

Endogenous growth factors as cosmeceuticals

RAHUL C. MEHTA* & RICHARD E. FITZPATRICK†

**Research and Development, SkinMedica, Inc., Carlsbad, California and*

†*La Jolla Cosmetic Surgery Center, La Jolla, California*

ABSTRACT: Growth factors play an important role in reversing the effects of skin aging mediated by chronological and environmental factors. Excessive oxidation of intra- and extracellular components result in breakdown of collagen and elastin network in the dermis and produce the effect of facial aging. Topical application of human growth factors in multiple clinical studies has been shown to reduce the signs and symptoms of skin aging, including statically significant reduction in fine lines and wrinkles and increase in dermal collagen synthesis. More double-blind and controlled studies are needed to confirm the preliminary clinical effects of growth factor products, and more controls on product quality and stability need to be established.

KEYWORDS: cosmeceuticals, growth factors, photoaging, wound healing

Introduction

Aging of the skin is mediated by a combination of the effects of time (intrinsic aging) and environmental factors (extrinsic aging) on cellular and extracellular infrastructure. These are two independent, clinically and biologically distinct, processes that affect the skin structure and function simultaneously (1,2). Growing evidence now suggests that the two aging processes have converging biochemical and molecular pathways that lead to photoaging of skin (3,4). The common mechanisms of the two aging processes may provide several unique opportunities to develop new antiaging therapies. Recent advances in understanding the role of endogenous growth factors in the aging process provide one such opportunity to develop novel antiaging cosmeceutic products.

Intrinsic and extrinsic aging of skin

Intrinsic or sun-protected aging of skin is mediated by the “biologic clock” that affects the skin in the

same manner as it affects the internal organs, i.e., by slow, irreversible tissue degeneration. Telomere shortening combined with metabolic oxidative damage is believed to play a major role in the intrinsic aging process (5). Intrinsic aging affects everyone at different rates based on genetic factors (6). Intrinsically aged skin is thinner, more evenly pigmented, shows higher laxity, and less fold accentuation as compared to photoaged skin. Extrinsic aging is mediated by environmental factors including exposure of skin to solar UV radiation and environmental pollutants. Photoaged skin usually shows coarseness, wrinkling, sallowness, discoloration, telangiectasia, elastosis, irregular pigmentation, and a variety of benign, premalignant and malignant neoplasm (3). Intrinsic and extrinsic aging are cumulative processes that occur simultaneously, and over time result in photoaging.

Histologic evaluations show that sun-protected skin contains a lower number of fibroblasts, flat epidermal–dermal interface with loss of the dermal papillae, elevated levels of partially degraded collagen, and irregularly thickened, fragmented, and disorganized elastic tissue network (7,8). It also shows a reduction in total elastin content and ability to synthesize type I procollagen as compared to young skin (8,9). Photoaged skin shows a statistically significant decrease (20%) in total collagen as compared to sun-protected skin

Address correspondence and reprint requests to: Richard E. Fitzpatrick, MD, c/o Ann-Marie Andrews, 5909 Sea Lion Place, Suite H, Carlsbad, California 92010, or email: fitzskin@pacbell.net.

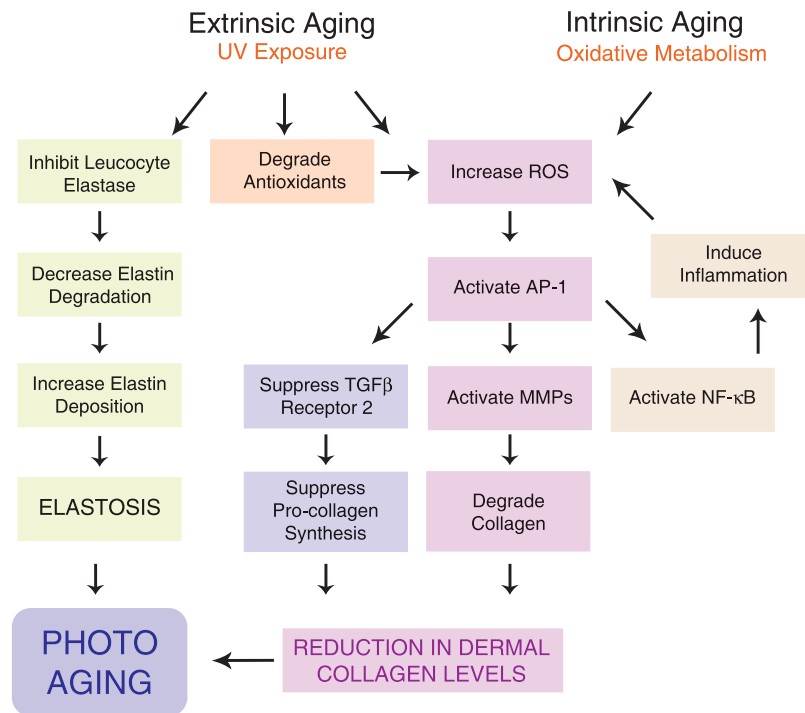


FIG. 1. Biochemical pathways of intrinsic and extrinsic aging leading to the symptoms of photoaging.

(10–12). Photoaged skin also shows marked elastosis with thickened, tangled, and amorphous elastic structures containing fragments of elastin and collagen that replace degenerating collagenous meshwork (8,13–17). The mean epidermal thickness appears to decrease with age in either sun-protected or photoaged skin (18,19).

Biochemical pathways of skin aging

Extensive research in the area of photoaging over the past decade has resulted in an improved understanding of the molecular mechanism of the aging process. FIGURE 1 shows a summary of major pathways involved in the aging process. Absorption of UV radiation by chromophores in the skin results in formation of reactive oxygen species (ROS) including superoxide anion and hydrogen peroxide. Normal oxidative metabolism (mitochondrial oxidative energy generation) also results in formation of excess ROS (20,21). ROS play a central role in intrinsic and extrinsic aging by increasing oxidative phosphorylation of cell surface receptors causing activation of one or more components of MAP kinase signaling pathways resulting in activation of transcription factors activator protein 1 (AP-1) and nuclear factor-kappa B (NF-κB) (22–25).

AP-1 stimulates transcription of matrix metalloproteinase (MMP) growth factor genes in fibroblast and keratinocytes, and inhibits type I procollagen gene expression in fibroblasts (10). Multiple studies have shown that activation of the MMP secretion as a result of intrinsic and extrinsic aging produces breakdown of dermal matrix (14,26,27). Different subtypes of MMP have different substrate proteins on which they act to produce a break in their primary sequence. MMP-1 (collagenase) produces cleavage at a single site in central triple helix of fibrillar type I and type III collagen. The cleaved subunits are further degraded by MMP-3 (stromelysin 1) and MMP-9 (gelatinase). Activity of MMP is decreased by binding with tissue inhibitors of metalloproteinase (TIMP). ROS inactivates TIMP thereby increasing MMP activity. AP-1 mediated reduction in synthesis of procollagen appears to result from two mechanisms, interference of AP-1 with type I and type III procollagen gene transcription and blocking the profibrotic effects of TGF-β by impairment of TGF-β type II receptor/smad pathway (28).

Activation of NF-κB stimulates transcriptions of proinflammatory cytokine genes including IL-1, TNF-α, IL-6, and IL-8 (29). Inflammation resulting from these cytokines increases secretion of ROS and more cytokines further enhancing the effect of UV exposure. Inflammation causes protease

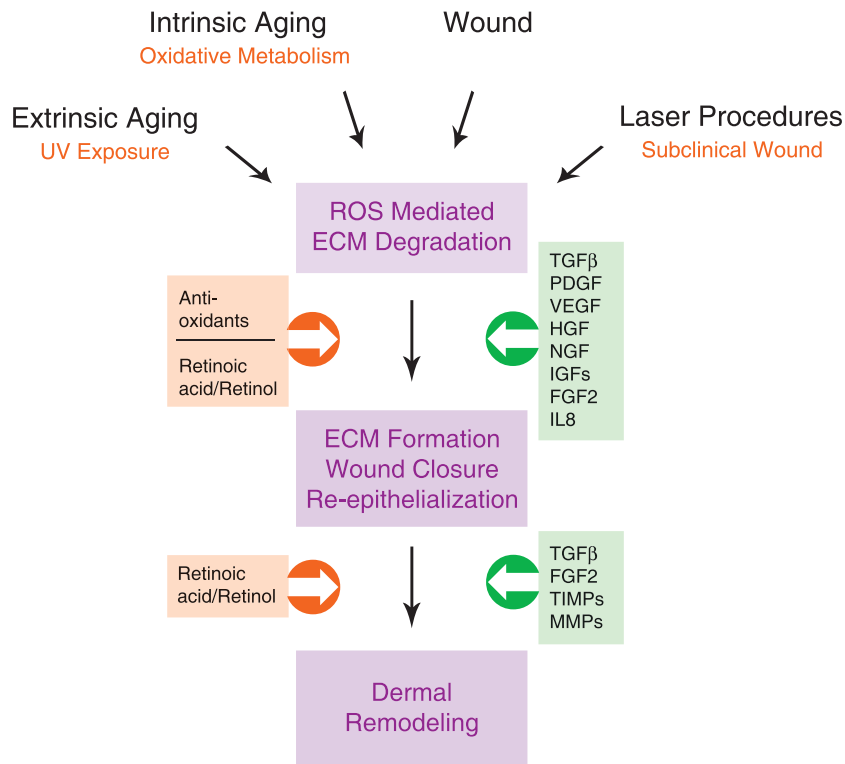


FIG. 2. Healing and remodeling of skin damaged by the effect of intrinsic aging, extrinsic aging, wound, or laser procedures.

mediated degradation of elastin and UV exposure causes formation of abnormal elastin by fibroblasts (19). UV light is also an inhibitor of leukocyte elastase thereby increasing accumulation of elastotic materials (30). The accumulation of elastotic materials is accompanied by degeneration of surrounding collagenous network (14).

The overall effects of these interlinked biochemical activities is reduction of procollagen synthesis, increase of collagen degradation in the dermal extracellular matrix, and increase in irregular elastin deposition.

Skin aging and wound healing

Some of the biochemical effects of intrinsic and extrinsic skin aging are similar to formation of a wound and the process of wound healing. FIGURE 2 shows a schematic of the process of photodamage and wound healing. Formation of a wound or UV damage induces inflammation via several pathways including NF-κB mediated activation of TNF-α and interleukins (29). Inflammation results in generation of ROS and proteolytic enzymes resulting in degradation of extracellular matrix. Successful wound healing requires a balance

between development of inflammation and its rapid resolution which includes involvement of growth factors and cytokines such as TGF-β, TNF-α, PDGF, IL-1, IL-6, and IL-10 (31). Intrinsic aging does not show the inflammatory component seen with healing of acute photodamage and wounds; instead, mitochondrial oxidative metabolism produces some of the key mediators of extracellular matrix degradation including ROS (20,21).

Transition from inflammatory phase of wound healing to granulation phase is mediated by a variety of growth factors and cytokines including PDGF, TGF-α, TGF-β, FGFs, IGF-1, CSE, ILs, and TNF-α (32–36). The growth factors and cytokines are derived from macrophages, epidermal keratinocytes, and fibroblasts. Multiple metabolic pathways lead to formation of new collagen and repair of extracellular matrix during the granulation phase.

The final stage of wound healing after granulation and wound re-epithelialization or peeling of sun-burned skin is the beginning of dermal tissue remodeling. During this stage low strength, unorganized type III collagen and elastin structures produced during the ECM production phase are replaced by stronger type III collagen and structured elastin fibers to provide strength and resiliency

Table 1. Growth factor signals at the wound site

Growth factor	Primary target cells and effect	Refs
HB-EGF	Keratinocyte and fibroblast mitogen	64
FGFs 1,2, and 4	Angiogenic and fibroblast mitogen	65,66
PDGF	Chemotactic for macrophages, fibroblasts; macrophage activation; fibroblast mitogen, and matrix production	67
IGF-1	Endothelial cell and fibroblast mitogen	36,68
TGF- β 1 and β 2	Keratinocyte migration; chemotactic for macrophages and fibroblasts	69
TGF- β 3	Antiscarring	69,70
IL-1 α and - β	Early activators of growth factor expression in macrophages, keratinocytes and fibroblasts	71
TNF- α	Similar to the IL-1s	71

to the dermis. This remodeling phase can last for several months and is the key to reversing the visible effects of skin aging (37).

Role of growth factors in reversing the effects of skin aging

The use of growth factors and cytokines in skin rejuvenation and reversal of photoaging is emerging as a novel antiaging treatment. Increased understanding of the role of growth factors and cytokines in wound healing has sparked great deal of interest, resulting in evaluating their role in repair and remodeling of dermal infrastructure. Table 1 lists some important growth factors and cytokines that affect the proliferation of dermal fibroblasts and extracellular matrix production. Providing some of these agents to cells responsible for extracellular matrix production and remodeling may benefit in rejuvenation of aging skin. Several cosmeceutic products containing either a single human growth factor or combination of multiple human growth factors and cytokines are currently marketed for skin rejuvenation. Clinical results are now available for some of these products and the results show that human growth factors when applied topically provide beneficial effects in reducing the signs of facial skin aging (37–39).

TNS Recovery Complex (SkinMedica Inc., Carlsbad, CA) contains NouriCel-MD, a proprietary mixture of growth factors, cytokines, and soluble matrix proteins secreted by cultured neonatal human dermal fibroblasts during production of extracellular matrix in an oil-free gel formulation. Neonatal fibroblasts have been used in the manufacture of several approved burn and wound-healing products including Dermagraft (40) and TransCyte (41) (Advanced BioHealing, La Jolla, CA). In a clinical study, 14 patients with Fitzpatrick class II or greater facial photodamage applied TNS Recovery Complex

twice daily for 60 days. The patients were evaluated for clinical grading of photodamage on a nine-point scale, optical profilometry and punch biopsy for Grenz-zone collagen measurements. The results show a statistically significant reduction in fine line and wrinkles and reduction in periorbital photodamage. Clinical grading showed a 12.2% improvement ($p = 0.0003$) in periorbital area after 60 days compared to baseline. FIGURE 3 summarizes

Optical Profilometry Showing Reduction in Depth and Number of Fine Lines

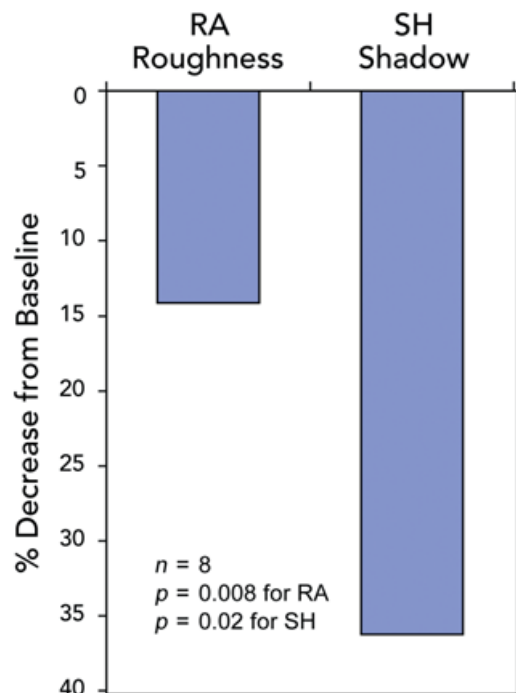


FIG. 3. Statistically significant reductions were observed in fine lines and wrinkles on the upper cheek using optical profilometry after using SkinMedica's TNS Recovery Complex twice daily for 60 days.

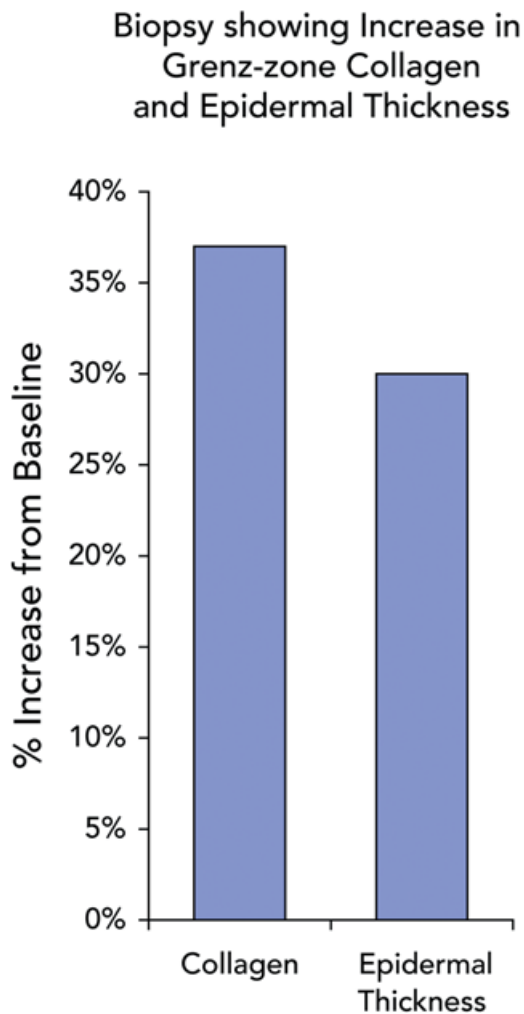


FIG. 4. Thickening of the Grenz zone, indicating new collagen formation (36% increase over baseline), as well as epidermal thickening (30% increase over baseline) is seen after using SkinMedica's TNS Recovery Complex twice daily for 60 days.

the reduction in fine lines and wrinkles measured by optical profilometry which show a 14.1% decrease in RA measurement ($p=0.008$) and a 36.2% decrease in shadow measurements ($p=0.02$). Measurements of Grenz-zone collagen and epidermal thickness measured from the biopsy show a 37% increase in Grenz-zone collagen and a 30% increase in epidermal thickness (FIG. 4) (37). Studies of longer durations show dramatic improvement in visible signs of aging. FIGURE 5 shows a case study of patient using TNS Recovery Complex twice daily for 6 months with a visible reduction in periorbital wrinkles in 3 months with continued improvements for 6 months.

Bio-Restorative Skin Cream (NeoCutis, Inc., San Francisco, CA) contains Processed Skin-Cell Proteins (PSP), a proprietary growth factor and cytokine

mixture extracted from cultured first trimester fetal human dermal fibroblasts in a moisturizing cream. Fetal fibroblasts have been used to construct skin grafts with horse collagen and have been clinically shown to produce complete closure of burn wounds in pediatric patients (42). Fetal fibroblasts express transient and lower amount of TGF- β 1 than adult fibroblasts, which may be resulting in scarless healing of skin during the first trimester of pregnancy (43). In a clinical study, 18 patients with Fitzpatrick class II or greater facial photodamage applied Bio-Restorative Skin Cream twice daily for 60 days. The patients were evaluated for clinical grading of photodamage on a five-point scale and facial photography. The results showed an improvement in average wrinkle score in periorbital and perioral areas. FIGURE 6 shows the clinical grading results from this study. A 17% decrease in periorbital area and a 13% decrease in perioral area were seen after 60 days compared to baseline. Improvements were also seen in the texture of cheek and chin areas (39).

Cell Rejuvenation Serum (CRS, Topix Pharmaceuticals, NY) contains liposome-encapsulated TGF- β 1, L-ascorbic acid and black cohosh (*Cimicifuga racemosa*) extract in a silicone base. Clinical studies with TGF- β 3 have shown increased wound healing rates in the treatments of pressure ulcers (44). In a clinical study, 12 patients with facial wrinkling applied CRS cream on one-half of the face and a CRS cream without TGF- β 1 on the other half of the face twice daily for 3 months. The patients were evaluated for clinical grading of photodamage on a five-point scale and facial photography. The results showed a statistically significant ($p < 0.05$) improvement in wrinkle score for TGF- β 1 contain cream as compared to cream with only L-ascorbic acid and black cohosh extract. In another arm of the same study, 19 patients with facial wrinkling applied CRS with TGF- β to one-half of the face and TNS Recovery Complex to the other half twice daily for 3 months. The patients were evaluated for clinical grading of photodamage on a five-point scale and facial photography. The results showed that both CRS and TNS Recovery Complex produced a statistically significant ($p < 0.05$) improvement in wrinkle score compare to baseline (38).

Growth factors as adjuncts to procedure

Laser resurfacing rejuvenates skin primarily by producing controlled wounds to localized area,

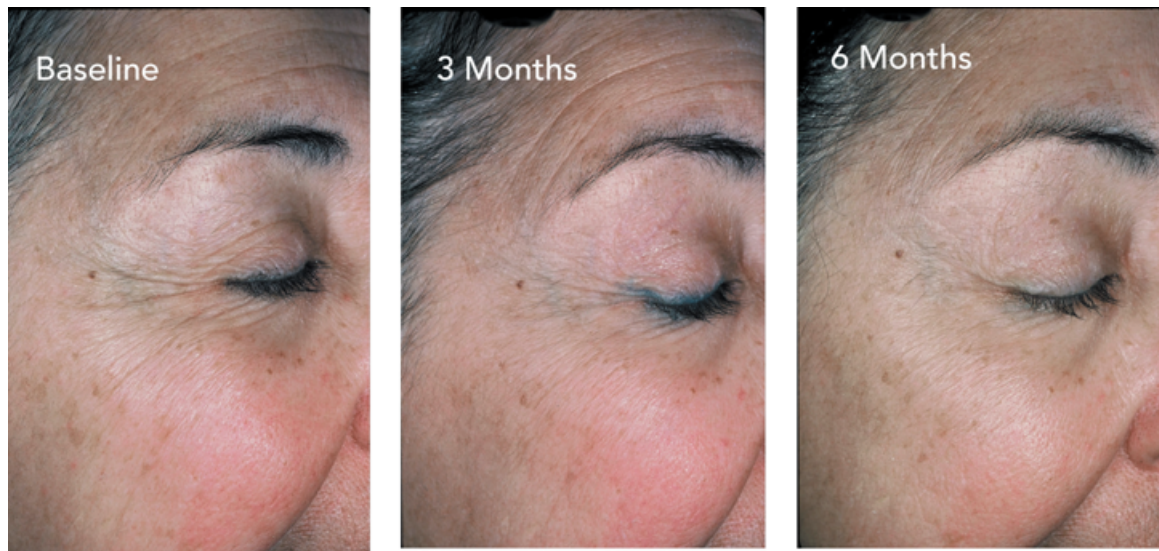


FIG. 5. Visible reduction in periorbital wrinkles in 3 months with continued improvements for 6 months in a patient using TNS Recovery Complex twice daily for 60 days.

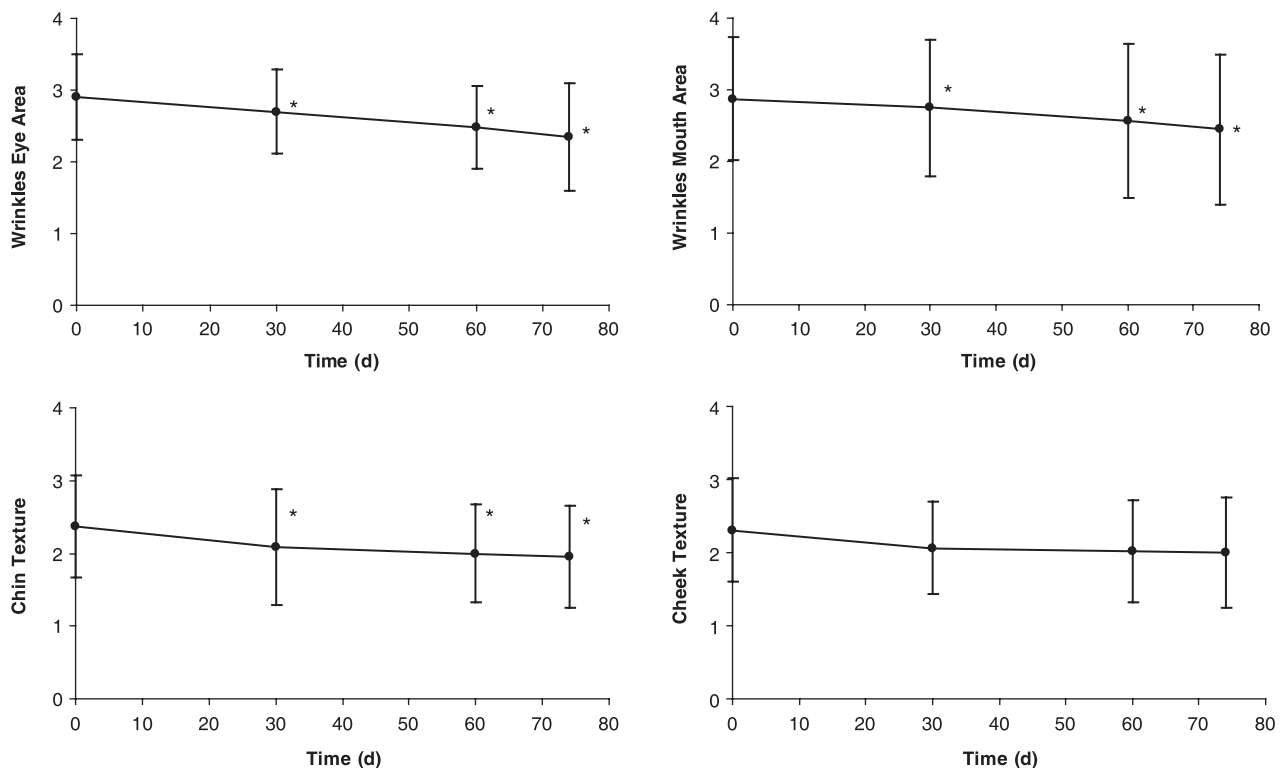


FIG. 6. Mean of facial wrinkle and texture scores as a function of time as assessed by investigators. The scores are baseline, after 30 days, and after 60 days of twice daily use of NeoCutis Bio-Restorative Skin Cream. ($n = 18$). (39)

which is followed by inflammation and healing resulting in stimulation of cytokine-mediated dermal collagen formation and structural remodeling of superficial dermis (45,46). Histologic studies have shown degrees of Grenz zone collagen with laser rejuvenation similar to those with treatment

using topical retinoids, vitamin C and growth factors. For noninvasive, nonablative laser resurfacing, the post-treatment application of growth factors in a topical formulation may provide benefit in accelerated or improved wound healing. Laser resurfacing also alters the barrier properties

of skin and may allow greater penetration of growth factors immediately postprocedure. Therefore, combining growth factors with laser resurfacing should not only improve postprocedure recovery time but also provide a synergistic effect on skin rejuvenation. Growth factors are also useful in the preprocedure period to condition the skin and allow for a more robust rejuvenating response to laser resurfacing.

Proposed mechanism of action of topical growth factors

Many studies have shown that hydrophilic molecules larger than 500 Da molecular weight have very low penetration through stratum corneum (47,48). Growth factors and cytokines are large hydrophilic molecules greater than 15,000 Da molecular weight and are unlikely to penetrate through the epidermis in measurable quantity to produce pharmacologic effects. However, results of the clinical studies described here clearly show that topical application of these macromolecules may have significant clinical benefits. The primary mechanisms by which the growth factors and cytokines can potentially exert their effect on the dermal matrix is by penetration through hair follicles, sweat glands, or compromised skin (49,50) followed by interaction with cells in the epidermis such as keratinocytes to produce signaling cytokines that affect cells deeper in the dermis such as fibroblasts (51,52). Skin may contain very small imperfections resulting from dryness, scratching, and use of products containing irritating chemicals that may allow small amount of large molecular weight materials to penetrate into the viable part of the epidermis. Aged skin is thinner, more susceptible to perturbations, and takes longer time to recover from loss of barrier function (3,53). Addition of lipophilic penetration enhancers or barrier-altering peptides may increase penetration of proteins through intact skin (54,55). Recent studies have shown that vaccines can exert immunologic response when applied topically, probably resulting from penetration of a very small amount of proteins through intact skin (56). Similar extent of penetration may also be sufficient for topically applied growth factors to produce an effect on epidermal cells.

Pathways of epidermal–dermal communications during wound healing may play a critical role in mediating the effects of topically applied growth factors and cytokines (57). Evidence strongly suggests presence of a double paracrine loop where

keratinocytes stimulate fibroblasts to synthesize growth factors that in turn stimulate keratinocyte proliferation, resulting in amplification of initial effect of topical growth factors (57,58). Keratinocytes express surface receptors for many growth factors and cytokines including KGF (FGF7), TGF- β , IL-1, TNF- α , EGF, IFN- γ , and GM-CSF (51,52,59–61), some of which are present in cosmetic products reviewed in the previous section. Penetration of small amounts of these molecules into the viable part of the epidermis after topical application can induce keratinocytes to produce growth factors 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 dermal extracellular matrix (51,62).

Future trends

Several pilot clinical studies now show the beneficial effects of topically applied growth factor on skin rejuvenation and reversal of signs and symptoms of skin aging. The next logical step in clinical research is to conduct double-blind, placebo-controlled studies to verify the findings of these preliminary results.

Another important step in formulation and quality control is to implement testing of quality, quantity, and activity of growth factors in the finished product. Growth factors and cytokines, like most proteins, are inherently unstable when not in their physiologic environment. Formulations containing surfactants, oils, and other excipients are likely to denature and inactivate the proteins. SkinMedica has initiated preliminary studies to detect and analyze key growth factors and cytokines in TNS Recovery Complex. Preliminary results (FIG. 7) show that the growth factors and cytokines

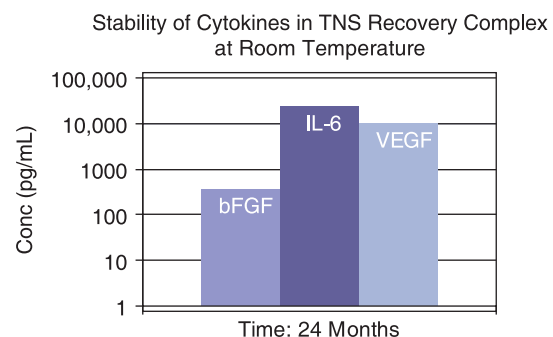


FIG. 7. Quantitative analysis of growth factors and cytokines in TNS Recovery Complex 24 months after manufacturing. Product was stored at ambient conditions for the duration.

in TNS Recovery Complex are stable for at least 24 months when stored at room temperature in the final product. Recent advances in the protein analysis research make it possible to extract, identify, and quantify increasingly smaller quantities of proteins from complex formulations (63). We encourage testing of other growth factor products to ensure that the activity of these fragile proteins is maintained during the manufacturing and storage of the final product.

Growth factors and cytokines may also work synergistically with antioxidants and retinoic acid. As shown in FIG. 2, antioxidants such as ascorbic acid can minimize production of reactive oxygen species, and retinoic acid and related agents can induce TGF- β to potentially counter the effects of AP-1 activation. Any antiaging regimen should include antioxidants and retinoic acid derivatives along with products containing growth factors.

Conclusion

Aging of skin mediated by the effect of time and environmental factors shows a common molecular pathway involving reactive oxygen species. Aging results in loss of dermal collagen and accumulation of unorganized collagen and elastin in the dermis, resulting in formation of wrinkles, elastosis, and loss of skin tone. The process of reversing some of the effects of aging can be accelerated by use of topically applied growth factors that accelerate wound healing. Although it is unclear how large proteins such as growth factors actually penetrate the site of action, results of multiple clinical studies and continued marketplace success of products containing growth factors show the beneficial effects of these cosmeceutic products on reducing the signs and symptoms of facial skin aging. More double-blind and controlled studies are needed to confirm the preliminary clinical effects of growth factor products and more controls on product quality and stability need to be established. Synergistic effects of growth factors with other noninvasive procedures and other cosmeceutic products should also be evaluated further.

References

- Krieg T, Hein R, Mauch C, Aumailley M. Molecular and clinical aspects of connective tissue. *Eur J Clin Invest* 1988; **18**: 105–123.
- Grove GL. Physiologic changes in older skin. *Dermatol Clin* 1986; **4**: 425–432.
- Gilchrest BA. Skin aging and photoaging: an overview. *J Am Acad Dermatol* 1989; **21**: 610–613.
- Oikarinen A. The aging of skin: chronoaging versus photoaging. *Photodermatol Photoimmunol Photomed* 1990; **7**: 3–4.
- Kosmadaki MG, Gilchrest BA. The role of telomeres in skin aging/photoaging. *Micron* 2004; **35**: 155–159.
- Gilchrest BA. Biochemical and molecular changes in photodamaged skin. In: Gilchrest BA, ed. *Photodamage*. Cambridge: Blackwell Science, 1995: 168–184.
- Dzubow L. The aging face. In: Coleman WP, Hanke WC, Alt TH, Asken S, eds. *Cosmetic surgery of the skin*. Philadelphia, PA: B.C. Decker Inc., 1991: 1–12.
- El-Domyati M, Attia S, Saleh F, et al. Intrinsic aging vs. photoaging: a comparative histopathological, immunohistochemical, and ultrastructural study of skin. *Exp Dermatol* 2002; **11**: 398–405.
- Varani J, Warner RL, Gharaee-Kermani M, et al. Vitamin A antagonizes decreased cell growth and elevated collagen-degrading matrix metalloproteinases and stimulates collagen accumulation in naturally aged human skin. *J Invest Dermatol* 2000; **114**: 480–486.
- Schwartz E, Cruickshank FA, Christensen CC, Perlish JS, Lebwohl M. Collagen alterations in chronically sun-damaged human skin. *Photochem Photobiol* 1993; **53**: 841–844.
- Oikarinen A, Kallioinen M. Biochemical and immunohistochemical study of collagen in sun-exposed and protected skin. *Photodermatol* 1989; **6**: 24–31.
- Smith JG Jr, Davidson EA, Tindall IP, Sam Wm Jr. Hexosamine and hydroxyproline alterations in chronically sun-damaged skin. *Proc Soc Exp Biol Medical* 1961; **108**: 533–535.
- Taylor CR, Stern RS, Leyden JJ, Gilchrest BA. Photoaging/photodamage and photoprotection. *J Am Acad Dermatol* 1990; **22**: 1–15.
- Fisher GJ, Wang ZQ, Datta SC, Varani J, Kang S, Voorhees JJ. Pathophysiology of premature skin aging induced by ultraviolet light. *N Engl J Med* 1997; **337**: 1419–1428.
- Kligman AM. Early destructive effects of sunlight on human skin. *JAMA* 1969; **210**: 2377–2380.
- Chen VL, Fleischmajer R, Schwartz E, Palaia M, Timpl R. Immunohistochemistry of elastotic material in sun-damaged skin. *J Invest Dermatol* 1986; **87**: 334–337.
- Mera SL, Lovell CR, Jones RR, Davies JD. Elastic fibres in normal and sun-damaged skin: an immunohistochemical study. *Br J Dermatol* 1987; **117**: 21–27.
- Branchet MC, Boissic S, Frances C, Robert AM. Skin thickness changes in normal aging skin. *Gerontology* 1990; **36**: 28–35.
- Lavker RM, Kligman AM. Chronic heliodermatitis: a morphologic evaluation of chronic actinic dermal damage with emphasis on the role of mast cells. *J Invest Dermatol* 1988; **90**: 325–330.
- Sohal RS, Weindruch R. Oxidative stress, caloric restriction, and aging. *Science* 1996; **273**: 59–63.
- Hensley K, Floyd R. Reactive oxygen species and protein oxidation in aging: a look back, a look ahead. *Arch Biochem Biophys* 2002; **397**: 377–383.
- Cobb MH. MAP kinase pathways. *Prog Biophys Mol Biol* 1999; **71**: 479–500.
- Fisher GJ, Talwar HS, Lin J, et al. Retinoic acid inhibits induction of c-Jun protein by ultraviolet radiation that occurs subsequent to activation of mitogen-activated protein kinase pathways in human skin in vivo. *J Clin Invest* 1998; **101**: 1432–1440.
- Senftleben U, Karin M. The IKK/NF- κ B pathway. *Crit Care Med* 2002; **30** (1 Suppl.): S18–S26.

25. Wlaschek M, Wenk J, Brenneisen P, et al. Singlet oxygen is an early intermediate in cytokine-dependent ultraviolet-A induction of interstitial collagenase in human dermal fibroblasts in vitro. *FEBS Lett* 1997; **413**: 239–242.
26. Varani J, Fisher GJ, Kang S, Voorhees JJ. Molecular mechanisms of intrinsic skin aging and retinoid-induced repair and reversal. *J Invest Dermatol Symp Proc* 1998; **3**: 57–60.
27. Brenneisen P, Wenk J, Klotz LO, et al. Central role of Ferrous/Ferric iron in the ultraviolet B irradiation-mediated signaling pathway leading to increased interstitial collagenase (matrix-degrading metalloprotease (MMP)-1) and stromelysin-1 (MMP-3) mRNA levels in cultured human dermal fibroblasts. *J Biol Chem* 1998; **273**: 5279–5287.
28. Quan T, He T, Kang S, Voorhees JJ, Fisher GJ. Solar ultraviolet irradiation reduces collagen in photoaged human skin by blocking transforming growth factor-beta type II receptor/smad signaling. *Am J Pathol* 2004; **165**: 741–751.
29. Yamamoto Y, Gaynor RB. Therapeutic potential of inhibition of the NF- κ B pathway in the treatment of inflammation and cancer. *J Clin Invest* 2001; **107**: 135–142.
30. Martin P. Wound healing – aiming for perfect skin regeneration. *Science* 1997; **276**: 75–81.
31. Eming SA, Krieg T, Davidson JM. Inflammation in wound repair: molecular and cellular mechanisms. *J Invest Dermatol* 2007; **127**: 514–525.
32. Kiritsy CP, Lynch SE. Role of growth factors in cutaneous wound healing: a review. *Crit Rev Oral Biol Medical* 1993; **4**: 729–760.
33. Moulin V. Growth factors in skin wound healing. Review article. *Eur J Cell Biol* 1995; **68**: 1–7.
34. Mateo RB, Reichner JS, Albina JE. Interleukin-6 activity in wounds. *Am J Physiol* 1994; **266**: R1840–R1844.
35. McCartney-Francis N, Mizel D, Wong H, Wahl L, Wahl S. TGF-beta regulates production of growth factors and TGF-beta by human peripheral blood monocytes. *Growth Factors* 1990; **4**: 27–35.
36. Rappolee DA, Mark D, Banda MI, Werb Z. Wound macrophages express TGF α and other growth factors in vivo: analysis by mRNA phenotyping. *Science* 1988; **241**: 708–712.
37. Fitzpatrick RE, Rostan EF. Reversal of photodamage with topical growth factors: a pilot study. *J Cosmet Laser Ther* 2003; **5**: 25–34.
38. Ehrlich M, Rao J, Pabby A, Goldman MP. Improvement in the appearance of wrinkles with topical transforming growth factor beta(1) and L-ascorbic acid. *Dermatol Surg* 2006; **32**: 618–625.
39. Gold MH, Goldman MP, Biron J. Efficacy of novel skin cream containing mixture of human growth factors and cytokines for skin rejuvenation. *J Drugs Dermatol* 2007; **6**: 197–201.
40. Naughton G, Mansbridge J, Gentzkow G. A metabolically active human dermal replacement for the treatment of diabetic foot ulcers. *Artif Organs* 1997; **21**: 1203–1210.
41. Noordenbos J, Dore C, Hansbrough JF. Safety and efficacy of TransCyte for the treatment of partial-thickness burns. *J Burn Care Rehabil* 1999; **20**: 275–281.
42. Hohlfeld J, de Buys Roessingh A, Hirt-Burri N, et al. Tissue engineered fetal skin constructs for paediatric burns. *Lancet* 2005; **366**: 840–842.
43. Cass DL, Meuli M, Adzick NS. Scar wars: implications of fetal wound healing for the pediatric burn patient. *Pediatr Surg* 1997; **12**: 484–489.
44. Hirschberg J, Coleman J, Marchant B, Rees RS. TFG-beta3 in the treatment of pressure ulcers: a preliminary report. *Adv Skin Wound Care* 2001; **14**: 91–95.
45. Rokhsar CK, Lee S, Fitzpatrick RE. Review of photorejuvenation: devices, cosmeceuticals or both? *Dermatol Surg* 2005; **31**: 1166–1178.
46. Omi T, Kawana S, Sato T, Honda M. Ultrastructural changes elicited by a non-ablative wrinkle reduction laser. *Lasers Surg Med* 2003; **32**: 46–49.
47. Bos JD, Meinardi MM. The 500 Dalton rule for the skin penetration of chemical compounds and drugs. *Exp Dermatol* 2000; **9**: 165–169.
48. Jakasa I, Verberk MM, Esposito M, Bos JD, Kezic S. Altered penetration of polyethylene glycols into uninvolved skin of atopic dermatitis patients. *J Invest Dermatol* 2007; **127**: 129–134.
49. Lademann J, Otbert N, Jacobi U, Hoffman RM, Blume-Peytavi U. Follicular penetration and targeting. *J Invest Dermatol Symp Proc* 2005; **10**: 301–303.
50. Jakasa I, Verberk MM, Bunge AL, Kruse J, Kezic S. Increased permeability for polyethylene glycols through skin compromised by sodium lauryl sulphate. *Exp Dermatol* 2006; **15**: 801–807.
51. Werner S, Krieg T, Smola H. Keratinocyte-fibroblast interaction in wound healing. *J Invest Dermatol* 2007; **127**: 998–1008.
52. Ansel J, Perry P, Brown J, et al. Cytokine modulation of keratinocyte cytokines. *J Invest Dermatol* 1990; **94** (6 Suppl.): 101S–107S.
53. Ghadially R, Brown BE, Sequeira-Martin SM, Feingold KR, Elias PM. The aged epidermal permeability barrier. Structural, functional, and lipid biochemical abnormalities in humans and a senescent murine model. *J Clin Invest* 1995; **95**: 2281–2290.
54. Cui CY, Lu WL, Xiao L, et al. Sublingual delivery of insulin: effects of enhancers on the mucosal lipid fluidity and protein conformation, transport, and in vivo hypoglycemic activity. *Biol Pharm Bull* 2005; **28**: 2279–2288.
55. Chen Y, Shen Y, Guo X, et al. Transdermal protein delivery by a coadministered peptide identified via phage display. *Nat Biotechnol* 2006; **24**: 455–460.
56. Skountzou I, Quan FS, Jacob J, Compans RW, Kang SM. Transcutaneous immunization with inactivated influenza virus induces protective immune responses. *Vaccine* 2006; **24**: 6110–6119.
57. Babu M, Wells A. Dermal-epidermal communication in wound healing. *Wounds* 2001; **13**: 183–189.
58. Florin L, Maas-Szabowski N, Werner S, Szabowski A, Angel P. Increased keratinocyte proliferation by JUN-dependent expression of PTN and SDF-1 in fibroblasts. *J Cell Sci* 2005; **118**: 1981–1989.
59. Finch PW, Rubin JS, Miki T, Ron D, Aaronson SA. Human KGF is FGF-related with properties of a paracrine effector of epithelial cell growth. *Science* 1989; **245**: 752–755.
60. Grossman RM, Krueger J, Yourish D, et al. Interleukin 6 is expressed in high levels in psoriatic skin and stimulates proliferation of cultured human keratinocytes. *Proc Natl Acad Sci USA* 1989; **86**: 6367–6371.
61. Freedberg IM, Tomic-Canic M, Komine M, Blumenbert M. Keratins and the keratinocyte activation cycle. *J Invest Dermatol* 2001; **116**: 633–640.
62. Brauchle M, Angemeyer K, Hubner G, Werner S. Large induction of keratinocyte growth factor expression by serum growth factors and pro-inflammatory cytokines in cultured fibroblasts. *Oncogene* 1994; **9**: 3199–3204.
63. Transdermal and Topical Delivery of Therapeutic Peptides and Proteins. In: Banga AK, ed. *Therapeutic peptides and proteins: formulation, processing, and delivery systems*, 2nd ed. Boca Raton, FL: CRC Press, 2005.

64. Marikovsky M, Breuing K, Liu PY, et al. Appearance of heparin-binding EGF-like growth factor in wound fluid as a response to injury. *Proc Natl Acad Sci USA* 1993; **90**: 3889–3893.
65. Werner S, Peters KG, Longaker MT, Fuller-Pace F, Banda MJ, Williams LT. Large induction of keratinocyte growth factor expression in the dermis during wound healing. *Proc Natl Acad Sci USA* 1992; **89**: 6896–6900.
66. Abraham JA, Klagsbrun M. Modulation of wound repair by members of the fibroblast growth factor family. In: Clark RAF, ed. *The molecular and cellular biology of wound repair*, 2nd ed. Springer Publisher, 1996: 195–240.
67. Eriksson A, Siegbahn A, Westermark B, Heldin CH, Claesson-Welsh L. PDGF alpha- and beta-receptors activate unique and common signal transduction pathways. *EMBO J* 1992; **11**: 543–550.
68. Mueller RV, Hunt TK, Tokunaga A, Spencer EM. The effect of insulin-like growth factor I on wound healing variables and macrophages in rats. *Arch Surg* 1994; **129**: 262–265.
69. Madlener FA, Werner S. Transforming growth factors beta1, beta2, and beta3 and their receptors are differentially regulated during normal and impaired wound healing. *J Biol Chem* 1996; **271**: 10188–10193.
70. Shah M, Foreman DM, Ferguson MW. Neutralization of TFG-beta1 and TFG-beta2 or exogenous addition of TGF-beta3 to cutaneous rat wounds reduces scarring. *J Cell Sci* 1995; **108**: 985–1002.
71. Hübner G, Brauchie M, Smola H, Madlener M, Fässler R, Werner S. Differential regulation of pro-inflammatory cytokines during wound healing in normal and glucocorticoid-treated mice. *Cytokine* 1996; **8**: 548–556.