

COSMECEUTICAL SCIENCE IN CLINICAL PRACTICE



Edited by
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Cosmeceutical Science in Clinical Practice

Series in Cosmetic and Laser Therapy

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Cosmeceutical Science in Clinical Practice

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A CIP record for this book is available from the British Library.
Library of Congress Cataloging-in-Publication Data

Data available on application

ISBN-13: 978 0 415 47114 5

Orders from

Informa Healthcare
Sheepen Place
Colchester
Essex CO3 3LP
UK

Telephone: +44 (0)20 7017 5540
Email: CSDhealthcarebooks@informa.com

Typeset by Exeter Premedia Services Private Ltd., Chennai, India
Printed and bound in Great Britain by MPG Books Ltd, Bodmin, Cornwall, UK

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Introduction

As we progress in the early stages of the 21st century, we can now offer our patients advanced scientifically and clinically proven cosmeceutical products; these have become a routine part of the therapeutic regimens utilized in conjunction with non-ablative, ablative, and surgical treatment programs carried out in the dermatologist's and plastic and cosmetic surgeon's offices. The changing paradigm of skin care has been protection of the skin utilizing broad-spectrum sun blockers and ultra potent antioxidants in the morning, followed by reversal agents which stimulate new collagen and reverse the global signs of photoaging (i.e., pigmentary dyschromias, vascular irregularities, and pilosebaceous aberrations) in the evening. The present book incorporates these principles, which are helpful to all patients and are best begun during the early decades of life.

The first chapters of the present volume discuss new approaches utilized in present skin care products incorporating new generation antioxidants, cytokines, and peptides. The discussion then broadens to novel delivery systems including nanotechnology, and specific problem-related targeted approaches

for the treatment of pigmentary dyschromias, as well as hair and nail disorders. One very important aspect of the book is its outline of how the aesthetic physician may incorporate cosmeceutical products into their clinical practices and how to combine these approaches with the technological procedures performed in the in-office setting. Finally, futuristic approaches to skin care—including stem cell research, targeting proteins and genetic modulation, and new generation cosmeceutical agents—complete this book, which hopefully will allow the practicing physician to understand new treatment paradigms for skin care, encompassing scientifically and clinically proven cosmeceutical agents which should be incorporated into all our patients' treatment programs to help lead to improved skin health and aesthetics.

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Disclosure

Contributors have acted as investigators or consultants for the following relevant companies:

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1 Ultrapotent Antioxidants and Anti-inflammatories

Laurel Naversen Geraghty, Diane S Berson, and Ranella Hirsch

INTRODUCTION

Antioxidants possess the unique ability to neutralize free radicals, which are highly reactive molecules that result from oxidative processes and may contribute to DNA damage, carcinogenesis, inflammation, and photoaging of the skin. Although the body is equipped with endogenous antioxidants, its supply may be overwhelmed by increased oxidative stress due to natural metabolic processes or exposure to ultraviolet light, pollution, or cigarette smoke. Free radicals damage dermal cells by promoting thymine dimer formation, causing the release of pro-inflammatory molecules, increasing apoptosis, and upregulating collagenase. By scavenging free radicals, antioxidants demonstrate anti-carcinogenic and anti-inflammatory activity and guard against photodamage and collagen breakdown.

By reinforcing the skin's endogenous supply of antioxidants, orally or topically administered antioxidants may help prevent an overload of oxidative stress. Antioxidants have become ubiquitous in the skin-care market because they have shown promise for preventing photoaging and protecting against cellular DNA damage. Although limited research exists to support the efficacy of many commercially available antioxidants, their effects are increasingly reproducible in scientific research. This chapter will address the evidence behind some of the best-studied antioxidants and anti-inflammatory ingredients used on the skin, including vitamins A and C, coenzyme Q-10, green tea, and soy, as well as newer ingredients that show promise, including CoffeeBerry™, idebenone, feverfew, and *Polypodium leucotomos*.

POLYPHENOL ANTIOXIDANTS

Polyphenols, or catechins, are plant-derived antioxidants with anti-inflammatory, photoprotective, and anti-carcinogenic properties (1). This family of compounds includes flavonoids, such as soy isoflavones, green tea extracts derived from the *Camellia sinensis* plant, grape seed extract, CoffeeBerry, and resveratrol.

Soy

Soy reinforces the skin's epidermal barrier, promotes collagen production and elastin repair, decreases hyperpigmentation, and inhibits hair growth (2–6). Among the active ingredients in soy are genistein and daidzein, isoflavones derived from fermented soy products, such as tofu and soy beans. These isoflavones are believed to possess chemopreventive qualities (7). Other active ingredients include essential fatty acids and amino acids; phytosterols, which strengthen and repair the skin's barrier function; and small protein serine protease inhibitors, such as Bowman–Birk inhibitor (BBI) and soybean trypsin inhibitor (STI). BBI and STI inhibit protease-activated receptor

2 to disrupt melanosome transfer to keratinocytes, thereby reducing melanin levels within the skin (2,8–10). BBI also inhibits the hair-growth enzyme ornithine decarboxylase (11).

Clinically, soy is touted for its gentle anti-inflammatory actions. In addition to moisturization, it is used to decrease inflammation, minimize hair growth, and effect photorejuvenation, photoprotection, skin lightening, and skin brightening (12,13). Clinical studies support the use of topical soy as a treatment for melasma and photodamage. Research suggested that, over 12 weeks, daily or twice-daily application of a topical soy formula improves overall skin tone and texture, hyperpigmentation, blotchiness, and dullness (4,5) (Fig. 1.1). In a study of six adults, genistein dose-dependently inhibited ultraviolet B (UVB)-induced erythema (14). Topical soy may also reduce the visibility and coarseness of hair, while increasing its softness and fineness (12) (Figs. 1.2 and 1.3).

Tea

Although all teas are derived from the tea plant *C. sinensis*, different varieties contain different active components, due to fermentation and other processes (8).

Green tea, the most well-studied type, contains significant amounts of polyphenols, which are known to reduce DNA damage, sunburn, inflammation, and erythema. The most potent catechin contained in green tea is epigallocatechin-3-gallate (EGCG) (15). Evidence suggests that EGCG and other green tea polyphenols regulate cell growth and disrupt the carcinogenic pathway at multiple points (16). In vitro and murine research showed that EGCG may inhibit activator protein 1 (AP-1)—a transcription factor—to disrupt UV-induced tumorigenesis, suppress enzymes related to carcinogenesis, and diminish edema and hyperplasia (7,17). In addition, green tea polyphenols may inhibit angiogenic factors and stimulate cytotoxic T cells in the presence of tumor cells (18).

An in vivo study suggested that topical green tea extract relieves sunburn and inhibits erythema in a dose-dependent manner (19). Topical EGCG has further been shown to reduce oxidative stress, pyrimidine dimer formation, and the infiltration of inflammatory leukocytes in humans (20). Orally consumed green tea also offers anti-tumor benefits (21). By promoting keratinocyte proliferation, green tea extracts may also increase epidermal thickness (22).

Black tea, a fermented type, has been less extensively studied for its cutaneous effects. Research suggests that oral or topical black tea inhibits tumorigenesis (23). Its mechanisms of action, as demonstrated in murine models, include the inhibition of cell proliferation, apoptosis, tumorigenic cell-signaling kinases, transcription factors, and the inflammatory protein cyclooxygenase 2 (24). Clinical study showed that the ingestion of black



Figure 1.1 Baseline visible (A) and enhanced (C) images clearly demonstrate an improvement in skin tone, texture, and clarity when compared with visible (B) and enhanced (D) images after 12 weeks of use of the total soy and SPF 30 facial moisturizer. *Abbreviation:* SPF, sun protection factor. *Source:* From Ref. 10.

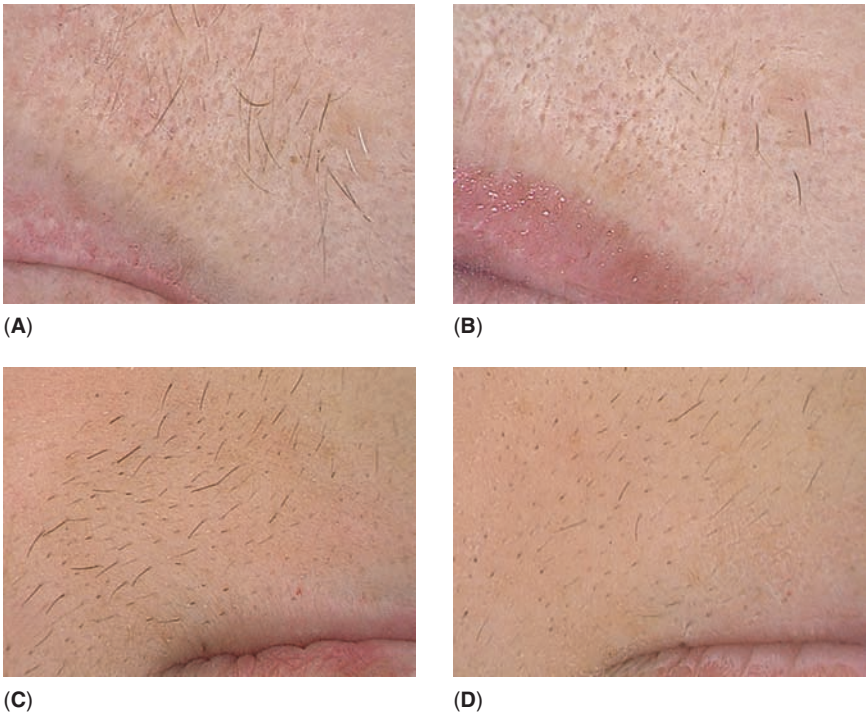


Figure 1.2 Video microscopic images before (A, C) and after (B, D) the use of natural soy and skin conditioner formulation. At the baseline visit, one week post-shaving, the unwanted facial hair on the upper lip area was very apparent. After eight weeks of twice daily usage of the natural soy and skin conditioner preparation and one week post-shaving, hair appeared finer and less noticeable. Video microscopic images further verify the improvements in the appearance of unwanted facial hair as observed by the dermatologist and study participants. *Source:* By permission of J&JCCI; J. Nebus.

tea reduced the incidence of squamous cell carcinoma and decreased photodamage (8,23,25). Topically applied black tea extract reduced the incidence and severity of erythema and inflammation in humans following UVB exposure (26).

Rooibos tea is derived from *Aspalathus linearis*, a plant native to South Africa that may be unfermented (“green rooibos”) or

fermented (“red tea”). Both varieties contain high levels of polyphenols, including flavonoids and phenolic acids (27). In vitro animal studies suggested that rooibos exerts antioxidant and anti-carcinogenic effects, and topically applied rooibos extract reduces the number and size of skin tumors (28,29). Rooibos has been used as a folk remedy for eczema, depression,

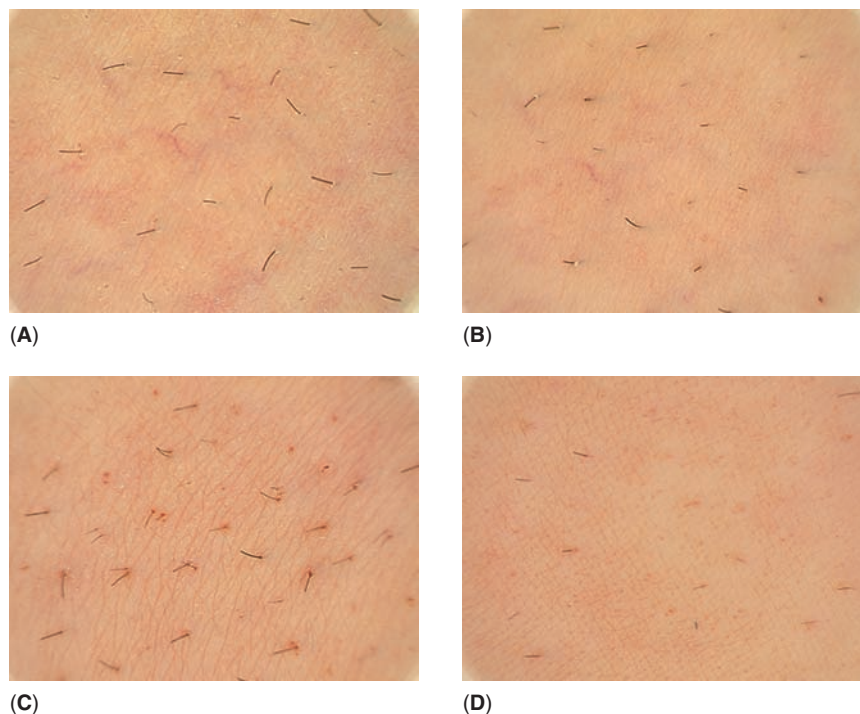


Figure 1.3 Video microscopic images before (A, C) and after (B, D) four weeks' use of natural soy and skin conditioner formulation. At the baseline visit, subjects enrolled in the study had a moderate appearance of unwanted leg hair four days after shaving. At the four-week visit, after twice-daily application of the moisturizer containing natural soy and skin conditioners and four days after shaving the hair appeared less noticeable and finer. Video microscopic images further verified the improvements in the appearance of the unwanted leg hair. Source: By permission of J&J CCI; W. Wallo.

nausea, and a variety of other conditions (28). However, the study of rooibos as a topical agent in humans remains limited.

Grape Seed Extract

Grape seed extract, derived from the seeds of red grapes, more effectively scavenges free radicals than vitamins C or E (30,31). Its considerable antioxidant capacity may be attributed to its high levels of proanthocyanidins, a type of flavonoid (32). Of the nine polyphenols contained in grape seed extract, procyanidin B5-3'-gallate has the most potent antioxidant activity (7). Murine models suggested that the extract possesses chemopreventive and photoprotective abilities (7). It also promotes dermal wound healing by upregulating vascular endothelial growth factor (VEGF) expression in keratinocytes (32). Topical grape seed, which is found in many anti-aging cosmeceuticals, increases the sun protection factor of sunscreens (31) and may be of benefit in the treatment of melasma. In a study of 12 women, melasma pigmentation diminished significantly after six months of oral intake of a grape seed extract, but did not improve over a longer period (33).

CoffeeBerry

Derived from the fruit of the coffee plant, *Coffea arabica*, CoffeeBerry extract (Johnson & Johnson) is rich in polyphenol

antioxidants, which prevent and repair damage caused by reactive oxygen species (ROS). In an oxygen radical absorbance capacity (ORAC) test, an assessment of the free-radical scavenging ability of antioxidants, CoffeeBerry demonstrated 10 times the free-radical quenching ability of green tea, and even greater potency compared to pomegranate, vitamin C, vitamin E, and ferulic acid (2,34).

Clinically, CoffeeBerry is used to combat signs of photoaging. In a proprietary trial of 30 adult women with moderate photoaging, subjects applied a 1% CoffeeBerry cream twice daily after washing with a 0.1% CoffeeBerry cleanser. Ten subjects applied CoffeeBerry formulas to one side of the face only and used a vehicle on the other half of the face, while 20 used CoffeeBerry on the entire face. After six weeks, blinded expert grading revealed a 30% mean overall skin improvement in both groups, compared to 7% improvement on the control side of the face in the split-face group; a 20–24% average reduction in fine lines and wrinkles (control: 3%) and 15% mean improvement in pigment (control: 5%) was also observed. Immunostaining demonstrated increased collagen synthesis relative to control and decreased levels of matrix metalloproteinase-1 (MMP-1), which promotes collagen breakdown. Side effects included erythema and transient sensation of burning (35–37).

Resveratrol

Resveratrol is a polyphenol that is abundant in the skin of grapes and in red wine. This antioxidant inhibits inflammation, carcinogenesis, and platelet aggregation, and upregulates sirtuin (silent information regulator) enzymes to slow the aging process in a manner similar to caloric restriction (38). Although clinical study was not available at the time of this publication, preliminary research supports resveratrol's promise as a topical treatment for UV-induced photoaging. In *in vitro* and murine trials, resveratrol inhibited cellular proliferation and dose-dependently reduced free radicals (39,40). In an ORAC test, a 1% resveratrol topical formula demonstrated approximately 17 times the antioxidant activity of a 1% idebenone formula (40).

VITAMIN, ENZYME, AND BOTANICAL ANTIOXIDANTS

Vitamin A

Vitamin A, also known as retinol, is the cosmeceutical cousin of the prescription topical drug tretinoin. Commonly used to treat acne and photoaging, and also for melasma and scars, tretinoin increases the vascularity of photoaged skin and upregulates collagen production to smooth fine lines and wrinkles (1,41). Although less potent and efficacious than tretinoin, retinol may be used for acne and photoaging, and it is generally less irritating and better tolerated. Retinol disrupts melanogenesis to help fade lentiginos and inhibits MMPs (1). Oral retinol has chemopreventive qualities (7).

As a treatment for photoaging, topical retinol reduces fine lines and wrinkles, upregulates collagen synthesis, increases dermal water content, and augments epidermal thickness (42). In a randomized, double-blind, vehicle-controlled study of 36 elderly subjects (mean age 87 years), topical 0.4% retinol was applied to one arm three times weekly. After 24 weeks, fine lines and rhytids were significantly improved in the retinol-treated arms. Histological evaluation revealed increased glycosaminoglycan expression and collagen biosynthesis compared to control (43).

Vitamin B

The vitamin B complex includes niacinamide (also known as niacin, nicotinamide, and vitamin B₃) and panthenol (provitamin B₅, dexpanthenol). B vitamins have been used to treat a variety of skin conditions, including acne, wound healing, blistering diseases, and other inflammatory conditions (1).

In an animal model, topical niacinamide prevented UVB-induced immunosuppression and skin cancer induction (44). In humans, topical niacinamide helps to reverse signs of photoaging through a variety of proposed mechanisms (45–47). First, it stimulates fibroblasts to increase collagen synthesis. Second, it inhibits melanosome transfer from melanocytes to keratinocytes, thereby decreasing hyperpigmentation. Third, it inhibits the oxidative glycation of proteins, reducing the sallow quality of photoaged skin (1). Finally, niacinamide bolsters levels of ceramides, free fatty acids, and the proteins keratin and filaggrin to enhance the skin's barrier function and reduce transepidermal water loss (1,47). Clinically, niacinamide is

non-irritating and improves skin tone and texture, decreases fine lines and wrinkles, and reduces hyperpigmentation (1,46).

Panthenol is a humectant with anti-inflammatory and anti-pruritic effects (48–50). As a precursor to pantothenic acid—a cofactor in lipid biosynthesis—panthenol increases lipid biosynthesis, improves stratum corneum hydration, and strengthens the skin's barrier function (50). *In vitro* and *in vivo* trials showed that panthenol enhanced wound healing via fibroblast proliferation and accelerated epidermal re-epithelialization (1,48,50). Clinical trials found that panthenol prevented and reduced signs of irritation, including dryness, roughness, scaling, pruritus, erythema, and stratum corneum damage (50,51).

Vitamin C

Vitamin C, which contains the active ingredient L-ascorbic acid, is a water-soluble, photoprotective, anti-inflammatory antioxidant not produced by the human body. Because the vitamin is a required cofactor and transcriptional regulator of collagen biosynthesis, it has implications for wound healing and photoaging (1,25). Vitamin C also reduces melanin formation via tyrosinase, inhibits elastin biosynthesis (possibly with the effect of diminishing solar elastosis in photodamaged skin), and is believed to stimulate sphingolipid production to reinforce the skin's lipid barrier (25,52–54).

Animal studies showed that topical L-ascorbic acid decreases skin erythema, sunburn, and immunosuppression following UV exposure (55,56). Clinically, topical vitamin C is used for photoprotection and the prevention and treatment of photoaging, hyperpigmentation, and erythema (57–59). In a small, double-blind, split-face trial, a topical solution containing 10% L-ascorbic acid and 7% tetrahexyloxydeyl ascorbate was compared with control. After 12 weeks, wrinkling and photodamage were significantly reduced on the cheeks, forehead, and perioral region on the treated side of the face (60). In a study of postmenopausal women, vitamin C increased pro-collagen mRNA and associated enzymes to bolster collagen synthesis (61). Because vitamin C inhibits tyrosinase and melanogenesis, it has potential clinical applications for the treatment of melasma and lentiginos (1,62).

Vitamin E

Vitamin E, which includes the molecular forms tocopherol and tocotrienol, is a lipid-soluble humectant and antioxidant known for its photoprotective actions. Some of these effects may be attributed to α -tocopherol's photoprotective absorption of ultraviolet light near 290 nm (63). By scavenging lipid peroxy and oxygen radicals, vitamin E prevents the oxidation of stratum corneum proteins and inhibits lipid peroxidation, helping to preserve the integrity of cell membranes. A carrier protein selectively shuttles α -tocopherol into lipoproteins, making it the most important form of vitamin E in humans (25).

Animal models revealed that topical vitamin E protects against UV-induced erythema, lipid peroxidation, photoaging, immunosuppression, photocarcinogenesis, and the formation of pyrimi-

dine dimers (25). In human cells, vitamin E reduced the number of sunburn cells following UV exposure and inhibits melanogenesis (1,25,64). Clinical reports suggested that orally administered vitamin E improves facial hyperpigmentation (64,65).

Topically applied vitamins C, E, and ferulic acid act synergistically to compound their antioxidant and photoprotective activities. Vitamin C regenerates the active form of vitamin E, while ferulic acid stabilizes vitamins C and E (25). In a clinical trial, a combination of orally administered vitamins C and E diminished the sunburn response following UV exposure. Individually, the vitamins were not efficacious (66).

Vitamin E has historically been used to minimize the appearance of scars. However, after a 12-week, double-blinded study comparing vitamin E to control, Baumann and Spencer concluded that the vitamin did not improve the cosmetic outcome of postsurgical scars, and found that it caused contact dermatitis in 33% of treated subjects (67).

Ferulic Acid

Ferulic acid is a potent antioxidant ubiquitous in plant, fruit, and vegetable species, including teas, CoffeeBerry, and *Polypodium leucotomos* (32,68). It is touted for its ability to absorb UV energy and photoprotect the skin, stabilize vitamins C and E, and reduce thymine dimers, which may lead to DNA mutations and skin cancer (69,70). Because ferulic acid's mechanisms of action are distinct from but complementary to the actions of sunscreens, the antioxidant may be a beneficial addition to sunscreen formulations (69).

In a small clinical study of a topical solution containing 15% L-ascorbic acid (vitamin C) and 1% α -tocopherol (vitamin E), 0.5% ferulic acid stabilized the vitamins and doubled the photoprotection of the formula, as determined by levels of erythema and sunburn cells. Ferulic acid also reduced thymine dimers, inhibited apoptosis, and suppressed four of five pro-inflammatory and immunosuppressive cytokines tested (69).

Alpha Lipoic Acid

Mitochondria synthesize alpha lipoic acid (ALA), a lipoamide that is both water- and lipid-soluble and has the ability to cross cellular membranes. ALA quenches free radicals, prevents lipid peroxidation, and reactivates other antioxidants, including vitamins C and E, glutathione, and ubiquinol. ALA also serves as an anti-inflammatory and an exfoliant (1).

Though clinical study showed that ALA is not effective in the photoprotection of skin (71), it may have clinical applications for photoaging. In a 12-week, double-blind, split-face study of 33 women (mean age 54 years), in which a 5% topical ALA product was applied twice daily, objective and subjective evaluation revealed a significant decrease in fine lines, lentigines, and skin roughness (72). ALA has also been used in the treatment of vitiligo. In a randomized, double-blind, placebo-controlled trial of 35 patients, oral supplementation with ALA, vitamins C and E, and polyunsaturated fatty acids was administered for two months before and the six months during narrow-band ultraviolet B (NB-UVB) therapy. The oral supplement

significantly improved the effectiveness of NB-UVB and diminished oxidative stress associated with vitiligo (73).

Kinetin

Kinetin (N^6 -furfuryladenine) is a botanical growth factor and antioxidant that is believed to be photoprotective. In vitro study showed that kinetin stimulates human fibroblasts and upregulates catalase, the enzyme that degrades hydrogen peroxide (74,75).

Clinically, kinetin may restore the skin's barrier function. It has been used in the treatment of rosacea, photodamage, and dry skin. One study found that a topical 0.1% kinetin product improved fine lines, color, texture, and blotchiness over 24 weeks (76). In another trial, 59% of subjects who applied a 1% kinetin lotion twice daily for 12 weeks demonstrated moderate to marked improvements in rosacea. Subjects showed significant improvement in skin roughness and mottling, and facial erythema decreased by an average of 32% (77).

Coenzyme Q-10

Coenzyme Q-10 (CoQ-10 or ubiquinone) is an endogenous, fat-soluble antioxidant that inhibits lipid peroxidation to prevent damage to cellular and mitochondrial membranes (78). It is believed to play a role in the prevention and treatment of photoaging. Vitamin E and CoQ-10 are the only lipophilic antioxidants found within skin surface lipids and act synergistically to reduce UV-mediated depletion of cholesterol, squalene, and unsaturated fatty acids, which help to maintain the skin's barrier function (79). In vitro study indicated that CoQ-10—particularly when combined with carotenoid antioxidants—protects fibroblasts from UV-induced inflammation and photoaging, and reduces MMP levels (80). Given that CoQ-10 levels diminish with age, topical formulations may replenish the antioxidant to generate improvements in photoaging (79). Application of a CoQ-10 solution decreased wrinkle depth and inhibited UVA-induced collagenase expression and oxidative stress in human keratinocytes (81).

Idebenone

Idebenone is a synthetic cousin of Coenzyme Q-10 that is believed to penetrate the skin more effectively (82,83). Idebenone exerts photoprotective and anti-inflammatory effects; reduces sunburn cells, thymine dimers, and immunosuppression following UV irradiation; and is thought to repair mitochondrial damage (1,84). Research demonstrated its potency relative to other common antioxidants: in a five-test comparison with tocopherol, kinetin, ubiquinone, ascorbic acid, and alpha lipoic acid, idebenone displayed the greatest overall protection against oxidative stress (84).

Idebenone may improve skin texture, hydration, and fine lines (1). In a clinical trial, 50 women with moderate photodamage were randomized to apply a 0.5% or 1.0% idebenone topical lotion twice daily. After six weeks, blinded experts noted improvements of approximately one-third in skin roughness, hydration, and fine lines and wrinkles in the 1.0% idebenone group, with more modest

results seen in the 0.5% group. Histological assessment showed increased collagen levels and decreased interleukins and MMP-1 for both groups (82). No adverse events were reported in the study. However, idebenone can cause contact dermatitis (85,86).

Polypodium leucotomos

Extract of the fern plant *P. leucotomos* is a phenolic antioxidant with photoprotective, chemoprotective, anti-inflammatory, and immune-modulating effects (87).

In murine models, orally administered *P. leucotomos* inhibited UVB-induced skin cancers, significantly diminished immunosuppression following UVB exposure, and reduced signs of photoaging, including dermal elastosis and skinfold thickness (88–90). In vitro study showed that, following UV exposure, *P. leucotomos* significantly improved membrane integrity, increased elastin, and inhibited lipid peroxidation and MMP-1 expression in human fibroblasts and keratinocytes. The researchers posited that *P. leucotomos* concentrations below 0.1% may protect against photoaging by reinforcing membrane integrity and inhibiting MMP-1, and concentrations greater than 0.1% may reverse elastin loss (90).

P. leucotomos has been used in the treatment of vitiligo, psoriasis, atopic dermatitis, and other inflammatory and immune-related skin conditions. It may also prove useful in the prevention and treatment of sunburn (32,91). In a study of 21 healthy human subjects, *P. leucotomos* administered orally or topically offered photoprotection to skin and Langerhans cells and increased the UV dose required for immediate pigment darkening, the minimal erythema dose, and the minimal phototoxic dose (87). Although oral *P. leucotomos* supplements are commercially available, the authors were unaware of any topical formulations at press time.

Catalase

Catalase facilitates the degradation of hydrogen peroxide and constitutes the body's primary defense against hydrogen peroxide damage (92). Increased oxidative stress, catalase deficiency, and hydrogen peroxide accumulation appear to contribute to photoaging and inflammatory skin conditions, such as psoriasis and vitiligo (93–98). Catalase may hold promise as a therapy for these conditions.

In vitro study of human skin cells demonstrated that catalase inhibits free radicals and cytokines induced by tumor necrosis factor α (TNF- α), which contributes to inflammatory skin disease (99). Catalase significantly reduces hydrogen peroxide transfer from keratinocytes to melanocytes, promoting melanogenesis and the progression of vitiliginous lesions (100). In a randomized, double-blind trial of 25 vitiligo patients, researchers found no significant differences in repigmentation after 4 and 10 months of consistent use of topical 0.05% betamethasone (a glucocorticoid) or a catalase/dismutase superoxide formula (95).

Additional evidence suggests that catalase may be beneficial in the prevention and treatment of dermal aging. In vitro study found significantly decreased catalase activity and increased hydrogen peroxide accumulation in fibroblasts obtained from photoaged skin. Catalase added to photoaged fibroblasts reduced hydrogen peroxide levels, suppressed

MMP-1, and reversed age-related alterations in the MAP kinase signaling pathway (101).

Açai

Açai fruit (*Euterpe oleracea*) is a palm species abundant in the Brazilian Amazon. Açai and açai oil are rich in antioxidants, catechins, procyanidins, sterols, and phenolic acids (102). Preliminary in vitro study suggests that açai protects against oxidative damage, inhibits lipid peroxidation, and reduces ROS and pro-inflammatory cytokines (103). Because of açai's high stability, rich phenol content, and antioxidant activity, it is considered a promising addition to future topical skin agents (102).

Cassia alata

The leaf extract of *Cassia alata* (ringworm bush) has been used as a treatment for tinea infections and bites, and has demonstrated anti-inflammatory and antimicrobial effects in laboratory studies (104–106). However, limited evidence exists to support its use as a topical agent in human skin. In a controlled study of 10 adults, an antioxidant formula containing *C. alata* extract and Oxynex-ST, a photostabilizer, offered significantly greater photoprotection following UV exposure compared to placebo, and an even greater level of protection when combined with sunscreen, as assessed by digital photography and immunohistochemistry. (Source: C Oresajo et al., Evaluation of the complementary effects of antioxidants and sunscreens in reducing UV induced skin damage as demonstrated by skin biomarker expression: clinical poster, 68th Annual Meeting of the AAD, 2010.)

ANTI-INFLAMMATORY BOTANICALS

While all antioxidants are anti-inflammatory, only a few anti-inflammatories (including feverfew, pycnogenol, and mushroom extract) possess antioxidant activity, and therefore have the ability to improve photodamage. However, all anti-inflammatories function to reduce cutaneous erythema and irritation.

Feverfew

Feverfew, an extract of the herb *Tanacetum parthenium*, has antioxidant, anti-inflammatory, anti-irritant, and photoprotective actions (107–109). In an ORAC assessment, feverfew exerted 16 times the free-radical scavenging effects of Coffee-Berry and 288 times the effects of vitamin C (110). Research demonstrated that feverfew may reduce DNA damage, apoptosis, and epidermal hyperplasia following UV exposure, and may restore cellular thiol levels after exposure to cigarette smoke (109,111).

Feverfew contains parthenolide, a lactone that inhibits inflammation, inflammatory cytokines, and platelet aggregation (107,109,112,113). Because parthenolide causes skin irritation and sensitization when ingested or applied topically, parthenolide-free feverfew extract (Feverfew PFE™) was developed as an alternative for use in skin care products (32,109,114,115). In vitro and in vivo studies suggested that PFE combats erythema after UV exposure and reduces irritation (101,111).

PFE is considered gentle enough for use on sensitive skin. Clinically, it is used as an anti-inflammatory and for photoprotection, rosacea, shaving irritation, and erythema reduction (114). In a randomized, double-blind trial, 31 women with sensitive skin applied moisturizer with PFE twice daily. After three weeks, significant improvements in erythema, roughness, and skin irritation were noted (116).

Pycnogenol®

Pycnogenol® is derived from the bark of the French maritime pine tree, *Pinus pinaster*. It contains high levels of proanthocyanidins, which have a range of antimicrobial, antioxidant, anti-inflammatory, photoprotective, and anti-carcinogenic effects (117,118). This extract accelerates wound healing, reduces scar formation, stabilizes elastin and collagen, and minimizes UV-induced pigmentation and erythema (119–122). Pycnogenol's anti-inflammatory mechanisms may include the inhibition of interferon-gamma (IFN- γ), an inflammatory cytokine, and the subsequent downregulation of ICAM-1 in keratinocytes. ICAM-1 is implicated in inflammatory skin conditions, including psoriasis and atopic dermatitis (123). Pycnogenol is also believed to convert the vitamin C radical to its active form, thereby regenerating vitamin E, and to raise levels of glutathione and other free-radical quenching enzymes (1,124).

In a mouse study, topical application of 0.05–0.2% Pycnogenol solution following UV exposure dose-dependently reduced edema, inflammation, immunosuppression, and tumor carcinogenesis (125). In another murine model, a 1–5% Pycnogenol gel significantly reduced wound-healing time compared to control (2,126). Clinical research remains limited, but a study of 21 adults showed that oral Pycnogenol supplements taken for eight weeks reduced UV-induced cutaneous erythema (122).

Licorice Extract

Licochalcone A is the primary active ingredient in the extract of the licorice plant, *Glycyrrhiza inflata*. Glycyrrhizin and liquiritin are active components of licorice root, *Glycyrrhiza glabra*. Extracts from both plant species are touted for their anti-inflammatory and anti-tumor activities (32,127). Evidence suggested that licochalcone A is an anti-inflammatory that reduces pro-inflammatory cytokines and prostaglandins following UV irradiation (128). *G. glabra* and glycyrrhizin inhibited skin tumors when administered orally or topically in murine models (129).

Licorice extract is used clinically for rosacea and may be found in commercial products advertising “redness relief” (1,32). In an eight-week study of 62 adults, a topical licochalcone A formulation significantly improved subjects' rosacea and quality of life ratings (130). A liquiritin cream applied twice daily for four weeks significantly diminished melasma in a split-face controlled study of 20 women (131).

Mushroom Extract

Extracts from *Lentinus edodes* (shitake), *Grifola frondosa* (maitake), *Ganoderma lucidum* (reishi, mannentake, and lingzhi), and other mushrooms contain a variety of compounds with antioxidant and anti-inflammatory properties (32,132). Among their many effects, mushroom extracts scavenge free radicals, stimulate epidermal cell growth, and inhibit inflammatory cytokines, the elastin-degrading enzyme elastase, and collagen-degrading MMPs and AP-1 (2,132,133). Mushroom extracts may be efficacious in the treatment of dermal aging. When topical mushroom extract formulations were used to treat 31 women with moderate facial photodamage, clinicians reported significant improvements in overall photoaging, fine lines, pigmentation, and skin texture (133) (Fig. 1.4). In a study of 45 human subjects who applied a mushroom extract product daily, cell turnover increased significantly compared to control (4).

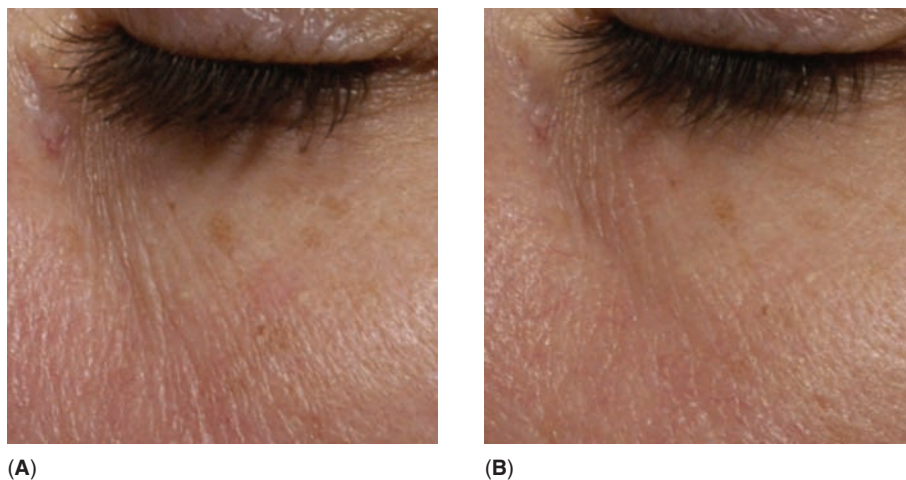


Figure 1.4 Digital photography before (A) and after (B) shows improvements in skin texture and fine wrinkling after 12 weeks of use of the mushroom complex serum. Source: By permission of J&J CCI; D. Miller.

Lycopene

Lycopene is a carotenoid responsible for the red color of many fruits, including tomatoes and watermelons (7). It exhibits considerable reductive potential and antioxidant activity (134). When administered orally or topically, lycopene exerts anti-carcinogenic effects, and when applied topically prior to UV exposure, it prevents apoptosis, reduces inflammation, and diminishes enzymes linked to tumor formation (7,135). In addition, lycopene has the ability to regenerate α -tocopherol (vitamin E) (134).

Clinical study of lycopene's dermal effects remains limited. In a trial of 10 volunteers, 6% topical lycopene reduced UV-induced erythema to a greater extent than a topical mixture containing vitamins C and E, and offered significantly more photoprotection than control (134). The formulation of lycopene may be important to its efficacy. In a study of human fibroblasts, lycopene was not shown to offer photoprotection or reduce UVA-induced levels of MMP-1 unless combined with vitamin E (136). In some experiments, lycopene enhanced rather than reduced UVA-induced oxidative stress in human and mouse fibroblasts (136,137).

Silymarin

Silymarin is a polyphenolic flavonoid from the milk thistle plant, *Silybum marianum*. It inhibits lipoprotein oxidation, scavenges free radicals (23), and exerts chemopreventive and anti-carcinogenic activity (7,25,138). It also reduces sunburn, apoptosis and edema following UVB exposure (139,140). Silymarin inhibits inflammatory cytokines and pyrimidine dimers, and reduces edema, hyperplasia, and proliferation (138,141).

Clinically, silymarin is used to alleviate symptoms of rosacea. In a double-blind, placebo-controlled trial, 46 subjects with rosacea were treated with a topical formulation of silymarin and methylsulfonylmethane. After one month, researchers noted statistically significant improvements in erythema, papules, itching, and hydration (142).

Chamomile

Chamomile (*Matricaria recutita*, *Chamomilla recutita*) is a member of the daisy family. This medicinal herb has been used as a soothing and moisturizing treatment for erythema, pruritus, sunburn, eczema, and allergic irritation, and it may improve skin texture and elasticity (143). Chamomile's anti-inflammatory effects may be due to its inhibition of cyclooxygenase, lipoxygenase, and histamine release (1,144). The literature contains one study that suggests that chamomile may also serve as an antioxidant (145).

Chamomile may be found in topical formulations that claim to combat inflammation, irritation, and dryness, particularly in sensitive skin. In a clinical study, topical chamomile cream proved more effective than 0.5% hydrocortisone for decreasing wound size and healing time following dermatitis and sunburn (146).

Aloe Vera

Aloe vera is a soothing, anti-inflammatory botanical with a variety of biological effects. Study suggests that it improves wound healing, diminishes pruritus, and possesses antifungal, antibacterial, viricidal, and antioxidant properties (144,147,148). The plant's active ingredients include salicylic acid and gel polysaccharides, which have anti-inflammatory activity, and magnesium lactate, an anti-pruritic (144). Like chamomile, aloe vera inhibits cyclooxygenase and lipoxygenase (1).

Aloe vera has been used in the treatment of burns, wounds, leg ulcers, frostbite, pruritus, and scarring related to radiation dermatitis (149). It may be an effective agent against psoriasis. In a double-blind clinical trial, patients with mild to moderate psoriasis who applied 0.5% aloe vera cream three times daily demonstrated a significant reduction in psoriatic plaques (83% vs. 8% for placebo) and increased rates of clinical cure (150).

Curcumin

Curcumin (diferuloylmethane) is the primary active agent within the herb turmeric (*Curcuma domestica*, *Curcuma longa*, or Zingiberaceae). This antioxidant possesses robust anti-inflammatory capabilities, resulting from its inhibition of lipoxygenase, cyclooxygenase, prostaglandins, and pro-inflammatory cytokines (1,143,151).

Animal models demonstrated curcumin's chemopreventive activities, which are attributed to increased apoptosis and the inhibition of tumorigenesis (7,143). According to other research, curcumin improves wound healing by bolstering collagen deposition, stimulating fibroblasts, and increasing angiogenesis and vascular density (151–153).

Preliminary study has explored the use of curcumin in the treatment and prevention of skin diseases, such as psoriasis, scleroderma, and skin cancer (151,154). Although curcumin inhibits collagenase, elastase, and hyaluronidase, suggesting that it may counteract photoaging, it has not yet been proven effective for this purpose (1,148).

Quercetin

Quercetin is an antioxidant and flavonoid found in many fruits and vegetables. Its anti-inflammatory actions may result from the inhibition of lipoxygenase, COX-2, and histamine release. By enhancing apoptosis in tumor cells, quercetin also exhibits anti-carcinogenic activity (1).

In a mouse model, quercetin reduced oxidative stress following UVA exposure (155). In vitro study found that quercetin inhibits melanoma cell growth (156). It also inhibits keloid fibroblast proliferation in a dose-dependent fashion, suggesting that it has promise for preventing and treating keloids and hypertrophic scars (157).

Allantoin

Derived from the comfrey root, allantoin is an anti-inflammatory antioxidant that accelerates the repair of cutaneous photodamage and reduces inflammation following UV radiation (1,148). Allantoin has been used in the treatment of psoriasis (158).

Sirtuins

Though not classified as antioxidants or anti-inflammatories, silent information regulators, or sirtuins, are a family of enzymes recently acknowledged as novel potential targets for anti-aging products. Sirtuins deacetylate histones to stabilize DNA and increase the longevity of organisms, including mammals, effectively mimicking the effects of caloric restriction (38). In an in vitro test of human skin keratinocytes, researchers found that UV exposure and ROS downregulate the sirtuin SIRT1 (silent mating type information regulation 2 homolog 1) (159), and theorized that reduced SIRT1 activity is linked to cellular damage caused by these insults. By acting as SIRT1 activators, resveratrol, yeast biopeptides, and other compounds may protect against this damage (159,160). In a clinical study of 33 women (mean age 51.6), daily application of a topical formula containing 1% *Kluyveromyces* yeast biopeptides, which are SIRT1 activators, resulted in improved fine lines and wrinkles, hydration, pigmentation, firmness, and texture after four weeks (160).

CONCLUSION

Antioxidants and anti-inflammatories comprise a rapidly expanding and ever-evolving area of study. New antioxidants are continually being explored, while longer-known compounds are undergoing continued research and adjustments to maximize their cutaneous effects (160,162). However, the U.S. Food and Drug Administration does not regulate the use of antioxidants and anti-inflammatories in cosmeceutical products or require skin-care companies to demonstrate the effectiveness of their ingredients or formulas. In vitro trials support the purported mechanisms of action outlined in this chapter, but few double-blind, vehicle-controlled studies exist to prove the benefits of antioxidants in the skin. A question that remains is the degree to which antioxidant efficacy hinges on emollient properties versus active therapeutic effects.

In summary, further study of the properties of antioxidants and anti-inflammatories is needed to elucidate their dermal effects, quantify the improvements that they may offer, and objectively review and compare the efficacy of antioxidant-containing oral and topical products.

DISCLOSURE

Dr. Berson has consulted for the following companies: Galderma, La Roche Posay, Neutrogena, and Stiefel.

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2 Proteins and Cytokines Used for Rejuvenation

Kenneth Beer

INTRODUCTION

Perhaps the most fundamental method of utilizing cosmeceuticals is to deliver the proteins and cytokines responsible for communication between cells. Since cellular rejuvenation requires communication between cells, the most rational target for this goal is the cytokines and peptides responsible for the majority of cellular interactions.

Convincing a senescent fibroblast to produce new collagen or a disorganized, atrophic epithelial cell to revert to a more organized and thicker one is accomplished by molecules that stimulate different cells. In this chapter, we will discuss existing methods of utilizing peptides and cytokines as cosmeceuticals and consider potential means of doing so in the future.

Cytokines are small molecules that serve as communication transmitters between cells. Typically, they signal cells to either up regulate or down regulate certain metabolic processes. Cytokines bind to receptors and trigger a conformational change in their structure. This triggers a cascade of molecular alterations that alter cellular behavior.

Although most cytokine research is geared towards treatment of diseases such as cancer, diabetes, and hypertension, cytokines are also an intrinsic messenger involved in cellular aging. As cosmeceuticals have advanced beyond the addition of vague extracts with amorphous claims to the point of genomic and cellular interventions, attention has turned towards the effects of cytokines and proteins as highly effective cosmeceuticals. Cytokines and proteins can have various functions in cosmeceuticals but it is reasonable to divide them into a few categories: stimulants for regenerative machinery, analogs of normal cellular proteins or proxy messengers.

PEPTIDES AS STIMULANTS FOR REJUVENATION

The breakdown of collagen fibers results in the production of short chains of amino acids (short chain peptides). These peptides act as feedback inhibitors for matrix metalloproteins (MMPs) including collagenase (1). Small peptides (3–5 chain amino acids) may stimulate fibroblasts to produce collagen. Several of these compounds have already found their way into cosmeceuticals. One such compound, Matrixyl[®], is comprised of five amino acids attached to a carrier molecule. This molecule represents a fragment of type 1 procollagen and it has been demonstrated to increase fibroblast production of collagen (2). This short chain peptide is part of a positive feedback loop that tells the fibroblasts to produce more collagen. As our understanding of these signal peptides improves, it is likely that signal peptides will play an increasing role in cosmeceutical formulas.

Senescent fibroblasts are known to have a diminished capacity to produce type 1 collagen (3). However, these fibroblasts can be stimulated to increase collagen production during the healing

phase that follows an injury or thermal stimulation. During this phase, several molecules, including numerous peptides signal the fibroblasts to up regulate their activity. For instance, following ablative laser resurfacing there is an increase in the amount of mRNA for collagen. Creating a cosmeceutical that harnesses these post-laser peptides and cytokines will be an opportunity to deliver resurfacing rejuvenating effects without the procedure.

Several of the peptides responsible for increased fibroblast activity have been elucidated. However, it is difficult to deliver larger molecules through the epidermal barrier. The ability to deliver large molecules through the epithelial layer has recently been enhanced. For instance, topical delivery of botulinum toxins has recently been achieved (4). This new delivery capability will enable peptides and cytokines responsible for wound healing to be increasingly effective cosmeceutical ingredients.

As the skin ages, there is an increase in the activity of enzymes that degrade collagen (1). Tipping the balance towards more youthful and plentiful collagen can involve inhibiting collagenase using cosmeceuticals or oral medications. Various types of inhibitors have been used to diminish the enzymatic activity of collagenase (matrix metalloproteinase 1, MMP-1). These range from low doses of antibiotics such as sub-antimicrobial doxycycline to small chain proteins used to inhibit matrix metalloproteinases. Presently, these molecules are delivered orally but they or the signal peptides they induce are potential candidates for cosmeceutical ingredients.

Another hallmark of senescent skin is fragmentation of the collagen matrix. This is due to the actions of matrix metalloproteinases including collagenase (5). The collapse of the dermal matrix causes a loss of support for fibroblasts which then regress and no longer produce significant amounts of collagen but instead produce enzymes that degrade the collagen. The authors of this study believe that rejuvenating procedures such as laser resurfacing and renovating medications such as tretinoin are able stimulate production of new collagen which in turn is able to anchor fibroblasts and restore a more youthful structure to the dermis. Key components of this cascade involve cytokines and/or peptide molecules and these would make exquisitely effective cosmeceuticals.

PEPTIDES INVOLVED IN RETINOID EFFECTS

Tretinoin and retinoic acid have been used for more than 20 years to rejuvenate the skin. Tretinoin has been demonstrated to inhibit the increase of metalloproteinases seen after ultraviolet light and this is one mechanism of its role in skin rejuvenation (6). Related molecules including retinol are used in cosmeceutical formulations to provide some of the benefits of the prescription product.

Although these molecules are not peptides or cytokines, the interactions with cellular mechanisms trigger a series of events that require peptides for their actions. The binding of the tretinoin molecule to a retinoid receptor begins a cascade of events that ultimately lead to decreased wrinkles, more organized epidermal layers and more youthful collagen. Targeted signal peptides amplify the actions of the retinoids and will be powerful additions to our cosmeceutical palette.

TOPICAL GROWTH FACTORS

Peptides that have gained widespread commercial acceptance include the various growth factors formulated as topical cosmeceuticals. Initially harvested from cells grown in culture and incorporated into topical creams, these cosmeceutical products have become more specific in their peptide contents as well as in their goals. The rationale for the incorporation of these molecules into cosmeceutical products is that they trigger the epidermis to respond to more youthful signals and thus, a more youthful appearance. A pilot study on one product demonstrated that “the application of a mixture of topical growth factors may stimulate the repair of facial photodamage resulting in new collagen formation, epidermal thickening, and the clinical appearance of smoother skin with less visible wrinkling” (7) (Fig. 2.1). Subsequent studies, using more specific growth factors and cytokines harvested from stem cell cultures rather than fibroblast cultures, demonstrated significant improvement of the wrinkles of the periorbital area (8) (Fig. 2.2). These products are among the first to directly utilize cytokines and growth factor proteins to stimulate cellular renewal. Their efficacy and popularity have prompted research into more targeted molecules which are gradually finding their way into cosmeceuticals. The future of cosmeceuticals will most likely contain peptides and cytokines that expand upon the effects obtained with the products already being used.

MECHANISM OF ACTION OF LASER/HEAT SHOCK PROTEINS

The use of lasers and intense pulsed lights has been a mainstay of skin rejuvenation for more than a decade. Fractionated versions are now being used to remodel the skin. Whereas some of the benefits of these treatments have been postulated to result from the shrinkage of collagen and elastic fibers, others are thought to derive from the repair of the thermal injury created. However, aspects of the benefits are due to elaboration of heat shock proteins (HSPs) including HSP70 (9). Topical products containing these peptides have the potential to deliver laser results. HSPs are a likely source of future cosmeceuticals.

PEPTIDES AS ANALOGS OF NEUROMUSCULAR INHIBITORY MOLECULES

The biochemical research surrounding the use of botulinum toxins has greatly expanded our knowledge of how these molecules work. Mechanisms of action for the various sub-types of botulinum toxins have been categorized and it is known that the type A toxin works by cleaving the SNAP 25 protein, preventing vesicle release of acetylcholine into the neuromuscular junction. However, it has been demonstrated that the entire molecule is not necessary for this reaction. Small fragments of these peptides have been shown to inhibit the release of neurotransmitters in a manner similar to the botulinum toxins (10). Although there have been cosmeceuticals that have compared themselves to botulinum toxins, to date, none have been shown to have the same activity in a published clinical trial. However, as mentioned previously, newer cosmeceutical delivery vehicles have the capability to deliver the small fragments capable of inhibiting neuromuscular release.

PEPTIDES AS REPLACEMENT MOLECULES FOR COLLAGEN AND ELASTIN

Collagen fibrils are complex structures comprised of collagen molecules. The collagen molecules are assembled from various

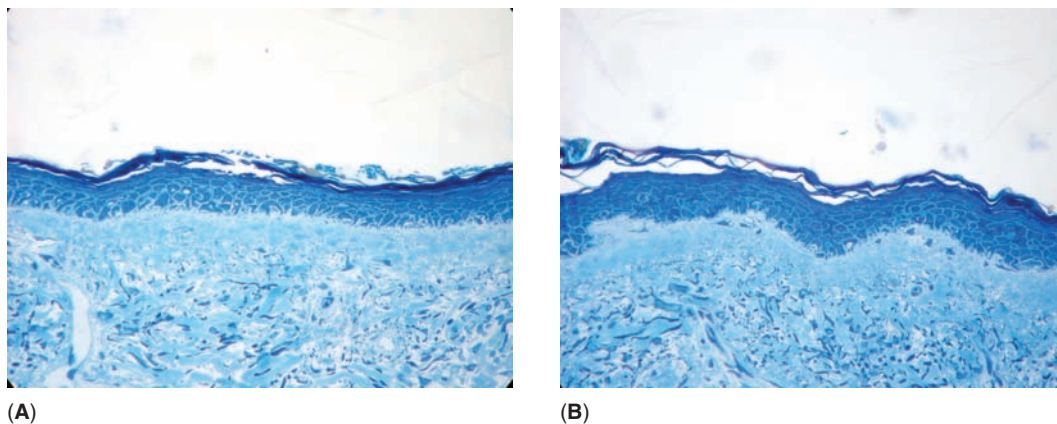


Figure 2.1 (A, B) Examples of histology obtained by light microscopy indicating a thickened epidermis, a slightly improved dermal–epidermal junction as well as a significantly increased cellularity, particularly in superficial dermis, reflecting new collagen synthesis at the end of the six-month treatment period with the human growth factor and cytokine containing skin cream. Both micrographs are at identical scale. *Source:* Courtesy of Dr. Robert Phelps, Dr. Mussarat Hussain, and Dr. David J Goldberg.

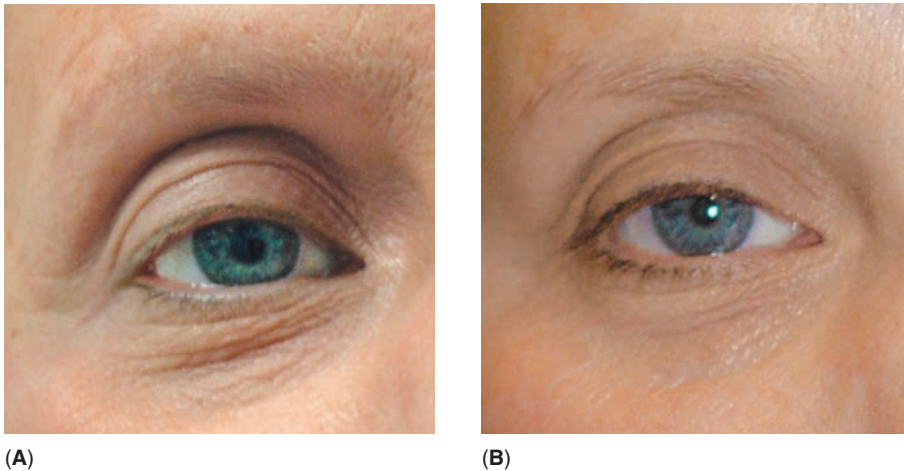


Figure 2.2 Photographs taken (A) before and (B) after six consecutive weeks of twice-daily application of the human growth factor and cytokine containing eye cream demonstrating reduced dark circles after the treatment period. Source: From Ref. 8. Courtesy of Dr. Mary L. Lupo, Dr. Joel L. Cohen, Dr. Marta I. Rendon, Neocutis, and the *Journal of Drugs in Dermatology*.

amino acids. Once again, the epithelial barrier has been the obstacle to getting intact collagen or large constituents into the dermis to replenish this layer and decrease wrinkles and creases (one major goal for any cosmeceutical). Delivery technologies for cosmeceuticals will deliver collagen in a clinically relevant manner. Elastin fibers will also be renovated in this manner. This will make cosmeceutical efficacy significantly better for treating wrinkles.

CYTOKINES AND SIGNAL PEPTIDES AS STIMULANTS OF MORE YOUTHFUL CELLULAR MACHINERY

Short chains of amino acids (short chain peptides) are elaborated after the breakdown of collagen and other structural fibers. These peptides may act as feedback inhibitors for MMPs (1). Short chain amino acids may stimulate fibroblasts to produce collagen and several have been utilized in commercial preparations. One such compound, Matrixyl[®], is comprised of five amino acids attached to a carrier molecule. This molecule constitutes a fragment of type 1 procollagen and it has been demonstrated to increase the production of collagen by fibroblasts (2). It is possible that this short chain peptide represents a positive feedback loop and tells the fibroblasts that they need to produce more collagen because at a cellular level, the fragments represent destroyed collagen which needs replacement. As these signals to the fibroblasts are intercepted and deciphered, more of these peptide molecules will find their way into cosmeceuticals.

Peptides may also serve as carrier molecules for a variety of substances (11). Copper is one such substance bound by peptides. Cosmeceuticals that deliver copper-peptide complexes are currently marketed and one such molecule (a glycyl-histidyl-lysine complex known as GHK) has been shown to have several beneficial cosmetic properties (12). Metals other than copper (such

as magnesium) are needed by cells responsible for healing and rejuvenation. Peptide complexes with other metals and catalysts will likely be developed to have more profound cosmeceutical activities.

CONCLUSION

Peptides and cytokines are potent effectors of skin rejuvenation. At the present time, they are included in cosmeceuticals in the form of growth factors and other mediators. Future versions will likely include botulinum toxin analogs, HSPs, and retinoid mediators. As our understanding of these molecules and our ability to deliver them through the epidermal barrier increase, so will our ability to effect changed to the appearance of the skin.

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3 Cosmeceutical Peptides

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INTRODUCTION

Cosmeceuticals are topical creams and lotions designed to improve the appearance of aging skin. These products are not tested and approved as drugs, but rather appear in the consumer arena based on theoretical benefits from *in vitro* studies of active ingredients. Peptides, which are short chain sequences of amino acids, are perhaps the most popular topical anti-aging product on the market. There are three main categories of cosmeceutical peptides: signal peptides, carrier peptides, and neurotransmitter-affecting peptides. The active ingredient must be delivered to the target in a stable form and be able to have the desired biologic effect *in vivo*.

BACKGROUND

Cosmeceuticals are topical product formulas designed to improve the appearance of the skin. The 1938 Food, Drug and Cosmetic Act differentiated clearly between drugs and cosmetics and charged the US Food and Drug Administration with regulating these products. This document allows the use of raw materials and ingredients in cosmetics for “cleansing, beautifying, promoting attractiveness, or altering the appearance” without approval by a government agency; however no therapeutic claims for these products may be made (1, p. 1). In 1984, Albert Kligman, MD, PhD, coined the term “cosmeceutical” to describe products that combine the concepts of “cosmetic” and a drug” (a product designed to mitigate or prevent disease). Dr. Kligman believes that a “cosmeceutical” should represent a product that “does something more than coloring the skin and something less than a therapeutic drug” (2, p. 890). Thus, cosmeceuticals have a unique niche in dermatology and some 90% of cosmetics now fall into this growing category.

The differences between cosmeceuticals and drugs may involve a simple difference in terminology. For example, although a drug and a cosmeceutical may contain the same active ingredient, the drug may be marketed as an anti-aging substance, while the cosmeceutical must be marketed as an agent that may “improve the appearance of wrinkles,” given that therapeutic claims may be made only for drugs (1, p. 2). More than cosmetics that adorns or camouflage, but not categorized as drugs that alter cellular functions, cosmeceuticals have grown in popularity in the past two decades. Both the peptides and their degradation products are key players in the field of cosmetics and dermatology. One of the most popular categories is the peptide group (3, p. 343).

Small sequence amino acid chains are being incorporated in cosmetic formulas to improve the signs of aging skin. According to Lupo (4), chronologically aged skin demonstrates lower procollagen type I messenger RNA and protein resulting in decreased production of new collagen (5, p. 1218). In addition, aging skin, and particularly aging skin that is exposed to ultraviolet

(UV) light, overexpresses proteolytic activity of matrix metalloproteinase-1, also known as interstitial collagenase (6, p. 43). Additionally, aging fibroblasts have a lower rate of proliferation than do fetal fibroblasts (7, p. 99). Much of the research demonstrating the role of amino acids and peptides in reversing the cutaneous signs of aging has been a secondary benefit of research on wound healing. These peptides are small sequence amino acid chains that may stimulate angiogenesis, production of granulation tissue, and new collagen synthesis. These classes of peptides have been designed with these goals in mind (Table 3.1).

SIGNAL PEPTIDES

Peptides with the ability to increase fibroblast production of collagen or decrease collagenase breakdown of existing collagen should potentially improve the clinical appearance of fine and course wrinkles visible in both chronologically and photoaged skin. Advanced research into the cellular and biochemical processes of aging and wounded skin is resulting in new strategies to manipulate these processes for therapeutic clinical effect. As wound healing and genomic research continues, certain bioactive amino acid chains have been discovered, which stimulate human skin dermal fibroblast growth *in vitro*. The use of signal peptides in commercial products has significantly risen in recent years. Peptides in the skin have been shown *in vitro* to: (i) stimulate the production of extracellular matrix components including collagen, elastin and fibronectin and (ii) decrease the production of glycosaminoglycans (GAGs) as illustrated in Figure 3.1.

Additionally, the variations in amino acid sequence, number of amino acids, and use of derivatives of these acids, there are limitless combinations of possible peptides. By testing the effect of multiple peptides on the synthesis of collagen in culture, scientists at the University of Tennessee narrowed the field of possible amino acid combinations to a pentapeptide fragment of procollagen, KTTS (lysine, threonine, threonine, lysine, serine). This peptide retained 80% of the collagen-stimulating activity of the original, much larger 34–44 amino acid procollagen peptide from which it was delivered (8).

According to Kamoun (9) and Tajima (10), the linking of valine-glycine-valine-alanine-proline-glycine (VGVAPG) peptide was discovered in one study of elastin-derived peptides, which significantly stimulated human dermal skin fibroblast production, and simultaneously down regulated elastin expression. Transdermal delivery of ionic peptides is likely to pose a significant issue for delivery of bioactive peptide into the skin. The signal peptide is combined with palmitic acid to aid in peptide penetration of the epidermis and is marketed currently in many cosmeceutical products under the name of palmitoyl oligopeptide (11).

Table 3.1 Cosmeceutical Peptides

Type	In vitro	Expected in vivo clinical benefit
Signal peptides	Triggers wound-healing mechanisms that activate fibroblasts in response to fragmented chains of elastin and collagen	Increased collagen production to improve skin appearance
Carrier peptides	To deliver copper into skin, resulting in activation of enzymatic wound-healing pathways	Enhanced copper production, resulting in smoother skin
Neurotransmitter-inhibiting peptides	Interferes with stabilization step in neurotransmitter release	Decreases muscle movement

Source: Adapted from Ref. 4.

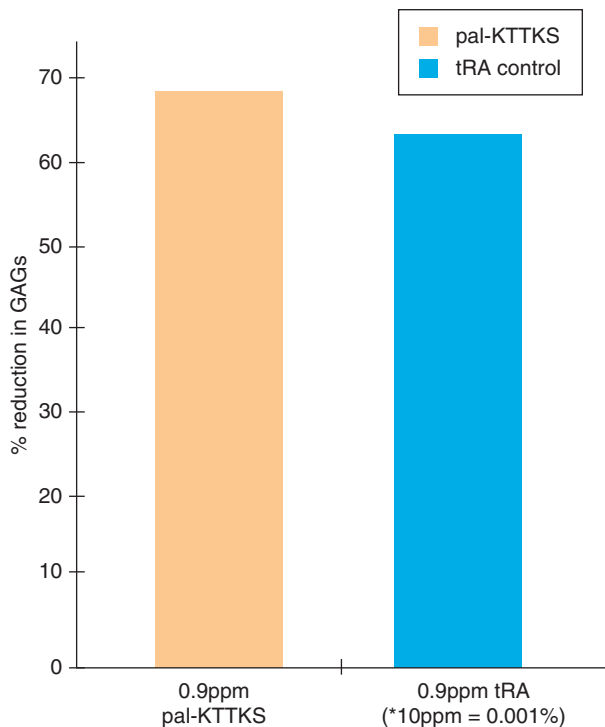


Figure 3.1 In vitro cell culture experiments, pal-KTTKS reduces glycosaminoglycan (GAG) comparably at 1/10th the dose of tRA (10 ppm = 0.001%). Source: From Ref. 8 and P&G Beauty.

In another study, Njieha et al. (12, p. 758) examined a different linking of peptides. Tyrosine-trosine-arginine-alanine-aspartame-aspartame-alanine sequence inhibited procollagen-C proteinase, which cleaves C-propeptide from type I procollagen, thus leading to decreased collagen breakdown.

The most prevalent and widely published signal peptide is the sequence lysine-threonine-threonine-lysine-serine (KTTKS) found on type I procollagen. This pentapeptide has been demonstrated to stimulate feedback regulation of new collagen synthesis and results in an increased production of extracellular matrix proteins such as types I and II collagen and fibronectin (3, p. 344). Like VGVAPG, KTTKS is linked to palmitic acid in order to enhance delivery through the epidermis for engagement in the dermis. In a 12-week, double-blind, placebo-controlled, split-face, randomized clinical study of 93 Caucasian women between 35 and 55 years of age, Robinson and others found that pal-KTTKS was well tolerated and significantly reduced fine lines by both qualitative technical and expert grader image analysis (Fig. 3.2) (3, p. 344). Of importance to mention, Robinson et al. (13) also found pal-KTTKS to be both efficacious and gentle on the skin barrier, as assessed by TEWL measurements. Maxtrixyl, the most common signal peptide currently on the market, is an ingredient in Olay Regenerist® and Stivectin-SD®.

The tripeptide glycyl-l-histadyl-l-lysine (GHK) is primarily known as carrier peptides, but it has also been shown to have some signal peptide effects. GHK without copper has been shown to enhance collagen production by stimulating fibroblasts (3, p. 344). GHK has also been linked with palmitic acid and marketed as Biopeptide-CL. In vitro and in vivo studies have been performed by the company (11).

CARRIER PEPTIDES

Carrier peptides function to stabilize and deliver important trace elements necessary for wound healing and enzymatic processes. In cosmeceuticals, the most common carrier peptide used is the delivery of copper into the cells. Copper is an elemental metal that enhances wound healing, enzymatic processes, and angiogenesis. There are several mechanisms whereby copper may have beneficial effects in the skin. Lysyl oxidase is an important enzyme in collagen and elastin production. It is dependent upon the action of copper. Tyrosinase and cytochrome-c oxidase require copper as well. Superoxide dismutase acts as an important antioxidant and requires copper and a cofactor. Copper is an essential cofactor for collagen and elastin formation, down regulate MMP's, and reduces the activity of collagenase. The tripeptide complex, glycyl-l-histidyl-l-lysine (GAK) spontaneously complexes with copper and facilitates the uptake of copper by cells (14, p. 715). This peptide sequence is found in proteins of the extracellular matrix such as the alpha chain of collagen, and it is believed to be released during wounding and inflammation. Prepared as a cosmeceutical, copper peptide is thought to improve skin firmness and texture, fine lines, and hyperpigmentation. GHK-copper complex increases levels of MMP/TIMPs and aids in dermal tissue remodeling (15, p. 2257). It also causes stimulation of collagen I, GAGs, cytochrome-c oxidase, and tyrosinase (16, p. 962; 17, p. 345). As mentioned earlier in this section, a feedback stimulation of collagen repair has also been proposed for this peptide (3), but the main benefit to photoaged skin is believed to be its ability to enhance delivery

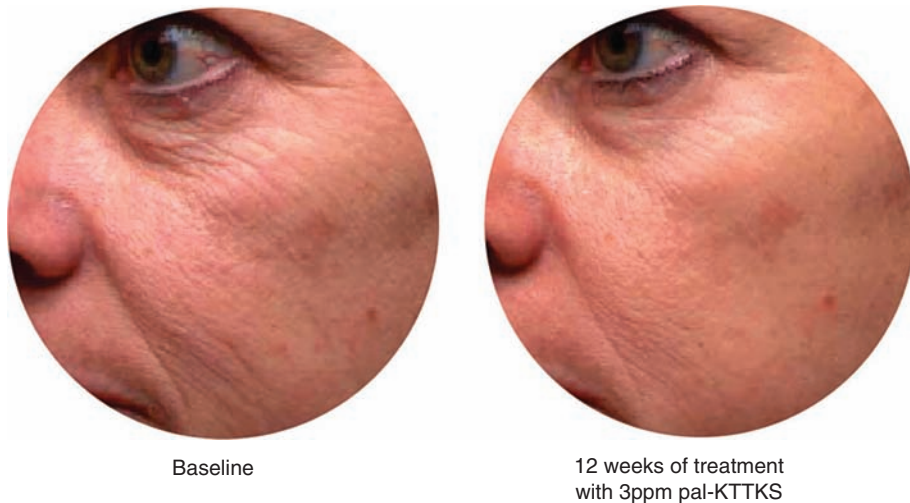


Figure 3.2 A significant improvement in the appearance of fine lines and wrinkles is observed following 12 weeks of treatment with moisturizer containing pal-KTTKS. *Source:* From Ref. 13 and P&G Beauty.

of copper. Both the tripeptide alone and copper tripeptide complex have been found to have beneficial effects on collagen stimulation. This carrier peptide–copper combination has also been found to increase levels on MMP-2 and MMP-2 mRNA, as well as TIMP-1 and -2. As such it could function in collagen remodeling (15, p. 2257). Experiments using GHK–Cu have demonstrated stimulation of both type I collagen and the GAG's dermatan sulfate and chondroitin sulfate in rat wounds as well as cultured rat fibroblasts (16, p. 962). Human fibroblast cultures showed increased synthesis of dermatan sulfate and heparin sulfate after addition of the tripeptide–copper complex (18, p. 1049). Limited clinical trials with patients using a facial cosmeceutical product containing the complex as the active ingredient did demonstrate improvement in the appearance of fine lines as well as increase skin density and thickness (19).

NEUROTRANSMITTER-AFFECTING PEPTIDES

Multi-chain peptides have generated interest for potential wrinkle-fighting properties. Pentapeptides may be more popular with patients and physicians, matrixyl acetate or Pal-KTTKS pentapeptide coupled with palmitic acid is a widely available drugstore product (Regenerist, Proctor & Gamble). However, the neurotransmitter-affecting peptides currently incorporated into cosmeceutical products were developed as topical mimics of the botulinum neurotoxins. Currently, only Botulinum neurotoxin type A (BTX-A) has been approved for subcutaneous, intradermal, and intramuscular injection for facial wrinkles (20, p. 543). All botulinum neurotoxin serotypes (A–G) are single chain polypeptides which inhibit acetylcholine release at the neuromuscular junction (NMJ) in a three-step process. The single chain polypeptides are activated by proteases and cleaved into a double chain consisting of heavy and light chain moieties. Upon cleavage, the heavy chain binds to a high-affinity receptor on the presynaptic nerve terminal, which enables

internalization of the bound toxin into the cell. The light chain moiety is a zinc-dependent endopeptidase that cleaves membrane proteins that are responsible for docking acetylcholine vesicles on the inner sides of the nerve terminal membrane. The cleavage of these proteins inhibits the fusion to the vesicles with nerve membrane, thereby preventing release of acetylcholine into the NMJ. The intracellular target of BTX-A is synaptosome-associated protein of molecular weight 25 kDa (SNAP-25), which is a protein essential for successful docking and release of acetylcholine vesicles with the presynaptic vesicle. In contrast, botulinum neurotoxin type B (BTX-B) cleaves vesicle-associated membrane protein (VAMP), which is known as synaptobrevin. Like SNAP-25, VAMP is essential for the docking and fusion of the synaptic vesicle to the presynaptic membrane for the release of acetylcholine. Use of these polypeptides inhibits the repetitive contraction of the intrinsic muscles of facial expression and thereby reduces hyperkinetic facial lines (21, p. 34). The topical neurotransmitter-affecting peptides that are currently marketed in cosmeceuticals reportedly function to decrease facial muscle contraction and thus reduce lines and wrinkles by raising the threshold for minimal muscle activity, requiring more signal to achieve movement and reducing subconscious muscle movement over time. Most of these peptides act on the soluble *N*-ethylmaleimide-sensitive factor attachment protein receptors (SNARE) complex, whereas others target different parts of the NMJ or certain neurotransmitters. Although it sounds like botulinum neurotoxin in a jar, it has yet to be proven whether these topical neurotransmitter-affecting proteins can penetrate to the level of the NMJ. The most popular cosmeceutical peptide in this category is acetyl hexapeptide-3 marketed as Argireline (McEit International Trade Co, Ltd; Tianjin, China).

Pentapeptide-3, a peptide of unpublished amino acid sequence currently marketed as Vialox® (Cellular Skin Rx), is

purported to act similarly to tubocurarine, the main active ingredient of curare (22). The peptide is a competitive antagonist at the acetylcholine postsynaptic membrane receptor. As the acetylcholine receptors are blocked, the sodium ion channel remains closed. Therefore, there is no sodium ion influx to depolarize the cell and lead to muscle contraction, and smooth muscles stay relaxed. Company-performed in vitro and in vivo studies have been performed to confirm efficacy at reducing muscle contraction and decreasing wrinkle size and skin roughness (Centerchem). Leuphasyl® (Lipotec S.A.), another pentapeptide of unpublished amino acid sequence, is proposed to modulate calcium channels by mimicking enkephalins. Enkephalins are endogenous opioids that inhibit neuronal activity. Their receptors are on the outside of neurons, couples to inhibitory G-proteins (Gi). The docking of enkephalins on these receptors results in the release of G-protein subunits (alpha, beta or gamma) in the cell. These subunits close calcium ion channels and open potassium ion channels. Preventing the entry of calcium ions into the neuron avoids vesicle fusion and consequently inhibits acetylcholine release across the synapse to the muscle (23,24). This enkephalin-like peptide couples to the enkephalin receptor on the outside of nerve cells and a conformational change initiates a cascade inside the neuron that results in a decrease of excitability and modulates the release of acetylcholine, thus diminishing muscle contraction. In vivo and in vitro placebo-controlled cosmeceutical studies performed by the company (Lipotec) reportedly confirm efficacy at reducing neurotransmitter release and decreasing wrinkle depth as assessed by skin topography analysis of silicon imprints. The studies also showed a synergistic effect when both Leuphasyl®

and Argireline® were applied together (Centerchem). Tripeptide-3(beta-Ala-Pro-Dab-NH-Benzyl*2AcOH), currently marketed as Syn®-Ake (Lipotec S.A.) (25), is proposed to act similarly to Walglerin-1. Walglerin-1 is a neurotoxin found in the venom of the temple viper, which causes reversible antagonism of muscular nicotinic acetylcholine receptors (mnAChR) at the postsynaptic membrane. It is proposed to bind to the epsilon subunit of the mnAChR which prevents binding of acetylcholine to the receptor, preventing it from opening. In the closed state, there is no uptake of sodium ions so that no depolarization takes place and the muscles remain relaxed (20). The company (Pentapharm) has performed their own in vitro and in vivo tests confirming its efficacy in decreasing muscle contraction and reducing wrinkle depth (Centerchem).

CLINICAL TESTING OF PEPTIDE PRODUCTS

In 2006, a randomized, investigator-blinded, parallel study of 77 female subjects compared four products that purported to improve wrinkles—BTX type A (BTX-A), StriVectin-SD, DDF Wrinkle Relax, and Hydroderm™—with a placebo injection (26, p. 191). This study, supported by Allergan, Inc, evaluated the products based on the safety and efficacy of treating moderate-to-severe glabellar rhytids. The products were assessed according to blinded investigators assessments of glabellar line severity on the facial wrinkle scale (FWS) and subjects' global assessments of overall change in appearance, ratings of glabellar-related self-perception before and after treatment, and satisfaction with the results. Figure 3.3 illustrates results based upon the FWS (26, p. 191). Statistically significant reductions in wrinkle severity on the FWS were found with

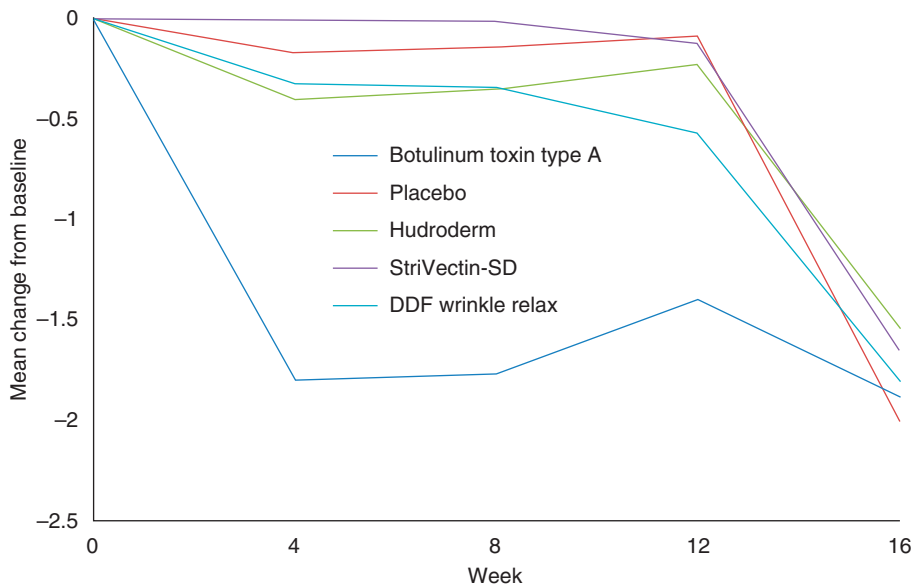


Figure 3.3 Changes in facial wrinkle scale. Source: From Ref. 26.

the use of BTX-A resulted in statistically significant improvements in subject reported outcomes and satisfaction.

NEW INNOVATIONS IN TECHNOLOGY AND CLINICAL TESTING

The past decade has witnessed remarkable advances in the field of biology. Disciplines such as genomics and proteomics have emerged to exploit our growing knowledge of the human genome and have drastically accelerated our ability to understand how the human body responds to its environment. Genomics is a branch of biotechnology which applies genetic and molecular biology techniques to the genetic mapping and DNA sequencing of sets of genes or the complete genomes of selected organisms, whereas proteomics applies the techniques of molecular biology, biochemistry, and genetics to analyzing the structure, function, and interactions of the proteins produced by the genes of a particular cell, tissue or organism. The development of genomics and bioinformatics capabilities today enables a comprehensive assessment of skin aging and photoaging at the fundamental level of gene expression. Bioinformatics tools also enable an integrated analysis of gene expression themes, pathways, which provide new insights into the mechanisms of skin aging and possible interventions (27). Robinson et al. conducted a combined study of chronologic skin aging and photoaging to address several questions: (i) How does gene expression compare between young and old skin? (ii) How does gene expression compare between UV-exposed skin and UV-protected skin? (iii) Can genomics help dermatologists understand the skin aging process; and (iv) Can genomics help dermatologists identify important biomarkers of skin aging and perhaps new targets to intervene in the skin aging process (p. 8). At the conclusion of the study, a strong knowledge base was derived that provided new insight into the mechanism of skin aging.

One of the most important technologies in the genomics toolbox is the gene chip or microarray. The gene chip allows dermatologists to determine which genes are turned on/up or turned off/down in response to different biological conditions. Previously, if researchers were interested in measuring these gene changes, it would have to be done one gene at a time. However, with the gene chip, tens of thousands of genes can be monitored in a single experiment; allowing the entire genome

to be examined in less than a week's time. If one gene at time was monitored, each trial would take nearly 150 years.

Genomics tools such as gene chips have become well established in the medical field of over the past 15 years and most universities have instituted some form of genomics curriculum in their biomedical programs. Additionally, this platform has proven to be very useful for learning how the skin responds to microbial infection including malassezia (28). Furthermore, pharmaceutical companies have seen the potential of this capability and exploiting it to develop new drugs and therapeutics. While the close connection between genomics technologies and medicine has been well established over the years, there are other realms of biology that are showing the benefits of incorporating genomics technologies into their research programs. The advent of next generation DNA and RNA sequencing technologies are poised to significantly impact the study of skin. The Next-Gen RNA platforms like RNA Seq (also known as Digital Gene Expression Profiling) promise to replace microarrays in the future by providing even more information about the transcription process (28). Once the information is received, dermatologists can provide greater individualized patient skin care.

PEPTIDES: WHAT IS TO COME?

To further explore the power of peptides for anti-aging applications, Procter and Gamble Beauty Science continues diligent research on the matter. Osborne et al. (29) indicated how human skin equivalents provide useful in vitro models to identify and evaluate cosmetic technologies based on knowledge gained via gene expression profiling of aged skin. A series of acyl-modified di-, tri-, and tetra-peptides were synthesized and screen in vitro for their capacity to stimulate production of collagen-I as well as other skin structural proteins, including procollagen-C, collagens III, IV, and VI, elastin, fibronectin, CD44, vimentin and laninins I and IV in both dermal fibroblasts and human skin equivalent cultures.

Of the peptides evaluated, palmitoyl-lysine-threonine (pal-KT) stimulated skin biomarkers to the greatest extent. RT-PCR analysis of mRNA from the human skin equivalent cultures revealed significantly increased expression of the skin structural proteins. The results illustrated in Figure 3.4 suggest

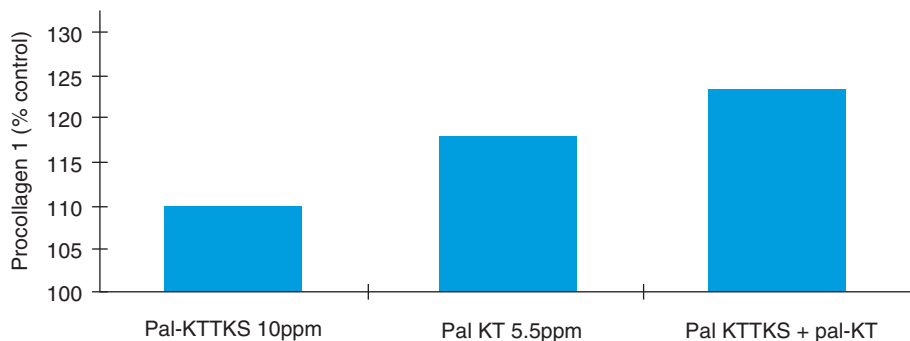


Figure 3.4 In vitro cell studies, pal-KT increases collagen synthesis to a greater extent than pal-KTTS. Further, the combination of both peptides has an even more profound effect on collagen synthesis that either peptide alone. Source: From Ref. 29 and P&G Beauty.

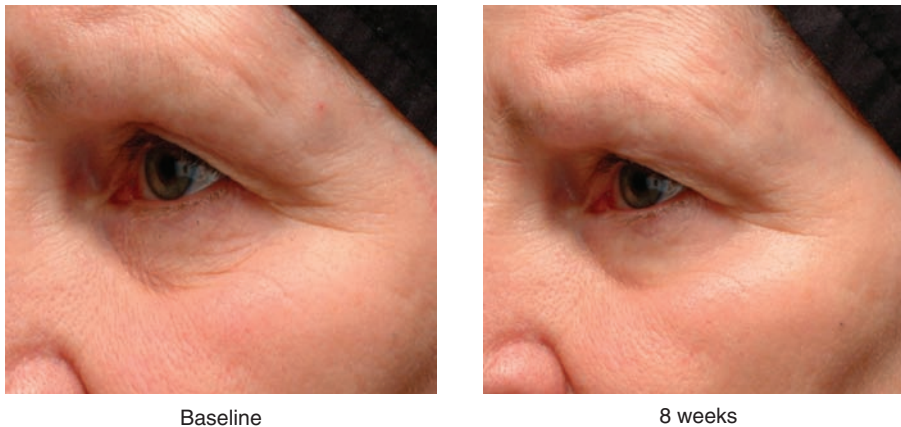


Figure 3.5 Used Olay Professional ProX 3-product wrinkle regimen for eight weeks: SPF 30 lotion during day; night cream at bedtime; wrinkle treatment twice daily. Source: From Ref. 31 and P&G Beauty.

that pal-KT is a promising candidate for cosmetic ingredients, either alone or in combination with other peptides.

According to a recent P&G genomic study (30), a regimen approach provides the greatest treatment flexibility as well as enhanced opportunity to use optimum levels of potentially incompatible anti-aging ingredients. After eight weeks of daily application, more than 70% of subjects showed some improvement. Computer image analysis showed that the periorbital region was significantly reduced (Fig. 3.5). Unlike the daily application of a tretinoin, considered the benchmark prescription, neither dryness or TEWL increased significantly during the study (31).

Cosmeceutical peptides are a major player in the anti-aging market. Dermatologists are trained adequately to guide patients in maintaining a youthful appearance and lessen any confusion over the bombardment of anti-aging claims. Clearly, peptides play various roles in combating the signs of aging. The potential for new treatment option, as represented by cosmeceutical peptides, is a developing field with most ongoing research occurring within the industry. In some of the marketing arenas, some peptide-containing cosmeceuticals cost much more than the average peptide bought at the drug store instigating many questions from patients on which product is better. It is important that the final marketed product is stable in formula, deliverable to its target dermal site and biologically active at this target site. As dermatologists well know, these results do not always translate into *in vivo* actions. It is not an easy task to penetrate the barrier of the skin. Double-blinded, placebo-controlled drug study is lacking, as it is with all cosmeceuticals as a result of regulatory concerns by industry (3, p. 348). Consumer demand for products that improve appearance and counteract the signs of aging likely will lead to more research and development into cosmeceutical peptides for primary and adjunctive treatment of the signs of aging.

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4 Growth Factors

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BIOCHEMISTRY OF SKIN AGING AND WOUND HEALING

Extensive research on skin aging in the last decade has resulted in an improved understanding of the pathophysiology of intrinsic (age-related) and extrinsic (UV-mediated photoaging) aging. Biochemical processes resulting in skin damage following exposure to UV radiation are now being identified and understood (1). A correlation between biochemical processes following photodamage and creation of wound is emerging. Of specific interest to cosmeceutical manufacturers are the effects of growth factors in the process of wound healing. Table 4.1 shows the stages of wound healing and role of growth factors in each stage. Growth factors are regulatory proteins that mediate signaling pathways between and within cells. After a wound has been inflicted, a variety of growth factors flood the wound site and interact synergistically to initiate and coordinate each phase of wound healing. They help recruit and activate fibroblasts to induce rapid production of the extracellular matrix to close the wound followed by stimulation and multiplication of keratinocytes to form the new epidermis. The overall process is complex and not completely understood (2).

Inflammation is induced as a result of formation of a wound or UV damage via several pathways including nuclear factor-kappa B (NF- κ B)-mediated activation of tumor necrosis factor- α (TNF- α) and interleukins (3). Reactive oxygen species (ROS) and proteolytic enzymes are generated as a result of inflammation which causes degradation of the extracellular matrix. ROS increase oxidative phosphorylation of cell surface receptors causing activation of transcription factors activator protein 1 (AP-1) and NF- κ B, two critical components of the MAP kinase signaling pathway (4). ROS therefore play a central role in intrinsic and extrinsic aging. AP-1 stimulates transcription of matrix metalloproteinase (MMP) growth factor genes in fibroblasts and keratinocytes, and inhibits type 1 procollagen gene expression in fibroblasts (5). Multiple studies have shown that increased secretion of MMPs resulting from intrinsic and extrinsic aging results in breakdown of the dermal matrix (6). 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). Tissue inhibitors of metalloproteinase (TIMP) decrease activity of MMPs providing a mechanism to balance MMP activity. ROS inactivate TIMPs by oxidation and indirectly increase MMP activity.

AP-1-mediated reduction in the synthesis of procollagen appears to result from two mechanisms, interference of AP-1 with type 1 and type 3 procollagen gene transcription and blocking the profibrotic effects of TGF- β by impairment of TGF- β type 2 receptor/Smad pathway (3). Activation of NF- κ B stimulates transcriptions of pro-inflammatory cytokine genes

including IL-1, TNF- α , IL-6, and IL-8 (4). Inflammation resulting from these cytokines increases secretion of ROS and more cytokines, further enhancing the effect of UV exposure. Inflammation causes protease-mediated degradation of elastin and UV exposure causes formation of abnormal elastin by fibroblasts. UV light is also an inhibitor of leucocyte elastase thereby increasing accumulation of elastotic materials (7). The accumulation of elastotic materials is accompanied by degeneration of the surrounding collagenous network. The overall effects of these interlinked biochemical activities is the reduction of procollagen synthesis, increase of collagen degradation in the dermal extracellular matrix, and increase in irregular elastin deposition. Successful resolution of damage to skin and 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 (8). 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 (9).

During wound healing, transition from inflammatory to granulation phase is mediated by a variety of growth factors and cytokines including PDGF, TGF- α , TGF- β , FGFs, IGF-1, CSF, ILs, and TNF- α (10). These growth factors and cytokines are derived from macrophages, epidermal keratinocytes, and fibroblasts. Multiple metabolic pathways lead to the 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 sunburned skin is the beginning of dermal tissue remodeling. During this stage, low strength, unorganized type 3 collagen and elastin structures produced during the ECM production phase are replaced by stronger type 1 collagen and structured elastin fibers to provide strength and resiliency to the dermis. This remodeling phase can last for several months and is the key to reversing the visible effects of skin aging (11).

SOURCES OF GROWTH FACTORS FOR COSMECEUTICAL USE

Human growth factors may be obtained from two major sources, cultured human cells or genetically engineered microorganisms. Human cells are capable of producing a wide variety of proteins in response to various stimuli. Fibroblasts or co-culture of fibroblasts and keratinocytes have been used to produce a mixture of growth factors and matrix proteins. Individual growth factors are commonly produced in bacteria and yeast. Stem cells derived from adipose tissue obtained from liposuction are being investigated for in vivo production of growth factors after intra-dermal injection.

Table 4.1 Role of Key Growth Factors/Cytokines in Wound Healing

Phase	Key growth factors	Activities
Hemostasis	TNF- α , inflammatory ILs	Neutrophils, platelets, and plasma proteins infiltrate the wound and initiate vasoconstriction Platelets release clotting factors to initiate coagulation Platelets then release cytokine and growth factors that attract neutrophils, macrophages, monocytes, and other cells necessary for cutaneous healing
Inflammation	TGF- β , TNF- α , IL-1, IL-6, IL-8, IL-10	Neutrophils initiate phagocytosis and attract macrophages Macrophages continue phagocytosis and release additional growth factors and cytokines which attract fibroblasts to the wound, promote angiogenesis, and stimulate keratinocyte growth
Granulation	TGF- β , PDGF, VEGF, HGF, NGF, IGFs, IL-8	Fibroblasts synthesize collagen New collagen fibers begin to form a matrix, or scaffold, for additional fibroblast attachment
Remodeling	TGF- β , FGF2, TIMPs, MMPs	Collagen fibers are remodeled, or cross-linked, into an organized matrix Additional collagen fibers attach to the matrix and are assembled into new tissue Wound contraction and tissue strengthening occur

Source: Modified from Ref. 21.

Natural Growth Factors

Human cells cultured in a three-dimensional network secrete a mixture of a large number of growth factors and other proteins capable of promoting wound healing (12). The composition of the growth factor mixture varies with cell phenotype and environmental variables. Cells growing under conditions resembling a wound are most likely to produce growth factors, cytokines, and matrix proteins that assist in wound healing. One commercial cell growth factor (NouriCel-MD, Skin-Medica, Carlsbad, CA) is collected from a three-dimensional matrix of dermal fibroblasts induced to produce collagen, the same protein they produce during wound healing. The associated combination of growth factors and cytokines (Table 4.2) naturally secreted during the collagen production phase of the tissue culture therefore represents the most appropriate combination to induce wound healing. Naturally secreted growth factors can also be obtained from fibroblast and keratinocyte co-cultures. Another method of collecting a mixture of growth factors and cytokines is to lyse fibroblasts and collect the intracellular components that include growth factors, cytokines, and other intracellular materials present at the time of cell lysis (13). Growth factors obtained from intracellular material may not have the final tertiary structure as many protein sequences

Table 4.2 Partial List of Growth Factors and Cytokines Identified in Active Gel and Their Function in Skin

Growth factor/cytokine	Skin-related functions
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 Bb	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: TGF- β 1, TGF- β 2, TGF- β 3	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: IL-1 α , IL-1 β	Early activators of growth factor expression in macrophages, keratinocytes, and fibroblasts
Interleukin: IL-2	Enhance epithelial wound healing
Interleukin: IL-6	Mediator of acute phase response to wound and has synergistic effect with IL-1
Interleukin: IL-10	Inhibits pro-inflammatory cytokines to reduce inflammation prevents scar formation
Interleukin: IL-4, IL-13	Stimulate production of IL-6
Interleukin: IL-3, IL-4, IL-5	Leukocyte maturation and degranulation during inflammatory phase
Interleukins: IL-7, IL-8, IL-15	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

Source: From Ref. 16.

require secretion through the cell membrane to form the final tertiary structure essential for its biological activity.

Growth Factors Secreting Stem Cells

Adipose-derived stem cells, a population of pluripotent cells, have been studied for promotion of wound healing by virtue of their ability to secrete growth factors. Preliminary studies show that intra-dermal injection of adipose-derived stem cell suspension can produce increased collagen production and reduce

Table 4.3 Synthetic Growth Factors Registered as Cosmetic Ingredients

INCI name	Growth factor
Human oligopeptide-1	Epidermal growth factor
Human oligopeptide-2	Insulin-like growth factor-1
Human oligopeptide-3	Basic fibroblast growth factor
Human oligopeptide-5	Keratinocyte growth factor
Human oligopeptide-7	Transforming growth factor beta-3
Human oligopeptide-8	Interleukin 10
Human oligopeptide-10	Platelet derived growth factor
Human oligopeptide-11	Vascular endothelial growth factor
Human oligopeptide-12	Fibroblast growth factor 10
Human oligopeptide-13	Acidic fibroblast growth factor
Human oligopeptide-14	Transforming growth factor alpha
Human oligopeptide-15	Interleukin 4
Human oligopeptide-19	Nerve growth factor
Human oligopeptide-20	Tissue inhibitor of metalloproteinases

Source: From Ref. 15.

signs of skin aging. These pre-clinical results warrant further evaluation in clinical studies after adequate testing and standardization of methods to obtain autologous adipose-derived stem cells (14).

Synthetic Growth Factors

Individual growth factors and cytokines can be made via recombinant technology using bacterial or yeast cultures modified to include the DNA sequence for specific growth factors. Many growth factors of cosmeceutical interest are produced by this technique including TGF-beta, VEGF, EGF, various FGFs, PDGF and more (15). Table 4.3 lists various growth factors registered with *International Cosmetic Ingredient Dictionary and Handbook* as cosmetic ingredients. While clinical studies have shown a beneficial effect of some of the individual growth factors, more studies must be done to understand the role of individual growth factors in skin rejuvenation. A combination of growth factors that complement effects of each other is likely to be more effective as multiple growth factors are involved in most biochemical processes including wound healing (8).

DESIRABLE ATTRIBUTES OF A TOPICAL GROWTH FACTOR PRODUCT

Growth factors produce anti-aging benefits by virtue of their biological role to maintain healthy skin structure and function. Together with cytokines, growth factors provide constant communication between cells of the immune system, keratinocytes, and fibroblasts throughout the process of wound healing and skin repair and regeneration. During the final remodeling phase of wound healing, a number of different growth factors and cytokines interact with each other and with the surrounding cells in concert to improve the quality of the extracellular matrix. Use of individual growth factors may not duplicate the complex interactions essential for remodeling of skin.

Therefore, a mixture of growth factors and cytokines proven to have a role in skin remodeling may yield higher efficacy.

Cosmeceutical products containing mixtures of natural substances such as cellular growth factors may be difficult to analyze for concentrations of active ingredients. Even products manufactured with a single growth factor are not labeled with the growth factor content which makes it difficult to compare product strengths. More analytical efforts are needed to ensure reliable quality standards. In addition, growth factors 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 further decrease product stability and compromise product efficacy during the claimed shelf-life of the product. A recent study shows presence of high levels of growth factors and cytokines in a commercial product stored at room temperature throughout its two-year shelf-life (16). If quantitative analysis is not possible due to complexity of the formulation, measurement of biological activity should be performed using appropriate techniques to ensure product stability throughout the labeled shelf-life.

CLINICAL EFFICACY OF PRODUCTS CONTAINING GROWTH FACTORS

The use of growth factors and cytokines in skin rejuvenation and reversal of photoaging is emerging as a novel anti-aging treatment. Table 4.1 lists some important growth factors and cytokines that affect the proliferation of dermal fibroblasts and extracellular matrix production (12). Growth factors, cytokines, and other agents that help rebuild the extracellular matrix are critical in the reversal of the signs of skin aging such as fine lines and wrinkles. Providing a physiologically balanced mixture of these growth factors and cytokines to cells responsible for extracellular matrix production and remodeling may benefit in rejuvenation of aging skin. Several cosmeceutical products containing either a single human growth factor or combination of multiple human growth factors and cytokines are currently marketed for skin rejuvenation. Several clinical studies show that topically applied human growth factors provide beneficial effects in reducing the signs of facial skin aging (2).

Cosmeceutical manufacturers have taken notice of the positive results of clinical studies showing accelerated wound healing and have begun to include growth factors in products designed to mitigate damage from chronologic aging and sun exposure (11).

Vehicle-controlled studies on cosmeceutical products are very difficult to conduct as topical vehicles can have benefits by virtue of skin hydration, reduced epidermal water loss or simply providing a physical barrier against the environment. Because of these effects, most vehicles cannot be classified as "inactive" and make it more difficult to achieve statistical significance in a vehicle-controlled double-blind study design. In spite of these difficulties, if the product contains any new active

combinations, the study must include a reasonably matched vehicle to scientifically validate effects of the new active (17).

In one of the first pilot clinical study with a product containing a natural combination of fibroblast-derived growth factor mixture, 14 patients with Fitzpatrick Class II or greater facial photodamage applied a growth factor-based moisturizer (TNS Recovery Complex, SkinMedica, Carlsbad, CA) twice daily for 60 days. The results show a statistically significant reduction in fine lines and wrinkles and reduction in periorbital photodamage by clinical grading and by optical profilometry. Figure 4.1 shows an example of a skin biopsy section with increased collagen after treatment with the study product. Measurements of Grenz-zone collagen and epidermal thickness from the biopsy show a 37% increase in Grenz-zone collagen and a 30% increase in epidermal thickness (11).

In a recent double-blind study, 60 subjects were randomly assigned to receive either the growth factor gel or vehicle and apply it twice daily for six months along with a moisturizing cleanser and sunscreen. Treatment with the growth factor gel for three months produced greater reduction in fine lines and wrinkles than vehicle treatment as measured by optical profilometry and assessment of photographs. The results were either statistically significant ($p \leq 0.05$) or trending towards statistical significance ($p \leq 0.1$). Figure 4.2 shows improvement in facial photodamage observed in this study. The study demonstrates that even when compared against a treatment with a good moisturizer and sunscreen, the product being tested showed significant benefits of reversal of signs and symptoms of skin aging (16). In another double-blind study, 18 patients with Fitzpatrick Class II or greater facial photodamage applied a growth factor moisturizer (Bio-Restorative Skin Cream, NeoCutis, Inc., San Francisco, CA) twice daily for 60 days. The results showed that while the average facial roughness did not decrease; a significant

improvement was seen in several other parameters of facial wrinkling. The measurements were conducted by a novel three-dimensional surface mapping technique (18).

RISKS ASSOCIATED WITH GROWTH FACTORS

Growth factors are key molecules that affect cellular proliferation and differentiation which, if unregulated, can lead to carcinogenic transformation of cells. Presence of receptors for some growth factors in melanoma cells and expression of certain growth factors by cancerous cells (19) has raised concerns about the potential for topically applied growth factors to stimulate the development of cancer. Whether presence of receptors or increased expression contributes to tumor growth is uncertain, however. A recent finding suggests that chronic administration of high concentrations of PDGF directly into debrided diabetic pressure wounds may result in increased mortality from cancer. It is unlikely that growth factors applied topically to intact skin would affect tumor proliferation as the protein molecules are too large to be absorbed in large quantities (16). In addition, it is unlikely that the levels of growth factors in skin after topical application is significantly higher than those following inflammation-causing event such as chemical peel, lasers or skin infections.

RELATED PRODUCTS

Phytokinins

Kinetin, a plant-derived growth hormone discovered almost 50 year ago, has been recently used in several cosmeceutical products as an anti-aging ingredient (20). Kinetin is found in the DNA of almost all organisms tested so far, including human cells. In plants, kinetin regulates cellular differentiation by an endocrine pathway with unknown mechanism, however, its function in human cells is not known. Kinetin and other

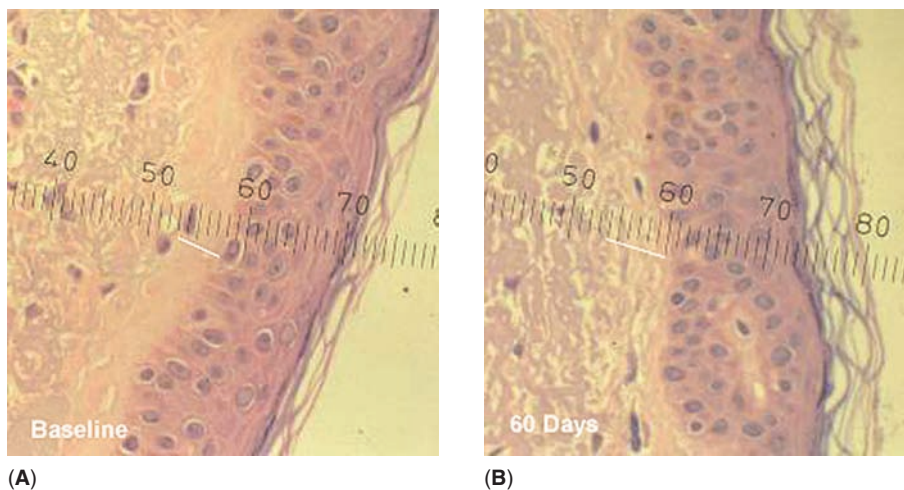


Figure 4.1 Increase in Grenz-zone thickness indicating increased production of collagen after treatment with TNS Recovery Complex: (A) histology baseline; (B) histology after 60 days.

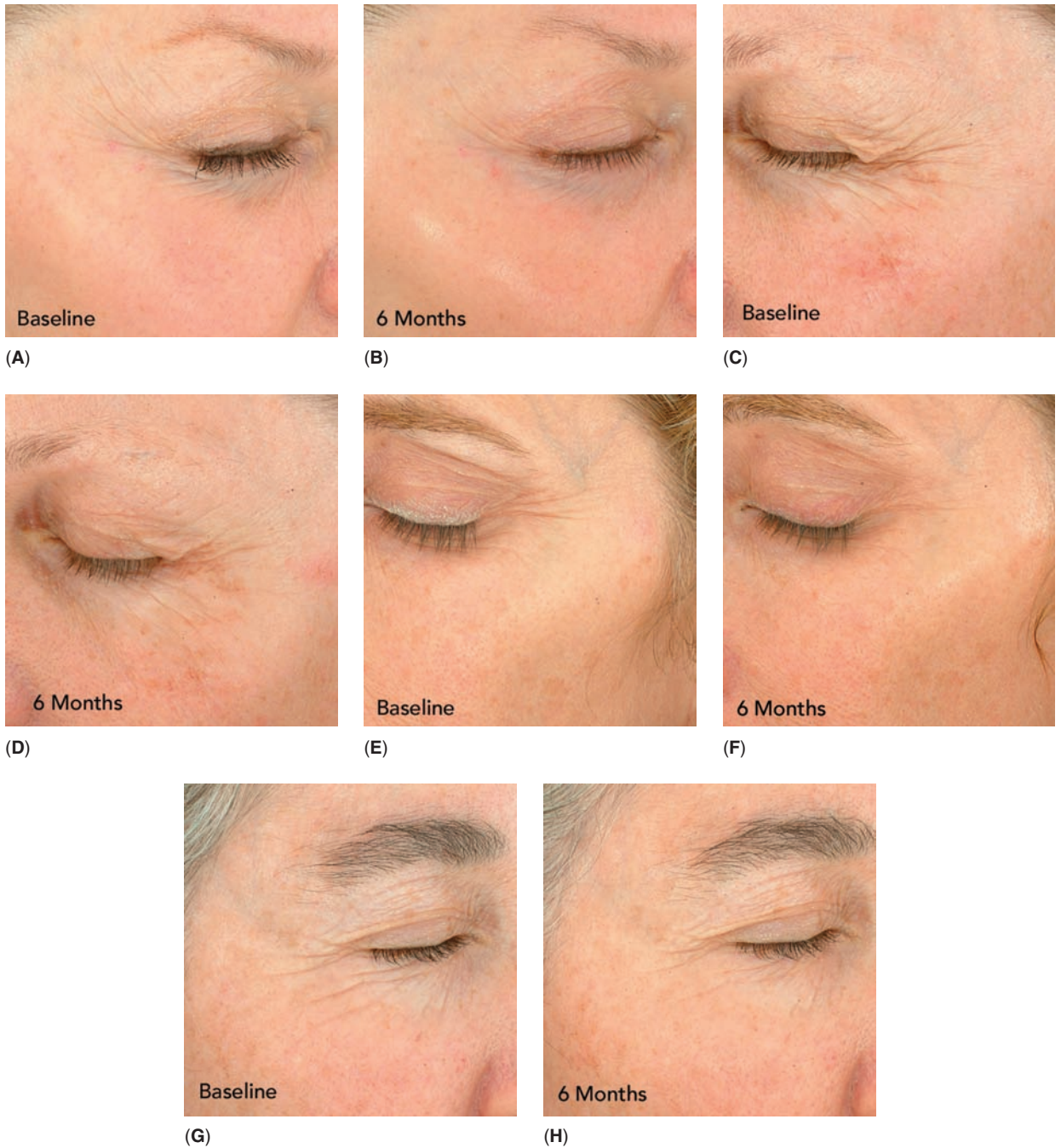


Figure 4.2 Examples of clinical effects observed after twice-daily use of TNS Recovery Complex: baseline appearance and after six months (A, B) Subject 1, women age 59; (C, D) Subject 2, women age 59; (E, F) Subject 3, women age 52; (G, H) Subject 4, women age 51.

cytokinins are products of oxidative metabolism of the cell. Kinetin is formed in the nucleus by reaction of hydroxyl free radicals with DNA, whereas reaction of hydroxyl radical with RNA results in the formation of zeatin, another cytokinin used in cosmeceutical products.

A large number of in vitro and in vivo studies in many species show a number of pharmacological actions leading to potential anti-aging effects of kinetin and other cytokines. The effects, however, are not related to the growth factor/hormone-related properties but are primarily due to their

strong intracellular antioxidant properties. A pilot study on 0.1% kinetin showed moisture retention and reduction in appearance of fine wrinkles and pigmentation; however, more controlled studies are required to substantiate the anti-aging benefits.

SUMMARY

Functions of growth factors in the natural wound healing process are complex and incompletely understood, however, it appears that wound healing is dependent on the synergistic interaction of many growth factors. Research suggests that growth factors used in combination stimulate the growth of collagen, elastin, and GAGs leading to reduction in fine lines and wrinkles. Studying the role of growth factors in cutaneous wound healing has led to research demonstrating positive cosmetic and clinical outcomes in photodamaged skin. Although the topical use of growth factors is an emerging treatment approach, clinical studies demonstrate that dermal collagen production and clinical improvement in photodamage appearance are significant. Further, the increase in dermal collagen produced by topical growth factors can be measured quantitatively by biopsy.

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5 New Delivery Systems for Novel Compounds

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INTRODUCTION

The ability of cosmeceuticals to induce the desired change in the skin is primarily dependent on the delivery system. The delivery system is the carrier that takes an active ingredient from a formulation and places it in the proper location to achieve an effect. Delivery systems are customized to keep the active ingredient in a certain form for a specified length of time. For example, the delivery system for a sunscreen should not be designed for stratum corneum penetration. Sunscreens function on the skin surface to either absorb or reflect ultraviolet radiation. They create a protective coating over the skin surface. Delivery of a sunscreen beneath the stratum corneum would be counterproductive; however, this is not the case for antioxidants. Oxidation occurs in the viable epidermis and dermis where cellular lipid membranes are present. Thus, an antioxidant that remains on the skin surface, such as vitamin E or green tea, provides little protection from oxidative damage. This need to place cosmeceutical ingredients in a specified compartment is the primary goal of delivery systems.

Delivery systems are also designed to maintain the active ingredient in a target location for a specified duration. For example, a superior sunscreen should remain on the skin as long as possible. Since consumers are unlikely to reapply sunscreen thoroughly every two hours as directed, extended duration sunscreens offer superior photoprotection and the delivery system should resist rubbing, sweat, humidity, and water contact. Conversely, it would be unaesthetic for a therapeutic moisturizer rich in petrolatum to use a delivery system that kept the petrolatum on the skin surface all day. The skin would feel greasy, appear shiny, and stick to clothing. A better delivery system would allow small amounts of petrolatum to be released onto the skin surface avoiding the aesthetic drawbacks.

Unwanted side effects can also be minimized through careful delivery system selection. Retinoids are excellent agents for the treatment of acne, but exhibit unwanted dryness and irritation, especially when the concentration on the skin surface is high. A possible solution is the delivery of lower concentrations of retinoid for a prolonged time creating a time-released reservoir. This type of delivery increases efficacy by allowing skin retinoid exposure for more hours while minimizing side effects.

A variety of delivery systems have been developed to carry novel compounds to the skin. The simplest and oldest delivery systems are creams, lotions, and ointments. These represent delivery emulsions. Newer forms include liposomes, nanodelivery, microsponges, and patches. This chapter evaluates the ability of delivery systems to lend efficacy to cosmeceutical ingredients.

EMULSIONS

The most basic delivery system is the emulsion (1). An emulsion is formed from oil and water, which are mixed and held in

solution by an emulsifier. Most emulsifiers are surfactants, or soaps, which dissolve the two nonmiscible ingredients. The most common emulsions are oil-in-water, where the oil is dissolved in the water (2). This emulsion is the most popular delivery system because the water evaporates leaving behind a thin film of oily ingredients. This is the basis for all moisturizers, the main method of transferring cosmeceuticals to the skin surface. If a large quantity of water is found in the emulsion, it is considered a lotion, while a thicker emulsion with less water is considered a cream. Creams typically contain a higher concentration of oily ingredients than lotions accounting for the thicker film produced.

Creams and lotions are the main cosmeceutical delivery systems because they are inexpensive to produce. They also impart moisturizing qualities to the skin surface, one of the main methods used by cosmeceuticals to improve skin texture and appearance. Thus, carefully constructed emulsions can accomplish moisturization and delivery of an active agent simultaneously. For an emulsion to function as an effective cosmeceutical moisturizer, 1% or more transepidermal water loss must occur (3,4). This is because transepidermal water loss is the signal for barrier repair, which must occur or the rehydration is temporary. Moisturizers do not rehydrate the skin, but rather form an environment optimal for barrier repair to occur, restoring the natural water balance in the skin. Water must be trapped in the skin that is drawn to the stratum corneum from the lower epidermal and dermal layers to achieve skin remoisturization (5).

The goal of all moisturizing emulsions is to accomplish skin remoisturization, which occurs in four steps: initiation of barrier repair, alteration of surface cutaneous moisture partition coefficient, onset of dermal-epidermal moisture diffusion, and synthesis of intercellular lipids (6). These steps must occur sequentially in order for proper skin barrier repair. Once the barrier has been repaired, there must be some substance that holds and regulates the skin water content. This substance has been termed the natural moisturizing factor (NMF). The constituents of the NMF have been theorized to consist of a mixture of amino acids, derivatives of amino acids, and salts. Artificially synthesized NMF has been constructed from amino acids, pyrrolidone carboxylic acid, lactate, urea, ammonia, uric acid, glucosamine, creatinine, citrate, sodium, potassium, calcium, magnesium, phosphate, chlorine, sugar, organic acids, and peptides (7). Ten percent of the dry weight of the stratum corneum cells is composed of NMF in well-hydrated skin (8). Cosmeceutical moisturizing emulsions try to duplicate the effect of the NMF.

Substances found in cosmeceutical oil-in-water emulsions include oily substances such as petrolatum, mineral oil, vegetable oils, and dimethicone. The water evaporates leaving the oil behind to place a water-impermeable barrier over the skin

surface. Substances that attract water to the skin surface and function as humectants are found in the water portion of the emulsion. Naturally occurring humectants in the skin include dermal glycosaminoglycans, which function to maintain skin hydration. Glycosaminoglycans are the first substances to see a burst in production following barrier damage. Common cosmeceutical humectants include glycerin, sorbitol, propylene glycol, polyethylene glycol, lactic acid, urea, and gelatin (9,10). Newer humectant substances include hyaluronic acid spheres, which hydrate on the skin surface to physically fill fine wrinkles on the face, especially around the eyes and on the lips. These spheres form the basis for the lip plumping and eye wrinkle minimizing cosmeceuticals, yet they are specialized emulsions.

Water-in-oil emulsions, where the water-soluble substances are dissolved in the oil-soluble substances, are less popular due to their greasy aesthetics. Most ointments are water-in-oil emulsions, but they leave the skin feeling warm and sticky. Ointments deliver higher levels of moisturization because the water phase is small leaving behind a proportionately larger concentration of ingredients capable of retarding transepidermal water loss. Even though their efficacy is greater, cosmeceutical ointment moisturizers are uncommon.

A specialized form of emulsion is a serum. Serums are usually low viscosity oil-in-water emulsions that deliver a thin film of cosmeceuticals to the skin surface. This delivery system delivers minimal moisturizing benefits, but efficiently places cosmeceutical ingredients on the skin surface. Typically, a sera is placed on the skin immediately after cleansing to function as a skin "treatment" suitable for all skin types followed by an additional moisturizer, if necessary. Both water- and oil-soluble ingredients can be delivered in serum formulations.

Examples of substances that can be delivered in cosmeceutical emulsions include: flavonoids, polyphenols, and carotenoids. These are all antioxidant substances that quench singlet oxygen and reactive oxygen species, such as superoxide anions, hydroxyl radicals, and hydroperoxides. Flavonoids possess a polyphenolic structure that accounts for their antioxidant, UV protectant, and metal chelation abilities. Polyphenols compose the largest category of botanical antioxidants. Carotenoids are chemically related to vitamin A. Cosmeceutical ingredients that fit into these categories include soy and silymarin as flavonoid-containing, curcumin and green tea as polyphenol-containing, and retinol as an example of a carotenoid.

Flavonoids

Soybeans and silymarin are examples of botanicals rich in flavonoids. Soy is a rich source of flavonoids called isoflavones, such as genistein and daidzein. These isoflavones function as phytoestrogens when orally consumed and have been credited with the decrease in cardiovascular disease and breast cancer seen in Asian women (11). Some of the cutaneous effects of soy have linked to its estrogenic effect in postmenopausal women. Topical estrogens have been shown to increase skin thickness and promote collagen synthesis (12). It is interesting to note that genistein increases collagen gene expression in

cell culture, however there are no published reports of this collagen-stimulating effect in topical human trials. This highlights the difference between cosmeceuticals and drugs.

Genestein has also been reported to function as a potent antioxidant scavenging peroxy radicals and protecting against lipid peroxidation in vivo (13). The problem is that antioxidant protection prevents damage to the cell that is yet to occur, making measurement of its efficacy difficult. Furthermore, long-term protection from oxidation is necessary for any clinical benefit. Mix soy-derived genestein in a carefully constructed oil-in-water emulsion and you get moisturization benefits to decrease facial wrinkles of dehydration in the short-term and possible long-term benefits from antioxidant effects. It is this combination of delivery system and activeness that accounts for the success of the cosmeceutical category.

Another example of a flavonoid-rich botanical is silymarin. Silymarin is an extract of the milk thistle plant (*Silbum marianum*), which belongs to the aster family of plants including daisies, thistles, and artichokes. The extract consists of three flavonoids derived from the fruit, seeds, and leaves of the plant. These flavonoids are silybin, silydianin, and silychristine. Homeopathically, silymarin is used to treat liver disease, but it is a strong antioxidant preventing lipid peroxidation by scavenging free radical species. Its antioxidant effects have been demonstrated topically in hairless mice by the 92% reduction in skin tumors following UVB exposure (14). The mechanism for this decrease in tumor production is unknown, but topical silymarin has been shown to decrease the formation of pyrimidine dimers in a mouse model, as well (15). Here again is a nice example of a cosmeceutical when silymarin is placed in a well-constructed emulsion. The emulsion repairs the barrier enhancing skin texture and appearance while delivering silymarin flavonoids to the skin surface. Is the silymarin improving skin appearance by decreasing pyrimidine dimers or by improving the skin barrier in an emulsion? Again, the cosmeceutical paradox is at work.

Polyphenols

Polyphenols are another category of botanicals popular in cosmeceutical moisturizer emulsions. Two currently popular examples include curcumin and green tea. Curcumin is a polyphenol antioxidant derived from the turmeric root, a popular natural yellow food coloring used in everything from prepackaged snack foods to meats. It is sometimes used in cosmeceuticals as a natural yellow coloring in products that claim to be free of artificial ingredients. Curcumin is consumed orally as an Asian spice, frequently found in rice dishes to color the otherwise white rice yellow. However, this yellow color is undesirable in cosmetic preparations, since yellowing of products is typically associated with oxidative spoilage.

Tetrahydrocurcumin, a hydrogenated form of curcumin, is off-white in color and can be added to skin care products not only to function as a skin antioxidant, but also to prevent the lipids in the moisturizer from becoming rancid. The antioxidant effect of tetrahydrocurcumin is said to be greater than vitamin E, based on its food preservation effects. Many

antioxidants are rated based on their ability to prevent the browning of foods, such as bananas, peaches, apples, and potatoes. Does this measure of antioxidant ability translate into cosmeceutical skin efficacy? It is unclear. Measuring the ability of a cosmeceutical to function as an antioxidant is difficult.

Probably the most accepted test to measure the ability of a product to prevent oxidative skin damage is the sunburn cell test. This test can be conducted with several experimental methods. One method is to apply the test product to one buttock and nothing to the opposite buttock for 8 to 12 weeks in subjects with Fitzpatrick skin type I who develop a consistent sunburn reaction to 2 MED of UVB and UVA radiation mimicking sunlight. The test is conducted on sun-protected skin to minimize the hardening effect of repeated solar exposure on the skin. Following radiation of both buttocks with 2 MED from a solar simulator, the skin is biopsied with a 3 mm punch to include the epidermis and superficial dermis. Following H&E staining, the specimen is sliced and the sunburn cells, which are apoptotic cells, are counted over several sections. Sunburn cell counts are compared between the untreated and treated sites to determine if the topical antioxidant reduced the number of apoptotic cells. Is this test medically relevant? Perhaps. However, it is the only standardized clinical test in human subjects that condenses years of oxidative damage into an 8- to 12-week realistic test period.

Are most topical antioxidants vetted with the sunburn cell test? Probably not. Most could not pass the test and this type of testing can only be performed by a clinician who can obtain and interpret the skin biopsies. This is the next challenge in cosmeceutical antioxidants, to demonstrate efficacy. In the mean time, skin benefits are achieved with the antioxidant in an emulsion that functions as a cosmeceutical vehicle.

Another topically applied food with antioxidant properties due to polyphenols is green tea. Green tea, also known as *Camellia sinensis*, is a botanical obtained from both the leaf and the bud of the plant. Orally, green tea is said to contain beneficial polyphenols, such as epicatechin, epicatechin-3-gallate, epigallocatechin, and epigallocatechin-3-gallate, which function as potent antioxidants (16). The term “green tea” refers to the manufacture of the botanical extract from fresh leaves of the tea plant by steaming and drying them at elevated temperatures, being careful to avoid oxidation and polymerization of the polyphenolic components. Green tea can be easily added to topical creams and lotions designed to combat the signs of photoaging, but it must be stabilized itself with an antioxidant, such as butylated hydroxytoluene.

A study by Katiyar et al, demonstrated the anti-inflammatory effects of topical green tea application on C3H mice. A topically applied green tea extract containing GTP ((-)-epigallocatechin-3-gallate) was found to reduce UVB-induced inflammation as measured by double skin-fold swelling (17). They also found protection against UV-induced edema, erythema, and antioxidant depletion in the epidermis. This work was further investigated by applying GTP to the back of humans 30 minutes prior to UV irradiation, which resulted in decreased myeloperoxidase activity and decreased infiltration of leukocytes as compared to untreated skin (18). This is a modified sunburn cell test

previously described. The application of topical green tea polyphenols prior to UV exposure has also been shown to decrease the formation of cyclobutane pyrimidine dimers (19). These dimers are critical in initiating UV-induced mutagenesis and carcinogenesis, which represent the end stage of the aging process. Thus, green tea polyphenols can function topically as antioxidants, anti-inflammatories, and anti-carcinogens making them a popular botanical for inclusion in cosmeceutical emulsions.

Carotenoids

The final major category of cosmeceuticals discussed is carotenoids. The major carotenoid found in cosmeceuticals is retinol, the naturally occurring form of vitamin A found in fruits and vegetables with a red, orange, or yellow color (20,21). Retinol and other carotenoids, such as lycopene, astaxanthin, and lutein, are found in tomatoes in high concentration and can be safely applied to the skin from these food sources. Topical retinol can function as an antioxidant, but exhibit more profound biologic activity following cutaneous enzymatic conversion of retinol to retinoic acid, also known as tretinoin. It is this cutaneous conversion of retinol to retinoic acid that is responsible for the biologic activity of some of the new stabilized vitamin A preparations designed to improve the appearance of benign photodamaged skin (22). Unfortunately, only small amounts of retinol can be converted to tretinoin by the skin, accounting for the increased efficacy seen with prescription preparations. Yet, carotenoids can be placed in cosmeceutical emulsions for effective skin delivery.

This discussion has focused on the diverse use of emulsion technology to deliver cosmeceuticals to the skin surface, but emulsions have profound limitations. For example, creation of a time-released delivery system is not possible with a simple emulsion. For this purpose, liposomes and multilamellar vesicles have been created, the next topic of discussion.

LIPOSOMES

Liposomes are spherical vesicles with a diameter between 25 and 5000 nm formed from membranes consisting of bilayer amphiphilic molecules, which possess both polar and nonpolar ends. The polar heads are directed toward the inside of the vesicle and toward its outer surface while the nonpolar, or lipophilic tails, are directed toward the middle of the bilayer (Fig. 5.1). This delivery system was discovered in by AD Bangham who reported his work in the 1965 *Journal of Molecular Biology*. His discovery was based on the observation that phospholipids could be dispersed in an aqueous solution to spontaneously form hollow vesicles, known as liposomes.

Liposomes are based on the natural structure of the cell membrane, which has been highly conserved through evolutionary change. The name is derived from the Greek word “lipid” meaning fat and “soma” meaning body. Liposomes are primarily formed from phospholipids, such as phosphatidylcholine, but may also be composed of surfactants, such as dioleoylphosphatidylethanolamine. Their functionality may be influenced by chemical composition, vesicle size, shape, surface charge, lamellarity, and homogeneity.

The liposome is an extremely versatile structure. It can contain aqueous substances in its core, or nothing at all. Hydrophobic substances can dissolve in the phospholipid bilayer shell, which allows liposomes to deliver both oil-soluble and water-soluble substances. This characteristic is used in drug delivery where an oil-soluble drug can be dissolved in water

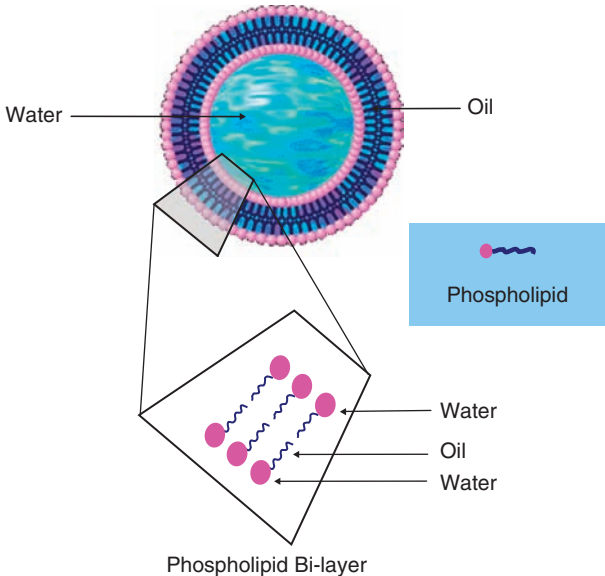


Figure 5.1 The architecture of a liposome.

if placed in the phospholipid shell. Similarly, water- and oil-soluble cosmeceutical ingredients can be dissolved in a stable solution without the use of an emulsifier with this technology.

It is unlikely that liposomes diffuse across the stratum corneum barrier intact. The corneocytes are embedded in intercellular lipids, composed of ceramides, glycosylceramides, cholesterol, and fatty acids, which are structurally different from the phospholipids of the liposome. It is postulated that liposomes penetrate through the appendageal structures. They may also fuse with other bilayers, such as cell membranes, to release their ingredients. This is the mechanism by which liposomes can function as moisturizers, supplementing deficient intercellular lipids.

Liposomes can be specially devised to release their internal contents under certain conditions. For example, liposomes can release when a desired pH or temperature is present in the stratum corneum. Liposomes can be made of a certain size such that they are a natural target for macrophage phagocytosis, releasing their payload inside the macrophage phagosome and inducing a desired change. Liposomes can also be coated with opsonins and ligands to encourage endocytosis in other cell types, but this mechanism would be more appropriate for drug than cosmeceutical delivery, at present.

Niosomes are a specialized form of liposome composed of nonionic surfactants. These are detergents, such as ethoxylated fatty alcohols and synthetic polyglycerol ethers (polyoxyethylene alkylester, polyoxyethylene alkylether). These liposomes do not deliver the moisturizing phospholipids to the skin surface.

Another variant of the liposome is a multivesicular emulsion (MVE) (Fig. 5.2). The MVE is created through a physical

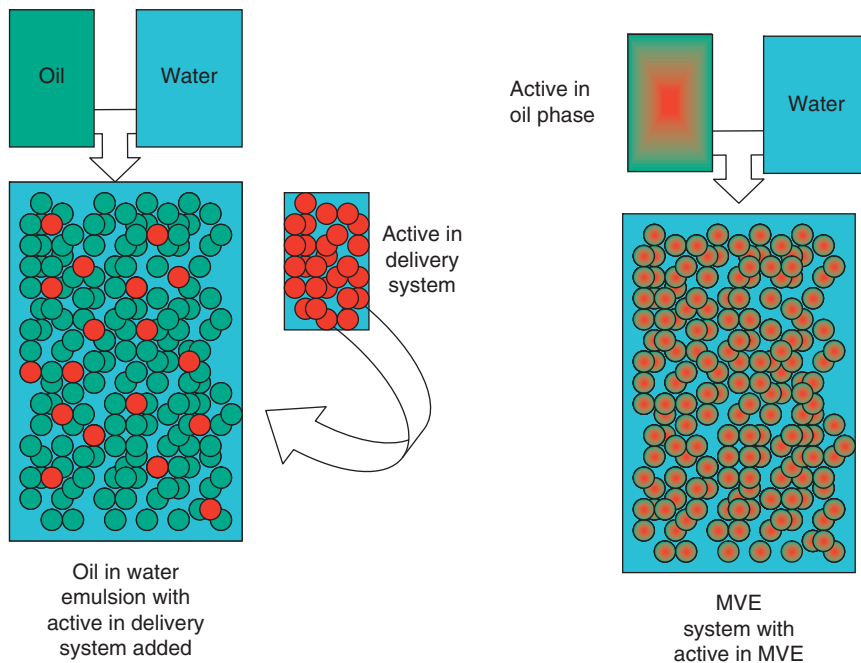


Figure 5.2 The left panel shows a traditional emulsion where the oil droplets are suspended in the water, while the right panel depicts a multilamellar vesicular emulsion (MVE) where the oil droplets are suspended in specialized liposomes.

mixing technique, which makes them more stable, but also less expensive to produce. An MVE can be thought of as a liposome within a liposome. Thus, with the release of each liposome, additional moisturizing ingredients can be deposited on the skin surface. MVEs can deliver glycerin, dimethicone, sphingolipids, and ceramides to the skin surface simultaneously.

In summary, liposomes represent an important cosmeceutical delivery system. Their functionality is dependent on where the active ingredient is stored, which may be in the vesicle, in the lipid bilayer membrane, or on the vesicle surface, and the chemical nature of the active ingredient. However, liposomes are inherently unstable. They are readily deformed and possibly lysed by the weight of a cover slip when viewed under a microscope. They are subject to fusion, aggregation, and precipitation. Even vigorous shaking of a liposomal solution may lyse all of the lipid bilayers. The cosmeceutical strength of liposomes is in their ability to deliver time-released moisturizers and other active ingredients to the skin surface. Some of these drawbacks have been overcome with MVEs. However, the need remains for delivery systems that can reliably penetrate the stratum corneum enhancing the effect of cosmeceutical actives. Many cosmeceuticals could be more effective if penetration kinetics were better. One method of enhancing delivery is to make the particles of the active ingredient so small that they can penetrate through and around the corneocytes. This delivery system, known as nanodelivery, is the next topic for discussion.

NANODELIVERY

Nanodelivery is predicated on the use of very small particles, known as nanoparticles, of a cosmeceutical ingredient to enhance skin penetration. By definition, nano means smaller than 100 nm. The creation of nanoparticles is not new. Nanoparticles have been found on the ninth century Mesopotamian pottery to create a glittery effect. Pottery from the Middle Ages and Renaissance with a copper metallic glitter contains copper nanoparticles dispersed homogeneously within the ceramic glaze. Michael Faraday first explained the optics of this nanoparticle effect in 1857.

Nanoparticles are found in the environment as a byproduct of fire or combustion. Automobile exhaust, airplane exhaust, and air pollution in general contain nanoparticles. It is the nanoparticles inhaled in cigarette smoke that deposit in the alveoli creating the chronic inflammatory process leading to emphysema. Yet, life has evolved to deal with nanoparticle insults. The anatomy of the lung largely prevents nanoparticles from penetrating lung tissue and entering the body. Otherwise, salt nanoparticles from an ocean mist, dust nanoparticles from volcanic eruptions, or fuel aerosols would prove toxic. In nature, free nanoparticles tend to agglomerate thus reducing their penetration abilities.

Yet, there is a growing concern over the presence of nanoparticles in the environment. These particles are invisible to the human eye and can penetrate the skin and lung tissues, gaining access to the lymphatics and blood circulation. From there, these particles can be widely distributed throughout the body.

Unfortunately, once these particles enter the body, they cannot be removed. Concern has been voiced in the medical community that nanoparticles of metals might be responsible for neurologic disease. Others have wondered if the chronic inflammation induced by nanoparticles might not cause other degenerative diseases.

At present, nanoparticles are being investigated for use in a variety of cosmeceutical products to include sunscreens, antiaging moisturizers, and pigmented cosmetics. This discussion focuses on the use of nanotechnology as it relates to cosmeceuticals.

Nanoparticles (Fig. 5.3)

Nanoparticle technology has been applied to vitamins, sunscreens, fragrances, and essential oils (23). Since nanoparticles possess a high surface area to weight ratio, they can impart new characteristics to previously existing materials. For example, normally ductile copper becomes extremely hard when nanoparticles smaller than 50 nm are created. The melting temperature of many raw materials is raised when placed in nanoparticle form. 10-nm nanoparticle ferroelectric materials can switch their direction of magnetization using room temperature thermal energy. This effect has been used to deliver multiple drugs into the body. Nanoparticles can be created that carry two different drugs with the first drug released by a small remotely generated electromagnetic pulse and the second drug released by a stronger pulse providing highly targeted delivery.

Nanoparticles also show promise as sunscreens. Nanoparticle zinc oxide and titanium dioxide are available to act as broad-spectrum inorganic filters by reflecting and to a lesser extent absorbing UVA and UVB radiation (24). Their concentration in sunscreen formulations is limited, however, by their white color. Nanoparticle zinc oxide and titanium dioxide are colorless conferring a large aesthetic advantage, but as of this writing

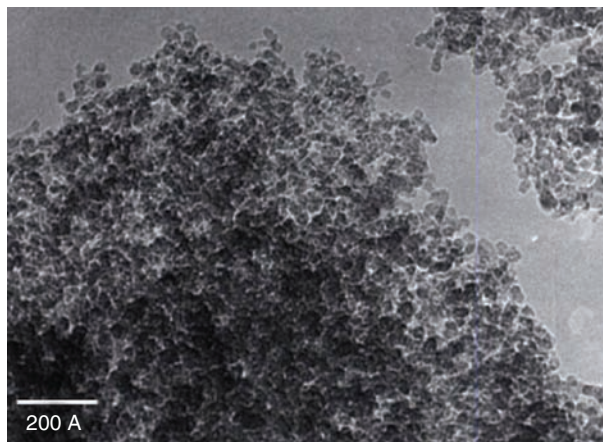


Figure 5.3 The appearance of diamond nanoparticles under a microscope. Source: Wikipedia.

the cosmetics industry has voluntarily agreed to suspend the use of nanoparticle technology sunscreens. An expert panel was convened by the US FDA in 2007 to gather information on the penetration of nanoparticles in the skin, but a final report is still forthcoming.

Nanoparticle sunscreens have health concerns of unwanted penetration, since sunscreen filters are intended to stay on the skin surface. Some experts believe that nanoparticle zinc oxide and titanium dioxide are unable to penetrate the stratum corneum, yet others are concerned about the potential health consequences. Both titanium dioxide and zinc oxide are chemically inert. They are theorized to remain in the body indefinitely either forming a reservoir within the dermis or spreading throughout the body via the circulation. Concern arose over the possibility that sunscreen nanoparticles were capable of absorbing and reflecting UV radiation within the skin causing the generation of oxygen radicals within the dermis and initiating the inflammatory cascade. It is currently unknown if a dermal nanoparticle sunscreen reservoir might enhance the photoprotective abilities of the skin or prematurely age skin due to chronic low grade inflammation characterized by unusually high levels of interleukins 8 and 12.

However, there are other cosmeceutical uses for nanoparticles where enhanced penetration may be desirable. Consider the ability of nanodelivered salicylic acid or benzoyl peroxide to enter the pilosebaceous unit. This would allow very small concentrations of OTC acne actives to reach the location of p. acnes and more efficiently kill the organism eliminating unwanted epidermal irritation. Nanoparticle topical antibiotics would create similar opportunities to decrease the incidence of skin infection. Very small ingredient quantities with targeted delivery would increase efficacy while reducing toxicity and side effects.

Thus, the nanoparticle controversy continues regarding cosmeceuticals. It is unclear whether nanoparticles represent the next great formulation frontier creating huge therapeutic opportunities or a looming danger. Perhaps with better understanding, nanoparticles will become an important cosmeceutical delivery system.

Nanoemulsions

A variant on nanoparticles is nanoemulsions, similar in construction to the emulsions previously discussed at the beginning of this chapter as the oldest delivery system. Nanoemulsions, just like simple emulsions, are liquids with an oil-loving hydrophobic phase and a water-loving hydrophilic co-existing as a single phase with the aid of an emulsifier. The difference is that the droplets in these emulsions are on the nano scale of 20-300nm. If the nano droplets are larger than 100nm, the emulsion appears white, while nanoemulsions with droplets of 70nm are transparent. Nanoemulsions offer the ability to deliver highly hydrophobic or lipophilic substances into the skin, which could not otherwise penetrate. The stratum corneum is an excellent barrier to lipophilic cosmeceuticals.

For example, new nanoemulsions of ubiquinone have been developed. Ubiquinone, also known as coenzyme Q10, is an important antioxidant manufactured by the body and found in all skin cells. It is found in both hydrophilic and hydrophobic cellular compartments, but topical delivery has been challenging. Nanoemulsions have successfully delivered higher concentrations of ubiquinone into the skin with the goal of enhancing the skin's natural antioxidant capabilities. Nanodispersed organic sunscreen formulations containing benzophenone or octylmethoxycinnamate have also been created, but are not yet in widespread use. However, two other methods of enhanced delivery, microsponges and transdermal patches, are widely used for cosmeceutical delivery as of this writing.

MICROSPONGES

Microsponges are a delivery system designed for controlled time extended release. They are macroporous beads 10 to 25 μm in diameter (25). Their active agent is released with temperature and rubbing as the sponge breaks down on the skin surface (26). Microsponges are presently used in prescription tretinoin formulations and OTC moisturizers and sunscreens. They are expensive to produce and leave a white residue on the skin. The main advantage of microsponges is to minimize irritation from active ingredients. For this reason, they can be effectively used to deliver cosmeceutical retinoids for antiaging therapy (retinol, retinaldehyde), benzoyl peroxide for acne treatment, and hydroquinone for pigment lightening.

The micro sponge is useful for the delivery of ingredients that require constant contact with the skin to achieve efficacy. Hydroquinone is an excellent example of a substance well suited for micro sponge delivery, since constant melanocyte pigment suppression is necessary for skin lightening. Hydroquinone, a phenolic compound chemically known as 1,4-dihydroxybenzene, functions by inhibiting the enzymatic oxidation of tyrosine and phenol oxidases. It covalently binds to histidine or interacts with copper at the active site of tyrosinase. It also inhibits RNA and DNA synthesis and may alter melanosome formation, thus selectively damaging melanocytes. These activities suppress the melanocyte metabolic processes inducing gradual decrease of melanin pigment production (27). The ability of the sponge to release hydroquinone over an extended period may increase the efficacy of 2% cosmeceutical preparations currently sold OTC. If extremely sustained delivery to a targeted area is desired, transdermal patches, may offer additional efficacy, discussed next.

TRANSDERMAL PATCHES

Transdermal patches, also known as skin patches, were originally developed for pharmaceutical delivery and then adapted to cosmeceutical delivery of actives to a targeted area. The first commercialized patch was approved in 1979 for the delivery of scopolamine for motion sickness with subsequent approval of nicotine, estrogen, and nitroglycerin patches. The patch contains four components: liner, active agent, adhesive, membrane, and backing materials. The liner protects the patch and

is removed before application. Removal of the liner exposes the drug, which is fixed to the skin with an adhesive. The membrane controls the release of drug onto the skin surface and the backing protects the patch from anything that rubs the skin.

This same patch technology has been adapted to cosmeceutical delivery. Patches containing vitamins C and E have been commercialized for application to wrinkles around the eyes, between the brows, and on the upper lip. These patches are an adaptation of an older product, known as frownies, which used adhesive to tape skin in place and minimize wrinkles overnight. The patch functions not only by immobilizing skin, but also by physically decreasing transepidermal water loss, delivering moisturizing ingredients, such as dimethicone to the skin surface, and placing vitamins or other cosmeceutical ingredients on the skin. The physical effect of the patch on the skin is just as important as the cosmeceutical delivered.

Transdermal patches are still a minor delivery method for cosmeceuticals. A variation of the transdermal patch is a film face mask. This delivery uses a polymer film to cover the face and deliver cosmeceutical moisturizers and other active ingredients without adhesive. The face is covered with the mask for 5 to 15 minutes, while reclining delivering vitamins and botanicals to the skin surface.

SUMMARY

This chapter has covered a variety of delivery systems for novel cosmeceuticals. As of this writing, cosmeceutical delivery is the biggest impediment to their efficacy. The stratum corneum is efficient at keeping substances out of the skin, a function consistent with life. Otherwise, hand washing could become a toxic event. Helping the skin maintain an intact barrier to prevent chemical entry and prevent disease is paramount, yet allowing the entry of cosmeceutical actives designed to minimize oxidation, inflammation, and protein glycation might slow and possibly reverse the skin aging process. Liposomes, nanodelivery, microsponges, and transdermal patches are a few of the currently available techniques to deliver novel cosmeceuticals.

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6 Nutracosmeceutical Drinks: Innovation in Skin Functional Drinks

Fredric S Brandt and Alex Cazzaniga

BACKGROUND

The current anti-aging revolution has scientists looking for ways to discover the fundamental nutritional beneficial compounds of fruits and vegetables. Consumers are looking for speedy ways to supplement their diets with proven active ingredients in order to prevent degenerative disorders associated with aging. Nutraceutical manufacturers are responding to this demand by incorporating a myriad of active ingredients in innovative functional drinks.

Since the era of Cleopatra's milk baths, believed to be used by her to keep her skin young and wrinkle-free, society has long had a passion for the preservation of youth. Everyone has a basic desire to have as much good health as realistically achievable, and maintaining a healthy lifestyle as long as possible while constricting into the shortest amount of time any chronic illness or disability. The way we look is increasingly associated with health, happiness, and an overall feeling of well-being, and there is growing recognition of the importance of a good diet for inner and outer health and beauty. There are several well-established intrinsic and extrinsic factors that provoke skin aging. Solar radiation, dryness, loss of tone, elasticity, hereditary predisposition, reduced capacity of cells to repair themselves in addition to diminished immune responses are all factors that slowly contribute to the process of aging throughout an extended period of time.

One of the main damaging events is the one caused by free radicals. These metabolically induced reactive oxygen compounds are formed in the skin just as in other organs. However, the skin is also exposed to high concentrations of exogenous oxygen, environmental oxidants, and, especially, UV light leading directly and indirectly via chromophores present in the skin to the formation of reactive oxygen species (ROS) (1). Therefore, it is essential to have a sufficient balance of antioxidants. An antioxidant is defined as a substance that is capable of preventing or significantly delaying the oxidation of an oxidizable substrate when present in lower concentrations than the substrate (2). Healthy aging and longevity depend on a successful and dynamic intracellular, molecular interaction among physiological, psychological, and environmental factors (3). Antioxidants help protect the skin against damaging free radicals. This increased cellular energy helps the skin repair, renew, and revitalize itself.

With the current fast-paced life-style and the high consumption of processed foods lacking quality and quantity of nutritional content, effective and easy to ingest products containing ingredients that interact with human living tissue and molecular targets to improve personal wellness and state of health are highly in demand. When most people think about how they can take care of their skin, they usually think about products they

might apply on their skin rather than what they could ingest to make their skin healthier. Although topical application of certain ingredients is essential, equally important is the nourishment of the skin from within. Everything from essential fatty acids, antioxidants, and other bioactive compounds we consume are critical in maintaining healthy skin that ages slowly.

As the global population is living longer and maintaining an active role in society, individuals must obtain healthy nourishments by consuming routinely a variety of nutrients. They are gradually demanding nutritional products containing a variety of beneficial scientifically proven ingredients. In addition, they are looking for convenient and effective ways to provide their bodies with faster absorption of these ingredients. Liquid nutracosmeceuticals contain the active ingredients in their simplified form, increasing the rate of absorption versus ingredients in solid form.

The younger generation wants to slow down the aging process and the older generation wants to reverse the aging process or at least retain their current status of well-being. Baby boomers—those born in the 1950s—comprise a large segment of the current U.S. population. They have been constantly more proactive and knowledgeable about their health-related issues and medical advances and are continually seeking if not demanding a better quality of life. Baby boomers as well as holistic health conscious individuals are turning away from drugs and surgery as the sole treatment of choice for most conditions; they have chosen prevention supplementation and alternative care modalities instead. Preventing the onset of age-related diseases by intervening in the basic process of aging is the best solution for improving the quality of human life.

The field of food science refers to functional drinks as beverages that are accompanied by health claims in general for marketing purposes. Examples of claims made for nutraceuticals are resveratrol from red grape products as an antioxidant, soluble dietary fiber products, such as psyllium seed husk for reducing hypercholesterolemia, broccoli (sulforaphane) as a cancer preventative, and soy or clover (isoflavonoids) to improve arterial health. Other nutraceutical examples are flavonoids antioxidants, alpha-linolenic acid from flax seeds, beta-carotene from marigold petals, anthocyanins from berries, etc. With the U.S. Dietary Supplement Health and Education Act (DSHEA), several other compounds were added to the list of supplements originally mentioned in the FDA notification. Thus, many botanical and herbal extracts such as ginseng, garlic oil, have been developed as nutraceuticals. The functionality spectrum of these drinks range from natural and simple to complex and medicinal. Bottled water, teas, and juices are categorized as belonging to the simple segment, while nutracosmeceuticals are more complex products containing a combination of ingredients.

The term was originally used in Japan in the 1980s where there is a government approval process for functional foods called Foods for Specified Health Use (FOSHU). Furthermore, the Foundation for Innovation in Medicine, an educational foundation established in 1976 by Stephen L. DeFelice, MD, in the United States in Mountainside, NJ, coined the term “nutraceutical” to encourage discoveries in medicine. “A nutraceutical can be defined as any substance that may be considered a food or part of a food and that provides medical or health benefits, including the prevention and treatment of disease. Such products may range from isolated nutrients, dietary supplements, and diet plans to genetically engineered designer foods, herbal products and processed foods such as cereal, soups, and beverages” (4).

Due to continuous changing of health claims, processing guidelines, rapid change in worldwide government policies, and science-based ingredient discoveries with clinical relevance value, the nutraceutical drink market is undoubtedly confusing. However, one can distinguish nutraceutical drinks by nature such as water and fruit juices from those made functional by man such as beverages with added ingredients such as vitamins. Therefore, nutraceutical drinks are also more likely to make health or nutrition claims. Functional beverages are further subdivided into enriched drinks (natural simple herbal drinks), sports drinks (isotonic, hypertonic, and hypotonic), energy drinks (functional energy caffeine, guarana, taurine) and nutraceuticals (mind and body drinks with specific purpose). Nutraceuticals are further categorized as nutracosmeceuticals drinks which target skin wellness from within. Nutracosmeceuticals are the direct result of the fusion between nutritional compounds and delivering technologies, such as nanotechnology, nano-encapsulation and microencapsulation technology. In the United States, the term nutracosmeceutical is commonly used for marketing purposes and bears no regulatory definition.

With the passage of the DSHEA in 1994, US congress freed dietary supplement manufacturers from stringent FDA guidelines used in the regulation of pharmaceuticals, provided that dietary supplements are not promoted for traditional pharmaceutical uses. This landmark legislation was passed with overwhelming support from the consumers. However, this act has resulted in some confusion regarding nutraceutical products advertising and labeling. In far too many instances, DSHEA has resulted in the dissemination of misleading information concerning product activity and quality. This has made it difficult for consumers and healthcare professionals to buy and recommend nutraceuticals with confidence.

However, a large numbers of individuals worldwide, from all cultures and walk of life are turning to nutraceuticals in the quest of attaining these nutrients. The nutraceutical marketplace is seeing younger consumers in their 20s and 30s seeking ways to keep the aging process at bay, in addition to the traditional older consumer group. In order to keep pace with the demand, nutraceutical manufacturers are taking advantage of technological advances and innovations in ingredients to allow

the inclusion into beverages of a wide variety of beautifying ingredients. This provides the increasingly time pressed consumer to have the desired potential quick beauty fix that a nutracosmeceutical drink might provide. Nutracosmeceutical drinks help with the replenishing of enzymes and other molecules such as antioxidants and micronutrients whose levels are diminished during aging. Unlike nutraceuticals, nutracosmeceuticals are products containing active ingredients that are taking orally combining nutritional value and cosmetic properties to enhance skin health. Bioactive ingredients such as vitamins, minerals, phytonutrients, and probiotics are used in cosmetic products for softening, firming, hydrating, clarifying, and replenishing the skin. An important aspect of effective use of an ingested nutrient is its absorption and ultimately bio-availability of the compound to the target tissues and/or receptors. Drinkable nutracosmeceuticals may have different rates of absorption and bio-availability depending on their chemical and biochemical forms. However, need to take into consideration that with age the absorption of nutrients is also diminished (5).

Most of the nutracosmeceuticals currently in the market contain high amount of antioxidants to counteract the deleterious effect of free radicals, as primary responsible molecules for premature aging of the skin.

Whereas pharmaceuticals have an explicit standardized regulatory definition and must be approved by authorities like the US FDA or the European Medicines Evaluation Agency (EMA) as being both safe and effective, cosmetic and food ingredients are not subject to this stringent review and approval process. (6). In the United States, the safety and the labeling of nutritional and cosmetic ingredients is regulated in parts by the Federal Food, Drug and Cosmetic Act (FD&C Act, 1997). In the EU, nutritional ingredients need to be approved following the Novel Foods Directive (EC258/97); cosmetic ingredients need to be approved according to the Council Directive 76/768 (a novel EU cosmetic directive is planned for 2010) (7).

Nutracosmeceutical drinks are defined as providing a health benefit beyond their basic nutritional content, by virtue of their physiologically active added components. Nutracosmeceuticals derive their functionality in most cases from a combination of different ingredients. This combination can differentiate nutracosmeceuticals targeted benefits such as:

- For moisturizing and skin suppleness: The main active ingredients are hyaluronic acid, vitamin C, collagen and polyphenols.
- For skin whitening or tanning: These products clearly demonstrate the cultural differences between the far east and the western cultures. Western consumers desire products to help them tan containing Aloe Vera extract, vitamin C, betacarotene and lycopene, while eastern customers seek products to whiten skin containing alpha-lipoic acid, vitamin C hesperidin.
- For clear complexion/radiance: The general public regards clear radiant skin as an indicator of general

health and well-being. White grape juice, aloe vera, green tea vitamins A, D, E, lactoferrin ingredients are combined to reduce the development of bacteria responsible for many skin impurities and help prevent the formation of blemishes and spots due to microbial effects.

- For hair and nails: Despite a growing number of dietary supplements specifically formulated for hair and nails health, there are few nutracosmeceuticals drinks targeting these areas. Oats and saw palmetto promote the production of keratin, strengthening hair and nails including gammaglutamylcysteine and flax lignans and aloe vera.

This chapter will describe briefly the functional food nutraceutical field with a concentration on the latest in nutracosmeceutical drink ingredients.

THE NUTRACOSMECEUTICAL MARKET

The world's consumption of carbonated soft drinks, loaded with sugar and artificial compounds, has dramatically declined since the new millennium. Consumers have moved beyond choosing drinks simply to maintain everyday hydration. They are now seeking to optimize performance and reduce the risk or delay the onset of diseases with nutraceutical-rich beverages. Nutraceutical beverages are commonly associated with sport athletes trying to maintain vitally and peak performance during training and competitions. However, lifestyle changes are resulting in younger consumers purchasing nutraceutical drinks with greater regularity in order to combat a numerous health problems faced on a daily basis such as fatigue. Changes in consumer demographics and advances in technology are the main stimulus for nutraceutical development. Dermatologists and cosmetic chemists try to use new active compounds and delivery systems capable of reaching the skin cells.

Nutracosmeceuticals are made possible by the increased understanding of skin physiology and food necessities. Innovation has the potential to contribute to new products capable of improving the quality of life (8).

Japan was the pioneering country to introduce ingredients traditionally used in topical skin care products, such as collagen and hyaluronic acid into beverages in the early to mid-1990s as a more holistic approach to beauty. This ground-breaking development created a reinforced interest for natural products and the personal care field quickly becomes an excellent source of inspiration for Japanese beverages, with skin and hair care ingredients quickly finding their way into drinks designed to increase beauty from within. Since then, companies are marketing natural products following a trend geared towards prevention rather than cure as the consumer acceptance for those is the highest and they are generally regarded as safe in view of the fact that they are of natural origin (9,10). This trend is forcing especially the nutracosmeceutical industry to search for and introduce natural products (11).

Even though the food industry as a whole has been traditionally characterized as having a low intensity of research and development, companies are increasing their research efforts to discovered novel, and effective compounds. Currently, they are about 250,000 natural compounds identified. Biotechnology is turning a traditionally low-tech industry, plain drinks, into a high-tech industry of nutracosmeceutical drinks.

Metagenomic techniques have now been well established to make use of this diversity and will further increase the accessibility of natural compounds.(12). The whole development process of novel nutracosmeceuticals is extremely fast. In this context, nutrigenomics, the science of the molecular relationship between nutrition or nutritional ingredients and the corresponding response of genes will soon uncover novel bioactive and physiological implications for one's healthiness. In the future, compounds will be identified not only for their general physiological properties but also for their genetic impact on the potential future disease status and actual wellness factor. Laboratory or histologic evidence of the efficacy of nutracosmeceuticals does not necessarily translate into clinical efficacy and these products do not have to undergo the rigorous testing that pharmaceuticals do before they can be sold to consumers (13).

Rigorous clinical trials may not be possible for many nutracosmeceutical products or ingredients because their desired effect may constitute subtle changes in the skin that, when measured do not achieve statistical significance. In order to exert their beneficial effects, active ingredients must consist of molecules that can reach the necessary skin layers (14).

The realm of nutraceuticals around the world had traditionally been confined to small, entrepreneur led businesses, each with a targeted and niche branded offering. Currently, the nutracosmeceutical category is one of the most dynamic sectors. Consumers are seeking higher quality of life and their choice of beverages and expectations are growing beyond health and beauty. The convenience of sipping a drink out of a bottle with the potential of looking younger and beautiful forever would be considered by many as priceless. Beyond this, the area of nutraceuticals opens up further opportunities as today's consumer becomes increasingly experimental and willing to invest in products that positively support health concerns. This migration of consumer demand to lifestyle products has motivated a large number of manufacturers to enter the nutraceutical arena.

Beverage companies big and small are diversifying their portfolios by acquiring nutraceutical brands or extending current lines with innovative ingredients variants or by developing their own nutraceutical brands in order to capitalize on this market opportunity. PepsiCo Inc., an international manufacturer and distributor of soft drinks and owner of the Pepsi brand acquired the beverage brand SoBe in October 2000 and later another popular nutraceutical brand called Naked Juice. PepsiCo has also introduced several other nutraceutical brands such as Aquafina, Tropicana and FruitWorks since then. In addition, PepsiCo has licensing agreements to market Dole juices, Ethos Water, Lipton tea drinks, and Ocean Spray juices.

These acquisitions have introduced PepsiCo into the functional beverage market ahead of the competition.

Following the business initiatives of the Pepsi Cola Company, the Coca-Cola Company, the world's largest beverage company with a portfolio of more than 450 beverage brands acquired Energy Brands, Inc., known as Glaceau and Fuze Beverages in 2007. With these purchases, the Coca-Cola Company obtained a full range of enhanced water brands, including Vitaminwater, Vitaminenergy, Smartwater, Fruitwater, Vitalize, Refresh, and Slenderize. The Coca-Cola Company, as the world's largest beverage distributor, operates in more than 200 countries. Recently it has also partnered with Nestle, Campbell, and L'Oreal to market novel functional drinks. Under the Minute Maid brand, the Coca-Cola Company offers several enhanced juice products claiming health benefits, thanks to the added nutrients and functional ingredients such as plant sterols, glucosamine, omega-3 fatty acids, and a variety of antioxidants. Moreover, companies have increased their research efforts and are taking advantage of the latest discoveries in taste modulatory factors to utilize the human sensory perceptions of taste sensation to enhance the customer experience by making their products more appealing. Color, taste as well as graphics and descriptions in their product labels are extensively researched to captivate the consumers' pleasant sensations and associations with beautiful skin (15). To garner maximum patient loyalty and continued use, nutracosmeceutical drinks need to be appealing (have a pleasant smell, texture, and flavor) while providing multiple short-term and long-term aesthetic benefits.

Capitalizing on the know-how, established markets and distribution channels, skin care companies are taking advantage of this trend supplementing their topical skin care product lines with skin enhancement beverages and antioxidant concentrates such as Dr. Brandt's Anti-oxidant Water boosters™. The emergence of nutracosmeceuticals beverages currently in the market taps into advances in dermatology research and published studies on antioxidants in order to reduce the amount of free radicals responsible for premature skin aging.

According to a 2008 Datamonitor report titled "New Consumer Insight Series. Functional Food, Drinks, & Ingredients: Consumer Attitudes & Trends," in the United States the market for functional foods, so-called "beauty foods," has grown 38% to \$40 billion in 2008. A Business Insight report published in May 2007 states that following the French Contrex Beaute brand of anti-aging and cosmetic waters first launched in 2000 a host of beverages are driving the anti-aging consumer beauty drinks trend with claims for beauty benefits, in addition to hydration.

Nutraceutical beverages are very much at the early adoption phase of the product life cycle in a number of countries. The most common product positioning amongst launches in 2006 was providing energy followed by cognitive health, digestive health, anti-aging, joint and bone health, blood sugar control, immune system health, and weight control. The development of multifunctional products is an important nutraceutical trend today.

The proliferation of these beauty drinks in the past 12 to 18 months is just starting. Their demand continues to grow worldwide fueled by the development of novel compounds and the current recognition of the benefits of their biological impact. In response to this demand, companies have increased the percentage of balanced, easy to digest active ingredients in their nutraceutical drinks. Global volume sales of nutraceutical drinks are projected to reach 1.42 billion gallons by 2013. North America will continue to lead the market ahead of Japan with a predicted 52.5% share of total volume compared to 26.6% for the Asian markets. The most popular products being enhanced waters. Beverage Marketing Corporation located in New York City reported that water sales grew from 600 million to 860 million in 2006 and the trend continues. The nutraceutical drinks market is expected to reach a value of 9.9 billion in the United States alone by 2010.

Nutracosmeceuticals try to contain potent bioactive ingredients that target cellular components affecting collagen production, pigmentation, erythema, and texture to promote the healthy appearance of skin. When antioxidants are incorporated into a daily routine they have the potential to counteract the harmful effects of solar radiation exposure and other environmental factors, as well as intrinsic factors such as chronological aging and the cellular production of free radicals.

Frequent and Innovative Ingredients of Nutracosmeceutical Drinks

Amino Acids, Peptides, and Proteins

Several nutracosmeceutical drinks contain essential and non-essential amino acids. Amino acids are critical to life and have a variety of roles in cell metabolism. They form short chains called peptides or longer chains called proteins. They are the building blocks of proteins, such as collagen. Amino acids are also important in many other biological molecules, such as forming parts of coenzymes. Therefore, they are the key elements in nutrition and they are commonly used in food technologies.

Amino acids are grouped as essential and non-essential amino acids. Non-essential amino acids are amino acids that your body can create out of other chemicals found in your body. Essential amino acids cannot be created, and therefore the only way to get them is through food.

Non-essential

- Alanine (synthesized from pyruvic acid)
- Arginine (synthesized from glutamic acid)
- Asparagine (synthesized from aspartic acid)
- Aspartic Acid (synthesized from oxaloacetic acid)
- Cysteine
- Glutamic acid (synthesized from oxoglutaric acid)
- Glutamine (synthesized from glutamic acid)
- Glycine (synthesized from serine and threonine)
- Proline (synthesized from glutamic acid)
- Serine (synthesized from glucose)
- Trypsine (synthesized from phenylalanine)

Essential

- Histidine
- Isoleucine
- Leucine
- Lysine
- Methionine
- Phenylalanine
- Threonine
- Tryptophan
- Valine

Alanine. Alanine is found in a wide variety of foods, but is particularly concentrated in meats, seafood, dairy products, egg, gelatin, beans, nuts, seeds, soy, whey, bran corn, legumes and whole grains. It is one of the simple amino acids and one of its functions is to metabolize glucose providing energy for muscle tissue, the brain and central nervous system. Alanine helps with the production of antibodies strengthening the body to fight disease.

Arginine. Arginine significantly contributes to insulin production, muscle metabolism, liver lipid metabolism, and is a component of collagen. It is found in dairy products, fish, legumes, meats, and seeds. It enhances the immune system, specifically by stimulating the thymus gland and the manufacture of T cells. This increase in T cell activity can be effective in fighting bacteria, viruses, cancer tumor cells, chronic fatigue, and other immune system-related health challenges.

Arginine is a factor for maintaining the nitrogen balance in muscles; and can enhance the lean tissue to fat tissue body fat ratio; a great factor for weight management. Arginine also neutralizes ammonia, which helps in liver detoxification and regeneration. As a component of collagen, it can assist with wound healing, skin problems, arthritis, and connective tissue problems.

Asparagine. This amino acid is found mostly in meat sources and sprouting seeds. Asparagine balances the central nervous system and prevents excess nervousness/anxiety or excessive calmness/depression.

Aspartic Acid. Aspartic acid assists in the expulsion of harmful ammonia from the circulatory system. It is mainly found in sprouting seeds. Aspartic acid increase stamina and endurance and helps to fight fatigue.

Carnitine. Carnitine plays an important role in fat metabolism. It helps with the transport of fat from fat cells to the mitochondria of muscle cells so it can be metabolized for energy. Carnitine body production, even though it is not an essential amino acid, it requires adequate supply of iron, vitamin B1, B6, and C, and the amino acids lysine and methionine (neither of these amino acids are obtainable in sufficient amounts from vegetable sources). Carnitine lowers triglycerides, improves organ muscle strength and enhances the antioxidant effectiveness of vitamins C and E.

Citruline. Citruline functions primarily in the liver. Like other amino acids, citruline detoxifies ammonia and it is involved in the energy cycle, and enhances the immune system.

Cysteine/Cystine. These amino acids are structured very closely and convert into each other as needed. They are involved in collagen production for skin elasticity and texture, and for alpha-keratin for fingernails, toenails, and hair. Hair and skin are made up of 10–14% cystine. They are found in broccoli, brussel sprouts, eggs, garlic, onions, and red peppers. Cysteine is a precursor to the liver detoxifying and antioxidant amino acid glutathione. Cysteine is a powerful antioxidant especially in combination with vitamin E and selenium. This functionality provides an anti-aging effect on the body—even reducing the accumulation of age spots.

Gamma Aminobutyric Acid (GABA). GABA functions in the central nervous system as a neurotransmitter; it occupies the nerve receptor sites for anxiety or stress-related messages so that they are restrained from reaching the brain. GABA can be taken as a tranquilizer to calm the body.

Glutamic Acid. Glutamic acid is the precursor of GABA but has somewhat the opposite function; it is an excitatory neurotransmitter. It is one of the few nutrients that crosses the blood-brain barrier and is the only means by which ammonia in the brain can be detoxified. It is considered to be nature's "Brain food" by improving mental capacities; and is used in the treatment of depression and chronic fatigue. It is mainly found in meats.

Glutamine. Glutamine enhances normal nervous system function by crossing the blood-brain barrier and increasing the amount of glutamic acid and GABA. Glutamine plays a role in the removal of this toxic ammonia from the brain. It improves thinking and mood.

Glutathione. The liver produces glutathione from the amino acids cysteine, glutamic acid, and glycine. Glutathione deficiency results in early aging and in the loss of coordination, balance, tremors, and mental disorders. Glutathione levels decline with age and if not corrected will accelerate the aging process.

Glycine. Glycine supplies additional creatine to muscles and is used to construct DNA and RNA. It functions in skin, connective tissues, and the central nervous system. A proper level of cellular glycine produces energy.

Histidine. Histidine is found abundantly in red and white blood cells and is a component of the myelin sheaths that protect nerve cells. It is used in the treatment of arthritis, allergies, and ulcers. It is found in cheese, pork, poultry, and wheat germ. Histamine, a chemical that functions in the immune system, is derived from histidine. Besides functioning in the immune system, histamine aids in sexual arousal, functioning and pleasure. To form histamine, histidine requires vitamins B₃ and B₆.

Isoleucine. Isoleucine, found in cheese, eggs, fish, lentils, meats, nuts and seeds provides energy, increase endurance, and aids in muscle tissue recovery and repair. It also lowers elevated blood sugar levels and increases growth hormone production.

Leucine. This essential amino acid stimulates protein synthesis, healing of bones, skin and muscle tissue. It is found

in almonds, brown rice, chickpeas, dairy products, lentils, meats, and a variety of nuts.

Lysine. Lysine is found mainly in dairy products, fish, lean meats, and potatoes and is especially needed for adequate absorption of calcium and bone development in children. It aids in the production of antibodies, hormones, and enzymes. A deficiency may result in tiredness, inability to concentrate, irritability, bloodshot eyes, retarded growth, hair loss, anemia, and reproductive problems.

Methionine. This amino acid is found in eggs, fish, lentils, meats, pumpkin seeds, sesame seeds, and dairy products. Methionine is a principle supplier of sulfur, which inactivates free radicals. Adequate methionine prevents disorders of the hair, skin, and nails; in addition it helps lower cholesterol levels by increasing the liver's production of lecithin; reduces liver fat and protects the kidneys. Methionine is a natural chelating agent for heavy metals and helps detoxify the body of these metals. It also influences hair follicles and prevents brittle hair.

Ornithine. Ornithine participates in the release of growth hormone, which then prompts the metabolism of excess body fat. This process is enhanced by the presence of arginine and carnitine. It is found in meats and dairy products.

Phenylalanine. It is processed by the brain to produce dopamine and norepinephrine. These two chemicals promote alertness, elevate mood, decrease pain, aid in memory and learning, and reduce hunger and appetite. It is found in meat and cheeses.

Proline. This non-essential amino acid is obtained primarily from meat and aids in maintaining collagen. Proline deficiency will cause a careless vegetarian to have early signs of skin aging. Proline also strengthens joints, tendons, connective tissue, and cartilage.

Pyroglutamate. A non-essential amino acid, pyroglutamate is obtained from dairy products, fruits, meats and vegetables. It helps stimulate cognitive functions and reduce anxiety.

Serine. A storage source of glucose by the liver and muscles; helps strengthen the immune system by providing antibodies; synthesizes fatty acid sheath around nerve fibers. Serine is found in meats, peanuts, wheat gluten, soy, and dairy products.

Taurine. Taurine, a non-essential amino acid, helps stabilize the excitability of cell membranes. Taurine is also thought to have antioxidant properties. Taurine and sulfur are considered to be factors necessary for the control of many biochemical changes that take place in the aging process as well as aids in the clearing of free radical wastes. A deficiency of zinc and taurine may impair vision.

Taurine is found in eggs, fish, meat, and milk, but not in vegetable proteins. It can be synthesized from cysteine and methionine as long as sufficient quantities of vitamin B₆ are present. Taurine is used in many energy drinks.

Threonine. This amino acid is an important constituent of collagen, elastin, and enamel protein; helps prevent fat build-up in the liver; helps the digestive and intestinal tracts function more smoothly. It is the precursor for the amino

acids glycine and serine. It is found in beans, dairy products, eggs, seeds, nuts, and meats.

Tryptophan. This essential amino acid is a natural relaxant, helps alleviate insomnia by inducing normal sleep; reduces anxiety and depression. Tryptophan aids the immune system, reduces the risk of artery and heart spasms and works with lysine in reducing cholesterol levels. It is mainly found in bananas, pineapple, poultry, and yogurts.

Tyrosine. A non-essential amino acid, tyrosine is found in dairy products, eggs, and meats. It promotes the healthy functioning of the thyroid, adrenal, and pituitary glands. Tyrosine suppresses the appetite and helps to reduce body fat.

Valine. Valine is one of the three branched-chain amino acids (the others are leucine and isoleucine) that enhance energy, increase endurance, and aid in muscle tissue recovery and repair. This group also lowers elevated blood sugar levels and increases growth hormone production. It is found in almonds, chickpeas, cottage cheese, fish, nuts, mushrooms, sesame seeds, and soy.

Dietary Fibers

Dietary fiber—found mainly in fruits, vegetables, whole grains, and legumes—is probably best known for its ability to prevent or relieve constipation. But fiber can provide other health benefits as well, such as lowering your risk of diabetes and heart disease. Dietary fiber, also known as roughage or bulk, includes all parts of plant foods that the body cannot digest or absorb. Fiber is often classified into two categories: those that do not dissolve in water (insoluble fiber) and those that do (soluble fiber). The amount of each type of fiber varies in different plant foods.

Insoluble Fiber

This type of fiber promotes the movement of material through the digestive system and increases stool bulk. Whole-wheat flour, wheat bran, nuts, and many vegetables are good sources of insoluble fiber.

Soluble Fiber

This type of fiber dissolves in water to form a gel-like material. It can help lower blood cholesterol and glucose levels. This fiber is found in oats, peas, beans, apples, citrus fruits, carrots, barley, and psyllium.

Betaglucan. Betaglucan is a soluble fiber found in oats and barley that nutritionally potentiates and modulates the immune response particularly by their ability to activate macrophage cells and NK-cells, T-cells, and B-cells including selected cytokines and complement (16).

Chitosan. Chitosan is particularly interesting in the nutritional industry when it was introduced into the market in the 1990s. Health food supplement manufacturers suggested its potential as a nutritional adjunct for weight management. Chitosan has a beneficial effect on weight loss, cholesterol, ulcers, osteoporosis and blood pressure, enhancing cholesterol excretion, along with its associated phospholipids, monoglycerides, and other fatty acids. Chitosan can be found in mushrooms, soft-shell crabs and yeast.

Inulin. Inulin is obtained from chicory root and promotes the growth of beneficial intestinal bacterium. It also enhances mineral absorption in the gut.

Oat Bran. This ingredient is rich in betaglucan, the soluble fiber found in oats that is effective in helping maintain healthy blood sugar levels. Oat bran is the edible, outermost layer of the oat kernel. Oats have been recognized as a food and a herb. Like oatmeal, oat bran contains B complex vitamins, protein, fat, minerals, and heart healthy soluble fiber. In 1997, the U.S. Food and Drug Administration passed a unique ruling that allowed oat bran to be registered as the first cholesterol-reducing food at an amount providing 3 g of beta-glucan per day.

Pectin. Pectin is found in apples and other fruits. It is a natural fruit fiber. It helps reduce cholesterol, regulates blood sugar levels and helps with the elimination of toxic body wastes.

Psyllium Seed Coat. It is a natural fiber composed mainly of hemicelluloses. It is found mainly in plantago, a type of plantain seed. Psyllium helps with the excretion of body wastes, lowers cholesterol, and regulates blood sugar levels.

Raffinose. A beet sugar has the ability of protecting against atopic dermatitis.

Fatty Acids

Fatty acids play an important role in maintaining health skin. Most nutraceuticals contain the following fatty acids: Alpha-linolenic fatty acid, arachidonic acid, docosahexaenoic acid, eicosapentaenoic acid, gamma linolenic acid, omega-3 fatty acids, omega-6 fatty acids.

HERBS

Acerola Cherry (*Malpighia glabra*)

The extract comes from juicy bright red fruits that grow on small trees or shrubs known as Acerola. Acerola is originated from the Yucatan peninsula and is rich in vitamin C and beta-carotene. As a potent antioxidant, Acerola prevents age-related disease by improving the immune system, having antifungal properties and preventing skin discoloration. In addition also contains calcium, vitamins, phosphorous, potassium, and minerals (17).

Alfalfa (*Medicago sativa*)

Alfalfa is a legume that has a long history of dietary and medicinal uses. Alfalfa leaves and sprouting seeds have been used for thousands of years as a nutritive tonic. Rich in anti-oxidants, chlorophyll and carotene, alfalfa sprouts are beneficial as anti-oxidants in breaking down toxins in the blood. Originally native to Asia, the herb was imported into the West by Darius, King of Persia (550-486 BC) during his battles in Greece. Alfalfa has therapeutic uses as both food and medicine. It is rich in vitamins, particularly A, B and C and also Vitamin K. It is believed to contain a higher mineral content than most grains and is a natural source of calcium, potassium, magnesium, and phosphorus. Its high mineral content promotes healthy bones

and teeth. The high chlorophyll content encourages the growth of connective tissue. This plant contents may aid tissue repair and be useful in the healing of wounds, varicose ulcers, and abscesses (18).

Aloe Vera

Aloe Vera has been used for centuries as a high quality moisturizer. It has been used to calm skin irritations, heal wounds, ulcers, and burns. It contains many essential oils, vitamins B1, B2, B6, vitamin E, folic acid, choline, betacarotene, enzymes, and glycoproteins (19,20).

American Ginseng (*Panax quinquefolius*)

Ginseng is an adaptogen. Adaptogens help the body fight the effects of just about any kind of stress, be it viral, bacterial, emotional, or physical. Asians consider ginseng to be the king of all herbs. American ginseng contains ginsenosides, which are thought to fight fatigue and stress by supporting the adrenal glands and the use of oxygen by exercising muscles (21).

Angelica sinensis

This herb native of China and also known as Dong Quai has coumarin derivatives, ferulic acid, iron, and polysaccharides. It helps in regulating the menstrual cycle, balancing blood sugar levels, and blood pressure.

Aronia Berry (*Aronia melanocarpa*)

A close cousin of the blueberry, it is known for its powerful antioxidant properties, especially from its high content of proanthocyanins and quinic acid. Quinic acid prevents urinary tract infections.

Artichoke (*Cynara scolymus*)

Artichoke can support overall health in two ways. Chlorogenic acid, an active ingredient in artichoke, maybe effective as an antioxidant. Another active component of artichoke extract, cynarin, has been reported to lower blood cholesterol levels by helping the body to excrete excess cholesterol instead of absorbing it. Artichoke leaf extract was found to reduce total cholesterol levels in patients with mild to moderate hypercholesterolemia. Artichoke has also been reported to have significant liver protecting and regenerating effects (22–26).

Ashwagandha

Ashwagandha is a plant used in traditional Indian and African medicine as an anti-inflammatory, for fever relief, and against infectious disease. Ashwagandha contains high amounts of flavonoids and includes withanolides. Withanolides are steroidal agents with powerful antioxidant properties similar to the active constituents of Asian ginseng (*Panax ginseng*) known as ginsenosides. As such ashwagandha, has anti-inflammatory, antitumor, anti-stress, antioxidant, mind-boosting, anti-aging, and rejuvenating properties (27,28).

Barberry (*Mahoni aquifolium*)

The root, stem, and bark of the barberry plant are used in a variety of herbal and medicinal preparations. Barberry is considered a strong antioxidant and as such is been used to treat a variety of skin conditions (29–31).

Barley Grass (*Hordeum vulgare*)

Barley grass is naturally rich in copper, potassium, manganese, and zinc. It is beneficial for ameliorating a variety of skin problems. Young barley grass contains concentrated nutrients more so than adult barley grass as well as, live enzymes, protein, vitamins, and minerals. Barley grass is also has very high chlorophyll levels. Chlorophyll has potential in stimulating tissue growth and in stimulating red blood cells in connection with oxygen supply. Many of the vitamins, minerals, and enzymes present in barley grass act as powerful antioxidants protecting our body from free radical damage, enhancing our immune system and improving cardiovascular health by reducing oxidative stress on cholesterol and the body as a whole.

Bearberry (*Arcostaphylos uva-ursi*)

Bearberry extract contains arbutin an inhibitor of melanin synthesis, allantoin an anti-inflammatory, antioxidant and keratolytic agent, betulinic acid a skin lightener and anti-cancer, ellagic acid, lupeol an antioxidant and anti-inflammatory compound, gallic acid an antibacterial agent and ursolic acid.

Bitter Orange (*Citrus aurantium*)

Bitter orange, also known as Bigarade Orange or Neroli has a complex chemical makeup to stimulate metabolic rate. Its oil that is extracted from the peel contains flavones, the alkaloids synephrine, octopamine, and *N*-methyltyramine, and carotenoids (32).

Black Caraway (*Nigella sativa*)

This herb has been used for medicinal purposes for centuries, both as a herb and pressed into oil, in Asia, Middle East, and Africa. It has been traditionally used for a variety of conditions and treatments related to respiratory health, stomach and intestinal health, skin conditions such as eczema and boils kidney and liver function, circulatory and immune system support, and for general well-being. According to Arab proverb, *Nigella sativa* “cures every disease except death.”

Black Chokeberry

Chokeberry fruit contains a variety of compounds such as sugars, malic and other organic acids, pectic substances, tannins, ascorbic acid (vitamin C), citrine (vitamin P), carotin, riboflavin, folic acid, nicotinic acid (vitamin PP), vitamin E, tocopherols, phyllochinon, pyrodoxine, niacin, thiamine, as well as amigdalin, coumarins, rutin, quercetine, quercitrine, hesperidins, catechines, cyanidin and its glycosides, sorbite and other compounds. Of macro- and microelements the following ones can be brought out: iron, manganese, iodine, as well as salts of molybdenum, boron, manganese, and copper. Chokeberry

juice has a vasorelaxant effect and it helps with eczemas and some other skin diseases.

Black Cohosh (*Cimicifuga racemosa*)

Black Cohosh, also known as Black Snakeroot, is a North American forest plant traditionally used to treat snake bites. North American Indians used this medicinal plant for gynecological disorders, kidney disorders, malaria, malaise, rheumatism, and sore throat. The active constituents in Black Cohosh include triterpene glycosides (e.g., acetin and deoxyactein) and isoflavones. Additional ingredients that may lend to its medicinal value include aromatic acids, resins, fatty acids, tannins, starches, and sugars (33–35).

Blackberry (*Rubus fruticosus*)

Its leaves and bark contains large amounts of tannins therefore blackberry has been used as an astringent and tonic. This herb contains high amounts of antioxidants.

Black Currant (*Ribes nigrum*)

Black currant seed oil is a rich source of an omega-6 fatty acid called gamma-linoleic acid (GLA). GLA is an essential fatty acid which the body converts into prostaglandin, a hormone-like substance. Prostaglandin is reported to offer anti-inflammatory and blood thinning properties. In many studies, it has been found that a deficiency in GLA attributes to poor skin condition, especially psoriasis and eczema.

Blessed Thistle (*Cnicus benedictus*)

Blessed thistle has been recommended as a treatment for stomach upset, indigestion, constipation, and gas. It has antimicrobial and anti-inflammatory properties.

Blue Cohosh (*Caulophyllum thalictroides*)

Blue cohosh contains a number of alkaloids and glycosides, of which the alkaloid methylcytisine and the glycoside caulosaponin seem to contribute most of the physiological activity. Caulosaponin constricts the coronary blood vessels.

Blueberry (*Vaccinium myrtillus*)

Blueberries have a variety of micronutrients, specifically high levels of dietary mineral manganese, vitamin B₆, vitamin C, vitamin K. In addition they contain high amounts of antioxidants, such as anthocyanins, proanthocyanidins, flavonols, and tannins. Their skins contain significant levels of the phytochemical, resveratrol (36).

Borage (*Borago officinalis*)

Borage oil (also known as starflower oil) is an ancient oil which has been used in the far east for thousands of years. It contains high amounts of essential fatty acids, calcium, and potassium. The seeds are a rich source of gamma-linolenic acid, which helps to regulate the hormonal systems and lowers blood pressure. It is used both internally and externally, helping to relieve skin complaints and pre-menstrual tension.

Buckthorn (*Rhamnus frangula*)

The fruits contain vitamins, mineral substances, and fatty acids and they have significant amounts of flavonoids, glycosides, and anthraquinones providing antioxidant protective and regenerative action. It has a laxative effect.

Buchu (*Barosma betulina*)

Buchu contains both diosmin and hesperidin, which provides it anti-inflammatory, hypolipidemic (blood cholesterol lowering), and vasoprotective actions.

Burdock (*Arctium lappa*)

Burdock is a root native to Europe and Asia. Burdock has been used as remedy for fevers and colds, urinary tract infections, and rheumatism. Burdock root has been described as alternative and a blood purifier. The primary active constituents in burdock include arctigen, calcium, chlorogenic acid, essential oil, flavonoids, iron, inulin, lactone, mucilage, polyacetylenes, potassium, resin, tannin, and taraxosterol. Fatty acids are contained in the seeds. Burdock seed oil may work as a diaphoretic, creating sweat, neutralizing and eliminating the body's toxins. The high amounts of inulin and mucilage in this herb likely explain why burdock exhibits soothing effects on the gastrointestinal tract. The burdock root was believed to clear toxins from the bloodstream. It may be applied externally as well as internally to relieve eczema and psoriasis. This herb contains polyacetylenes that have antibacterial and antifungal properties (37,38).

Cat's Claw (*Uncaria tomentosa*)

Cat's claw, also known as the Healing Vine of Peru and Uña de Gato, has become very popular because of the many therapeutic benefits. The main health effects are primarily in its ability to boost the body's immune system. The active chemical compounds found in cat's claw are alkaloids, tannins, and several other phytochemicals. The major alkaloid rhynchophylline also exhibits anti-hypertensive properties and may help to reduce the occurrence of stroke and heart attack by lowering blood pressure, increasing circulation, reducing heart rate, and controlling cholesterol levels (39–41).

Catuaba (*Erythroxylum catuaba*)

Catuaba is considered a central nervous system stimulant with aphrodisiac properties. The chemical constituents found in catuaba include alkaloids, tannins, aromatic oils and fatty resins, phytosterols, cyclolignans, sequiterpenes, and flavonoids (42).

Cayenne Pepper (*Capsicum anuum*)

Cayenne's medicinal and culinary applications date back as far as 9000 years with Native Americans. Taken orally, it was believed to help restore a poor appetite, heal digestive problems, and support circulatory problems. Cayenne's benefits are derived from the active ingredient capsaicin. Capsaicin gives cayenne its heat. It helps relieve pain, both when applied

topically and taken internally, and can help reduce blood platelet thickness. Cayenne holds promise of other benefits which are still being explored. Early indications point to its ability to boost energy as well as lower stress-related fatigue and depression (43).

Celery Seed (*Apium graveolens*)

This herb contains a series of beneficial compounds, such as alpha-linolenic acid, boron, calcium, chlorophyll, coumarins, flavonoids, iron, magnesium, phosphorus, potassium, vitamins A, C and B, as well as volatile oils, including apiol, and zinc.

Chamomile (*Matricaria chamomilla*)

This herb contains flavonoids and polyenes as well as alpha bisabolol. Among its benefits, it has a calming and smoothing effect to skin irritation, in addition it has antispasmodic properties due to its level calcium.

Chaya (*Cnidocolus chayamansa*)

Chaya, also known as Tree Spinach, is native to the Yucatán Peninsula of Mexico. Chaya contains good levels of protein, vitamins, calcium, and iron.

Chlorella

A green algae known as Chlorophyll Nature's Greatest Cleanser. It is one of the greatest food substances for cleansing the bowel and other elimination systems, the liver and the blood is chlorophyll, as found in all green vegetables, especially the green, leafy vegetables. In addition, the mysterious chlorella growth factor speeds up the healing rate of any damaged tissue. A clean bloodstream, with an abundance of red blood cells to carry oxygen, is necessary to a strong natural defense system.

Coltsfoot (*Tussilago farfara*)

The Coltsfoot name refers to the shape of the leaves. The plant has been used since pre-history to relieve coughs and other respiratory problems. It is made into herbal teas and is found in commercial cough preparations. It is an ingredient in concoctions used to treat diarrhea. The leaves are sometimes smoked for relief of congestion. The crushed leaves or a leaf decoction is used externally where it may be applied to sores, injuries, rashes, and painful joints. It contains tannins, salts, sterols, and inulin several other active components.

Cordyceps Mushroom (*Cordyceps sinensis*)

It is also known as Chinese caterpillar fungus. This herb has anti-aging and anti-oxidant properties. It is also used in the treatment of fatigue and weakness.

Cranberry (*Vaccinium macrocarpon*)

The active ingredients in cranberry include chemical compounds called proanthocyanidins. Proanthocyanidins are potent antioxidants that appear to be able to decrease bacterial adherence to the bladder epithelium cells. The main benefit of this action is that bacteria have less likelihood of grouping

together to cause bladder infection, urinary track infections and other related conditions (43,44).

Damiana (*Turnera aphrodisiaca*)

Damiana is a yellow-flowering plant that is frequently found growing in climates that are relatively hot and humid including Central and South America and in a few regions in the United States. This herb contains arbutin, cyanogic glycoside, damianin, tannin, and volatile oils. It is considered an aphrodisiac. It also has diuretic, antidepressant, tonic, and mild laxative properties (45).

Dandelion (*Taraxacum officinale*)

The dandelion leaves contain substantial levels of carotene, vitamins A, C, D, and B complex as well as iron, magnesium, zinc potassium, manganese, copper, chlorine, calcium, boron, and silicon. The substances eudesmanolide and germacranolide are the active constituents in dandelion and are unique to this plant.

Devil's Claw (*Harpogothytum procumbens*)

Devil's claw is a plant native to southern Africa. The name devil's claw comes from the herb's unusual fruits, which are covered with numerous small claw-like appendages. The roots, or tubers, of the plant are used in herbal preparations.

Devil's claw is used as a tonic to relieve arthritis, rheumatism, reduce fever, ease sore muscles, and to reduce cholesterol. It is also used to cleanse the lymph system and to remove toxins from the blood. Devil's claw contains harpagoside which reduces inflammation that is responsible for irritation, injury, or infection. Inflammation generally results in pain, redness, and swelling in the area of the damage. It can occur within body tissues as well as on the surface of the skin.

Devil's claw also has some effectiveness for increasing appetite and for improving digestion. Extracts of devil's claw are thought to reduce blood sugar levels somewhat, and several of the chemicals in devil's claw appear to affect blood pressure, heart rate, hearth rhythm and contraction of the heart. However, no reliable scientific evidence supports the use of devil's claw for diabetes or heart conditions (46).

Dong Quai (*Angelica sinensis*)

Known as Chinese Angelica, or Angelica Root, is used mainly to treat menstrual cramps, regulate menstrual periods, and lessen menopausal symptoms. The main beneficial ingredients of this herb are tannins, vitamin A and vitamin B, iron, and coumarin (47,48).

Echinacea (*Echinacea purpurea*, *Echinacea angustifolia*, *Echinacea pallida*)

Echinacea, also known as coneflower, is a wild flower that grows naturally in meadows and moist low-lands throughout the mid-west. All three varieties are used to boost the immune system and fight infections, but only the purpurea and pallida varieties have been shown to be effective. This popular herb contains alkamides,

chicoric acid, glycoproteins, and polysaccharides. Echinacea is thought to serve as a stimulant to the body's immune system by activating white blood cells. Topically, Echinacea has been used in connection with eczema and psoriasis. It has been added to topical preparations for hemorrhoids and it is included in other topical products used on insect bites. It may also protect skin from damage due to sunlight (49).

Elder (*Sambucus nigra*)

As berries they contain vitamins A, C and B, flavonoids, carotenoids, tannins, and amino acids. It stimulates sweating and reduces congestion to treat feverish colds. It is a detoxifying herb (50).

Epimedium (*Epimedium grandiflorum*)

Epimedium, also called Horny Goat Weed is an ivy-like shrub native to the higher and drier areas of China and Tibet. The benefits of epimedium have something to do with increasing the production of the pleasure chemical dopamine and decreasing production of the stress hormone cortisol. The constituents of epimedium are the following: fats, saponins, and essential oil, with trace amounts of epimedins, epimedosides, icariin, magnflorin, and quercetin.

Eucalyptus (*Eucalyptus globules*)

Eucalyptus is native to Australia. Its primary active constituent in eucalyptus leaves is the volatile oil euclyptol which is a decongestant and antiseptic.

Fennel (*Foeniculum vulgare*)

Native to Europe, Fennel is now cultivated in many regions in North America, Western Asia, China, and Iraq. The major chemical compound found in fennel, terpenoid anethole, is responsible for its ability to inhibit spasms in smooth muscles such as those in the intestinal tract. This is thought to contribute to fennel's use as a carminative, a gas-relieving and gastrointestinal tract cramp-relieving agent.

Fenugreek (*Trigonella foenum*, *Trigonella graecum*)

Fenugreek is commonly found growing in Mediterranean regions of southern Europe, where both the seeds and leaves are used primarily as a culinary spice. The active constituents in fenugreek are alkaloids, lysine and l-tryptophan. It also contains steroidal saponins (diosgenin, yamogenin, tigogenin, and neotigogenin) and mucilaginous fiber which are responsible for many of the beneficial effects fenugreek exhibits. The chemical compounds found in fenugreek have the ability to aid the digestive process.

Feverfew (*Chrysanthemum parthenium*)

Feverfew offers a variety of medicinal benefits including pain relief from arthritis, reduction of menstrual cramping, ability cure asthma, stimulation the appetite, and relieving migraines. Feverfew is also rich in nutrients including Iron, niacin, vitamin A, and vitamin C.

Fo-Ti (*Polygonum multiflorum*)

The root of the fo-ti plant is used medicinally in traditional Chinese medicine in connection with premature aging, weakness, vaginal discharges, a variety of infectious diseases, angina pectoris, and erectile dysfunction. The primary chemical compounds found in the fo-ti herb are phospholipids, anthraquinones, tannins, and tetrahydroxystilbene glucoside.

Garcinia (*Gracinia cambogia*)

Garcinia cambogia, also known as malabar tamarind and brindle berry, is a relatively small purple fruit that contains a chemical compound called hydroxycitric acid (HCA). HCA is much like a derivative of the same citric acid that can be found in many other citric fruits. HCA assists in blocking the conversion of sugars and starches into fats

Gentian Yellow (*Genciana lutea*)

This herb is useful to combat exhaustion from chronic disease. Strengthen the circulatory and digestive systems.

Ginger (*Zingiber officinale*)

Ginger helps to improve digestion, reduce nausea caused from motion sickness and stimulates the circulatory system. It also has anti-inflammatory properties.

Ginkgo Biloba

One of the oldest living species of trees, it helps to increase blood flow to the brain reducing memory loss, vertigo, tinnitus, disorientation, headaches, and depression. Ginkgo offers antioxidant effects as well, which may protect both the central nervous system and the cardiovascular system from the negative effects of aging, specifically age-related mental deterioration (51).

Ginseng

Ginseng is an adaptogen. Adaptogens help the body fight the effects of just about any kind of stress, be it viral, bacterial, emotional, or physical. Asians consider ginseng to be the king of all herbs. American ginseng contains ginsenosides, which are thought to fight fatigue and stress by supporting the adrenal glands and the use of oxygen by exercising muscles, because ginseng demonstrates blood-thinning effects.

Goldenseal (*Hydrastis canadensis*)

Goldenrod is used to treat urinary tract inflammation and to prevent the development of kidney stones. Goldenseal may also be used as a topical application to aid in healing injuries and skin conditions such as eczema. The main active ingredients are alkaloids hydrastien and berberine.

Gota Kola (*Centella asiatica*)

Gotu Kola contains as active compounds glycosides, as well as vitamin K, magnesium, sodium, and calcium. Gotu kola has been used to treat a variety of conditions including syphilis, hepatitis, stomach ulcers, mental fatigue, epilepsy, diarrhea,

and fever. Today gotu kola is used to relieve symptoms of poor circulation in the veins of the legs and to speed the healing of wounds and burns. Gotu kola has gained some popularity as a remedy for disorders that cause connective tissue swelling such as scleroderma, psoriatic arthritis (arthritis occurring in conjunction with psoriasis), ankylosing spondylitis (arthritis of the spine), and rheumatoid arthritis (52).

Grapevine (*Vitis vinifera*, *Vitis coignetia*)

The main active ingredients of grapevines are polyphenols, resveratrol. The seeds contain bioflavonoids complex called procyanidolic oligomers—proanthocyanidins, essential fatty acids. They are powerful antioxidants that protect and enhance capillary strength, immune system, reducing allergic reactions, and skin aging.

Griffonia (*Griffonia simplicifolia*)

This herb alleviates anxiety, depression, and insomnia. The main active ingredient is 5-hydroxy-tryptophan.

Guanábana

The Guanábana is a sweet fruit that comes from a broadleaf flowering evergreen tree native to the Caribbean and Brazil. The fruit contains significant amounts of vitamin C, vitamin B1, and vitamin B2. The fruit, seeds, and leaves have a number of herbal medicinal uses among indigenous peoples of regions where the plant is common, including treatment of skin concerns.

Guarana (*Paullina cupana*)

It produces caffeine-like effects which include stimulating the central nervous system, increasing metabolic rate, and having a mild diuretic effects. Guaranine (which is nearly identical to caffeine) and the closely related alkaloids theobromine and theophylline make up the primary active agents in guarana. Guarana also provides antioxidant benefits, improves memory, and prevents the formation of blood clots (53).

Gymnema (*Gymnema sylvestre*)

This exotic herb main characteristic is as a regulator of blood sugar levels. It also has the ability of suppressing cravings for sweets. Its main active ingredients are gymneric acid and gurmarin (54).

Hawthorn (*Crataegus oxyacanthas*)

The Hawthorn plant produces small berries, called haws. These berries are rich in amines, bioflavonoids, and saponins. Due to the high amount of these compounds, hawthorn is a powerful antioxidant responsible for the herbs health benefits. In addition, it increases blood circulation to the brain and heart (55).

Heartsease (*Viola tricolor*)

Heartsease is named because of its long use as an herbal remedy for heart disease. However, it has also been used as a poultice in the treatment of eczema. The seeds are considered to have the same therapeutic activity as the leaves and flowers. A strong

decoction of syrup of the herb and flowers is recommended by the older herbalists for skin diseases (56).

Jasmine (*Jasminus officinale*)

Jasmine has traditionally been considered an aphrodisiac and a calmative. The root and leaves are used to treat headaches, insomnia, and pain due to dislocated joints and broken bones and skin diseases.

Juniper (*Juniperus communis*)

Juniper berry oil is used to alleviate acne and greasy skin and clears the body of acid waste. It contains high amounts of flavonoids, volatile oils, and vitamin C.

Kava Kava (*Piper methsticum*)

One of the main uses of kava root is to reduce stress-related anxiety due to chemicals called kavapyrones.

Knotgrass (*Polygonum aviculare*)

Knotgrass contains many antimicrobial compounds. The leaves and flowers of knotgrass contain high amounts of antioxidants, such as flavonoids, kaempferol, myricitrin, quercitrin, and avicularin.

Korean Ginseng (*Panax ginseng*)

Ginseng is the dried root of one of several species of the Araliaceae family of herbs. Ginseng contains compounds known as adaptogens. Adaptogens are helpful for people dealing with physical and/or emotional stress. It is also effective in lowering the sugar content in the blood and lower cholesterol levels. Ginseng is a popular added ingredient in sports drinks or supplements as an attempt to increase athletic performance. However, there is no clinical evidence to substantiate all of its purported uses.

Kudzu (*Pueraria lobata*)

This herb contains high amounts of isoflavones and isoflavone glycosides. It helps in combating colds and high blood pressure due to its ability to dilate blood vessels.

Lady's Mantle (*Alchemilla vulgaris*)

Tannins are the main ingredients of this herb. It is used to regulate menstrual cycle and to ease the symptoms of menopause.

Lapacho (*Taebuia impetiginosa*)

Native to the rainforests of Argentina, Brazil, and Paraguay, it is commonly used as a tea for candida fungal infections, inflammation, and other infections. The active ingredient lapacho has the ability to block fungal infections.

Lavender (*Lavendula officinalis*, *Lavendula vera*)

Lavender is primarily used in connection with insomnia, anxiety, depression, and mood disturbances. Its active ingredients are coumarins, flavonoids, tannins, triterpenoids, and volatile oils. This plant has strong antibacterial properties.

Lemon Balm (*Melissa officinalis*)

The leaves, stems, and flowers of lemon balm are used medicinally due to high amounts of catechins, flavonoids, rosmarinic acid, tannins, and triperpenes. Lemon balm is used to relieve pain and discomfort associated with indigestion and offers relief for such symptoms as gas and bloating. Lemon balm holds some sedative properties found specifically in some of the chemicals it contains in volatile oils, including citronellal, and citrals A and B.

Lemongrass (*Cymbopogon citrates*)

Lemongrass has been considered a carminative and insect repellent. Lemongrass is used in herbal teas and other nonalcoholic beverages.

Lion's Mane Mushroom (*Hericium erinaceus*)

This herb contains erinacines, amino acids, and potassium. It enhances the immune system and the function of the cardiovascular system.

Liquorice (*Glycyrrhiza glabra*)

Licorice is traditionally used to soothe coughs and the skin. The plant is composed of a glycoside called glycyrrhizin, flavonoids, amino acids, essential oils, saponoids, and sterols. Licorice exhibits anti-inflammatory effects and it is commonly used to treat the upper respiratory track.

Maca (*Lepidium meyenii*)

The active ingredients of Maca are alkaloids, amino acids, essential fatty acids, iodine, saponins, sterols, and tannins. This herb provides energy, and acts as an aphrodisiac.

Maitake Mushroom (*Grifota frondosa*)

A very large mushroom, maitake can be found growing in the mountains of Northeastern Japan and in a few regions of North America and Europe. Maitake contains complex polysaccharides. These polysaccharides help support immune function in the body and consequently are sometimes referred to as immunomodulators. The complex polysaccharides found in maitake are among the most potent polysaccharides found in any other mushroom or herb. Beta-d-glucan, the primary polysaccharide in maitake, is well absorbed when taken orally.

Marigold (*Calendula officinale*)

Calendula flowers have been considered beneficial in reducing inflammation wound healing and used as an antiseptic. Calendula has been used to treat a variety of skin diseases and has been seen effective in treatment of skin ulcerations and eczema.

Marshmallow (*Althea officinalis*)

The root and leaves of the plant contains a high amount of mucilage. This ingredient smooths and heals inflamed mucous membranes.

Meadosweet (*Filipéndula ulmaria*)

This herb has analgesic, antacid, astringent, antiseptic, anti-inflammatory, diuretic, diaphoretic, and relaxant properties. Its main active ingredients are flavonoids, mucilage, phenol glycosides, salicin, and tannins..

Mikan

This herb is also known as Japanese tangerine and aids with fatigue. The main active ingredients are the betacryptoxanthin antioxidants.

Milk Thistle (*Silybum marianus*)

Milk thistle helps relieve the symptoms of hepatitis, cirrhosis, and inflammatory liver conditions. It is one of the most effective herbs known for relieving liver disorders. Milk thistle contains a chemical substance, silymarin, which is purported to protect liver cells due to its antioxidant properties.

Muira Puama (*Ptychopetalum olacoides*)

Muira puama is also called ‘potency wood.’ It is an aphrodisiac with coumarins, fatty acids, sterols, tannins, and alkaloids including muirapamine.

Myrrh

Myrrh is known to relieve inflammation and is approved by the U.S. Food and Drug Administration as a flavoring, fragrance, or stabilizing ingredient in beverages, cosmetics, drugs, and foods.

Nettle (*Urtica dioica*)

Historically, nettle has been used to relieve coughs, tuberculosis, arthritis, and to stimulate hair growth. It contains acetylcholine, flavonoids, serotonin, chlorophyll, vitamins, and minerals.

Noni (*Morinda citrifolia*)

Native to Polynesia, the Noni Plant contains exceptional amounts of vitamin C and niacin (vitamin B3), iron, potassium, vitamin A, and calcium as well as proxeronines and xeronines. Noni juice is also very good for the skin. Noni juice has several key ingredients that have been scientifically proven to be beneficial for good skin health and skin healing. Some of these key ingredients are linoleic, linolenic, and arachidonic—three essential polyunsaturated fatty acids for the body.

Nopal (*Opuntia indica*)

Most species of Opuntia contain a range of phenethylamines, mucilage, vitamin C, and pectin in ample quantities. It is beneficial for the pancreas and liver.

Oat Straw (*Avena sativa*)

Described by the genus name, *Avena*, which means “nourishing.” Oat straw helps build healthy bones, skin, hair, and nails. Oat straw also nourishes the nerves and has antiviral properties. The primary chemical constituents of oat straw includes saponins, flavonoids, starch, alkaloids (trigonelline, avenine), steroids, calcium, iron, B-vitamins, lysine, and methionine.

The fruits (seeds) contain alkaloids, such as gramine, as well as saponins, such as avenacosides A and B. The seeds are also rich in iron, manganese, and zinc.

Passion Flower (*Pasiflora incarnate*)

This herb is widely used as a sedative, antispasmodic and nerve tonic. Its main active ingredients are alkaloids, flavonoids and glycosides. Like benzodiazepines and other herbs, the passion flower increases the levels of the neurotransmitter gamma-aminobutyric acid (GABA).

Peppermint (*Mentha piperita*)

It is commonly used to relieve many ailments like gastritis, irritable bowel syndrome, motion sickness, cramps, and many other conditions. It contains azulene, betaine, carotenoids, flavonoids, tocopherols, and volatile oils.

Perilla (*Perilla frutescens*)

It is commonly used as an anti-allergy agent.

Pomegranate (*Punica granatum*)

Pomegranate is now well known for antioxidant properties. Pomegranate is a rich source of polyphenolics, which have been shown to exert anti-inflammatory, antioxidant, and anticarcinogenic activity in numerous in vivo and in vitro studies (57).

Primrose (*Primula vulgaris*)

Primroses have a very long history of medicinal use and have been particularly employed in treating conditions involving spasms, cramps, paralysis, and rheumatic pains. The plant contains saponins, which have an expectorant effect, and salicylates which are the main ingredient of aspirin and have anodyne, anti-inflammatory and febrifuge effects.

Red Clover (*Trifolium pretense*)

Red clover has been used in connection with cancer, mastitis, joint disorders, asthma, bronchitis, spasmodic coughs, jaundice, and skin inflammations such as psoriasis and eczema. Its main active ingredients are coumarins, cyanogenic and phenolic glycosides, flavonoids, salicylates, and mineral acids.

Redbush (*Aspalathus linearis*)

This plant contains highly beneficial flavonoids, especially quercetin, and nine trace minerals which can refresh and rehydrate. Redbush can be a potent aid for skin complaints.

Reishi Mushroom (*Ganoderma lucidum*)

Reishi is cultivated in North America, China, Taiwan, Japan, and Korea for its medicinal value. The fruiting part of the reishi mushroom is used medicinally in connection with a variety of conditions. It contains coumarin, polysaccharides, sterols, triterpenoids called ganoderic acids which suppresses blood pressure and lowers cholesterol while fating fatigue.

Rosehip

Rosehip is good for the skin and fights infection and stress. Its main active ingredients are bioflavonoids and vitamin C.

Rosemary (*Rosmarinus officinalis*)

The leaf of the plant is used to treat digestive problems, circulatory problems, pain, neuralgia, mild spasms, wounds, eczema, muscle pain, sciatica, rheumatism, and depression as well as parasites. It may also improve memory, relieve muscle pain, and stimulate the circulatory and nervous systems. Rosemary contains flavonoids, phenolic acids, tannins, triterpenoid acids, and volatile oils.

Sage (*Salvia officinales*)

The leaves of the sage herb serve both medicinal and culinary purposes. It has been used in connection with sprains, swelling, ulcers, and bleeding. As a tea, sage has been administered for sore throats and coughs. Its active ingredients are flavonoids, glycosides, phenolic acids, sterols, tannins, rosmarinic acid, and volatile oils.

St. John's Wort (*Hypericum perforatum*)

Best known for use as a mild antidepressant, St. John's wort is also being studied for its possible effects on other mood disorders such as anxiety and obsessive-compulsive disorder. The major active ingredients in St. John's Wort include hyperforin, and flavonoids, hypericin and other dianthrones, flavonoids, xanthenes, and hyperforin (58,59).

Sandalwood (*Santalum album*)

Its volatile oils help for bronchitis and cystitis as well as for relieving a variety of skin conditions.

Sarsaparilla (*Smilax officinalis*)

Sarsaparilla is a natural herb that has been used for treating skin complaints such as eczema and psoriasis. It is also used as a general tonic, aphrodisiac and a blood purifier. It contains steroidal saponins, such as sarsapogenin, which can duplicate the action of some human hormones. Sarsaparilla also contains beta-sitosterol, a phytosterol, which may contribute to the anti-inflammatory property of this herb.

Saw Palmetto (*Sabal serrulata*)

Saw palmetto is a small palm tree used to relieve the symptoms of benign prostatic hyperplasia. Saw palmetto contains a number of compounds including plant sterols and fatty acids. One of the few herbs that are considered anabolic; it strengthens and builds body tissue.

Schizandra (*Schizandra chinensis*)

This herb contains acids, essential oils, and lignans. It is recognized as an adaptogen, capable of increasing the body's resistance to stress and disease.

Sea Buckthorn (*Hippophae rhamnoides*)

Sea buckthorn is rich in antioxidants, mainly flavonoids.

Shiitake Mushroom (*lentinan edades*)

The key chemical ingredient found in the Shiitake mushroom is a polysaccharide known as lentinan. Shiitake mushrooms also contain complex carbohydrates, proteins, fats, soluble fiber, vitamins, and essential minerals.

Siberian Ginseng (*Eleutherococcus senticus*)

Siberian ginseng is widely known as an adaptogen, a substance that may help assist people in dealing with physical and emotional stress. The main active ingredients are glycosides called eleutherosides. They can help to increase concentration, focus, and physical stamina.

Slippery Elm (*Ulmus rubra*)

It is easily digested and is high in carbohydrates and mucilage. It is used to clam coughs and colds. It also has anti-inflammatory properties.

Spirulina (*Spirulina maxima, Spirulina platensis*)

Spirulina is one of hundreds of algae species and is commonly known as blue green algae. Spirulina, contains high amounts of chlorophyll and antioxidants such as beta-carotene and zeaxanthin.

Stevia (*Stevia rebaudiana*)

It is used for the treatment of rheumatism, eczema, arthritis, gout, and anemia. It is also used in connection with kidney stones, urinary tract infections, and urinary problems. The main active ingredient is stevioside.

Suma (*Pfaffia paniculada*)

This herb is rich in beta-ecdysterone, amino acids, minerals, pfaffic acids, saponins, and vitamins. Also called Brazilian ginseng, it is an adaptogen helping with resistance to stress.

Tea (Black, Green, Oolong)

Unlike black and oolong tea, green tea is not fermented, so the active constituents remain in the herb. Green tea contains a variety of chemical compounds, minerals, vitamins, volatile oils, and essential nutrients, but the primary compounds that are believed to provide green tea with its health and medicinal effects are polyphenols, particularly epigallocatechin gallate (EGCG). Recent studies have linked EGCG to skin cell rejuvenation. It is touted as the "fountain of youth" for skin cells. Green tea can lower cholesterol levels and reduce amounts of harmful LDL cholesterol. Green tea has also been shown to be a strong blood antioxidant and detoxifier that can reduce oxidative damage to LDL (60,61).

Valerian (*Valeriana officinalis*)

Best known for its calming effect, valerian is used in connection with insomnia, nervousness, and related conditions including anxiety, nervous restlessness, anxiety-induced headache or upset stomach. The main ingredients are essential oils, glycosides, sesquiterpenes, and valepotriates.

Vervain (*Verbena officinalis*, *Verbena hastate*)

Contains high amounts of glycosides and tannins, vervain is used as tonic for the nervous system. It is also a wound healing agent.

Wheatgrass

This herb contains high amounts of vitamins, minerals and other easily assimilated nutrients. It boosts the immune system by the presence of abscisic acid, amino acids, enzymes and phytonutrients.

White Willow Bark (*Salix alba*)

The main ingredient of this herb is salicylic acid which is used as pain relief and fever reduction agent.

Wild pansy

The important phytochemicals of wild pansy are violutosite, rutin, violanthin, scoparin, orientin, violaxanthin, triterpene saponins, methyl-salicylic acid, flavonoids, tannins, coumarin, vitamins E and C. It is used in a variety of skin conditions such as diaper rash and even eczema.

Wolfberry (*Lycium barbarium*)

Wolfberry contains zeaxanthin, an important dietary carotenoid antioxidant as well as lutein, lycopene and cryptoxanthin, a xanthophyll, 18 amino acids, flavonoids, and vitamin C. This herb is used for the treatment of inflammatory and some types of skin diseases

Yerba Mate (*Ilex paraguayensis*)

It is a species native to subtropical South America. Mate contains xanthines, which are alkaloids in the same family as caffeine, theophylline, and theobromine. Mate also contains elements such as potassium, magnesium, and manganese. It is used as a general tonic for the whole body. It invigorates the body and reduces appetite (62,63).

Ylang Ylang (*Cananga odorata*)

Ylang ylang is a tropical tree that grows in Indonesia and the Philippines. It is used as an aphrodisiac and a tonic to soothe the nervous system. It is also an antiseptic, and it is used to help control the production of sebum, smoothes the skin, and stimulates new cell growth. It works on oily and irritated skin, acne, dandruff, eczema, and wrinkles.

Yucca Root

The root contains high amounts of the steroid saponin, it is used as a gentle foaming cleanser. In addition, it is used as a pain reliever for arthritic joints and a blood purifier.

MINERALS AND VITAMINS

Nutraceutical drinks contain a fairly amount of minerals, mainly calcium (Ca), chromium (Cr), copper (Cu), iodine (I), iron (Fe), magnesium (Mg), manganese (Mn), phosphorus (P), potassium (K), selenium (Se), sodium (Na) and zinc (Zn) and vitamins. The main vitamins available in nutraceutical

drinks are vitamin A, B-complex, vitamin C, vitamin D, vitamin E, and vitamin K.

All of these minerals and vitamins play an important role in skin rejuvenation and overall wellness. However, the most beneficial vitamins for the skin are vitamin B3 called niacinamide. It performs several important functions in maintaining healthy skin, including improving the lipid barrier component of the epidermis and reducing melanin content in the skin by inhibiting melanosome transfer from melanocytes to keratinocytes. These functions result in improved skin tone and texture, diminished fine lines and wrinkles, and reduced hyperpigmentation. In addition, vitamin C, a water-soluble antioxidant is necessary for collagen biosynthesis and has photoprotective antioxidant and anti-inflammatory effects. Vitamin C does more than inhibit skin damaging free radical activity. It is also required for collagen synthesis, which declines markedly with aging skin. Vitamin C regenerates vitamin E in the skin. An antioxidant like vitamin E can only suppress a limited number of free radicals before it runs out of electrons to donate. Vitamin C regenerates vitamin E and enables vitamin E to provide sustained antioxidant protection in the skin elastin fibers. Vitamin C also plays a vital role in skin repair. When skin is injured its vitamin C content is used rapidly in the scavenging of free radicals and in synthesizing collagen to speed healing.

OTHER FUNCTIONAL INGREDIENTS**Apple Vinegar**

This compound improves metabolism and aids with weight control, regulates blood pressure, and fights infections. It contains several minerals, vitamins, and pectins.

Arabinoxylan

It is a derivative of rice bran and it is used to increase energy levels while strengthening the immune system.

Bee Pollen

Bee pollen has been called nature's perfect food because it is rich in vitamins and contains almost all known minerals, trace elements, enzymes, and 18 amino acids. It is used to increase energy and to strengthen the immune system.

Caffeine

It has a stimulating effect on the central nervous system. It acts as a mild diuretic and helps fight fatigue and increases alertness.

Chondroitin Sulfates

Chondroitin sulfate is a natural component of the cartilage that cushions joints. It is composed of repeating chains of mucopolysaccharides. It is helpful for the relief of osteoarthritis or rheumatoid arthritis.

Colostrum

Colostrum is the first milk a newborn receives from its mother. It strengthens the immune system and supports the healing cascade and cellular regeneration.

Creatine

Synthesized by the body, it serves as an energy resource.

Dehydroepiandrosterone (DHEA)

DHEA is an anti-stress hormone found in skin. It helps to increase the immune system's function and restore mental ability. It has also been studied for the treatment of conditions such as Addison's disease, Alzheimer's disease, AIDS, chronic fatigue syndrome, erectile dysfunction, depression, and Parkinson's disease. While research continues for several of these indications, no definitive results prove that DHEA is effective for these conditions.

Deep Sea Water

Deep sea water is used for its purifying effects and richness of minerals and trace elements.

Glucosamine Sulfate

This compound is obtained from animal cartilage. It is composed of mucopolysaccharides called glycosaminoglucon. It helps with osteoarthritis (64).

Glucuronolactone

The metabolite of glucose, it stimulates the metabolism and detoxifies the body.

5-Hydroxy-tryptophan (5-HTP)

It is the precursor of serotonin. It is used to treat depression and anxiety.

Kombucha

Rich in amino acids, glucuronic acid, lactic acid, usnic acid, vitamin C and B complex. Kombucha helps with the detoxification of the body and the regulation of the intestinal flora.

Lecithin

As active ingredients of lecithin, choline and inositol, help with arteriosclerosis protecting the cardiovascular system and brain function.

Loquat Leaf Extract (Folium eribotryae)

It contains a compound called corosolic that reduces peaks in blood sugar levels.

Melatonin

It is a powerful antioxidant produced as a natural hormone by the pineal gland.

Nigari

The main ingredient is magnesium chloride. It helps with the immune system.

Octacosanol

It is consumed to increase endurance and muscle strength while lowering cholesterol. Its main ingredients are sterols, phosphatides, stearines, and fatty acids.

Phosphatidylserine

This nutrient is found concentrated in brain cells and helps with cognitive functions.

Propolis

It is a natural antibiotic used by bees to protect their hives. Its active ingredient is phytotonizides.

Pyruvate

Pyruvic acid is formed in the body during several metabolic processes. It helps with athletic performance.

Resveratrol

Resveratrol is one of the major antioxidative constituents found in the skin of red grapes. It is a polyphenol that has several anti-aging properties. It is a potent antioxidant and as such it protects endothelial cells from oxidative functional damages.(65,66).

Royal Jelly

This ingredient has antibacterial and antifungal properties. It lowers cholesterol and it is high in amino acids, fatty acids, minerals, B-complex vitamins. It is the result of the salivary secretion of bees.

S-Adenosyl Methionine (SAM-e)

S-Adenosyl methionine is a cofactor involved in methyl group transfers and occurs in every living cell. It plays an important role in a variety of cellular reactions. In healthy individuals, it supports joint health and is beneficial against depression, anxiety, and liver damage.

Sesame Lignan

It is one of the most abundant lignans in sesame seeds. Its metabolites have vasodepressing properties by controlling nitric acid production which helps with the control of high blood pressure.

Trehalose

It is a natural disaccharide non-cariogenic sweetener. This compound has a suppressive effect on osteoporosis.

Whey

Rich in minerals, vitamins, trace elements and lactose, whey helps maintain the body's acid-alkaline balance. In addition, whey helps regulate and reduce spikes in blood sugar levels.

Xeronine

This compound is produced in the large intestine from proxeronine and proxeronase and allows proper function of cells. It regulates specific proteins to repair damaged cells and enhance the immune system.

Xylitol

This sugar alcohol is extracted from birch trees. It is used as a non-cariogenic sweetener. It is low in calories and has antimicrobial properties.

PHYTOCHEMICALS**Anthocyanin**

There are more than 20 varieties of berries such as acai berries, blueberries, strawberries, elderberries, and blackberries. These berries contain high amounts of anthocyanin. Anthocyanins are naturally occurring compounds that impart color to fruits, vegetables, and plants. They are probably the most important group of visible plant pigments besides chlorophyll. Apart from imparting color to plants, anthocyanin also has an array of health-promoting benefits, as they can protect against a variety of cellular oxidants. Anthocyanin belongs to a parent class of molecules called flavonoids. Anthocyanins occur in all tissues of higher plants, including leaves, stems, roots, flowers, and fruits. As of 2003, more than 400 anthocyanins had been reported (67–69).

Astaxanthin

It is a powerful antioxidant, more potent than carotenoids. It belongs to a larger class of phytochemicals known as terpenes. It is obtained from microalgae, yeast, salmon, trout, krill, shrimp, crayfish, and crustaceans.

Beta-carotene

Potent antioxidant found in carrots, pumpkins, sweet potatoes, apricots, mangos, oranges, tangerines, grapefruit, and lemons. As antioxidant it supports the health of the heart, immune system, and vision.

Bioflavonoids

These are the natural pigments found in fruits and vegetables. Bioflavonoids are also referred to as vitamin P. Many bioflavonoids prevent the cellular damage caused by free radicals and are useful in enhancing the antioxidant action of certain nutrients.

Carnosine

A dipeptide found in high concentrations in the brain and muscle tissues. Carnosine has been proven to scavenge ROS.

Catechins

These are antioxidant metabolites of plant origin. They belong to the flavonoids family and are abundantly found in teas made from *Camellia sinensis* including white tea, green tea, black tea, and oolong tea as well as cocoa. Epigallocatechin gallate (EGCG) is the most abundant catechin in tea.

Coenzyme Q10

CoQ10 are components of many cell membranes where they safeguard the integrity of a cell. Besides their role in electron-transfer reactions they may also act as free radical scavengers.

Coix Seed Extract (Yokuinin)

The main active ingredient of Yokuinin is a compound of triglycerides containing four kinds of fatty acids. It is used as anti-cancer treatment in China and to regulate gastric functions and to reduce pathological water retention (70).

Daidzein

It is one of the many isoflavones isolated from soybeans. As antioxidants, isoflavones counteract the damaging effects of free radicals.

Flavan-3-ols

This compound is found in berries, fruits, teas, and wines. They include anthocyanidins, proanthocyanidins, catechins, and tannins. They protect the circulatory system. Not to be confused with flavonols.

Genistein

It is a soy isoflavone and as such a potent antioxidant. It modulates vascular inflammation.

Glucosinolates

They are a class of organic compounds responsible for the bitter taste of many foods such as cabbage, radish, broccoli, and rapeseed. They have antimicrobial properties.

Glycetein

An isoflavone isolated from soy beans.

Hesperidin

A bioflavonoid isolated from citrus fruits. This antioxidant reduces cholesterol and blood pressure as well as having anti-inflammatory properties.

Hydroxytyrosol

Phytochemical with antioxidant properties. Its oxygen radical absorbance capacity is 10 times higher than green tea. It is isolated from olive oil.

Indoles

Member of the organosulfuric compounds and as such responsible for the foul smell found in cabbage. It activates detoxifying body enzymes.

Isoflavones

Many of the benefits of soy are due to the fact that soy contains high amounts of isoflavones which are potent antioxidants.

Isoprenoids

Isoprenoids are organic compounds that contribute to the scent of eucalyptus, the flavors of cinnamon, cloves, and ginger, and the color of yellow flowers. They have antioxidant properties.

Isothiocyanates

They are sulfur-containing antioxidants that inhibit carcinogenesis.

Kaempferol

Kaempferol is a natural flavonoid that has been isolated from tea, broccoli, grapefruit, brussel sprouts, apples, and other plant sources. They possess anti-cancer properties.

Lemon Verbena

Lemon verbena is also known as *Aloysia triphylla*. It strengthens the nervous system. It is antispasmodic, expectorant, and helps with digestion.

Limonoids

A phytochemical abundant in citrus fruits with antiviral, anti-fungal, antibacterial, antineoplastic, and antimalarial properties.

Lotus Extract (Nelumbo nucifer)

Potent antioxidant with radical scavenger activity.

Lutein

A carotenoid found in yellow and orange fruits and green leafy vegetables.

Lutein was found to be concentrated in the macula and as an antioxidant helps protect against degeneration of the retina.

Lycopene

Lycopene is a bright red carotenoid pigment, a phytochemical found in tomatoes, and other red fruits. Lycopene is the most common carotenoid in the human body and is one of the most potent carotenoid antioxidants.

Narigeninin

A compound that naturally occurs in grapefruit and other citrus fruits may be able to block the secretion of hepatitis C virus from infected cells, a process required to maintain chronic infection

Phenols

Phenols are found in fruits, herbs, vegetables, and soy. They have antioxidant and antiseptic properties.

Phytochemical

Phytochemicals are non-nutritive plant chemicals that have protective or disease preventive properties. There are more than thousand known phytochemicals. It is well known that plants produce these chemicals to protect themselves, but recent research demonstrate that they can protect humans against diseases. Most phytochemicals have antioxidant activity and protect cellular activity against oxidative damage and reduce the risk of developing certain types of cancer. Phytochemicals modulate hormonal action, stimulate enzymes, interfere with DNA replication, and some have antibacterial properties.

Phytosterols

These compounds are plant sterols that reduce dietary cholesterol absorption. They are mostly isolated from corn, rice, soy, wheat, and oats.

Polyphenols

Polyphenols have antioxidant characteristics. Notable sources of polyphenols include berries, tea, beer, grapes/wine, olive oil,

chocolate/cocoa, coffee, walnuts, peanuts, borojo, pomegranates, yerba mate, and other fruits and vegetables. High levels of polyphenols can generally be found in the fruit skins (71).

Proanthocyanidin

The major proanthocyanidins (flavanols) are present in whole fruit, juice, and pulp of strawberry, Saskatoon berry, raspberry, wild blueberry, chokecherry, seabuckthorn, and some other plants. Proanthocyanidins deserve their stellar reputation as antioxidants that quench free radicals and potentiate other antioxidants (72).

Quercetin

Quercetin is a phytochemical that is part of the coloring found in the skins of apples and red onions. Quercetin is a powerful antioxidant. It is also a natural anti-histamine, and anti-inflammatory.

Rutin

Rutin has strong antioxidant properties and stabilizes vitamin C. Rutin is a bioflavonoid that strengthens capillaries and has anti-inflammatory properties.

Saponins

Saponin has an effect on blood cholesterol levels, cancer, bone health, and stimulation of the immune system. The non-sugar part of saponins have also a direct antioxidant activity, which may result in other benefits such as reduced risk of cancer and heart diseases. Saponins are phytochemicals which can be found in most vegetables, beans, and herbs.

Tannins

Several fruits such as pomegranates, herbs, nuts, teas, and red wines are good sources of tannins. They belong to the flavan-3-ols group of polyphenols. They have anti-cancer properties. The anti-inflammatory effect of tannins helps control all indications of gastritis, esophagitis, enteritis, and irritating bowel disorders. They also have anti-infective properties.

Terpenes

Vitamin A is a class on terpenes. Terpenes are compounds of single and multiple hydrocarbons and they help protect the heart and circulatory system.

Thio-sulfonates

Mainly found in onions, garlic, chives, leeks and shallots, these phytochemicals protect the body from heart disease and cancer. Some thio-sulfonates even have antifungal, antibacterial, and antiviral properties.

Xanthophylls

These compounds are found in the leafy green vegetables and are responsible for providing the characteristic yellow and orange colors. They are potent antioxidants with anti cancer properties.

Para-aminobenzoic acid (PABA)

PABA is used to improve the protein used in the body, it relates to red blood cell formation as well as assisting the manufacture of folic acid in the intestines. PABA is found in liver, kidney, brewer's yeast, molasses, whole grains, mushrooms, and spinach.

Prebiotics and Probiotics*Prebiotics*

Prebiotics are carbohydrates that act as food for the nonpathogenic bacteria. Prebiotics are not destroyed, digested or absorbed in the upper GI tract and therefore reach the gut where our beneficial bacteria strains reside and hence contribute fully in promoting their effectiveness. Prebiotics provide a natural way of increasing the number and activity of the non-pathogenic bacteria already resident in the colon. Prebiotics increase in levels of non-pathogenic bacteria, and in turn reduce the levels of pathogenic bacteria. They increase mineral absorption, and provide relief from occasional constipation, particularly in the elderly.

Probiotics

Probiotics, which means, "for life," have been used for centuries as natural components in health-promoting foods. This microorganisms are beneficial for the treatment of irritable bowel syndrome, hypertension and elevated blood cholesterol as well as maintaining a healthy digestive system.

Currently the adopted definition by FAO/WHO, probiotics is: 'Live microorganisms which when administered in adequate amounts confer a health benefit on the host'. Lactic acid bacteria are the most common type of microbes used as probiotics. Probiotics strengthen the immune system to combat allergies. The probiotics strains used are primarily species of *Lactobacillus* or *Bifidobacterium*, or *Streptococcus thermophilus* bacteria or yeast *Saccharomyces boulardii* (73).

Other Ingredients Found in Nutraceutical Drinks*Red Clover (Trifolium pratense)*

It is a flower extract and part of about 230 species of legumes. Red clover relieves gastrointestinal ailments and skin irritations. It also has anti-cancer properties. It contains phytoestrogens.

Chromium Picolinate

Chromium is a mineral that humans require in only trace amounts. It is found in yeast, calf liver, whole grains, meats, and cheese. It is needed for the normal function of insulin. As the picolinate form, chromium is better absorbed by the body. It has weight loss properties if ingested in recommended amounts.

Calcium Lactate Pentahydrate

This salt has antacid properties and it is used to treat calcium deficiencies. Calcium lactate is added to sugar-free foods to prevent tooth decay.

Hyaluronic Acid

Hyaluronic acid, also called hyaluronan or hyaluronate is a non-sulfated glycosaminoglycan distributed widely throughout

connective, and neural tissues. Hyaluronan is also a major component of skin, where it is involved in tissue repair. It is one of the chief components of the extracellular matrix, contributes significantly to cell proliferation and migration. Oral administration of hyaluronan has a positive effect on osteoarthritis. Due to its ability for drawing water into the skin giving, hyaluronic acid provides volume while binding collagen and elastin fibers into a supportive and protective matrix that gives the skin its structure.

Fucoxanthin

Fucoxanthin is a carotenoid found as an accessory pigment in the chloroplasts of brown algae and most other heterokonts, giving them a brown or olive-green color. Brown seaweed which is rich in fucoxanthin, promotes fat burning within fat cells surrounding internal organs. In addition, fucoxanthin has strong anti-diabetes effects and, by a separate biological mechanism, it appears to kill prostate cancer cells in culture.

Policosanol

Policosanol (or polycosanol) is the generic term for a natural extract of plant waxes. It is used as a nutritional supplement to lower (bad) LDL cholesterol and increase (good) HDL cholesterol and to help prevent atherosclerosis.

Cassia Nomame

Cassia Nomame is a lipase inhibitor that is believed to impair the enzyme responsible for the breakdown of fat, therefore blocking its absorption.

Cinnamon Extract (Cinnamon aromaticum Cass)

Cinnamon is known to contain manganese, iron, and calcium and a host of volatile oils. It is beneficial for the treatment of colds, diarrhea, and other problems of the digestive system. One of its components is cinnamaldehyde which has an anti-clotting action and is also anti-inflammatory.

Collagen

Collagen is the most abundant protein in the animal kingdom. It is found in all of our connective tissues, such as dermis, bones, tendons, and ligaments, and also provides for the structural integrity of all of our internal organs. There are more than 28 types of collagen described in literature. Over 90% of the collagen in the body, however, is of type I, II, III, and IV.

Hoodia

Hoodia cactus is native to the semi-deserts of South Africa. Of the 20 species in this cacti family, *Hoodia gordonii* is the variety of Hoodia that contains appetite suppressant properties.

Salvia hispanica, Chia

Chia (*S. hispanica*) is a plant of the genus *Salvia* in the mint family. It originated in the central Valley of Mexico. Chia is very rich in omega-3 fatty acids.

Theobromine

Theobromine, also known as xantheose, is a bitter alkaloid of the cacao plant, and is therefore found in chocolate. This ingredient has vasodilator and diuretic properties.

Citicoline

Citicoline is an important intermediate in the making of phospholipids in cell membranes. It protects nerve cells in conditions where there is low oxygen availability. Citicoline helps in age-related cognitive decline and degenerative neurological diseases

Fucoidan

Fucoidan is distributed in the intercellular matrix in brown algae. They have anticoagulant and antithrombotic effects. Fucoidan helps regenerate healthy skin tissue. Fucoidan supports the normal disposal of non-native cells. In addition, anti-tumor activity of fucoidan is related to the activation of macrophage-mediated tumor cell killing and interferes cancer cell metastasis by inhibition of physical interaction between the tumor cell and basement membrane

Long Jack (Eurocoma longifolia, Tongkat Ali)

It is a flowering plant used to treat fever and as a tonic after childbirth. In addition, it has testosterone enhancing properties.

Natto Extract

Natto is a traditional Japanese food made from fermented soybeans, popular especially at breakfast. A rich source of protein, natto and the soybean paste miso formed a vital source of nutrition in Japan. Natto contains pyrazine which provides its distinct smell and reduces the likelihood of blood clotting. Natto contains high amounts of vitamin K and pyrroloquinoline quinone which are important for healthy skin.

Pine Nut Extract

Also called Indian nut, piñon, pignoli and pignolia, this high-fat nut comes from several varieties of pine trees. Pine nuts contain thiamine, vitamin B1 and protein and appetite control properties.

S. boulardii

S. boulardii is a tropical strain of yeast used for prevention and treatment of gastrointestinal disorders.

Grape Seed Extract

It helps to prevent and treat heart disease such as high blood pressure and high cholesterol. Grape seed extract contains procyanidins which have strong antioxidant properties and play an important role skin health (74,75).

Black Elderberry (Sambucus nigra)

The flowers and berries contain flavonoids such as quercetin making it a powerful antioxidant. It helps in combating common conditions of modern living (76).

Bee Propolis

It stimulates the formation of antibodies to build body resistance to many diseases. It also helps the body in releasing substances against cellular deterioration. In addition, propolis stimulates enzyme systems, cell metabolism, circulation, collagen formation, and improved healing of burn wounds.

Oryzanol

Oryzanol is one of the several minor components found in rice bran oil. Most of the biological activities of oryzanol are because of its excellent antioxidant property. This phenolic antioxidant scavenges oxygen-free radicals.

Eicosapentaenoic Acid (EPA or Also Icosapentaenoic Acid)

This ingredient is an omega-3 fatty acid with skin wellness properties.

Aspalathus linearis (Rooibos)

This tea is high in antioxidants such as aspalathin and nothofagin. It does not contain caffeine, and its low in tannin levels compared to fully oxidized black tea or unoxidized green tea leaves. Rooibos also contains a number of phenolic compounds, including flavanols, flavones, flavanones, flavonols, and dihydrochalcones (77).

Rhodiola rosea

R. rosea stimulates the immune system. It is a powerful anti-aging phyto supplement with adaptogenic and anti-stress activity (78).

Mangosteen (Garcinia mangostane Linn)

This plant is rich in prenylated xanthenes. Xanthenes exhibited a variety of biological activities containing anti-inflammatory, anti-bacterial, and anti-cancer effects (79,80).

CONCLUSION

A wide array of nutracosmeceutical drinks exists in today's market. Most of these products contain multiple active ingredients that help restore and maintain the health and beauty of the skin and protect it from the damaging effects of natural aging by the solar radiation end product, free radicals. Research studies have demonstrated that certain ingredients have the potential to improve the skin from within.

These are exciting times for the development of innovative nutracosmeceutical products marked by the constant introduction of new innovative ingredients with the potential to provide multiple short-term and long-term aesthetic wellbeing benefits. The aging population is more active and health conscious than ever before; they are demanding products containing ingredients with proven health benefits. Proper nutrition daily plays an important role in the preservation and rejuvenation of the skin.

The current nutracosmeceutical drink revolution represents an enormous opportunity for growth and expansion taking advantage of moving from topical application to benefits from within to supplement and amplify the healthy effects of these

nutritional compounds. Looking towards the future, the likelihood is that nutraceuticals will remain a high value. The future may see beauty beverages marketed as part of inside and outside beauty systems, sold next to branded topical creams and personal care products to create a total skincare package.

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7 Cosmeceuticals for Hair and Nails

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INTRODUCTION

The hair and nails play important roles in the self-esteem and psychological well-being of humans. In the last few years, the range of cosmeceuticals available for hair and nail care has rapidly expanded. Natural hair and nail care products are very popular in the community, as is what may be a false belief in the scientific benefit, safety and lack of side effects of these natural products. Cosmeceuticals (a combination of the words “cosmetic” and “pharmaceutical,” originally introduced by Kligman (1)) are topical cosmetic products that claim a biological benefit or improvement resulting from an improved pharmacologic action when compared to an inactive cosmetic (2). Cosmeceuticals contain active ingredients such as antioxidants, botanicals, minerals, vitamins, enzymes or essential oils, as well as components including moisturizers, fragrances, preservatives, emulsion stabilizers, surfactants, and viscosity controlling substances (3). Although some cosmeceuticals may provide a benefit, given the range of ingredients in cosmeceuticals, like medicinal drugs, the potential for misuse and adverse side effects exists. As clinicians, it is important to understand if any scientific basis underlies these compounds and be familiar with these compounds which our patients will use in hair and nail care products.

The term “cosmeceutical” is not recognized by the US Food and Drug Administration (FDA) (2). The definition of a cosmetic in the FDA Food, Drug and Cosmetic Act

is a compound intended to be applied topically to any part of the human body for cleansing, enhancing attractiveness or for changing the appearance without altering structure and function (2,4–7). A drug, however, is defined as a compound intended for use in the diagnosis, treatment or prevention of diseases or used to affect the structure and/or function of the body (2,4). A cosmeceutical then is an intermediate between a cosmetic and a drug, having pharmaceutically active compounds affecting a cosmetic improvement by a physiologic action on the skin (7). Cosmeceuticals are not subject to the extensive scientific testing that drugs approved by the FDA must be (3). The ingredients in cosmeceuticals need only to be tested as safe, and the beneficial claims of the active ingredient do not need to be proven (3). Similarly, there are few biological studies and even fewer randomized double-blind placebo-controlled trials testing on humans the efficacy and safety of these products. Attractive characteristics of a cosmeceutical are safety, effectiveness, stability of formulation, cheap manufacturing, metabolism within the skin, and novelty and patent protection (7,8).

Botanicals or natural plant extracts, essential oils, amino acids, vitamins, and other active ingredients are incorporated into many cosmeceutical preparations for hair care such as

shampoos, conditioners, styling products, and colors. Some cosmeceutical ingredients also have a therapeutic value in hair conditioning, growth promotion, and even treating scalp infections such as seborrheic dermatitis and head lice (3). Nail cosmeceuticals also can contain botanical extracts and vitamins and claim to have a value in nail strengthening and conditioning. To review and ensure the ingredients of cosmeceuticals are safe, the Cosmetic Fragrance Toiletory Association (now the Personal Care Products Council) established the Cosmetic Ingredient Review (CIR), supported by the FDA and the Consumer Federation of America, which has been operating for over 30 years reviewing the safety of cosmetic ingredients (3). The difficulties encountered by the CIR regarding botanical ingredients used in cosmeceuticals are lack of data on the part of the plant used, the origin of the plant and any possible contaminants such as heavy metals or pesticides in the plant or soil, and the actual documented biological activity of the botanical/plant on human skin (3,9). Plant biology is complex and differs with each species. The chemistry of different parts of the plant is also specific and can alter the final botanical extract (3). The extraction, processing and storage of a botanical or plant-based ingredient is also not standardized and impurities in the processing techniques can potentially contaminate the final extract and vary from batch to batch (3,10). The cosmetic industry has endeavored to follow the recommendations of the CIR, ensuring that these products are as safe as possible for the community (3).

HAIR COSMECEUTICALS

Hair condition is affected by age, cosmetic grooming practices, and diseases such as androgenetic alopecia and seborrheic dermatitis. Hair cosmeceuticals claim to improve the appearance of hair by promoting growth (Table 7.1) and influencing hair color, volume, and conditioning. Hair cosmeceuticals can include both botanical and non-botanical products. Some botanicals and active ingredients may potentially affect the regulation of the hair cycle and hair growth. Other botanical products are also available to remove excess or unwanted hair.

The Hair Cycle

Anagen, the active growth phase of the hair cycle lasts two to six years (11). Catagen, a transition phase follows, ending with telogen, a resting phase that lasts two to three months (11) (Fig. 7.1). Approximately 80–85% of scalp hair follicles are in anagen, with 10–15% in catagen and telogen (12), with a normal loss of 50 to 150 telogen hairs per day (11). The hair cycle has a complex regulation and the trigger for each phase is thought to involve the interaction of growth factors and hormones (11).

Growth factors such as insulin like growth factor -1 (IGF-1) are important in hair follicle cycling and IGF-1 increases hair follicle growth in vitro (11). Other studies have shown that the transforming growth factor β 3 (TGF- β 3) and basic fibroblast growth factor (bFGF) are present in anagen, but their levels are much lower in catagen (13). Hormones such as androgens,

estrogens, and thyroxine can also influence hair growth (11). Testosterone is converted at the level of the hair follicle into the more potent dihydrotestosterone (DHT) by type II 5- α -reductase (11). Androgens act on the dermal papillae to produce growth factors to either stimulate or inhibit growth of the hair follicle depending on the body site (13). For example, in the axillae and pubic area at puberty, vellus hairs enlarge to terminal pigmented hairs. On the scalp, androgen-susceptible follicles miniaturize and revert back to vellus hairs.

Table 7.1 Botanical Cosmeceuticals with Hair Growth Promoting Properties

<i>Serenoa repens</i> (saw palmetto)
<i>Ginkgo biloba</i>
Aloe (<i>Aloe barbadensis</i> , <i>A. vera</i> , <i>A. capensis</i>)
<i>Asiasari radix</i>
<i>Sophora flavescens</i>
<i>Illicium anisatum</i>
<i>Ginseng radix</i>
<i>Hydrangea macrophylla</i>
<i>Vitis vinifera</i>
<i>Hibiscus rosa-sinensis</i>
Bergamot
Dabao Chinese herb
Amino acids
Lygodii Spora
Unsaturated fatty acids
Green tea and polyphenols
Pinacidil and potassium channel openers

Hair Growth Promoters

Serenoa Repens (Saw Palmetto)

Serenoa repens extract originates from the berries of a small evergreen palm tree native to North-Eastern America (14) (Fig. 7.2). The name derives from its saw-shaped leaves (14). It has properties of a 5- α -reductase inhibitor and has been used for prostate conditions such as benign prostatic hyperplasia (10). In a randomized double-blind placebo-controlled trial over 4.6 months using an oral liposterolic extract of *serenoa repens* and β -sitosterol, 6 out of 10 patients improved on a 7-point investigator rating scale compared to only 1 out of 9 patients in the placebo group (15). Some over-the-counter topical hair loss products contain saw palmetto as the active ingredient, but no studies have been done using this topical extract to determine whether it has any benefits topically (3).

Ginkgo biloba

Ginkgo biloba has long been used in traditional Asian medicine (Fig. 7.3). The extract originating from the tree's leaves is a mixture containing flavone glycosides including quercetin and kaempferol, and terpenes (16). As well, as anti-inflammatory properties, a study has shown that *Ginkgo biloba* extract promoted hair growth in vitro by promoting follicular proliferation

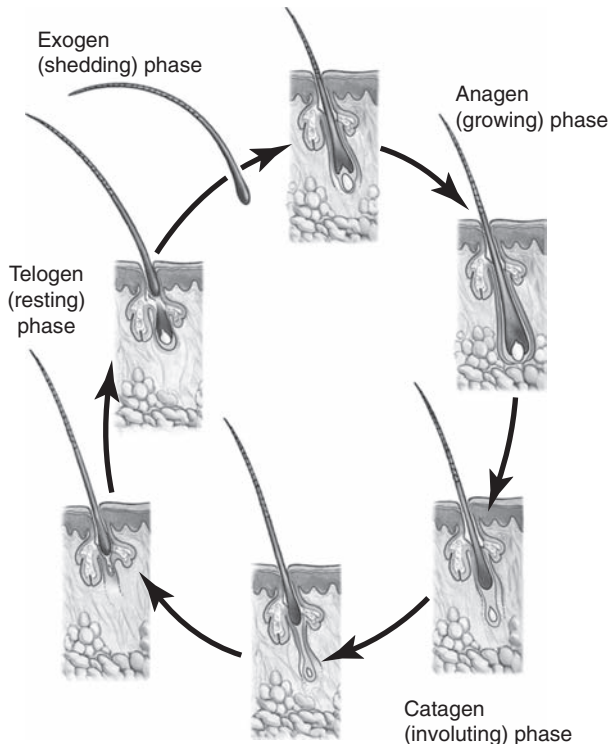


Figure 7.1 The hair growth cycle.

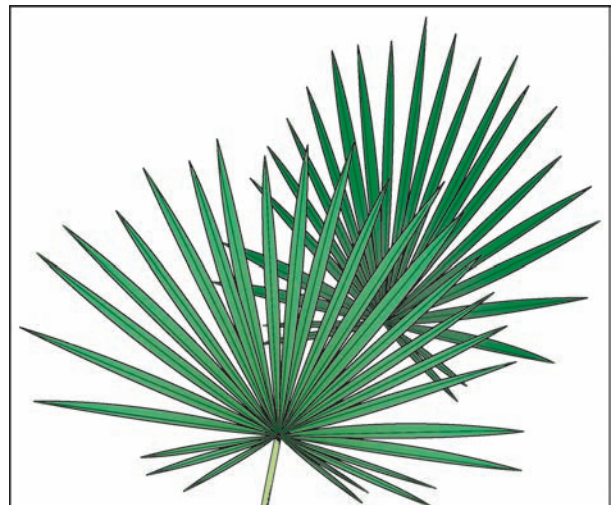


Figure 7.2 *Serenoa repens* (saw palmetto).

and inhibiting apoptosis (17). It is used commercially in several hair care products (3).

Aloe (Aloe barbadensis, Aloe vera, Aloe capensis)

Aloe is a traditional Asian therapy used for its antibacterial, antiviral and antifungal activity (18). Apart from anti-inflammatory activities, it is believed to have hair growth promoting activity (19). Aloe is a succulent plant found in Florida, USA, and South Africa (14) (Fig. 7.4). Aloe gel is derived from broken down leaves, particularly, the pericyclic cells below the plant's skin and in the inner central leaf region (3). The gel contains mucopolysaccharides, allantoin,

anthracenes such as aloin, alkylchromone, flavenoids, amino acids, minerals, hydroxyquinine glycosides, and carboxypeptidases (18). CIR safety data exists only for certain aloe species. The anthraquinone levels in *Aloe barbadensis/Aloe vera* should be kept below the industry established level of 50 ppm in cosmetic formulations (20). Inadequate data for the extracts from other aloe species exists (3). The CIR has also recommended that the total polychlorinated biphenyl pesticide contamination of plant-derived cosmetic components should be restricted (3,20). Possible side effects include allergic contact dermatitis, phototoxic reactions, and mutagenicity (18). It is contraindicated in pregnancy and lactation (18).

Asiasari radix

Asiasari radix has hair growth promoting potential. Topical *A. radix* dried root extract induced transition from telogen to anagen and increased protein synthesis in mice hair follicles, stimulating hair growth (21). In cultured human dermal papilla cells *A. radix* extract increases cellular proliferation and upregulates expression of growth factors such as VEGF (21). Vascular endothelial growth factor is important in angiogenesis and may be an autocrine growth factor for dermal papilla cells (22). Similarly, minoxidil, a useful drug treatment for hair loss may also upregulate the expression of VEGF in dermal papilla cells of anagen follicles (23). *A. radix*, however, does not have an inhibitory effect on 5- α -reductase (21). At present this extract is not available in any cosmetic preparations (3).

Sophora flavescens

Sophora flavescens has hair growth stimulating potential. Topical *S. flavescens* dried root extract stimulated transition of telogen to anagen in mice hair follicles and induced growth factors such as IGF-1 and keratinocyte growth factor (KGF) in cultured human dermal papilla cells (24). Unlike *A. radix*, *S. flavescens* has an anti-androgen effect with an inhibitory effect on type II 5- α -reductase (24). This ingredient is presently not available in any cosmetic preparations (25).

Illicium anisatum

Hair growth depends on a supportive vascular supply for the anagen hair follicles. *Illicium anisatum* extract increases subcutaneous blood supply in mice and supports hair growth (26). *I. anisatum* water-soluble extract derived from leaves, roots and fruits on an organ culture system of mouse vibrissae hair follicles demonstrated hair growth longer than controls (26). This water-soluble extract is believed to contain shikimic acid, but the exact compounds are not clearly known, and shikimic acid induced IGF-1, VEGF, and KGF mRNA expression in hair follicles (26). Extract in acetone inhibited hair follicle growth and was found to contain toxic sesquiterpene compounds (26). Although the potential dermatologic implications are that *I. anisatum* extract may be helpful as a hair promoting compound, it has not yet been used in any hair products (27).

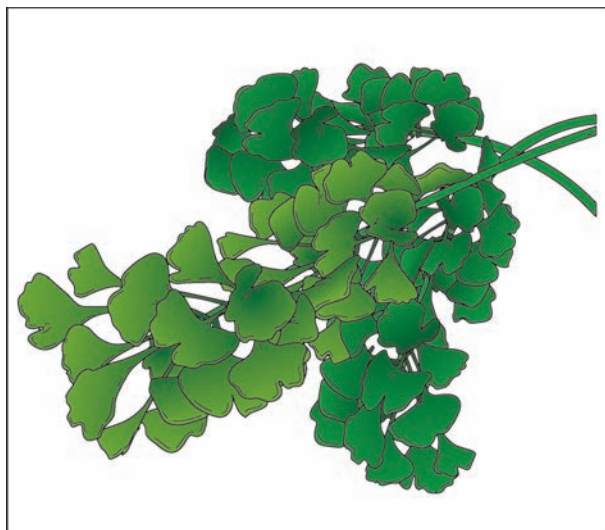


Figure 7.3 *Ginkgo biloba*.

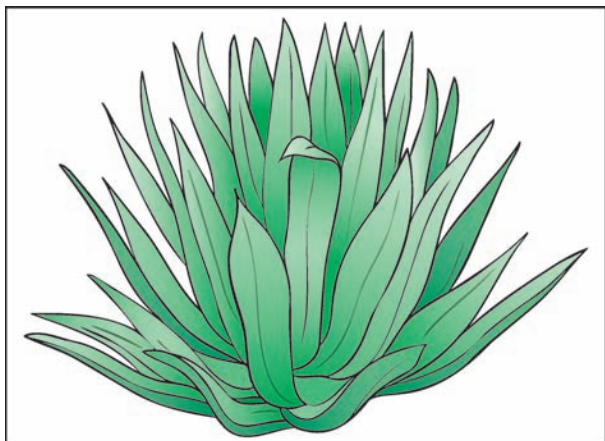


Figure 7.4 *Aloe vera*.

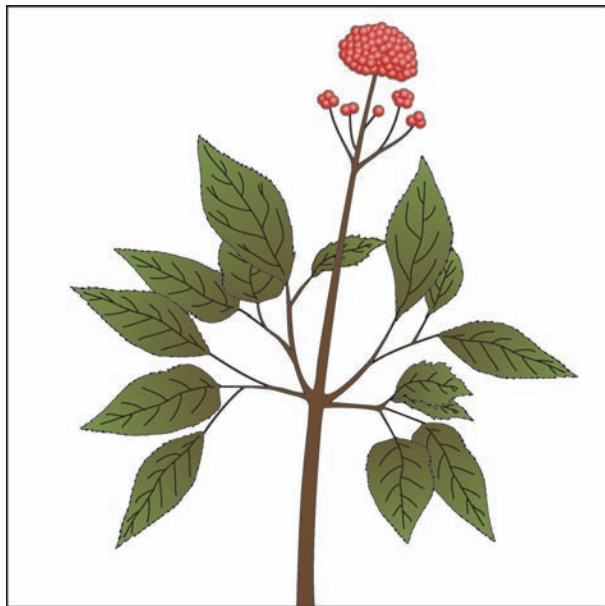


Figure 7.5 *Ginseng radix*.

Ginseng radix

Ginseng has been used for years by China and Russia for its medicinal value (Fig. 7.5). The dried root is the medicinal part, but the root can take at least six years to reach marketable size (14). The plant can live up to 100–400 years and the older the root, the smaller amount or dose of ginseng that should be used (14). The major active constituents of ginseng are the ginsenosides, claimed to have anti-aging, anti-inflammatory and antioxidant properties (16). One dermatologic benefit is purported to be hair growth. A study on cultured mice vibrissae hair follicles and a 70% methanol extract from red ginseng (steamed and dried roots) showed hair growth promoting activity (28). This function of red ginseng is better than white ginseng (28). Ginsenoside–Rb (1) or G-Rb (1) exhibited hair promoting activity as did the bioactive component 20(S)-ginsenoside (28). Ginseng extract is used in some hair care products (25).

Hydrangea macrophylla

Hydrangea macrophylla extract has potential hair growth activities. In the hair cycle, catagen, the involution phase, is characterized by an increase in TGF- β 2 (29). *H. macrophylla* extract reduced synthesis of an inducible TGF- β 2 protein and helped suppress the apoptotic caspase cascade, thus elongating hair growth and delaying catagen progression (29). This may provide an avenue for new treatments for hair loss. Currently this is not an ingredient in any cosmetic preparations (25).

Vitis vinifera

Vitis vinifera or grape seed extract activity is derived from the proanthocyanidins. Proanthocyanidins have good antioxidant

activity (18). Grape seed oil also contains other active compounds such as flavenoids, stilbenes, fruit acids, tocopherols, essential fatty acids, and phenylacrylic acids (18). Proanthocyanidins promote hair follicle growth and initiate anagen (30), likely by inhibition of TGF- β 1 (31). Resveratrol is strong antioxidant and anti-inflammatory present in grape seed oil and grape skin (16). Resveratrol has phytoestrogen activity, as well as antiviral activity (18). A topical solution of grape seed, jojoba, lavender, rosemary, and thyme was successfully used for alopecia areata and statistically significant hair regrowth was seen after seven months of daily use compared to placebo (32). Proanthocyanidins are presently not listed in any hair care products (25).

Hibiscus rosa-sinensis

Hibiscus rosa-sinensis in murine models promotes hair growth (33). Petrolatum ether extracts of the leaves and flowers of *H. rosa-sinensis* found that the leaves are the more potent hair promoter (33). Hibiscus-derived extracts are in several available hair care products (25).

Bergamot

Bergamot extract, a citrus derived oil from the rind of the bergamot orange, has a pleasant fragrance, which until a few years ago was found in many perfumes (34). It has been restricted due to its phototoxicity due to its furocoumarins (34). However, bergamot and boxthorn in murine studies applied topically for 42 days has shown hair growth promoting activities (35). Bergamot oil is used in some hair care products (25). Phototoxic reactions have been reported with oil of bergamot in aromatherapy oils (34).

Dabao Chinese Herb

A Chinese herb extract “Dabao” has been used as a hair restoring lotion (36). The dabao preparation contains water, ethanol and extracts of mulberry leaves, saffron flowers, stemona root, sesame leaves, ginger root, fruits of the hawthorn, fruits of the pepper plant, Chinese angelica root, bark of pseudolarix, and the skin of a Sechuan pepper fruit (36). In a randomized, placebo-controlled study, dabao applied topically to the scalp twice daily for six months resulted in increased hair in men with androgenetic alopecia compared to placebo of 133 hairs and 109 hairs, respectively in a studied 5 cm² area ($p < 0.03$) (36). However, allergic contact dermatitis has been reported and folliculitis was a potential side effect with treatment (36). Dabao is not available in any cosmetic hair products (3).

Amino Acids

High sulfur proteins, arginine/L-arginine and cysteine/L-cysteine are thought to be involved in hair growth promotion as they are an important component of the keratin intermediate filaments (10). Cysteine is a cosmetic ingredient in some topical hair care products with activities that theoretically comprise hair conditioning and hair waving/straightening (3,25,27).

Lygodii Spora

Lygodii Spora is the spore of *Lygodium japonicum* and a 50% aqueous ethanol extract in vitro demonstrated 5- α -reductase inhibitory activity and in vivo anti-androgenic properties (37). It showed hair regrowth after shaving in testosterone treated mice and suppressed the growth of the flank organ in castrated Syrian hamsters (37). The main active ingredients with anti-5- α -reductase activity were the fatty acids: oleic, linoleic, and palmitic (37).

Fatty Acids

Certain unsaturated fatty acids can inhibit 5- α -reductase in cultured cells and cell-free systems (38). A study has found that γ -linolenic acid has the most potent 5- α -reductase inhibitory activity followed in decreasing order of potency by arachidonic acid and α -linolenic acid, followed by linoleic acid, palmitoleic acid, oleic acid, and myristoleic acid (38). Other fatty acids such as erucic acid and undecylenic acid had no 5- α -reductase inhibitory function (38).

Another Chinese herb used for hair growth, the seeds of *Sesamum indicum* contain large amounts of fatty acids and this mechanism of action may be how *S. indicum* influences hair growth (37). Another botanical, *Boehmeria nipoonivea* in acetone extract also inhibits 5- α -reductase and encourages hair growth in mice (39). Fractionation of the leaves extract revealed six fatty acids including α -linolenic acid, linoleic acid, palmitic acid, elaidic acid, stearic acid, and oleic acid (39). α -Linolenic acid, elaidic acid, and stearic acid, in addition to the acetone extract of *B. nipoonivea* showed hair growth promotion (39). Other *Boehmeria* species such as *B. longispica* and *B. plantanifolia* have similar 5- α -reductase inhibitory effects (39). The CIR has examined the safety of fatty acids and have approved them in cosmetics at the current available concentrations and uses (40,41).

Green Tea and Polyphenols

Green tea, as well as black tea, is derived from the leaves of *Camellia sinensis* (42). Green tea comprises 2–4% caffeine and 8–12% polyphenols (18). Topical and oral green tea in studies has been found to be antibacterial, anti-cancer and promote keratinocyte differentiation (42). Epigallocatechin gallate (EGCG) is the most potent antioxidant and the largest polyphenol catechin in green tea, with green tea having the highest concentration of EGCG of any tea (18). EGCG has been shown to inhibit 5- α -reductase, with the type I being more affected than the type II 5- α -reductase (43). Other polyphenols such as biochanin A, daidzein, and myricetin were also found to have inhibitory effects on 5- α -reductase activity (43). Furthermore, EGCG in vitro increased hair growth and dermal papilla cell proliferation (44). Kaempferol, found in green tea seed extract, has been shown to also inhibit type I and II 5- α -reductase enzyme activity in cell lines (42). Green tea oral intake also may influence sex hormone binding globulin (SHBG) concentration (45). With higher levels of SHBG, more testosterone is bound in circulation, therefore the free and active testosterone

fraction decreases, reducing the androgen effect on hair follicles. These functions of green tea may suggest a possible role in the treatment of hair loss (43). It is important to note that high intake of naturally occurring 5- α -reductase inhibitors may have adverse effects on male fetus sexual development in pregnant women (43).

Pinacidil

Minoxidil is a FDA-approved drug for the treatment of hair loss. The exact mechanism by which minoxidil promotes hair growth is unknown, but is believed to influence potassium channel opening, blood supply and hair cycling (46). Other agents that influence potassium channel opening such as pinacidil, P-1075 (an active pinacidil analogue), cromakalim, and nicorandil have been shown to support growth of cultured hair follicles (46). Some over-the-counter hair growth promoters claiming to be natural, contain minoxidil as an active ingredient (3).

Hair Removal or Hair Growth Inhibitors*Thioglycollate Preparations*

Chemical depilatory creams are usually thioglycollate preparations, which can break down disulfide bonds, removing hairs at the level of the skin surface (47). They are painless and inexpensive, however, they can cause irritant dermatitis (47).

Soy Protein

Topical soy claims to delay hair regrowth and have anti-aging properties (16). Soy contains phospholipids such as phosphatidyl choline and essential fatty oils (18). Soy also contains small amounts of isoflavones, saponins, essential amino acids, phytosterols, calcium, iron and the proteases, Kunitz-type trypsin inhibitor or the soybean trypsin inhibitor (STI) and the Bowman-Birk protease inhibitor (BBI), which are the most active soy components (18). The strongest isoflavonones are genistein and daidzein (18). A study has shown that topical fresh soymilk, BBI, and STI decrease the rate of hair growth and reduce hair shaft dimensions in mice and in a small human study (48). The pigmentation of hair was also reduced with soybean protease inhibitors and soybean extracts (48). It is believed that the STI is the main agent in soymilk that influences hair growth and dimensions (48). The soy isoflavone, genistein, has also been shown to reduce hair growth on cultured hair follicles, likely due to its phytoestrogen effect (49). These natural proteins may have a role in managing unwanted hair in patients with hirsutism. Side effects of soy rarely include gastrointestinal upset, asthma, dermatitis, and itch (18). Patients with a family or personal history of breast or other estrogen-dependent cancers should avoid soy-derived products (16).

Natural Hair Colorants

Hair colors can improve the appearance of the hair by enhancing color, eliminating grey hairs, and improving the apparent hair volume.

Henna

Henna is well known as a red-brown natural hair colorant/dye and is available in hair care preparations for this function. It also has antibacterial properties and has been used to treat wounds (50). Different types of henna are available to give the hair colors of red, neutral, brown, and black. Egyptian henna characteristically causes the red-brown hair color (51). Henna is derived from the leaves of *Lawsonia alba*, *Lawsonia spinosa*, and *Lawsonia inermis* (50) (Fig. 7.6). The shrubs grow in India, North Africa, and Sri Lanka (51). The chemical dye is lawsone, a naphthoquinone (2-hydroxy-1,4-naphthoquinone), which attaches to keratin of the hair, probably via thiol group binding, as a substantive dye and gives it the red color (50,51). It does not penetrate the hair shaft (50,52). This red color can also be found in walnut, *Juglans regia* (50). Other colors can be produced by adding other dyeing agents to the henna or by treating the hair with another rinse from different plants including apigenin from Chamomile flowers to produce a yellow color, onion (*Allium cepa*) to give a copper color and curcumin to develop colors from yellow to deep orange (3,51,53,54). It is also an ingredient of shampoos, conditioners, and other products that do not alter the hair's color to add volume, control split ends, and aid manageability (51).

Adverse effects of natural henna are staining of the skin, nails, and clothes if accidentally contacted (51). These natural hair dyes have low allergic potential, but both irritant and allergic contact dermatitis have been reported to occur (51,55,56).

Hair Shampoo, Conditioning Agents and Anti-dandruff Agents

Hair products can improve the texture, volume, and tensile strength of the hair, in addition to aiding manageability (57).



Figure 7.6 *Lawsonia alba* (henna).

Hair shine and texture are dependent upon the hair surface, whereas hair structural integrity depends on the hair cortex (57).

Many varieties of shampoos and conditioners exist for hair care. Shampoos are formulated to cleanse the hair by eliminating sebum, sweat, yeast, scale, dirt, and styling products (57). Shampoos are also directed at hair type such as dry or oily, the frequency of hair shampooing and also underlying conditions like seborrheic dermatitis (7,58).

Shampoos may contain up to 30 ingredients including cleansing agents, stabilizers, preservatives, fragrances, conditioning agents, and special care ingredients with active properties to treat specific hair conditions such as oily hair or an inflamed scalp (7).

Oily or greasy hair is due to accumulation of sebum from the sebaceous glands of the scalp and sweat gland secretions (7). Oily hair needs to be washed frequently to reduce this build-up of natural grease and over-conditioning should be avoided. Some herbal ingredients in shampoos claim to help oily hair such as rosemary. Growth promoting ingredients in shampoos are unlikely to have an effect on the hair due to their short duration of contact with hair during shampooing and their dilution with water (7,59).

Seborrheic dermatitis is caused by *Malassezia* species, previously known as *Pityrosporum* species (59,60). Seborrheic dermatitis presents with scalp pruritus, erythema, and yellowish scale (60). Treatment consists of prescription and over-the-counter drugs such as zinc pyrithione, ketoconazole, imidazoles, selenium sulfide, and tar (61). The botanical agents reported to be useful for seborrheic dermatitis are extracts of sage, rosemary, thyme, garlic, and walnut (3) (Table 7.2).

With age, the hair greys and growth slows. The hair and scalp are exposed to physiologic aging and environmental factors causing oxidative stress such as ultraviolet light, smoking, poor diet, and pollution (59,62). Topical anti-aging hair compounds would therefore require antioxidants and photoprotectors (59,62). The current antioxidants in shampoos such as ascorbic acid (vitamin C) and photoprotectants shield the shampoo from oxidative degradation and not the hair and scalp (59,62). Possible future antioxidants and anti-aging compounds for shampoos are green tea and phytoestrogens (59,62). Cinnamidpropyltrimonium chloride, a UV absorber, is an ingredient in shampoos that has conditioning and photoprotective effects (59,62). Hair dyes also provide a photoprotective effect for the hair (59,62). Lipid nanoparticles of UV blockers are being developed for photoprotection in shampoos for hair (63). Shampoos with amino acids are also being created (59,62).

Table 7.2 Natural Anti-dandruff Agents

<i>Rosmarinus officinalis</i> (rosemary)
<i>Salvia</i> species (sage)
<i>Thymus vulgaris</i> (thyme)
<i>Allium sativum</i> (garlic)
<i>Juglans regia</i> (red walnut)
<i>Melaleuca alternifolia</i> (tea tree)

Hair conditioners improve cortical and cuticular function (57). Shampoos can also contain conditioning agents (62). Cuticular degeneration and cortex hair damage occurs due to weathering and exposure to chemical and hair grooming practices. Conditioners should leave the hair smooth, glossy, and hydrated (57). Conditioners also help with the manageability of the hair and reduce frizz and fly-aways (57,58). Weathered and dry hair obtain more benefit from conditioners than normal hair (59). Conditioning agents may be wax, lanolin compounds, vegetable oils, silicones, quarternary ammonium compounds, and protein hydrolysates (62). Panthenol is a potent humectant and is absorbed into the hair shaft adding moisture and condition (58,62). Panthenol is used in hundreds of hair care products at concentrations up to 6% and has been found safe by the CIR (64). Intensive conditioners are available which can moisturize the hair and restore conditions that have dimethicones, gum extracts and poly-quarternium polymers (57).

Oligosaccharides

Oligosaccharides such as 1% Cotton Honeydew (*Gossypium hirsutum*) extract rinse-off mask has been shown to be effective in softening the hair surface by smoothing the cuticle cells and protecting from cuticle lifting compared to placebo (65). The extract contains oligosaccharides such as fructose, glucose, inositol, melezitose, saccharose, trehalulose, and trehalose suggesting a role for them in hair conditioning (65).

Essential oils, such as chamomile, rosemary and West Indian Bay, are incorporated into hair care products for their fragrance and conditioning effects on the hair (66). They can be extracted from different parts of the plant including the leaves, the flowers, and the root (66). The mechanism of action of these oils is not clearly understood, but is believed to be the combined effect of both the active and inactive ingredients that modulate the action (66).

Other plant-derived substances can also condition the hair. Coconut oil, predominantly containing a triglyceride of lauric acid, reduced protein loss in damaged and undamaged human hair samples in both pre- and post-wash grooming products, while no reduction was observed in applications with sunflower and mineral oils (67). Using secondary ion mass spectrometry with time-of-flight mass spectrometry, it was found that coconut oil was able to penetrate into the hair cortex and reduce swelling of the hair fiber when the hair is exposed to water, protecting hair hygral fatigue (68). Coconut oil also functions as a fragrance ingredient in cosmetic formulations (27). The CIR (2008) recently completed a safety assessment of coconut oil (termed *Cocos Nucifera* (Coconut) Oil on cosmetic product labels) and found it to be safe in the present practices of use and concentration (69).

Rosmarinus officinalis (Rosemary)

In traditional folk medicine, rosemary is used as a hair rinse to promote hair growth, body and shine (66). It contains rosmarinic acid and caffeic acid, which are believed to have

antioxidant properties (66). It is also used as a conditioner for oily hair and used as a rinse for treatment for dandruff (66). The effect on hair growth will need further study. Rosemary extract, flower extract, flower/leaf/stem extract, and leaf extract are found as ingredients in hair care products (3,25).

Salvia Species (Sage)

Salvia officinalis, also known as common sage or garden sage, is believed to condition hair, add shine particularly to dark, curly hair and to promote hair growth (66) (Fig. 7.7). Sage extract is also used for dandruff as a massage oil into the scalp (66). Sage contains tannins, camphor, borneol, saponins vitamins A, C, and B-complex, calcium, and potassium (3,66). The effect on hair growth will need further study. Sage extract, flower/leaf/stem extract, and leaf extract are used in hair care products (3,25).

Thymus vulgaris (Thyme)

Traditionally, thyme has been used as a massage oil for the treatment of dandruff and hair shedding and a rinse with sage and rosemary to promote hair condition (66) (Fig. 7.8). The effect on hair growth will need further study. Thyme flower/leaf extract and leaf extract are used in hair care products (3,25).

Allium sativum (Garlic)

Garlic lotion has been used as a dandruff treatment in folk medicine (66). It also has antiseptic and antioxidant properties (70), in addition to antibacterial effects (71). Irritant and allergic contact dermatitis can develop when neat garlic is applied to the skin (66). Garlic bulb extract and oil are listed as ingredients in hair care products (3,25).

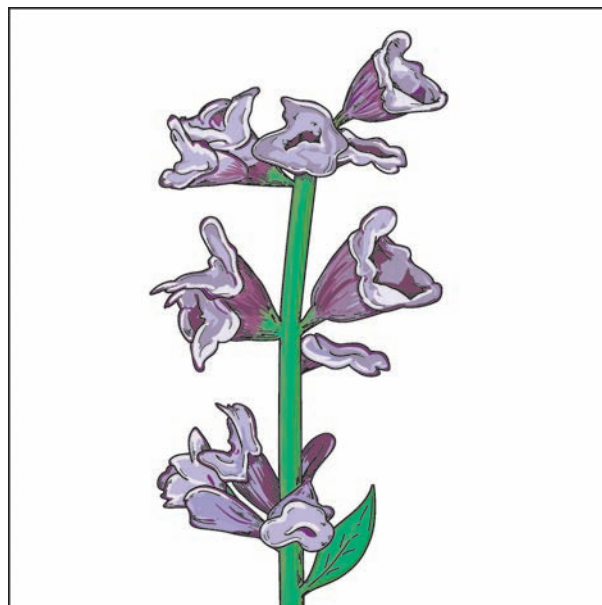


Figure 7.7 *Salvia officinalis* (sage).

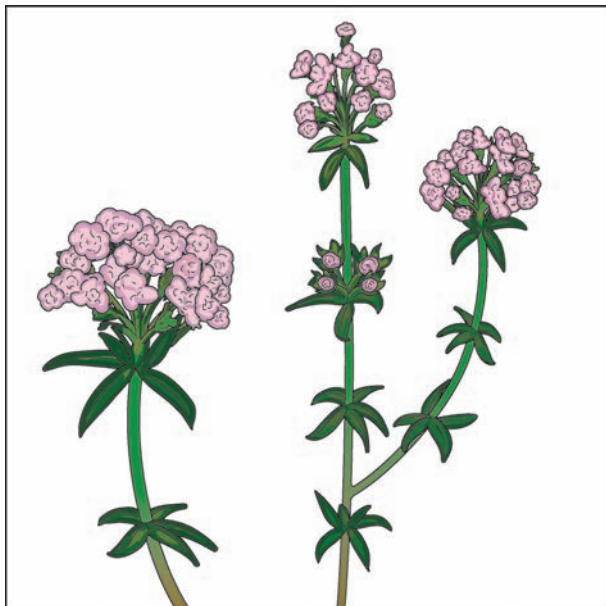


Figure 7.8 *Thymus vulgaris* (thyme).

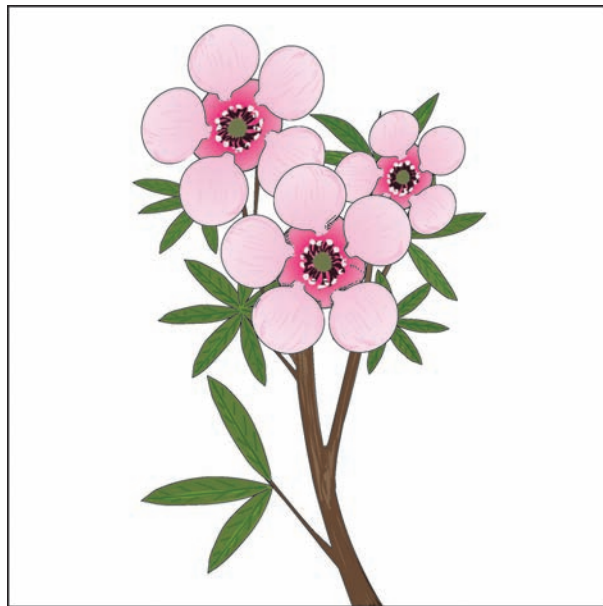


Figure 7.9 *Melaleuca alternifolia* (tea tree).

J. regia (Walnut)

Walnut leaves have been used for hair loss and dandruff. It also has emollient properties (66). Black walnut (*Juglans nigra*) is used mainly for dandruff (71). *J. regia* (Walnut) leaf extract is found in hair care products (3,25,27).

Melaleuca alternifolia (Tea Tree)

Tea tree oil (malaleuca oil) has long been used as an antiseptic and antimicrobial agent (16). It is an essential oil extracted from the leaves of the Australian *Melaleuca alternifolia* (3) (Fig. 7.9). Tea tree oil contains hydrocarbons and terpenes such as cineole (18). A study has shown that a 5% tea tree oil shampoo has antimicrobial activity against *Pityrosporum ovale*, and showed a 41% improvement compared to placebo for seborrheic dermatitis (73). Adverse effects are irritant and allergic contact dermatitis. The monoterpene, terpinen, is the significant sensitizing agent in tea tree oil (18). Prepubertal gynecomastia has been reported in young boys receiving topical formulations of lavender and tea tree oils (74). *Melaleuca alternifolia* (tea tree) leaf oil is listed in hair care products (3,25,27).

Natural Anti-head Lice Agents

Natural alternatives to medicinal head lice treatment are becoming more popular and in demand by the public.

Annona squamosa (Custard Apple)

Traditionally, in Thai medicine, *Annona squamosa* (custard apple) seeds have been used for the treatment of head lice. The hexane extract of *A. squamosa* contains oleic acid and triglyceride with one oleate ester (75). The hexane extract and the

triglyceride with one oleate ester have anti-head lice activity (75). It has been shown that a 20% w/w oil in water cream applied for three hours was effective in clearing the lice compared to a 25% benzyl benzoate emulsion (76).

Herbal Head-lice Shampoo

Paw paw (*Asimina triloba*), *Thymus vulgaris*, and *M. alternifolia* are anti-sarcoptic agents. The mechanism of action involves the inhibition of adenosine triphosphate (ATP) production, resulting in a depletion of ATP stores, which kills the lice (77). A herbal lice removal shampoo containing a standardized extract of 0.5% paw paw, 1.0% thymol, and 0.5% tea tree oil was successful in clearing 16 individuals with head lice after three applications of one hour, eight days apart in conjunction with a lice comb (78). The active ingredients of paw paw are bullatacin, asimicin, and trilobacin (77). The active constituent of tea tree oil, *M. alternifolia*, is 1-terpinen-4-ol (77). Thymol is the active ingredient of the essential oil of *T. vulgaris* and has been studied by the CIR. Thymol is safe for use in cosmetics at concentrations up to 0.5% (78) and is found in cosmetic hair products (3,25).

NAIL COSMECEUTICALS

Nail Biology

The normal nail has not only a cosmetic function, but also is also important in touch, fine manipulation, and protection for the distal phalanx (79). Environmental factors such as water, soap, and detergents can damage the nail.

The colorless, translucent nail plate is the creation of the nail matrix. Hard and soft keratins form the bulk of the nail plate (80).

Its tensile strength derives from the disulfide cystine bonds crosslinking the keratin fibers (80). The nail is inserted into the proximal and lateral nail folds. The matrix is inferior to the proximal nail plate and forms most of the nail plate (81). The nail bed is from the lunula to the hyponychium (81). The cuticle is formed by the stratum corneum of the ventral and dorsal surface of the proximal nail fold fusing and is loosely attached to the nail plate (81). The cuticle gradually desquamates and seals the nail for protection from bacteria and fungi (81).

Cuticle and Nail Care Products

Cuticle emollients can be creams, lotions, oils, waxes or ointments (79). They soften the keratin of the nail plate and surrounding skin. Emollients used in cuticle care preparations include lanolin, mineral oil, urea, and petroleum jelly (79). Plant oils such as safflower oil, wheat germ oil, tea tree oil, grapeseed oil, apricot kernel oil, and avocado oil can be added (79). Multiple other ingredients such as vitamin C (ascorbic acid), vitamin E (tocopherol acetate), aloe vera, amino acids, collagen, wheat protein, and salicylic acid can also be added (80). Green tea products are also now available for nail care claiming to increase nail strength and act as an antioxidant. The efficacy of these additives has not been evaluated in human randomized controlled trials.

Cuticle Removers

The cuticle plays an important protective function for the nail. In manicures, the cuticle is softened by a cuticle remover and pushed proximally with a metal or wooden cuticle pusher (79). Occasionally a cuticle trimmer is used to remove excess cuticle (82). If this manipulation of the cuticle is too forceful, injury to the cuticle and proximal nail fold can occur and lead to acute and chronic paronychia (79). Cuticle removers are placed over the cuticle and the proximal edge of the nail plate for a few minutes to soften the cuticle and then removed (79). Cuticle removers contain substances to break the cystine sulfide bonds, which soften the cuticle, such as 2–5% sodium hydroxide or potassium hydroxide in a liquid or cream base (79,83). The cuticle removers may also contain propylene glycol and glycerol to decrease irritation and evaporation (83). Organic bases like triethylamine and inorganic salts such as trisodium phosphate and tetrasodium pyrophosphate are weaker and less helpful cuticle removers (83). Irritant dermatitis can occur if the cuticle remover is left too long on the nail, however, allergic contact dermatitis is rare (83).

Nail Hardeners

Nail hardeners assert to make the existing nail harder and stronger and are applied like nail polish (79). They can contain Teflon, zirconium/silicon/titanium polymers, nylon, acrylic resins, and silk (79). In addition, some claim to make the growing nail stronger with supportive ingredients such as minerals and vitamins like biotin and calcium (79). Other types of nail hardeners contain up to 5% formaldehyde, which can cause allergic contact dermatitis, painful onycholysis, subungual hyperkeratosis, and hemorrhage as well as nail plate

discoloration (79). In the USA, these formaldehyde resins are for use on the free edge of the nail only, while the skin is protected (79). Formaldehyde causes crosslinks of the keratin and increases strength and hardness of the nail, but reduces the flexibility of the nail leading overall to a brittle nail (83). Vitamin supplements orally of biotin daily may assist brittle nails (80).

CONCLUSION

Cosmeceuticals have experienced exponential market growth, popularity and use in the community. As clinicians, it is important to be aware of the botanical ingredients and other active chemical ingredients incorporated into these products for hair and nail care in order to counsel patients on the safety of products and the efficacy of the marketing claims. Since cosmeceuticals are considered an intermediate between an OTC product and a drug, they usually contain higher concentrations of the active ingredients. The FDA does not directly monitor their use, but claims of biological activity can promote an investigation of the product and on occasion withdrawal of a product from the market. Few available clinical studies support the use of active botanicals and chemical ingredients in hair and nail care products, however, unpublished and published safety data reviewed by CIR, can support the safety of some chemical ingredients and can apply any necessary restrictions in their current practices of use. The botanicals and other active cosmeceutical ingredients, like other topical products, have potential for side effects, such as contact dermatitis. Clinicians should be familiar with the panoply of cosmeceutical ingredients which patients will use in hair and nail care products and be alert for side effects.

ACKNOWLEDGEMENT

Dr. S. Harrison was funded for 2008 by the F.C. Florance Bequest and the Australasian College of Dermatologists.

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8 Cosmeceuticals for Hyperpigmentation

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Even skin tone is idealized by individuals of all racial and ethnic groups. It represents youth, health, and beauty, and individuals will go to great lengths to maintain or achieve it. As such, disorders of pigmentation, especially hyperpigmentation, are among the most common dermatologic concerns treated by dermatologists. Cosmeceutical agents are an increasingly important part of the therapeutic armamentarium for hyperpigmentation and can be used in conjunction with or as an alternative to prescription products and in-office cosmetic procedures.

The cosmeceuticals available to treat hyperpigmentations can be divided into three major groups based on mechanism of action: tyrosinase inhibitors, melanosome transfer inhibitors, and skin turnover accelerators. However, there may be overlap between groups (Table 8.1).

TYROSINASE INHIBITORS

Agents that inhibit tyrosinase include hydroquinone (HQ), arbutin, aloesin, kojic acid, mulberry extract, licorice extract, ascorbic acid, vitamin E, *N*-acetyl glucosamine, and pomegranate extract. By blocking tyrosinase, conversion of DOPA to melanin is prevented.

Hydroquinone

HQ (1,4-dihydroxybenzene) remains the mainstay of treatment for hyperpigmentation. HQ is a competitive inhibitor of tyrosinase, likely acting at its copper binding site (1). It also reduces pigmentation via destruction of melanocytes, degradation of melanosomes, and inhibition of DNA and RNA synthesis (2,3). Several formulations, both over-the-counter (OTC) and prescription, are available. In the United States, the maximum allowed concentration is 2% for OTC products and 4% for prescription products. Higher concentrations, up to 10%, are available from compounding pharmacies. Hydroquinone formulations available by prescription are often combined with other ingredients to enhance efficacy. These include tretinoin, retinol, vitamin C, glycolic acid (GA), and fluocinolone. Improvement in hyperpigmentation is usually seen by eight weeks, but varies depending on the severity and the specific formulation that is used. A triple-combination product containing HQ 4%, tretinoin 0.05%, and fluocinolone 0.01% has been shown to be more efficacious than HQ combined with either tretinoin or fluocinolone alone (4).

Most adverse events to topically applied HQ are mild and transient and include contact dermatitis, which can result in paradoxical PIH, and a “halo” of hypopigmentation when perilesional skin is lightened (1). More serious effects include exogenous ochronosis and permanent depigmentation, which may be related to chronic use, higher HQ concentrations, or combination use with other substances (1,2). Furthermore,

oral HQ has been found to be carcinogenic in rodent models, but this has not been confirmed in humans. Although malignancy related to topical HQ use has not been reported, HQ is a byproduct in the metabolism of benzene, a known leukemogenic agent (5). The potential risks associated with its use led to a 2006 proposal by the FDA to ban all OTC products containing HQ and to require the manufacturers of prescription HQ products to submit investigational new drug applications. However, due to the lack of data supporting a significant risk of using FDA-approved topical HQ formulations, as well as opposition from the dermatologic community, there appears to be no movement to apply this proposed ban at the time of this writing. Nevertheless, the rare risk of exogenous ochronosis, the proposed ban by the FDA, and the recent removal of OTC HQ products from European, Japanese, and South African markets has encouraged the search for alternative pigment-lightening agents.

Mequinol

Only available in prescription formulation, mequinol (4-hydroxyanisole), a derivative of HQ, is approved in the US under the trade name of Solag  for the treatment of solar lentigines (6).

Azelaic Acid

Azelaic acid, a prescription product (available as 15% or 20% gel), is often used off-label for hyperpigmentation as it is FDA approved only for rosacea and acne. Azelaic acid is a dicarboxylic acid which exhibits antityrosinase effects (7). In a 24-week, double-blind study of 329 women with melasma 20% azelaic acid cream was shown to have comparable efficacy to 4% hydroquinone (8).

Rucinol

4-*n*-Butylresorcinol, or rucinol, inhibits both the activity of tyrosinase and tyrosine related protein-1 (TRP-1) (9). These authors showed an improvement in melasma with a three-month study using 0.3 serum (9).

Arbutin

Arbutin, a natural extract from the bearberry and pear tree, is a beta-D-glucopyranoside of HQ that competitively and reversibly blocks tyrosinase activity by interacting with the L-tyrosine binding site, so that tyrosine hydroxylation is inhibited, without affecting the mRNA expression (10,11). It also blocks the oxidation of DOPA but has a lower affinity for the DOPA oxidase catalytic site on tyrosinase (12). Compared to HQ, it is much less cytotoxic to melanocytes (10). Its efficacy is dose-dependent, thus, higher concentrations are more effective but may also be associated with a greater irritation and risk of paradoxical

Table 8.1 Cosmeceutical Ingredients in the Management of Hyperpigmentation: Mechanism of Action

Cosmeceutical agent	Tyrosinase inhibition	Melanosome transfer inhibition	Increased skin turnover	Epidermal melanin removal	Melanin dispersion	UV pigment inhibitor
Hydroquinone	X					
Mequinol	X					
Azelaic acid	X					
Rucinol	X					
Arbutin	X					
Aloesin	X					
Kojic acid	X					
Mulberry extract	X					
Licorice extract	X			X	X	
Ascorbic acid	X					
Vitamin E	X					
N-Acetyl glucosamine	X					
Pomegranate extract	X					X
Ergothioneine	X					
3-Hydroxyphloretin	X					
Oligopeptides	X					
Undecylenoyl phenylalanine	X					
Soy proteins		X				
Niacinamide		X				
Alpha hydroxy acid (AHA): glycolic acid			X	X	X	
Beta hydroxy acid (BHA): lactic acid and salicylic acid			X	X	X	
CoffeeBerry®			X			
Linoleic acid	X		X			
Retinoids	X		X		X	
Ferulic acid						X
Pycnogenol						X

(post-inflammatory) hyperpigmentation (2). Because naturally derived arbutin has been shown to be relatively ineffective, a synthetic form known as deoxyarbutin (dA) has been developed. Data from a 12-week, paired, controlled double-blind trial comparing 3% dA to placebo demonstrated that dA significantly reduced skin color and improved the appearance of solar lentigines when applied to the forearm. The authors did note that the basal skin color of dark-skinned subjects was not as responsive to dA compared to Caucasian subjects; therefore, pigmented skin may require higher concentrations of dA or longer treatment periods (13).

Aloesin

Aloesin is a natural C-glycosolated chromone derived from the leaves of the *Aloe vera* plant (14). It competitively inhibits both tyrosine hydroxylation and DOPA oxidation by tyrosinase in a dose-dependent manner (12). It does not demonstrate cytotoxicity in cell cultures, mutagenicity in the Ames assay, or skin irritation in preliminary human studies; however, its hydrophilic nature limits its ability to penetrate the skin (12). Choi et al. (15) investigated the inhibitory effect of aloesin on ultraviolet (UV)-induced pigmentation compared to those of vehicle, arbutin, and aloesin plus arbutin. Compared to the control (vehicle), the three treatment regimens were found to significantly suppress

pigmentation ($p < 0.05$). The efficacy of aloesin alone was lower than that of arbutin or combination treatment, 34% vs. 43.5% and 63.3%, respectively. However, the authors did not comment on statistical significance of these differences. The dose effect of aloesin was also studied; 50 and 100 mg/g PEG, but not 10 mg/g PEG, showed statistically significant reductions in pigmentation compared to vehicle ($p < 0.05$) (15). A recent study using pigmented skin equivalents in vitro confirmed that aloesin directly inhibits tyrosinase activity and reduces melanin content in a dose-dependent manner (16).

Kojic Acid

Kojic acid (KA; 5-hydroxymethyl-4H-pyran-2-one), a derivative from *Aspergillus* and *Penicillium* fungal species, is the second most effective OTC agent in the treatment of hyperpigmentation (2,14). It inhibits tyrosinase via copper chelation. It also acts as an antioxidant. One major disadvantage is that KA has been established as an irritant and can cause a true hypersensitivity. Irritant effect may be countered by combinations with other cosmeceuticals or topical steroids (17). In addition, skin penetration is limited due to its hydrophilic nature. KA dipalmitate, a modified lipophilic form, has improved skin penetration and stability with maintained inhibitory activity (1). Data on the efficacy of KA monotherapy for hyperpigmentation has

not been published. It has been studied in combination with other treatments to treat facial hyperpigmentation. In a 12-week split-face trial of 40 Chinese women with melasma treated with GA 10% and HQ 2% ± KA 2%, the addition of KA resulted in greater improvement compared to GA/HQ alone. Sixty percent of the KA-treatment group had greater than 50% improvement in pigmentation compared to 47.5% in the non-KA-treatment group. Complete clearance was noted only in the KA treated side (two patients). Overall, 45% of subjects responded equally to both regimens; the KA-containing regimen was more effective in 42.5% and in 12.5% GA/HQ alone was more effective. These differences were not statistically significant ($p = 0.9$). Adverse effects included transient redness, stinging and exfoliation on both sides of the face (18). Garcia and Fulton (19) compared GA 5% plus either KA 2% or HQ 2% in a split-face trial of 39 patients. Fifty-one percent of patients responded equally to both regimens; 28% and 21% demonstrated greater improvement with KA/GA and HQ/GA, respectively. The differences were not significant and the authors concluded that both regimens similarly effective. Of note the KA formulation was found to be more irritating (19).

Mulberry Extract

In vitro investigations of mulberroside F isolated from mulberry leaves (plant *Morus alba* L.) have shown it to inhibit the DOPA oxidase activity of tyrosinase and melanin synthesis in vitro, as well as scavenge superoxides. However, it was found to be less potent than KA in these studies (20). Although mulberry extracts may potentially be used to lighten hyperpigmentation, in vivo studies are lacking.

Licorice Extract

Licorice extract (glabridin) is likely the safest lightening agent and is widely used in the cosmetics industry (2). The active ingredients in licorice extracts are flavonoids, such as glabrene, liquiritin, and isoliquiritigenin. Mechanisms of action in addition to tyrosinase inhibition include melanin dispersion, epidermal melanin removal, and cyclo-oxygenase inhibition (anti-inflammatory activity) (21). The efficacy of topical liquiritin in reducing pigmentation was investigated in a split-face trial of 20 women with idiopathic epidermal melanin. After four weeks of twice-daily application, 80% of the treatment group had an "excellent" response defined as no difference between the previously pigmented skin and the adjacent normal skin, whereas 90% of the vehicle treated side had a "poor" response. Two patients experienced transient erythema and slight burning. For clinical results, the authors suggested topical application 1 g daily for four weeks (22).

Ascorbic Acid

L-Ascorbic acid, or vitamin C, is a natural antioxidant that serves to reverse and prevent hyperpigmentation on many levels. First, it reduces tyrosinase activity by interacting with copper ions at the active site (2). In addition, it acts as an anti-inflammatory agent; thus, it has the potential to reduce

subsequent post-inflammatory hyperpigmentation (23). It also inhibits the transfer of melanin. Active L-ascorbic acid is unstable and undergoes rapid oxidation. Its esterified derivatives, ascorbyl-6-palmitate and magnesium-L-ascorbyl-2-phosphate (VC-PMG; MAP), have improved stability, but are minimally metabolized to the active form in the skin (23). An effective cosmeceutical must contain at least 10% L-ascorbic acid and must be stable (23). Although adverse effects are minimal, ascorbic acid is relatively ineffective when used alone; therefore it is usually combined with other agents, such as licorice extracts (2). In a study of 34 patients with various pigmented dyschromias, twice-daily application of VC-PMG 10% cream resulted in significant improvement in 55.9% (24). One author noted that daily application of 15% topical vitamin C for two months resulted in clinical improvement of melasma and solar lentiginos (unpublished data) (23). A 16-week, split-face, double-blind trial comparing 5% ascorbic acid cream and 4% HQ cream for melasma found that HQ resulted in significantly better subjective improvement ($p < 0.5$); however, calorimetry showed no statistically significant differences. In addition, adverse effects were present in 68.7% (11/16) with hydroquinone compared to only 6.2% (1/16) with ascorbic acid (25).

Vitamin E

Vitamin E, alpha-tocopheryl, is a naturally occurring lipophilic antioxidant that reduces pigmentation by interfering with lipid peroxidation of melanocyte membranes and indirectly inhibiting tyrosine hydroxylase activity of tyrosinase (26,27). Oral vitamin E intake has been shown to effectively reduce hyperpigmentation, especially when combined with vitamin C. In vitro studies have shown that the compound alpha-tocopheryl ferulate has been found to significantly suppress melanogenesis. It is thought that ferulate, an antioxidant, stabilizes vitamin E and results in sustained antioxidant properties (26,27). Vitamin E may be combined with vitamin C to enhance efficacy as has been shown in a double-blind study in which vitamin E plus vitamin C was compared with each alone for the treatment of melasma and pigmented contact dermatitis. The combination treatment resulted in a significantly higher improvement compared to either monotherapy (1,28).

N-Acetyl Glucosamine

Glucosamine and its derivative N-acetyl glucosamine are amino-monosaccharides that serve as substrate precursors for the biosynthesis of glycosaminoglycans such as hyaluronic acid and proteoglycans (29). Glucosamine inhibits the enzyme tyrosinase activation by inhibiting the glycosylation of tyrosine. This action inhibits melanin production in the skin. It shows promise in the treatment of disorder of pigmentation.

In an eight-week double-blind, placebo-controlled left-right randomized, split-face clinical trial conducted by Bissett et al (30), topical 2% N-acetyl glucosamine was effective in improving the appearance of facial hyperpigmentation in a small cohort of Japanese female subjects (30). Glucosamine has also been combined with niacinamide for the treatment of facial

hyperpigmentation. As of this publication, *N*-acetylglucosamine is currently available as an OTC product.

Pomegranate Extract

Pomegranate extract contains ellagic acid, a polyphenol, that inhibits tyrosinase by chelating copper at the active site (17). It is also a potent antioxidant and has been reported to improve the efficacy of topical sunscreens (31).

Ergothioneine

Ergothioneine, a water-soluble amino acid found in grains, is a stable antioxidant. A 4% HQ formulation containing ergothioneine has been found to have improved stability and tolerability compared to commercially available 4% HQ formulations (32).

3-Hydroxyphloretin

3-Hydroxyphloretin, a constituent of the Formosan apple (*Malus doumeri* var. *formosana*) indigenous to Taiwan, demonstrated in vitro inhibition of tyrosinase activity in human epidermal melanocytes as well as potent antioxidant activity (33). This compound has recently been included in a cosmeceutical product and may be useful in the treatment of hyperpigmentation. At the time of this writing, peer-reviewed published clinical trials evaluating the efficacy of this agent in the treatment of hyperpigmentation are not available.

Oligopeptides

In a recent split-face, double-blind, randomized and placebo-controlled pilot study investigating the efficacy of a proprietary oligopeptide (Lumixyl™), five female patients with recalcitrant epidermal melasma demonstrated statistically significant improvement in melasma (using a 10-point scale) at 16-weeks of treatment (34). This oligopeptide was previously shown in vitro to competitively inhibit human and mushroom tyrosinase without melanocyte cytotoxicity (35). Additional studies with this agent are warranted.

MELANOSOME TRANSFER INHIBITORS

Soy Proteins

Soy extract contains two serine protease inhibitors, serine trypsin inhibitor (STI) and Bowman-Birk inhibitor (BBI), which inhibit activation of the protease-activated receptor-2 pathway (PAR-2). This pathway is required for keratinocyte phagocytosis of melanosomes and melanosome transfer (36). Fatty acids and fatty acid esters in soybeans can inhibit trypsin which may further inhibit the PAR-2 pathway. In addition, isoflavones, antioxidants contained in soybeans, may reduce the DOPA oxidase activity of tyrosinase (1). Application of STI has been shown to result in reversible skin lightening over a three-week period without adverse effects as well as prevent UV-induced pigmentation (37).

Niacinamide

Niacinamide is a stable water-soluble derivative of the vitamin niacin, a precursor for the enzymes NAD and NADP, which are important for cell metabolism. Niacinamide has been shown to

inhibit melanosome transfer by 35–68%, without an effect on melanogenesis or cytotoxicity (38). In a clinical study, 5% topical niacinamide resulted in a reduction in hyperpigmentation; however, its effectiveness leveled after four weeks. In addition, 2% niacinamide plus sunscreen lightened baseline skin color. Its effects were noted to be dose-dependent and reversible (38).

SKIN TURNOVER ACCELERATORS

Alpha Hydroxy Acid

Alpha hydroxy acids (AHAs) have been used to treat a variety of skin conditions including acne, photodamage, and pigmentation. GA and lactic acid (LA) are the most widely studied and used AHAs. AHAs likely exert their effects by causing thinning of the stratum corneum and dispersion of melanin. GA has also been shown to act as an antioxidant, therefore, providing anti-inflammatory actions (39). As noted above, AHAs may be used in combination with other topical treatments; the addition of an AHA to the regimen may enhance penetration, thus, efficacy. Most published studies regarding the efficacy of AHAs for hyperpigmentation have primarily investigated their use as chemical peeling agents and found them to be effective for hyperpigmentation when used in this manner.

Beta Hydroxy Acid

The lightening or whitening effect of salicylic acid has been observed frequently especially in darker skin types (40,41). These effects can be very helpful in treating hyperpigmentary disorders. Salicylic acid, the prototypical beta hydroxy acid (BHA), effects hyperpigmentation by loosening the keratinocytes and the associated removal of melanin which results in lightening the skin. A new derivative of salicylic acid is lipohydroxy acid which is used in 5–10% strengths as a chemical peeling cosmeceutical which has demonstrated skin lightening (Fig. 8.1A and B).

CoffeeBerry

CoffeeBerry is a proprietary extract of the subripe berry of the plant coffee Arabica. CoffeeBerry is abundant in polyphenolic antioxidants which include proanthocyanidins, chlorogenic acid, guinic acid, and ferulic acid and thus qualifies as the most potent topical antioxidant as measured on the oxygen radical absorbance capacity (ORAC) test. A 2007 double-blind proof of concept study in a cohort of 30 women showed a three-fold improvement in skin pigmentation with twice-daily application of coffee berry (42). CoffeeBerry is available commercially as RevalSkin Cream™.

Linoleic Acid

Linoleic acid is an unsaturated fatty acid that increases epidermal cell turnover and, therefore, desquamation of melanin from the epidermis. It also reduces tyrosinase activity without affecting mRNA levels (43). Linoleic acid has demonstrated melanogenesis inhibition in brown guinea pigs (43,44). Published data in humans is limited with only one small trial showing that linoleic acid significantly reduced

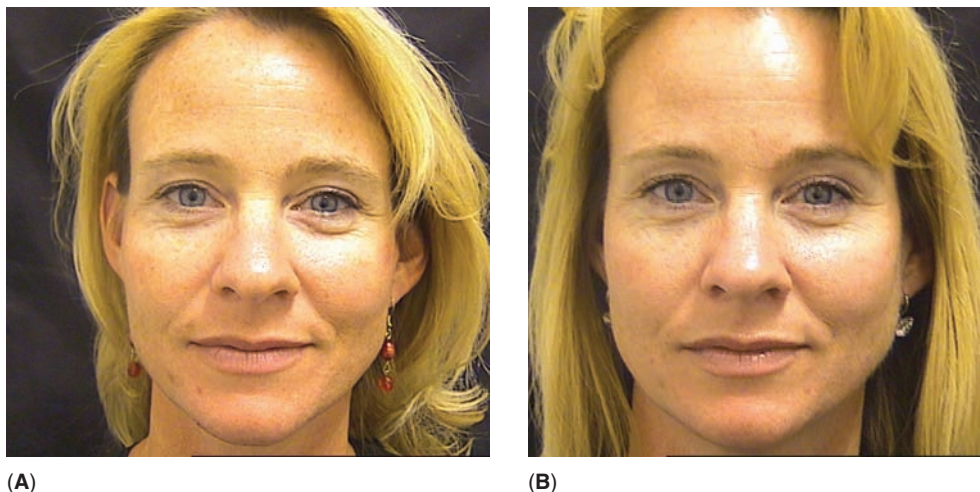


Figure 8.1 Reduction of solar lentigines with salicylic acid derivative (lipohydroxy acid) peel and moisturizers: (A) before and (B) after treatment.

UVB-induced hyperpigmentation on the upper arm after twice-daily application for two months (44).

Retinoids

Retinoids are derivatives of vitamin A, a fat-soluble vitamin. They are available in both prescription and non-prescription topical formulations and are commonly used to treat various dermatologic conditions. In addition to increasing the rate of skin turnover, retinoids promote epidermal melanin dispersion, interfere with pigment transfer, and inhibit tyrosinase activity (45,46). Retinol, the dietary form of vitamin A, is available as an OTC product and is thought to produce effects similar to those produced by retinoic acid, but with less irritation. It is converted to retinoic acid in the skin through a two-step oxidation process. Although several studies have documented the efficacy of prescription retinoids for the treatment of hyperpigmentation, similar studies investigating retinol monotherapy are limited. Two 12-week, open-label trials examined the efficacy and safety of a formulation of 4% HQ with 0.15% retinol and reported significant improvement of hyperpigmentation. The combination was well tolerated in both studies (47,48). Draelos conducted a 16-week trial in which 4% HQ/0.3% retinol cream was compared to 0.05% tretinoin cream and noted that HQ/retinol cream more effectively reduced signs of photoaging including hyperpigmentation (49).

UV PIGMENT INHIBITORS

Pycnogenol®

Pycnogenol®, a French maritime pine (*Pinus pinaster*) bark extract which contains phenolic compounds and condensed flavonoids, acts as a strong antioxidant and anti-inflammatory. It has been shown to protect against UV radiation-induced effects in vitro and in vivo. A 30-day open-label trial of 30 women with melasma treated daily with 75 mg of oral Pycnogenol® decreased the mean pigment intensity ($p < 0.001$) and mean affected area ($p < 0.001$). No adverse effects were reported (50).

Ferulic Acid

Ferulic acid, a hydroxycinnamic acid, is a plant antioxidant that has been shown to stabilize vitamins C and E. This triple combination has been found to provide substantial UV protection in vivo (51).

COSMECEUTICALS USED IN COMBINATION

There is evidence which indicates that one or more cosmeceuticals used in combination or simultaneously during a treatment period may provide increased efficacy. As the search for a possible replacement for hydroquinone continues, these investigative efforts will probably increase. One example of a non-hydroquinone compound made from synergistic skin lightening agents is a formulation containing kojic acid and emblica fruit extract. In a study involving 70 female subjects with facial dyschromia, a formulation containing emblica extract and kojic acid was shown to have comparable efficacy to hydroquinone 2% (52). A more recent study evaluated a combination skin lightener containing kojic acid, emblica fruit extract, and glycolic acid and found it to have comparable efficacy to 4% hydroquinone in the treatment of facial dyschromia. (Source: Z Draelos et al., Evaluation of a kojic acid, emblica fruit extract, and glycolic acid skin lightener as compared to 4% hydroquinone: clinical poster, 68th Annual Meeting of the AAD, 2010.) Such combination products may serve as useful alternatives to hydroquinone. Further study is warranted to elucidate their comparative efficacy in specific disorders of hyperpigmentation.

Table 8.2 is a listing of synergistic cosmeceuticals.

N-undecylenoyl phenylalanine, an alpha-melanocyte-stimulating hormone receptor antagonist which has been shown to reduce melanin production in cultured melanocytes, was studied in combination with niacinamide in recent trials. In two double-blind, 10-week (2-week washout + 8-week treatment), left-right randomized, split-face clinical studies, 5% niacinamide plus 1% N-undecylenoyl phenylalanine was more effective than 5% niacinamide alone in reducing facial

Table 8.2 Cosmeceuticals in Combination for Hyperpigmentation

Glucosamine and niacinamide
Azelaic acid 20% and glycolic acid 15% or 20%
Azelaic acid 20% and hydroquinone 2%
Kojic acid and hydroquinone 2%
Kojic acid, hydroquinone 2%, and glycolic acid 10%
Glycolic acid 5% and hydroquinone 2%
Glycolic acid 10% and hydroquinone 2%
Glycolic acid 10%, hydroquinone 4%, antioxidants, and sunscreen
Glycolic acid 2%, hydroquinone 4%, ascorbyl palmitate, and tocopherol acetate
Salicylic acid 20–30% peels and hydroquinone 4%
Retinol 0.15% and hydroquinone 4%
Mequinol 2% and retinoic acid 0.01%
Ferulic acid, vitamin C, and vitamin E

hyperpigmentation. Further study of this combination in specific disorders of hyperpigmentation is warranted (53).

CONCLUSION

Hyperpigmentary disorders especially those affecting the face range from mild to severe. It may be the most common condition worldwide for which dermatologic help is sought and can be associated with genetics, aging skin, trauma, and sundamage. These disorders are often a cause of significant psychological distress to individuals and commonly encountered by dermatologists. Hydroquinone, traditionally the mainstay of therapy, has recently been under scrutiny by the FDA and other national health agencies due to safety concerns from animal data and the rare complication of exogenous ochronosis. In this context, identifying alternative skin lightening agents is of increasing interest to both consumers and manufacturers. Although several of these products are commercially available, the majority of them have not been rigorously investigated and data regarding their safety and efficacy is limited. However, preliminary data has been promising; and in the future, these newly emerging cosmeceuticals will likely play an integral role in the treatment of hyperpigmentation.

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9 Cosmeceuticals in Conjunction with Lasers, Light Sources, and Energy-based Devices

Vic A Narurkar

INTRODUCTION

Patients undergoing treatments with energy-based devices require multiple and complex skin care needs. While there is a plethora of cosmeceuticals and many anecdotal reports of using cosmeceuticals in combination with lasers, light sources, and other energy-based devices, there is very little evidence-based peer-reviewed literature on this subject. Cosmeceuticals have multiple roles in this capacity including preparing the skin for better outcomes with devices; enhancing the effects of devices with skin care regimens and accelerating recovery in post-procedure phases. This chapter will systematically review how cosmeceuticals can play multiple roles with energy-based devices. Future directions include the simultaneous use of cosmeceuticals with devices and home-based combination devices.

SKIN REJUVENATION WITH DEVICES

Devices for skin rejuvenation fall into ablative, nonablative, and fractional categories (1). Ablative skin resurfacing is losing popularity due to significant downtime, recovery and risks. Nonablative and fractional skin resurfacing comprise the majority of device-based resurfacing procedures. Fractional resurfacing is further divided into nonablative fractional and ablative fractional technologies. The skin care regimens for the aforementioned modalities are unique. With a compromise of the stratum corneum (as in traditional ablative and fractional ablative resurfacing), post-procedure care is initiated initially with emolliation until there is complete re-epithelialization. With an intact stratum corneum (as in nonablative fractional resurfacing and nonablative laser/light treatments), skin care regimens can be initiated immediately post resurfacing (2).

COSMECEUTICALS IN PREPARATION OF THE SKIN FOR SKIN REJUVENATION

Deep cleansing and gentle exfoliation assist in acceleration of cell turnover without skin disturbance and are essential for effective penetration of the procedure. Preparation of the skin strengthens the skin prior to ablative and nonablative resurfacing. Prescription retinoids have been studied in this modality for preparing the skin prior to resurfacing and act by stimulating new collagen preparation. Hydroquinone preparations are used, especially in darker skin tones, to act synergistically with devices to reduce dyschromias, lentigines and prevent post-inflammatory hyperpigmentation. Recently, cosmeceuticals alone or in combination with prescription products are being studied prior to nonablative, fractional, and ablative procedures (3).

Exfoliant ingredients include salicylic acid, glucosamine, and vitamin A. Antioxidants such as topical vitamin C and vitamin E,

green tea, CoffeeBerry, mangosteen extracts, and durian extracts are used prior to resurfacing to protect the skin from damaging free radicals and promote even skin tone. Alpha-hydroxy acids can speed up exfoliation, improve skin texture and tone and reduce dyschromias and lentigines and enhance new collagen production. These products can be used in conjunction with prescription retinoids and hydroquinones for synergistic effects.

COSMECEUTICALS IN PREPARATION FOR TREATMENT OF DISORDERS OF HYPERPIGMENTATION AND PREVENTION OF POST-INFLAMMATORY HYPERPIGMENTATION

Energy-based devices are very popular for the treatment of dyschromia, lentigines, and also for therapy-resistant melasma. Nonablative devices include Q switched 532 and 755 nm lasers, pulsed 510 and 595 nm lasers, long pulsed 755 and 800 nm lasers and intense pulsed light. Fractional nonablative devices include 1440, 1540, and 1550 nm lasers. It is critical to prepare the skin for the treatment of pigmentary devices with devices for several reasons—greater efficacy in clearance of lesions, regardless of skin type, and prevention of post-inflammatory hyperpigmentation, especially in darker skin tones. Moreover, conditions such as melasma which are chronic, absolutely necessitate preparing the skin. While prescription hydroquinones and retinoids are highly effective, cosmeceuticals play a vital role, especially in patients intolerant or allergic to prescription regimens.

Soy-based ingredients have been shown to improve hyperpigmentation by interfering with melanin transfer by inhibiting the protease-activated receptor-2 (PAR2) pathway. Licorice extracts are used in correction of dyschromias with active ingredients glabridin and licochalcone A, which has been shown to have bleaching activity and UVB-induced hyperpigmentation inhibition. Topical niacinamide is an inhibitor of melanosome transfer to keratinocytes and can also assist in reducing hyperpigmentation. Topical vitamin C, in addition to the antioxidant activity, has also been shown to reduce hyperpigmentation and solar lentigines (4).

COSMECEUTICALS FOR IMMEDIATE POST-TREATMENT REGIMENS BY ENERGY-BASED DEVICES

The ultimate outcomes of any laser, light source or energy-based device procedure are dependent on optimal healing. The first week post-procedure is critical to achieving optimal aesthetic outcomes. Immediately after the procedure, appropriate care should be given to optimize the healing process and manage potential side effects such as erythema, excessive inflammation, irritation, and unwanted post-inflammatory hyperpigmentation.

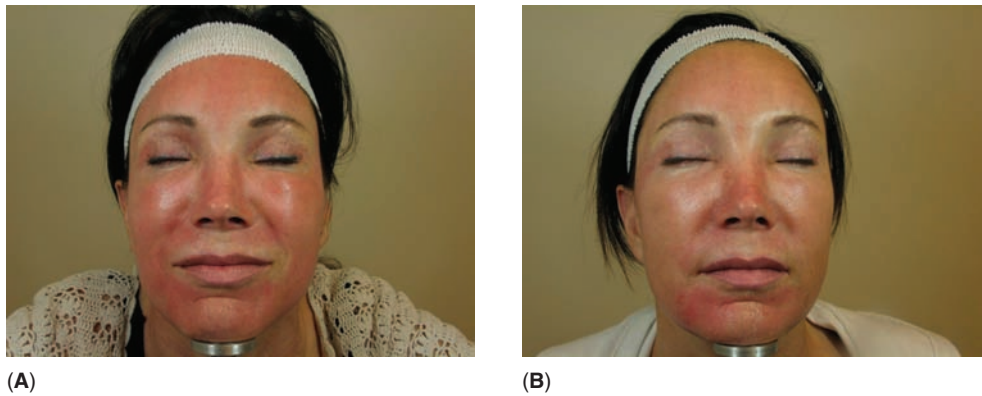


Figure 9.1 Patient 48 hr after 1550 nm fractional laser resurfacing without (A) and with (B) post skin-care regimen with probiotics.

Probiotics such as lactobacillus ferment help maintain the barrier of the skin and produce a range of antimicrobial peptides that work to eliminate potential cutaneous pathogens (5). Figure 9.1 shows an example of a patient who underwent 1550 nm fractional laser resurfacing with and without a post skin-care regimen consisting of probiotics, with the untreated aspect showing greater erythema and edema. The lipid-rich protective barrier, created by probiotics, protect against external substances that could compromise the healing process. Colloidal oatmeal which has a high concentration of polysaccharides forms a gelatinous hydrocolloid in water, leaving a protective film which helps to repair and maintain the epidermal barrier.

COSMECEUTICALS FOR LONG-TERM POST-TREATMENT BY ENERGY-BASED DEVICES

After the initial recovery phase, a maintenance skin care regimen is imperative to sustain the effects achieved acutely by energy-based devices. This field is in its infancy and is lacking well controlled and long-term clinical studies. However, it is intuitive that there is some synergy between devices and cosmeceuticals. The most exciting group of cosmeceuticals in this category is peptides and growth factors.

Peptides have multiple mechanisms of actions and delivery into the skin, with their small molecular size and assisted delivery systems, can assist in their clinical effects. Signal peptides such as palmitoyl pentapeptide may assist in neocollagenesis. Copper peptides, made with the addition of copper, have a dual role as collagen stimulators and antioxidants for wound healing. Neuropeptides such as argireline have been promoted to act as neuromodulators, but with no real published data. Modification of peptides can manipulate their characteristics, allowing for increased specificity and increased skin penetration. Therefore, peptides may be synergistic in ablative and nonablative resurfacing, for sustained neocollagenesis, with the hope of making the effects of these procedures last longer.

Growth factors and growth factor stimulators play a vital role in wound healing and tissue regeneration. Examples include

fibroblast growth factors, transforming growth factors, interleukins, colony stimulating factors, and proteins of Wnt signaling pathway. The controversy surrounding the actual efficacy versus penetration of growth factors relates to the limitation of penetration of most growth factors through the epidermis based on their molecular weight. It has been speculated that regardless of the actual quantity of absorption, natural communication mechanisms between epidermis and dermis may amplify the effect of topically applied growth factors with minimal penetration. Of all the cosmeceuticals, the greatest controlled clinical studies which demonstrate skin regeneration and repair, have been with growth factors. Growth factors, however, also pose risks because if unregulated, they could mediate carcinogenic transformation of cells.

Synergistic combination of peptides, growth factors, antioxidants, and skin conditioners are most likely the optimal cosmeceutical product, as they address multiple modalities of skin regeneration and repair (6).

FUTURE DIRECTIONS

The limitation of most cosmeceutical agents has been optimal delivery. One approach to enhance the delivery of cosmeceuticals is to combine it with an energy-based device. Current research is being conducted to optimize such delivery. For materials to successfully enter the epidermis, three potential routes can be taken—intercellular, transcellular, and appendageal. The intercellular route is the most challenging, as the lipids occupying the spaces between the flattened cornified cells are an effective barrier to hydrophilic and hydrophobic substances. The transcellular route requires penetration through the cellular wall and is used by hydrophilic substances. The transappendageal route consists of the pilosebaceous unit and eccrine unit. Studies are underway with devices utilizing fractional resurfacing to transiently open up these channels to deliver topical agents. Another approach is photopneumatic therapy with profusion. Photopneumatic profusion devices use a vacuum to stretch the skin, causing the intercellular layers of

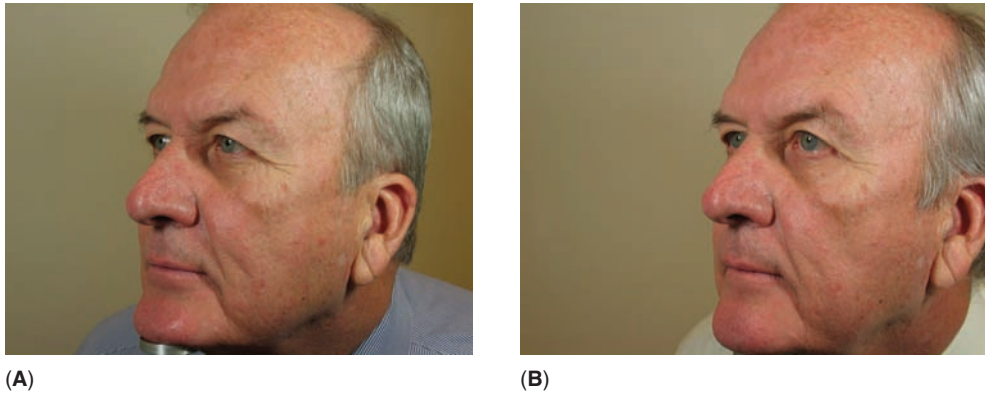


Figure 9.2 Pre- (A) and post-treatment (B) using topical growth factors and a profusion photopneumatic device.

the stratum corneum to separate. In addition, the pilosebaceous unit is placed closer to the skin surface and the differential pressure between the outside and inside of the skin causes the pilosebaceous gland to be pushed outward, allowing for easier entry of topical agents. Topical growth factor containing cosmeceuticals have been delivered through this method and show some impressive early clinical results (Fig. 9.2). Controlled clinical studies are underway finally, if these devices have the potential to be home-based. Thus, after a procedure, patients can continue long-term treatments with a true “combination therapy” of devices and cosmeceuticals.

CONCLUSIONS

The role of cosmeceuticals in conjunction with lasers, light sources, and energy-based devices is in its infancy. While it is intuitive that a comprehensive pre-procedure regimen to condition the skin, immediate post-procedure regimen to accelerate healing and reduce complications, and a long-term skin-care regimen to prolong the effects of a device, is essential, there are little or no controlled studies to document these benefits. Moreover, this is furthered by the fact that there are limited controlled studies on cosmeceuticals themselves. What is reality is that most dermatologists employ pre- and post-device skin care regimens and it is at least anecdotally evident

that these regimens are highly effective. The future of devices and cosmeceuticals employs a true “combination therapy,” whereby simultaneous delivery of a cosmeceutical through a device is performed, both in the office of the physician and potentially as a home-based regimen.

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10 Incorporating Skin Care Products into Your Practice

Mary P Lupo

INTRODUCTION

All dermatologists make skin care recommendations to their patients. Medical dermatologists diagnose and treat a myriad of skin conditions that require adjunctive topical care to complement the prescription treatments. Atopic dermatitis, contact dermatitis, psoriasis, seborrheic dermatitis, and rosacea are just some of the most commonly diagnosed conditions that should have strict protocols to help treat and then prevent flares of these skin diseases. Acne treatment with retinoids can lead to dry, inflamed skin if the dermatologist does not properly instruct the patients with skin care recommendations that help to hydrate and protect the epidermal barrier. Retinoid irritation can result in non-compliance and treatment failure if skin care instructions are not outlined by the treating dermatologist. Surgical dermatologists who diagnose and treat skin cancers always admonish these patients to protect their skin from the risk of future skin cancers by using skin creams that provide adequate ultraviolet (SPF) protection. Finally, cosmetic or “procedural” dermatologists utilize cosmeceutical protocols to prepare the skin for treatments such as chemical peels and laser, to complement the benefits and speed healing of these procedures, and use skin care to maintain the benefits of these treatments. Using products such as retinoids and hydroquinone is a gold standard in pre- and post-laser and light-based treatments (1). For these reasons, it is a natural extension of the dermatologists’ care to include these topical cosmeceutical products in treatment recommendations and to incorporate them into their practices. Patients look to their dermatologists for direction on which products to purchase for their individual needs and goals. The decision of which product to recommend, ethical business and medical practices of selling cosmeceuticals, and the logistics of adding this service are important considerations that must be resolved. This textbook will extensively review cosmeceutical products so that the treating physician can best address the needs of their patients and help the dermatologist decide which products to incorporate into their practices.

HOW TO CHOOSE PRODUCTS

In order to make cosmeceutical recommendations to patients, it is important to understand the potential benefit that a product can deliver and which medical or cosmetic condition may respond to that product. Some of the clinical diagnoses that dermatologists treat which can be improved with the addition of appropriate complementary cosmeceuticals include skin cancer, photoaging, acne, rosacea, melasma, and any skin condition that results in lipid barrier disruption (i.e., atopy). Some

topical prescription remedies such as 5-fluorouracil, imiquimod, and aminolevulinic acid for actinic damage and adapalene, tretinoin, and tazarotene for acne or photoaging, all require complementary topical care to protect from ultraviolet light and epidermal barrier disruption.

If a dermatologist wants to offer skin care products for sale out of their office, there are common categories of products that would likely be included. Photoprotective products, antioxidants, collagen-stimulating products, pigment lightening, exfoliants, area-specific products, cleansers, and barrier-restorative would be good categories to offer. Many products do multi-tasking such as retinol, which is both pigment lightening and collagen stimulating, and alpha hydroxy acids which exfoliate, thereby improving acne as well as dyschromia.

Photoprotection creams, or sunscreens, are the most important recommendation that dermatologists can make to patients. Rosacea, skin cancers, melasma, photoaging, and light-sensitive dermatoses like polymorphic light eruption, are just some of the medical conditions needing regular sunscreen use. It is critical that pulsed light, peel, and laser patients use sun protection before procedures to begin the process of correction, and after the procedure to prevent post-inflammatory hyperpigmentation immediately post-procedure and consistently over time to maintain the benefit of these paid-out-of-pocket and costly cosmetic procedures. The benefits of using retinoids and hydroquinone both pre- and post-treatment have been proven to enhance results and these products can irritate the skin, so adequate sun protection enhances their tolerability. Much progress has been made recently in improvement of broad spectrum sunscreens, especially with improved protection from ultraviolet A rays and improvement in photo-stability of final product formulations.

Antioxidants purportedly complex free radicals that have various deleterious effects on the skin. Free radicals cross-link collagen and accelerate its breakdown. It would make sense to use this product class to protect from increases of matrix metalloproteinase I (MMP-1, collagenase) and sunburn cell formation that result from ultraviolet light exposure. UVR also causes NK- κ B activation and the production of pro-inflammatory mediators (2). Antioxidants have anti-inflammatory actions against these mediators and may protect against DNA damage. Table 10.1 lists some of the most popular antioxidant ingredients found in mainstream product lines. Since no sun protection cream can block all ultraviolet light, the use of antioxidants in the morning to complement the protective benefits of sunscreens, would be a reasonable recommendation to patients.

Table 10.1 Popular Antioxidants

Green tea extract
Grape seed extract
Genistein
CoffeeBerry
Co-enzyme Q10
Idebenone
Vitamin A
Vitamin C
Vitamin E
Pycnogenol
Alpha lipoic acid
Dimethylaminoethanol
Glutathione
Catalase

Table 10.2 Collagen-stimulating Ingredients

Peptides (copper peptide, pentapeptides)
Amino acids
Human growth factors (transforming growth factor β epidermal growth factor)
Retinol
Niacinamide
CoffeeBerry

Collagen-stimulating products are typically recommended for older skin in which wrinkles and fine lines are the primary concern. Table 10.2 lists some of the ingredients with cosmeceutical studies documenting *in vitro* or *in vivo* increases of collagen. These increases in collagen are the result of fibroblast stimulation (3). Besides collagen, other components of the dermis, such as elastin and glycosaminoglycans are produced by fibroblasts. Improvement in skin texture is often the result of all of these fibroblast-stimulating effects. Aging skin and fine lines are the complaints for which these types of products are recommended (4).

Irregular pigmentation is a primary concern for many patients across all ethnic lines. Photoaging, post-inflammatory hyperpigmentation, and melasma are all medical conditions that may benefit from adjunctive use of skin care products (5). Primary recommendations include daily sun protection daytime and a hydroquinone plus retinoid prescription combination product at bedtime. Supplementing these agents with pigment reducing cosmeceuticals is a very popular protocol. Table 10.3 outlines ingredients that are prescribed for the lightening of pigment and reduction of sallow discoloration with regular use.

Products that facilitate keratinocyte turnover and maturation, or act as keratolytics can improve the appearance of photoaged skin, discoloration, and acne (6). Regular exfoliation using certain exfoliation actives increases dermal matrix hyaluronic acid and glycosaminoglycans, improving dermal

Table 10.3 Skin-lightening Agents

Magnesium ascorbyl phosphate
Ascorbyl phosphate
Ascorbic acid
Ellagic acid
CoffeeBerry
Retinol
Soy extracts
Ferulic acid
Niacinamide
<i>N</i> -Acetyl glucosamine
Kojic acid
Azaleic acid
Glabridin
Arbutin
Aloesin
Hydroquinone

Table 10.4 Common Exfoliating Ingredients

Glycolic acid
Lactic acid
Malic acid
Polyhydroxy acids (gluconolactone, lactobionic acid)
Salicylic acid

Table 10.5 Area-specific Products

Eye creams/gels
Lip plumper/softener
Hand creams
Cuticle oils
Neck creams
Décolleté creams
Firming body lotions
Anti-cellulite creams
Foot creams

thickness (7). Exfoliation can aid in the turnover of keratinocytes in the pilosebaceous unit and diminish microcomedo formation thus improving acne. Table 10.4 lists cosmeceutical ingredients that act as exfoliants. Products that produce a mechanical exfoliation rather than a chemical exfoliation using abrasive particles can also improve the appearance of aging or discolored skin and enhance penetration of prescription or dispensed topical skin-lightening agents.

Sometimes, patients' complaints are specific to a particular region of the skin. Table 10.5 lists common area-specific cosmeceuticals. Due to the very thin skin around the eyes, fine lines and discoloration can occur in even young patients. This area is often the first to concern a patient. Since lip augmentation is a very popular procedure performed in dermatologists'

Table 10.6 Barrier Repair Agents

Linoleic acid
Glycerin
Borage oil
Safflower oil
Ceramides
Omega oils
Allantoin
Fatty acids
Cholesterol

office, lip products to plump or smooth lips is another popular skin care product. The body may have areas of blotchy pigmentation and loss of elasticity, especially on the neck and décolleté. Finally, no area other than the face shows the ravages of cumulative ultraviolet light exposure over a lifetime more than the hands. For all these reasons, many patients are desirous of area-specific products to address such issues. Consumer demand results in delivery of product when beauty is the issue, so neck creams, hand creams, and other treatments for the body (such as cellulite) are becoming more popular for sale to the public.

Dermatologists also make cleanser recommendations, especially for patients with eczema, contact dermatitis, and acne. Ingredients that hydrate and soothe, formulas that do not remove surface lipids and are pH balanced, and those containing non-alkaline surfactants are typically offered by dermatologists. Procedures such as intense pulsed light, chemical peel, laser resurfacing, microdermabrasion, and aminolevulinic acid photodynamic therapy treatments all necessitate non-irritating cleansing to prevent redness, irritation, and flaking. One new trend is probiotic cleansers that claim to strengthen the immune system of the skin are now being offered for patients undergoing peels and nonablative laser resurfacing.

The stratum corneum is very important to the health and beauty of the skin. Simply hydrating this layer of the epidermis will improve the appearance of fine lines and improve skin's elasticity. The exfoliation of dead corneocytes and replenishing the lipids between the living keratinocytes gives the skin suppleness and a healthy glow. Barrier repair and protection is very important for many skin diseases as well as before and after such procedures as chemical peel and laser resurfacing. Emollients that replenish the lipid layer of the skin can be essential to the success and long-lasting improvement of several procedures and to complement prescription treatments of many diseases such as eczema and psoriasis. Aging skin often experiences dryness as a result of lipid barrier disruption. Barrier repair products are typically applied at bedtime since they can be heavier and do not contain necessary sun protection that is required for daytime products. Table 10.6 lists several effective ingredients known to repair and protect the epidermal lipid barrier.

ETHICAL BUSINESS PRACTICES

The decision of whether to incorporate cosmeceutical suggestions into your patient treatment plan is usually easy and necessary. There are very good mass market products that, as cosmeceuticals, improve skin condition and make prescription remedies work even better. The decision of whether one should sell cosmeceuticals in the office can be more difficult. The ethical side of this issue has many opponents and proponents. Each physician must decide how best to handle product sales. The American Academy of Dermatology has a formal position on the subject. In their medical practice booklet they specifically highlight their position (8). In summary, the decision to dispense must be in the best interest of the patient, with dermatologists never placing their personal financial interest above the welfare of patients. Patients should never be coerced into making a purchase. Listing of ingredients, advising the patient of less expensive mass-marketed options and fair pricing are also mandated. Products cannot be misrepresented as exclusive or unique unless it is true and all products should have valid claims of potential benefit. These recommendations are not only in line with the AAD, but also recommendations that will not diminish the reputation of dispensing dermatologists with patients, fellow physicians, and the general public. It is important to work with the patient so that they understand the product, use it as directed, and communicate reasonable expectations for what the cosmeceutical can realistically accomplish. It is just common sense and good business practice to conduct ones business practice in such an honorable way. Most dermatologists are comfortable dispensing cosmeceuticals because they feel it improves compliance with skin care regimens and offers patients significant convenience.

Products offered should be safe, effective, and able to be incorporated into the patient's lifestyle so compliance can be achieved.

Once a decision to dispense is made, the dermatologist must choose a company that manufactures the desired products. Published data to support claims is essential in order to believe you are offering products that will help the patients. Customer support issues such as brochures, credit for product returns, employee training, and volume purchase discounts are all important to financial success of adding cosmeceutical dispensing to the practice. Storage of products, purchase of correct amount to avoid expiring before sale as well as local and state regulations on sales tax collection, and submission are all part of the internal logistics of retail sales. Employee training and data collection will take time from medical part of the practice and must be efficiently managed. In general, the sale of physician dispensed cosmeceutical products is an ethical and practical way to increase income while providing a benefit to patients.

SUMMARY

If accomplished in an ethical, knowledgeable and responsible manner, the incorporation of cosmeceuticals into the practice of dermatology is good for both patients and doctors. Deciding

if to dispense, what to dispense, and how to do it ethically and profitably are all necessary for success.

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11 Futuristic Approaches to Skin Care

Neil S Sadick

THE SCIENCE OF EPIDERMAL STEM CELLS

Introduction

Embryonic stem cells can differentiate into all types of cells or they can divide many times without differentiating. Like embryonic stem cells, adult stem cells can renew themselves and divide many times, but they can give rise to only several cell types. Adult stem cells retain their high capacity for renewing themselves throughout adult life, and they can usually produce daughter cells, called transit amplifying (TA) cells, that undergo terminal differentiation (1,2).

Adult stem cells are found in animal tissues whose cells must be replaced by continual cell division. In such tissues, which include epithelial cells of the skin, cells are replaced not by proliferation of fully differentiated cells, but by replication of the less differentiated stem cells. Stem cells can divide symmetrically to produce two identical daughter cells or asymmetrically to produce an identical stem cell or a more differentiated TA cell (3). In this way, stem cells provide a source of differentiated cells throughout the life of the organism.

The destiny of a stem cell is determined by the intercellular signals of its environment. Adult stem cells are surrounded by “niche” cells that shelter the stem cells from stimuli for differentiation, apoptosis, and other activities that would compromise stem cell reserves (4). Niche cells also protect stem cells against uncontrolled production that could lead to malignancy (3).

Epidermal Stem Cells

Epidermal stem cells maintain tissue homeostasis and repair injured tissue (5). Homeostasis is maintained by replication of keratinocytes (derived from stem cells) in the basal layer attached to the basement membrane. A stem cell in the basal layer divides asymmetrically to produce an identical stem cell and a TA cell. Daughter cells of the TA cell undergo terminal differentiation and divide only about five times (3). As the TA cells differentiate and stratify, they detach from the basement membrane and progress upward to the spinous layer, the granular layer, and finally to the stratum corneum (5). These three epidermal cell layers represent early versus late stages of differentiation (6). Stratum corneum cells are constantly shed from the skin surface and must be replaced by differentiating cells from below (1,5). The epidermis, hair follicles, and sebaceous glands each have distinct stem cells that can renew themselves, generate all cell types in their resident tissue, and maintain homeostasis in their tissues (7).

As stated earlier, stem cells are influenced by intercellular signals. One important pathway is “Notch” signaling, whose genes are named after the notched wing phenotype of the *Drosophila* fruit fly (8). Notch signaling is associated with the commitment of keratinocytes to differentiate and the suppression

of tumorigenesis (9,10). Notch signaling also promotes differentiation of the sebaceous glands and hair follicle (11). The Notch pathway includes receptors, negative and positive modifiers, ligands, and transcription factors. Four Notch receptors (Notch 1–4) have been found in mammals (8).

P63, a close homolog of the p53 tumor suppressor protein, is associated with keratinocyte cell fate and self renewal of epithelial cells (9,12). The p63 transcription factor is necessary for regenerative proliferation in the development of epithelium (13,14). In the mature epidermis, p63 has two roles: (1) to initiate epithelial stratification during development and (2) to maintain the proliferative potential of basal keratinocytes (14). Since p63 also counteracts the promotion of differentiation by Notch signaling, a mutual antagonism between p63 and Notch signaling may be an important factor in epidermal homeostasis (9,11).

Aging and Stem Cells

As people age intrinsically, the renewal rate of epidermal cells decreases. Cellular turnover in the skin requires 40 to 60 days in the elderly, compared to 28 days in young adults (15,16). Reduced cellular turnover reduces epidermal thickness and compromises skin barrier function. Cell exfoliation is also adversely affected. The dermal–epidermal junction becomes flattened, increasing skin fragility. In the dermis, the number of fibroblasts decreases, as does the synthesis of collagen and elastin. Glycosaminoglycans (GAGs) and fibronectin (3) are also reduced as the functional ability of fibroblasts diminishes. Subdermal fat tissue decreases, microvasculature is reduced, and sebaceous glands are lost. The overall result is wrinkling, loss of elasticity, dryness, thinning, and increased susceptibility to trauma and bruising (15,17,18).

Extrinsic aging is essentially due to prolonged exposure to ultraviolet (UV) light, particularly longer wavelengths (340–400 nm). Collagen synthesis in photodamaged skin is reduced compared to sun-protected skin (19). UV radiation also damages keratinocyte stem cells (20).

With aging, the regenerative potential of their adult stem cells declines (3,21), possibly due to deletion of a DNA-damage response gene (3). The number of stem cells and their ability to renew themselves does not necessarily change with aging, but the ability of stem cells to produce precursors and differentiated effector cells decreases (22). Wound healing is also impaired in aged skin, suggesting that either mobilization of stem cells is slower or the number of stem cells that respond to replicative signals is low. The latter would be consistent with the findings of Barrandon and Green (23) in which colony-forming keratinocytes from older donors, compared to similar cells of younger donors, gave rise to a lower proportion of clones of cells with

the greatest proliferative capacity (holoclones) and a higher proportion of clones of cells with reduced proliferative capacity (paraclones).

People seeking to improve the appearance of aging skin spend more than \$230 per year worldwide on cosmetics and over-the-counter cosmetics and cosmeceuticals (15). Cosmeceuticals are the most rapidly growing segment in the skin care market (15,24). At the time of this writing, stem cell-based skin care products include Amatokin (Voss Laboratories), SimulCell (NV Perricone), and Dior Capture Totale XP Ultimate Wrinkle Restoring Cream.

The importance of clinical testing to substantiate claims cannot be overestimated. The remainder of this chapter focuses on the application of scientific methods in the development of products to combat aging and cellulite.

CELL LONGEVITY GENES IN SKIN CARE

A study showing the efficacy of a cosmetic formulation in activating a cell longevity gene (SIRT1) in human skin cells has recently been reported (25). This section presents an overview of how enzyme activators of the SIRT1 gene enhance cell longevity and summarizes the results of this study.

Aging and Skin Care

Aging has traditionally been considered part of a genetic program as well as a 'wear and tear' phenomenon. The genetically programmed component has been partially discredited, and current thought attributes aging to an organism's declining ability to repair damage caused by environmental stress.

As the skin ages, proteins, DNA, lipids, and glycans are damaged, primarily by free radicals induced by UV radiation and internal metabolism (25,26). First-generation skin care products were therefore formulated to include natural scavengers of free radicals. As research progressed, more sophisticated products emerged with ingredients that went one step further: they reinforced natural cellular enzymes that detoxified free radicals. Examples of these cellular enzymes are superoxide dismutase, which detoxifies oxygen free radicals; catalase; and glutathione peroxidase, which detoxifies hydrogen peroxide. Subsequent formulations activated chaperone proteins (27) that protect cellular proteins or they activate detoxifying proteins such as proteasome (28), which assists in recycling damaged proteins.

The most recent scientific upgrade makes use of ingredients that activate the expression of master genes that affect life span and improve resistance to stress (25). These activators are enzymes called sirtuins, which are involved in gene silencing, fatty acid metabolism, apoptosis, and cellular life span regulation (25,29). Up to seven sirtuin enzymes (Sir1–Sir7) have been described in humans (30).

The Role of Sirtuins

The importance of sirtuins in aging is supported by a study in which a yeast cell's life span was increased 30% after an extra copy of a sirtuin gene was added to the cell. A later study showed

that extra copies of the same gene extended the life span of roundworms by up to 50% (31). Additional support comes from the increased activity of sirtuin enzymes observed in yeast when food is reduced. This is important because caloric reduction is known to increase life span as well as to lower the incidence and postpone the onset of age-related diseases (25,31). Finally, recent human genomic research shows that high-activity sirtuin genes are significantly increased in humans with the greatest longevity (32).

Sirtuins exert their anti-aging effects by adding acetyl groups to and removing them from histones, a group of proteins that wrap around cellular DNA (31). Adding acetyl groups to histones allows transcription to occur, and removing them from histones inhibits transcription. Sir1, for example, regulates gene silencing by removing acetyl groups from histones. (Genes are "silenced" when they are in a region of the genome that cannot be activated due to deacetylation (31).) Sir1 also regulates chromatin remodeling and the activity of a large range of transcription factors (e.g., p53) involved in the regulation of apoptosis, cell repair, cell cycle progression, and senescence (33).

The Skin Care Product

Sirtuins occur in keratinocytes and fibroblasts of the lower epidermis, upper dermis, and the epidermal–dermal junction. In these three layers, collectively called the aging-responsible interface (ARI), are located 20 "youth markers" (Table 11.1) involved in wrinkle formation, irregular pigmentation, dry skin, loss of firmness, and other signs of aging (25).

The skin care product of this study is formulated to reduce wrinkles, dark spots, loss of firmness, and loss of radiance. It contains an extract of seeds of *Aframomum angustifolium* (a plant found in Madagascar), nine anti-aging ingredients that reverse the visible signs of photoaging by interacting with the 20 youth markers, and *Kluyveromyces* biopeptides that increase sirtuin expression in the ARI cells (25).

Table 11.1 Youth Markers Found in ARI

Aquaporine-3
Chondroitin 4S
Collagen (types I, III, IV, VII)
Cytokeratin 1
Elastic fibers
Fibrillin
Hyaluronic acid
Integrin (Beta 1 and Beta 4)
Laminin (Type I)
Matrix metalloproteinase 1 and 9
P63/p73 genes
Platelet-derived growth factor
Tissue inhibitor of metalloproteinases 1
Tocopherol
Tyrosinase

Source: From Ref. 25.

Expression of the SIRT1 gene was evaluated by immunofluorescence, Western blot analysis, and cytometric studies of skin cells. Preliminary experiments showed that SIRT1 expression could be increased in cultured human fibroblasts by adding biopeptides of the yeast *Kluyveromyces* (25) to the culture medium. This finding is important because fibroblasts are associated with aging-related loss of biomechanical properties of human skin. When the biopeptide activators were added, SIRT1 expression was increased by 176% over control cultures, suggesting that human fibroblasts responded to the yeast biopeptides. Other experiments showed that SIRT1 expression also increased (compared to untreated controls) in epidermal cells enriched with biopeptide. A third line of evidence emerged when cultured human fibroblasts irradiated with UV-B energy were treated with the same biopeptide, resulting in reduced degradation of DNA. This suggested that maintenance of nuclear chromatin had increased and that the integrity of the genetic information in the nucleus had increased. Collectively these results showed conclusively that the yeast biopeptide had stimulated SIRT1 expression in human skin cells (25).

The next step was to evaluate SIRT1 expression in normal human skin cells when a skin care formulation containing the same yeast biopeptide (1% in an oil–water emulsion) was applied once daily to the faces and necks of 33 women (aged 37–64 years) for four weeks. Cellular integrity and aging were monitored by measuring DNA fragmentation and the activity of beta galactosidase, a marker of senescence. Reductions in cell senescence (as shown by decreases in beta galactosidase

activity) and UV-induced DNA fragmentation were observed. Using a scale of 1 to 9, dermatologists graded fine lines and wrinkles, hydration, pigment color intensity, complexion radiance, skin density, firmness, complexion homogeneity, and texture of the skin before and after the first application and again after four weeks of use. Objective data of age-related changes in the extent and intensity of skin imperfections were obtained by analyzing numerized pictures of faces taken by a patented Pixel Skin device (34). Improvements in fine lines and wrinkles, hydration, pigmented spot color intensity, complexion radiance, firmness, complexion homogeneity, and texture were apparent at the end of the study. Pixel data showed significant reductions in skin surface imperfections (–30.4%).

Subjects continued to apply product daily for an additional four weeks. The eight-week results on the nasolabial fold on one patient are shown in Figure 11.1.

Conclusions

The yeast *Kluyveromyces* biopeptides activate SIRT1 expression in human skin cells and improve DNA resistance and senescence. A formulation enriched with these same biopeptides improves multiple signs of skin aging.

ANTI-AGING BY GENE MODULATION

Introduction

To meet the demand for topical anti-aging therapies, investigators may first identify epidermal markers that change with aging, and then measure changes in the expression of those markers after treatment with a prospective anti-aging product (35).

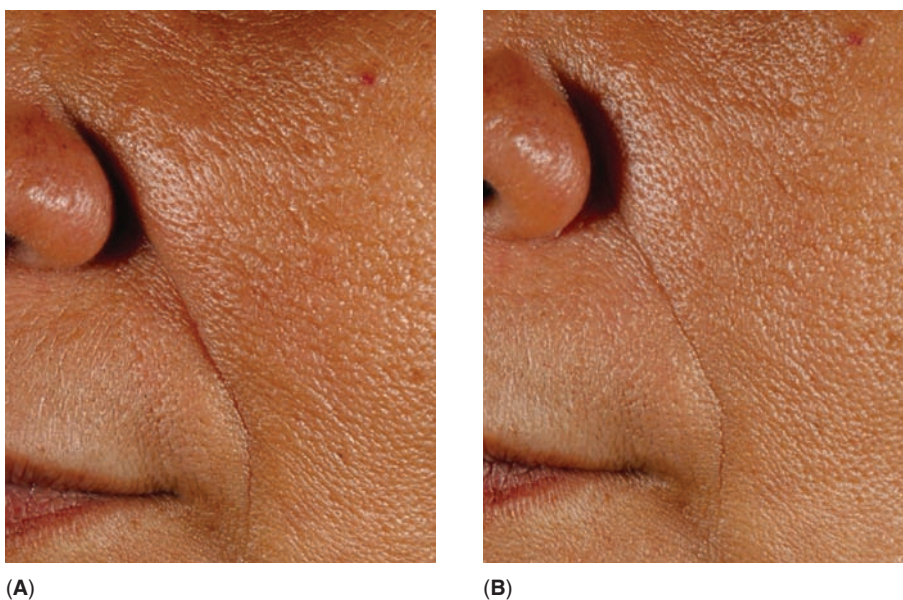


Figure 11.1 Visible effect of oil–water emulsion skin care product containing yeast biopeptides SIRT1 activator after eight weeks of once-daily skin treatment (nasolabial region): (A) before treatment; (B) after treatment.

For example, an aging-associated epidermal marker has been identified by Bonnet-Duquenois et al. (36) These investigators used real-time quantitative reverse transcriptase-polymerase chain reactions (RT-PCR) to quantify transcripts for β 1A integrin, a marker of basal keratinocyte proliferation, and involucrin, a marker of keratinocyte differentiation. When they compared the transcripts of these two markers in photodamaged skin with their counterparts in non-exposed skin, they found fewer transcripts for β 1A integrin in the photodamaged skin than in the non-exposed skin. This suggested that changes in keratinocyte function were associated with photoaging. In contrast, transcripts for involucrin showed no such changes, indicating that alterations in involucrin transcripts were not related to sun exposure. The authors concluded that transcripts for β 1A integrin could be used to evaluate the efficacy of potential anti-aging skin care products.

Once the appropriate epidermal markers have been identified, the next step is to measure changes in the expression of these markers after treatment with a prospective anti-aging product. This review summarizes the results of studies (35) to evaluate the in vitro transcriptional effects of an extract from *A. angustifolium* seeds on normal human fibroblasts (NHF) and keratinocytes (NHKs), the in vitro transcriptional effects of a *Malva sylvestris* extract on reconstituted human epidermis, and the in vivo effects of a facial skin care product containing *A. angustifolium* seed extract on photodamaged skin. *A. angustifolium* is a flowering plant and *M. sylvestris* is a herb found in various geographical locations (35).

Transcriptional Effects

A. angustifolium Seed Extract

This extract was selected for study because it inhibits the production of anti-inflammatory enzymes, suggesting that it has potential as an anti-aging treatment. Messenger RNA profiles of NHKs and NHFs were identified in vitro using low-density DNA array technologies. Then the cells were treated for up to 48 hr with the extract. In NHKs, the expressions of three antioxidant genes, metallothionein 1, metallothionein 2, and thioredoxin, were increased, while in NHFs, expression of a single antioxidant gene, glutathione peroxidase, was increased. Other upregulated genes were associated with dermal-epidermal

junction components and epidermal renewal, suggesting that the *A. angustifolium* seed extract has anti-aging potential.

M. sylvestris Extract

This extract was evaluated by DNA microarray and RT-PCR technologies. Anti-aging properties of the extract were compared with those of retinoic acid (RA), a well-known topical formulation for the treatment of wrinkles and photodamage. Experiments were performed in reconstituted human epidermis and the genes studied were known to be affected by RA. The post-treatment results showed that at the mRNA level, most RA-modified genes were also modified by the *M. sylvestris* extract, and that the gene expression profiles were similar, suggesting that the extract has potential for the treatment of photodamaged skin.

Facial Skin Care Product

In a four-week, single-center study, a facial skin care product containing the *A. angustifolium* seed extract was applied to the forehead, cheek, crow's feet area, and nasolabial groove of 30 volunteers. Image analysis of high-resolution photographs taken at the beginning and end of the study period showed significant reduction in areas of skin imperfection and an improvement in skin homogeneity at the end of the treatment period. In a two-center study, 100 subjects applied a facial cleanser and cosmetic formulation (in blind packaging) to the face and neck twice daily for eight weeks. The cosmetic cream was an oil-water emulsion with ingredients that included the *A. angustifolium* seed extract. Photographs were obtained before treatment and after four and eight weeks of treatment. A greater than 50% overall global improvement in their skin was reported by 11% and 28% of subjects after four weeks and eight weeks of treatment, respectively. More than 75% of subjects said they would buy the cream. Clinical examples are shown in Figures 11.2 and 11.3.

Conclusion

Extracts of *A. angustifolium* and *M. sylvestris* have anti-aging effects on human skin as shown by treatment-induced changes in gene expression profiles; a facial skin care product enriched with *A. angustifolium* extract produces improvement in skin irregularities and in global rejuvenation parameters.

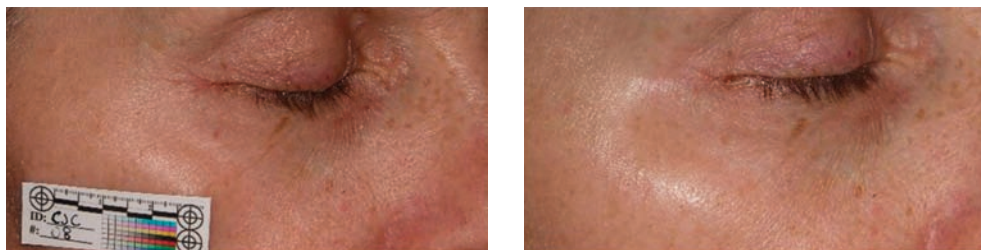


Figure 11.2 The right periorbital area of a 54-year-old woman at baseline (left) and 56 days after twice-daily application of cream containing extract of *A. angustifolium* (right).

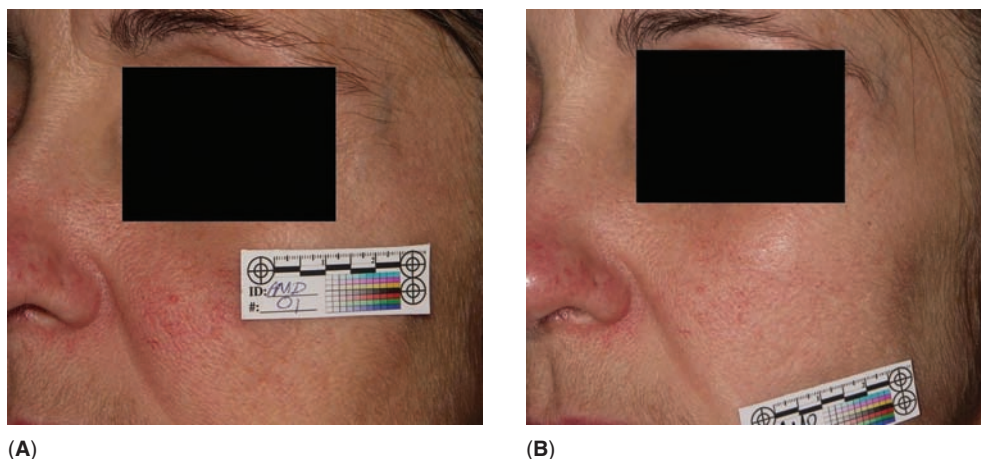


Figure 11.3 The left cheek area of a 50-year-old woman at baseline (A) and 56 days after twice-daily application of cream containing extract of *A. angustifolium* (B). The post-treatment image shows a reduction in redness compared to the pre-treatment image.

TOPICAL TREATMENT OF CELLULITE WITH RETINOL, CAFFEINE, AND RUSCOGENINE COMBINATION

A safe, effective treatment to improve the appearance of cellulite is needed because the condition is widespread and cosmetically distressing among women. Although the pathophysiology of cellulite is not completely understood, a variety of medical, mechanical, light-based, and surgical therapies have been described (37,38). This chapter summarizes the evidence supporting the efficacy and safety of a new topical formulation containing retinol, caffeine, and ruscogenine.

Retinol

Also known as vitamin A, retinol has important regulatory functions in normal growth, reproduction, vision, and cellular (e.g., keratinocyte) differentiation and proliferation (39). Most of its biological activities are mediated by all-*trans* RA (tretinoin) which activates nuclear RA receptors. These receptors, through a series of binding reactions, reduce or enhance gene transcription. Retinol penetrates the skin more rapidly and it is less irritating to the skin than RA (38,40,41). Because of its superior penetration, retinol, though a weaker retinoid than RA, delivers a stronger retinoidal effect than RA (41). Retinol has also been shown *in vitro* to weakly inhibit proliferation of human adipocyte precursor cells but strongly inhibit their differentiation to mature adipocytes, suggesting that it may prevent cellulites (42).

The landmark study of Kligman et al. (38) showed that topical retinol (Johnson & Johnson, New Brunswick, NJ) improves the appearance of cellulite, probably by acting as a prodrug that the skin metabolizes to RA which, in turn, increases collagen deposition in photodamaged dermis (43–45). Increased collagen synthesis is important because it thickens and firms up the dermis, thus “capping” the underlying fat whose mobility, Kligman and colleagues postulate, is associated with the dimples of cellulite. Retinol also stimulates synthesis of GAGs in the

space between collagen bundles. Since GAGs are hygroscopic, an increase in GAG synthesis would, in theory, increase dermal firmness by binding larger volumes of water (38).

In their randomized, double-blinded study of 20 patients with moderate cellulite, Kligman and colleagues treated the lateral thighs twice daily for six months, one thigh of each subject receiving 0.3% stabilized retinol cream and the other thigh receiving vehicle. Efficacy was evaluated in three ways: (i) measuring dermal thickness by ultrasound, (ii) determining blood flow by laser Doppler velocimetry, and (iii) assessing global improvement (none, fair, good, or excellent) by subjects and a dermatologist. On the retinol-treated thighs, dermal thickness increased from 1.44 to 1.60 mm; on the vehicle thighs, thickness was 1.47 mm before treatment and 1.44 mm after treatment. On the retinol side, blood perfusion increased from 30.8 to 35.2 perfusion units, whereas on the vehicle side, it remained essentially the same (31.1–31.6 perfusion units). Thirteen subjects (68%) rated the retinol side more improved (1: excellent; 7: good; 5: fair) than the vehicle side, five rated improvement the same on both sides. One subject rated improvement on the vehicle side higher, and one withdrew for reasons not related to the study. Assessments by the dermatologist and investigators were in agreement with these results. These results showed that retinol appears to be effective in improving the appearance of cellulite.

Caffeine

In addition to having diuretic effects, caffeine increases nervous system excitability, alertness, basal metabolism, and cardiac work (46). Its role in mobilization of free fatty acids has also been shown (46) and explained by the following mechanism: cyclic 3'5'-AMP, which facilitates lipolysis in fat cells, is inhibited by phosphodiesterase which, in turn, is inhibited by caffeine. As 3'5'-AMP accumulates in adipose tissue (due to caffeine-induced inhibition of phosphodiesterase), lipolysis is increased. Catecholamines also increase the concentration of

cyclic 3'5'-AMP, and thus also promote lipolysis (47–49). Since caffeine affects the release of catecholamines (49), caffeine influences lipolysis by a second mechanism as well. Caffeine has also been shown to affect the gene expression of lipoprotein lipase, an early indicator of adipocyte differentiation (50).

Ruscogenine

Ruscogenine extract has been shown to inhibit elastase activity (but not hyaluronidase) in vitro (51–53), a desirable property in anti-aging cosmetic products because elastase degrades elastin in connective tissue. Ruscogenine and neoruscogenine are the primary ingredients of *Ruscus aculeatus* (Butcher's Broom), a medicinal plant found in Africa and Mediterranean Europe. The ability of ruscogenine to reduce vascular permeability has led to its use in the treatment of chronic venous insufficiency (52,53), a condition with signs and symptoms similar to those of cellulite (45). Ruscogenine extract improves blood vessel tone by an adrenergic-type action associated with increased lymphatic and venous flow (54). Adverse effects of the topical formulation are limited to occasional irritation (52).

Inhibition of elastase activity facilitates the restoration of extracellular matrix integrity, thus improving the exchange of nutrients between the ground substance (proteoglycans, glycoproteins, and hyaluronic acid) and vessels of the microcirculatory system (arterioles, venules, capillaries, lymphatic and interstitial tissues) (45). The ground substance permits diffusion of nutrients, hormones, and metabolites from blood vessels to the interstitial fluid and into tissue cells (45). Poor communication between cells and ground substance may be associated with the development of cellulite (45,54).

These reports of the effects of retinol, caffeine, and ruscogenine laid the foundation for launching a study to evaluate a topical combination of these ingredients for improving the appearance of cellulite (55). The rationale for using the combination was also supported by an earlier study (56) in which the authors found a significant reduction in the anthropometric measurement of the thigh after topical administration of isoproterenol, a beta-agonist; aminophylline, a phosphodiesterase inhibitor like caffeine; and yohimbine, an alpha agonist; the reduction (after four weeks of three to five times weekly application) was greatest when the three drugs were used in combination.

In their 2001 report (55), Bertin et al. described their double-blinded, randomized, placebo-controlled study in which 46 healthy women with moderate cellulite of the thighs applied active product containing retinol, caffeine, ruscogenine extract, and alcohol, and placebo with alcohol to the other thigh. Active product and placebo were applied uniformly over the entire thigh twice daily for three months. Participants presented with body mass index (BMI) between 20 and 25 and gave informed written consent treatment.

Treatment effects were compared to baseline after 28, 56, and 84 days of application and results were evaluated objectively and noninvasively by measuring skin macrorelief (topography), dermal and hypodermal parameters, skin mechanical characteristics, and cutaneous microcirculation. Skin macrorelief

measurements were based on profilometric analysis of digital images of the treated skin surface as the thigh was pinched under standardized conditions of compression and lighting. Dermal thickness, dermal and hypodermal echogenicity, dermal and hypodermal texture, hypodermal cellulite ratio, and dermal–hypodermal interface were evaluated by 3D ultrasound image analysis. Skin mechanical characteristics (elasticity, plasticity, immediate and total retraction, immediate recovery ability after strain, viscoelasticity and recover rate, and residual deformation [to check for hysteresis phenomenon]) were measured by cutometry. Cutaneous microcirculation and its homogeneity for each thigh were evaluated by laser Doppler flowmetry.

After 84 days of application, skin macrorelief with the active product decreased 53.1% compared to 14% with placebo. Improvement at each time point was statistically significant. At day 28, dermal thickness, echogenicity, and texture, and hypodermal echogenicity and texture were significantly improved compared to baseline. After day 56, dermal texture, dermal echogenicity, and hypodermal texture were significantly improved. Product and placebo both significantly improved dermal texture and hypodermal echogenicity and texture. Day 84 measurements were not reported due to technical difficulties with the ultrasound apparatus. Regarding mechanical characteristics of the skin, both product and placebo firmed the skin and no significant differences in effect were observed. Mean blood flow increased with both active product and placebo, but was greater and more persistent with active product, probably due to the presence of ruscogenine extract (51,54). Differences did not achieve statistical significance.

The placebo effect in skin firmness and mean blood flow may be attributed to massage alone as active product and placebo were applied (57). Another factor was the presence of ethyl alcohol in both active product and placebo; ethyl alcohol may qualitatively alter free fatty acids in plasma (58,59).

The authors concluded that active product was more effective than placebo in decreasing skin macrorelief, which is responsible for the “orange peel” appearance of the skin, and that the skin microcirculation was more improved by active product than by placebo. The combination of different methods of evaluation showed that the active product was generally superior to placebo in improving the appearance of cellulite.

On the basis of the results of this study, a topical formulation (ROC®, Johnson & Johnson) containing retinol, caffeine, and ruscogenine in an oil–water emulsion has been developed and released as a non-prescription therapy for the treatment of cellulite. It can be used in all skin types and is the first anti-cellulite treatment containing retinol.

COMPARISON OF IMPROVEMENTS IN THE APPEARANCE OF FINE LINES AND WRINKLES WITH A COSMETIC VERSUS A PRESCRIPTION PRODUCT

Background and Objectives

Years of exposure to solar UV radiation are believed to cause breakdown of extracellular collagen and to prevent localized synthesis of new collagen. Clinical photodamage is speculated

to result from the accumulation of this damaged collagen (60). Manifestations of photodamage include fine and coarse wrinkling; changes in elasticity, texture, and thickness; and irregular pigmentation (61), telangiectasias, and premalignant and malignant neoplasms (62). Extensive research has shown that topical tretinoin, or retinoids, stimulate collagen synthesis (43) and that tretinoid is the only agent known to repair photo-damaged skin (44,63–65). Three to four months of tretinoin therapy are normally required for fine wrinkles to improve noticeably (63). Skin irritation, or the retinoid skin reaction, is a side effect associated with early tretinoin usage (44,63,66) in more than 90% of patients (65).

This section summarizes the results of a published study (67) in which the primary objective was to compare the efficacy of a nonprescription cosmetic moisturizer regimen (Olay Professional Pro-X Intensive Wrinkle Protocol) with that of the leading prescription treatment for improving the appearance of fine lines and wrinkles.

Materials and Methods

Caucasian women ($n = 196$, skin types I–III) aged 40 to 65 years enrolled in the study. All subjects had moderate to moderately severe periorbital wrinkles. After a two-week washout period, subjects were divided into two treatment groups. One ($n = 99$) applied a new topical regimen (Olay Professional Pro-X Intensive Wrinkle Protocol, Procter & Gamble Company, Cincinnati, OH) (68) to the face while the other applied topical tretinoin (0.02%) in an emollient base to the same areas according to the manufacturer's package directions (69) and investigator guidance. The Olay protocol consisted of a morning application of an SPF 30 moisturizing lotion (niacinamide, peptides, and antioxidants), an evening moisturizing cream niacinamide and peptides, and an anti-wrinkle product (niacinamide, peptides, retinyl propionate) (70–77). The tretinoin regimen consisted of a morning application of an SPF 30 sunscreen and an evening application of tretinoin. After eight weeks of use, a cohort consisting of 25 subjects from each treatment group (self-selected before treatment began) continued treatment for an additional 16 weeks (total of 24 weeks). Written informed consent for participation was obtained from all subjects and the protocol was approved by Schulman Associates IRB, Inc., Cincinnati, OH.

At baseline, 8, and 24 weeks, standardized high-resolution digital images were taken of both the left and right sides of the subjects' faces to capture the changes in periorbital fine lines and wrinkles. The images were evaluated by the investigators, expert graders, and additional collaborating dermatologists. Images were also assessed by computer image analysis for changes in fine lines and wrinkles. Erythema and dryness were graded at baseline, 8, and 24 weeks. Skin barrier (trans-epidermal water loss, TEWL) measurements were made on each side of subjects' faces at baseline, 8 and 24 weeks. Expressed in $\text{g}/\text{m}^2/\text{hr}$, TEWL is a non-invasive measurement of water vapor loss through the skin and acts as an indicator of stratum corneum barrier function in the absence of sweat.

Results and Discussion

After eight weeks, expert visual grading and wrinkle image analysis both showed that the Olay Professional Pro-X Intensive Wrinkle Protocol was as effective as the 0.02% tretinoin regimen for reducing the appearance of fine lines and wrinkles. The appearance of facial fine lines and wrinkles improved in both groups as measured by expert visual grading of the Rapid Evaluation of Anti-aging Leads (REAL 3.0) (78) images ($p = 0.05$, tretinoin regimen; $p < 0.01$, Pro-X Intensive Wrinkle Protocol). Additionally, the Olay Professional Pro-X and tretinoin regimens produced a 17% and 11% reduction in visibility of fine lines and wrinkles, respectively, based on wrinkle image analysis. Among the 50 subjects who continued to use their respective regimens for a total of 24 weeks, there was over 20% reduction in fine lines and wrinkles for both treatment groups. The Olay Professional Pro-X Intensive Wrinkle Protocol was well tolerated. There was no sign of increased skin dryness/flakiness throughout the 24 weeks. Subjects using the Olay Professional Pro-X Regimen also showed significant improvement in facial stratum corneum barrier function (as shown by TEWL) after 24 weeks of treatment.

Conclusions

When used for 24 consecutive weeks, the Olay Professional Pro-X Intensive Wrinkle Protocol did not increase skin dryness or flakiness and was comparable to a 0.02% tretinoin regimen for improving the appearance of periorbital fine lines and wrinkles.

COMBINATION OF NATURAL PLANT EXTRACTS AND SYNTHETIC HEXAPEPTIDE FOR THE TREATMENT OF CELLULITE

Introduction

Cellulite occurs primarily in the thighs, hips, and buttocks. The condition is difficult to treat with diet alone because these gynoid areas are genetically programmed to be the first to store and the last to release their fat (79).

Topical formulations are advantageous because they facilitate fat release by a local rather than a systemic approach. When developing these products, the cosmetic industry tends to use plant extracts as active ingredients (80). One example is a *Plectranthus* complex vine extract hexapeptide, a combination of active ingredients in hydroalcoholic gel designed to improve cellulite by promoting lipolysis, limiting lipogenesis, inhibiting differentiation of preadipocytes to adipocytes, and facilitating the differentiation of precursor cells into fibroblasts rather than adipocytes. The *Plectranthus* complex vine extract hexapeptide is indicated for mild to moderate cellulite and approved by the European Union as a non-prescription product. The formulation includes three natural products: a vine extract containing eviniferin (EV), a *Plectranthus barbatus* extract, and extracts from *Prunella vulgaris* and *Celosia cristata*, as well as a synthetic hexapeptide.

This review discusses the scientific basis for including each ingredient in the *Plectranthus* complex vine extract hexapeptide and presents the results of a study to evaluate the product for the treatment of cellulite.

εViniiferin

EV is extracted from the *Vitis vinifera* grape vine and has been shown to have antitumor, antioxidant, hepatoprotector, and anti-inflammatory effects (80). In a study to identify biological targets for EV, Do and colleagues (80) found that EV inhibits the activity of cyclic nucleotide phosphodiesterase 4 (PDE 4), an enzyme that inhibits the release of tumor necrosis factor α (TNFα). By inhibiting PDE 4 (an anti-inflammatory action), the release of TNFα is free to occur, which is important for the treatment of cellulite because TNFα is a cytokine that stimulates lipolysis, suppresses lipogenesis, induces adipocyte dedifferentiation, and impairs preadipocyte differentiation in vitro (80,81). EV also inhibits the enzymatic degradation of cyclic adenosine 3'5'-monophosphate (cAMP) (82). Since lipolysis is stimulated by agents that increase the concentration of cAMP (83,84), EV's ability to prevent the degradation of cAMP should encourage lipolysis. EV therefore stimulates lipolysis in two ways: by inhibiting the activity of PDE 4 (thereby facilitating the release of TNFα) and by inhibiting the degradation of cAMP.

***P. barbatus* Extract (Forskolin)**

More commonly known as forskolin, *P. barbatus* extract activates adenylate cyclase in cell membrane preparations and thus increases cAMP levels (and lipolysis) in intact cells of many types (85), including adipocytes in rats (84), hamsters (86), and humans (87). The forskolin-stimulated increases in cAMP production (thus increasing lipolysis) is dose-dependent (87). Forskolin also limits lipogenesis and storage of new fat by inhibiting the activity of glucose-3 phosphate dehydrogenase (G3PDH) (82). For these reasons *P. barbatus* extract is included in the *Plectranthus* complex vine extract hexapeptide for the treatment of cellulite.

***C. cristata* and *P. vulgaris* Extracts**

Celosia is an annual plant native to tropical Africa and Latin America. The exotic shape of its flowers led to its common name, crested cock's comb. A symbol of immortality during the Middle Ages, *Celosia* has been used to treat shortsightedness and liver disease (88). Antiviral proteins have been extracted from its dried leaves (89), some shown to have deoxyribonuclease and ribonuclease activities (90).

P. vulgaris is commonly called as self heal and has a variety of similar names (91). Oral formulations have been used to treat inflammatory bowel disease while topical preparations are used to treat leukorrhea, gynecological disorders, bruises, and wounds. Self heal contains triterpenes, tannins, rosmarinic and caffeic acids, thiamine, and vitamins K and C (91).

An extract of *C. cristata* in combination with *P. vulgaris* extract promotes the breakdown of fat and reduces the rate at which fat cells develop, the latter property associated with the orange peel appearance of cellulite (88). The effectiveness of this combination also involves peroxisome proliferator activated receptor γ (PPARγ), a nuclear receptor found only in adipose tissue. PPARγ is a key regulator of two adipocyte genes and a positive regulator of adipocyte differentiation (92).

In the *Plectranthus* complex vine extract hexapeptide, the association of *P. vulgaris* and *C. cristata* extracts inhibits PPARγ expression, thus inhibiting adipocyte maturation from preadipocytes (82).

The final active ingredient of the *Plectranthus* complex vine extract hexapeptide is Peptamide 6, a synthetic hexapeptide used to firm skin (93). Peptamide 6 mimics the action of transforming growth factor β (TGFβ) which facilitates the maturation of adipocyte precursor cells into contractile fibroblasts (rather than adipocytes) that express alpha-smooth muscle actin (SMA) (82). In this case, SMA is a marker for the differentiation process. By stimulating differentiation into fibroblasts, differentiation into adipocytes is prevented, thus contributing to the treatment of cellulite.

Evaluation of the *Plectranthus* Complex Vine Extract Hexapeptide

Areas of the upper thighs of 18 women with mild to moderate cellulite were treated twice daily with product by massage or were not treated. Each subject served as her own control. Thickness of adipose tissue was measured by an ultrasound technique before treatment and after one and two months of treatment. The results are shown in Table 11.2. All subjects completed the protocol, and 83% achieved a measurable reduction in adipose tissue thickness.

Reductions in circumferences of the upper and middle thighs of 23 women were determined after two months of treatment. Treated and untreated areas were compared. Results are shown in Table 11.3. Among subjects who completed the protocol, 15 achieved a measurable reduction in circumference of the upper thigh and the middle thigh.

The immediate smoothing effect after a single application of product was evaluated by three experts in skin care. Among the

Table 11.2 Reduction in Adipose Tissue Thickness of Upper Thigh (n = 18)

	Reduction (%)		Reduction (mm)	
	One month	Two months	One month	Two months
Adipose				
Mean	-2	-5	-0.75	-1.33
Maximum	-7	-16	-1.82	-3.27
Minimum	0	0	0	0

Table 11.3 Reduction in Circumference of Thighs (cm) After Two Months of Treatment

	Area of thigh	
	Upper	Middle
Mean	-0.7	-0.5
Maximum	-1.8	-1.1
Minimum	0	0

19 subjects, 82% showed noticeable increases in smoothness of the treated area.

In rare cases, patients have experienced mild itching or stinging.

Conclusion

The combination of a vine extract containing EV, a *P. barbatus* extract, extracts from *P. vulgaris* and *C. cristata*, and a synthetic hexapeptide improves the appearance of cellulite in the thighs with minimal adverse effects.

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COSMECEUTICAL SCIENCE IN CLINICAL PRACTICE

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Published in association with the *Journal of Cosmetic and Laser Therapy*

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healthcare

Telephone House, 69-77 Paul Street, London EC2A 4LQ, UK
52 Vanderbilt Avenue, New York, NY 10017, USA

www.informahealthcare.com

Y008470

ISBN 978-041547114-5





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