultant, and is on the Advisory Board for SkinMedica, Inc.

Dr. Rahul Mehta is an employee of SkinMedica, Inc.

Josie Norine is an employee of SkinMedica, Inc.

Dr. Hema Sundaram is an advisor and speaker for SkinMedica, Inc., and does media work for Senetek.

Dr. Philip Werschler is a consultant and speaker for SkinMedica, Inc.

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