Cosmeceutical Agents: A Comprehensive Review of the Literature

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Abstract: Cosmeceuticals represent one of the most promising, yet challenging treatment options available to physicians. They are the fastest growth segment in the skin-care market, and a number of topical cosmeceutical treatments for conditions such as photoaging, hyperpigmentation, and wrinkles have come into widespread use. This comprehensive review attempts to examine the current literature of the more commonly encountered cosmeceutical agents in order to determine their utility in treating various dermatologic conditions, as well as their potential use in the area of wound healing. Each section, dealing with a different agent, provides a brief chemical background, a review of the published research studies, and finally concludes with a prediction about its potential role in skin regeneration. Although further research needs to be conducted, adjuvant cosmeceutical therapy may help in prevention of skin cancer, photoaging, and the rejuvenation of skin during wound healing.

Introduction

"Cosmeceuticals"—a fusion of the terms "cosmetic" and "pharmaceutical"—represent one of the most promising, yet challenging treatment options available to physicians. The term is attributed to the dermatologist Dr. Albert Kligman, who defined a cosmeceutical as a cosmetic product that exerts a pharmaceutical therapeutic benefit but not necessarily a biologic therapeutic benefit.[1] Currently available cosmeceutical agents exert their effects through a variety of mechanisms, acting on keratinocytes, fibroblasts, as well as melanocytes (Fig. 1). Pragmatically, to the patient these compounds appear as over-the-counter drugs in the U.S., cosmetics in the EU, and as quasi-drugs in Japan, which is the only country that provides regulatory oversight of cosmetics harboring active pharmaceutical compounds. In the U.S., because cosmeceuticals fall short of the legal definition of a drug but can exert therapeutic effects above and beyond those of simple cosmetics, they reside in a gray area of the 1938 Federal Food, Drug, and Cosmetic Act governing the established categories of drugs and cosmetics. Accordingly, cosmeceuticals are not regulated by the FDA. Yet, cosmeceuticals represent the fastest growth segment in the skin-care market, and a number of topical cosmeceutical treatments for conditions such as photoaging, hyperpigmentation and wrinkles have come into widespread use.[2, 3] In 2005, the U.S. cosmeceutical market was estimated to be $12.5 billion and projected to grow to over $16 billion by 2010.[4] Since clinical trials are not mandated for cosmeceuticals, the efficacy of these compounds is often questioned by physicians and the public alike. Especially as the public becomes more knowledgeable about these compounds, it is imperative for all physicians to become familiar with the research and clinical trials evaluating cosmeceuticals that do exist in order to provide the best recommendations for their patients.

While cosmeceuticals have become established tools in the treatment of photoaging in dermatologic practices, their general application to wound healing has yet to be fully explored. Wound healing is a complex process that, when impaired, results in many untoward effects such as ulcers, dehiscence, hypertrophic scars and keloids.[5] Furthermore, wound healing post-operatively not infrequently results in poor aesthetic outcomes and functional deformities causing a significant amount of physical or psychological distress in the patient. The anti-inflammatory and skin regeneration effects of various cosmeceuticals may provide additional benefits to traditional management of wound healing, potentially augmenting the aesthetic outcomes in the wound healing process.

There has been an increasing discussion of the role of cosmeceuticals in the dermatologic literature, yet increasing patient interest warrants broader awareness by physicians, in general.[1–3, 6–9]

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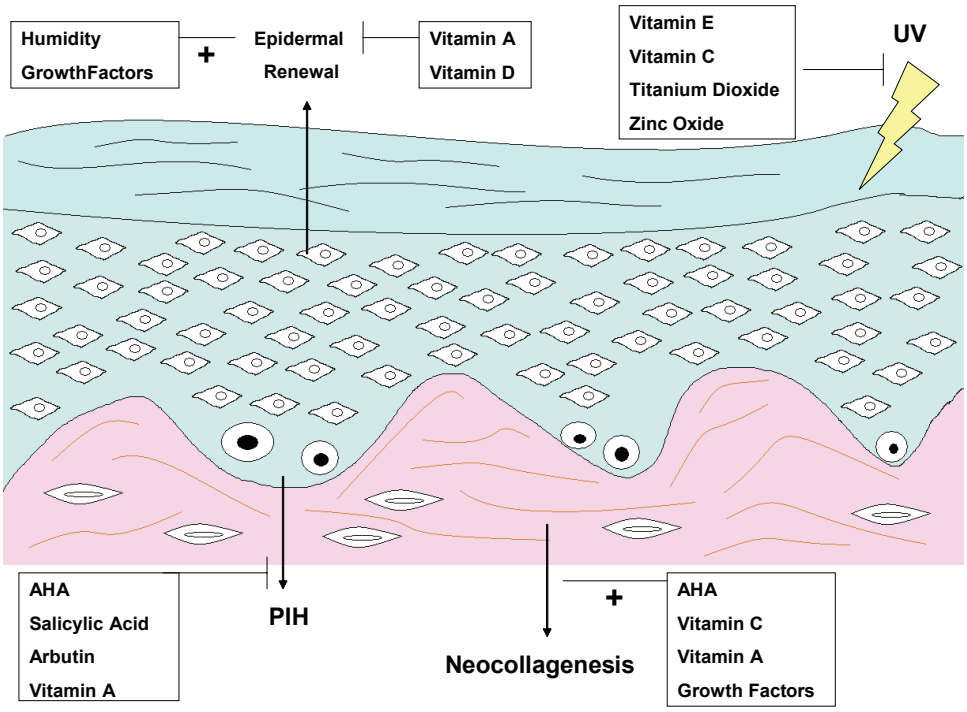

Figure 1. Mechanisms of current cosmeceutical agents. Alpha-hydroxyacid (AHA), post-inflammatory hyperpigmentation (PIH), ultraviolet light (UV).

This comprehensive review attempts to examine the current literature of the more commonly encountered cosmeceutical agents in order to determine their utility in treating various dermatologic conditions, as well as their potential use in the area of wound healing (Table 1).

Each section, dealing with a different agent, provides a brief chemical background, a review of the published research studies, and finally concludes with a prediction about its potential role in skin regeneration. Although further research needs to be conducted, adjuvant cosmeceutical therapy may help in prevention of skin cancer, photoaging, and the rejuvenation of skin during wound healing.

Anti-Inflammatory Agents

Salicylic acid

Salicylic acid (SA) has been used in the treatment of skin disorders for over two millennia.[10] As early as the 1st century AD, SA was used for the treatment of corns and calluses.[11] SA occurs naturally in willow bark, wintergreen leaves and sweet birch.[12] The structure of SA was discovered in the early part of the 19th century, thus facilitating its chemical synthesis in a laboratory allowing its use in dermatology. SA, also known as 2-hydroxybenzoic acid or orthohydrobenzoic acid, is a phenolic aromatic acid with both a hydroxyl and carboxyl group attached to the benzene ring, distinguishing it from other β-hydroxy acids. SA is lipophilic and easily enters the epidermis and membrane of adnexal structures such as sebaceous glands via hair follicle pores. This property is in sharp contradistinction to α-hydroxy acids such as glycolic and lactic acid, which are more hydrophilic. The lipophilic nature of SA helps limit its clinical effect to the superficial epidermis leading to fewer side effects in comparison to α-hydroxy acids, which penetrate deeper into the dermis.[12] In order to produce a favorable ratio of free acid (active) to salt (inactive) form, SA must be formulated at a pH near its pKa of 2.98, where it has a tendency to irritate skin. Using an ionic as opposed to an anionic surfactant can reduce irritancy by controlling the rate at which SA permeates skin in large doses.[13]

SA at concentrations between 0.5% and 5% weight per volume has been shown to possess numerous dermatological effects. SA is most commonly employed in the treatment of acne, where its lipophilicity allows penetration into the pore and subsequent exfoliation of dead skin;

Table 1. Clinical trials evaluating cosmeceuticals agents.

Agent	Clinical indication	Study type	Patient #	Results	Reference #
Salicylic Acid	Acne	DB, R	30	16.6 to 10.9 comedones	16
	Acne	DB, R	180	68% of patients improved	15
Glycolic Acid	Acne	DB, R	70	45% of patients improved	28
	Acne	DB, R	40	90% of patients improved	31
	Photodamage	DB, R	74	76% of patients improved	34
	Discoloration	DB, R	75	76% of patients improved	39
	Stretch marks	OL, Cl	10	68% of patients improved	40
	Hyperpigmentation	DB, R	65	25% of patients improved	41
	Melasma	DB, R	39	75% of patients improved	44
Ceramide	Atopic dermatitis	OL, Cl	24	92% of patients improved	59
	Atopic blepharitis	DB, R	8	10.6% more hydrated	62
Ascorbic acid	Hyperpigmentation	DB, R	16	62% of patients improved	93
	Wrinkling, hyperpigmentation	DB, R	10	40% of patients improved	100
	Wrinkling, photoaging	DB, R	19	84% improvement	75
	Stretch marks	OL, Cl	10	48% improvement	40
	Melasma	DB, R	29	1.9 units better than control.	91
Phosphatidyl-choline	Lower-lid bulging	OL, Cl	30	100% of patients improved	66
Oligopeptide	Wrinkles, skin aging	OL, Cl	90	80% reduction in wrinkles	169
Peptide Primers	Wrinkling	OL, Cl	14	57% of patients improved	168
TGF-β1	Wrinkling	DB, R	32	87% of patients improved	166
Pal-KTTKS	Wrinkling	DB, R	49	37% less wrinkle volume	166
	Winkling, fine lines	OL, Cl	92	13% length reduction	166
Retinaldehyde	Acne	OL, Cl	1,709	61% of patients improved	156
	Acne	DB, R	87	86% of patients improved	153
Vitamin K	Post-laser purpura	DB, R	20	0 vs. 0.6 degrees of disclrtn	83

Abbreviations: DB: double blind; R: randomized; OL: open-label; CL: closed-label.

excess oil build up is also diminished. Numerous studies have shown that SA decreases follicular plugging and progression to inflammatory acne lesions.[14–17] For example, Shalita and colleagues compared SA to benzoyl peroxide, another common acne treatment, in an open-label 4-week cross-over study of 15 patients. Topical application of SA 2% for 2 weeks resulted in a reduction of mean comedone count from 16.6 to 10.9; there was no significant change in the control group of benzoyl peroxide 10%. When controls were switched to SA, the average comedone count decreased from 18.6 to 13.6 after 2 weeks.[16]

Its anti-inflammatory role was shown in a study of 180 patients treated with SA 2% pads.[15] Response was measured based on reduction of inflammatory lesions and open comedones. The investigators reported a favorable response in 68% of patients compared to only 11% in the vehicle arm. The reduced skin irritation observed with SA relative to conventional topical acne treatments has been attributed to its anti-inflammatory effects.[18] Similarly, β-hydroxy acids, derivatives of SA, were shown to exert anti-inflammatory effects as well as increase epidermal cell turnover resulting in exfoliation of skin.[1]

SA also has been shown to protect against ultraviolet (UV) B radiation and photoaging, as well as improve the appearance of photo-induced skin dyspigmentation.[19–21] Consistent with this, SA was shown to enhance the effect of bleaching agents such as hydroquinone.[22] In a study of 268 patients, weekly facial peels of SA 30% used for 8 weeks improved the appearance of melasma, post-acne scars, post-inflammatory hyperpigmentation (PIH), fine lines and wrinkles.[22] In the author's (BMH) experience, SA is an effective topical treatment for mild cases of acne. A component of its benefit can be attributed to the natural history of acne, with a tendency for mild cases to spontaneously remit. It is also important to keep in mind that most topical anti-acne medications do not resolve existing acne lesions but instead help minimize future outbreaks.

While there is moderate support for SA as a cosmeceutical, there has been scant research investigating its direct effects on wound healing (Table 2). Similar to corticosteroids, any positive influence on wound healing may have been countered by its anti-inflammatory effects. Thus, the benefits of SA in wound healing may be restricted to prevention of PIH, which often results after cuts, scrapes or burns, especially in patients with darker skin.

Glycolic acid

Alpha-hydroxy acids have been used as therapeutic agents for more than a quarter of a century.[23] In contrast to SA, α-hydroxy acids are a family of carboxylic acids that are found naturally in food products, including fruits, sugar cane and milk. The best studied of this family are glycolic and lactic acid, which are commonly used to acidify topical products with a high pH that would otherwise irritate skin. Glycolic acid (GA) is the smallest of the α-hydroxy acids with only 2 carbon molecules, giving it the advantage of increased stability, water-solubility and decreased toxicity. Alpha-hydroxy acids function as keratolytics, detaching keratinocytes from the superficial skin layer, or stratum corneum, creating a smoother skin texture.[24] This can be achieved clinically by using a once-daily application of GA 10% buffered to a pH of greater than 3.[25] It is important to note that the efficacy of GA diminishes above pH 3.8.[26] Higher concentrations at a similar pH range can be utilized for office peels.[27]

Table 2. Role of cosmeceutical agents on wound healing.

Agent	Results	Date	Reference #
Salicylic acid	No efficacy	1989	170
Glycolic acid	25% increase in skin thickness	1998	40
	Clinical and histological improvements	1998	28
Ceramides	Cathepsin D activated	2004	63
Vitamin C	Induced production of hepatocyte growth factor	1998	107
	No effect on healing rates of rat tympanic membrane wounds	2003	171
	Increased radiation wound contraction rates in rats and mice	1998	108
Oligopeptides	Induces growth of collagen and fibronectin	1993	167
Vitamin A	Activated the TGF-β1 in an in vitro, porcine study	2004	158
	Pre-treatment in guinea pigs encourages faster wound healing	1995	159
	Induced production of an epidermal growth factor from keratinocytes	2003	160
Vitamin E	Palm vitamin E enhances wound healing in rats	2005	139
	Increased rate of laser wound healing in pre- or post-treated pigs	1995	140
	No significant difference in range of motion, scar thickness or cosmetic appearance of burn wounds	1997	138
	Decreased tensile strength and hydroxyproline content of wounds	1998	47

Like SA, GA possesses anti-inflammatory activity. Several clinical studies have examined the effect of GA on the treatment of acne. In a 12-week multicenter, randomized, double-blinded clinical trial, GA 15% in combination with azelaic acid 20% reduced inflammatory acne lesions by 55% in comparison to only a 29% reduction with nightly retinoic acid (RA) 0.025%.[28] This series also demonstrated that the two treatment arms had equal efficacy (about a 45% reduction) in the treatment of non-inflammatory lesions. However, a significant amount of dryness, redness, and peeling was experienced by the patients in the RA arm compared to virtually no side effects for the GA/azelaic acid arm as reported by patient surveys. Furthermore, 45% of patients using GA/azelaic acid reported improvement in skin appearance at the end of the 12-week treatment, compared to only 10% for RA.

Several reports have indicated that GA can also be used as an adjunct to RA for the treatment of acne.[29, 30] In one double-blind clinical trial, GA 8% daytime application was combined with RA nightly application. The investigators showed that GA enhanced skin tolerance and patient comfort with RA treatment.[29] Furthermore, a head-to-head randomized double-blind study comparing RA 0.05% and GA 12% in 40 patients found that 83% versus 90%, respectively, reported a significant improvement in their acne.[31] Unlike RA, GA does not induce angiogenesis and neovascularization in the dermis.[32] This can be exploited for patients suffering from photo-induced telangiectasia or rosacea, conditions where RA is contraindicated.

Additionally, GA has also been studied as a treatment for photodamaged skin. It is believed that GA and other α-hydroxy acids induce the epidermis to remodel and accelerate desquamation, thereby whitening sun-damaged skin while also promoting dermal neocollagenesis.[33–35] A topical application of GA 8% cream significantly decreased photodamage, sallowness, mottled pigmentation and roughness of the forearms in 76% of patients versus 40% of those treated with vehicle.[34] Researchers attributed this effect to several possible factors, including the exfoliative properties of GA, as well as its ability to activate fibroblasts. The latter leads to a dose-dependent increase in cell proliferation and collagen production, effects not observed with other α-hydroxy acids such as malic acid.[36, 37] This increase in collagen production may explain why rhytides, or fine facial wrinkles, improve after treatment with GA.

Long-term use of GA may improve skin texture and provide a more youthful appearance. This can be partially explained by its ability to induce glycosaminoglycan and hyaluronic acid synthesis in the dermis, leading to improved dermal hydration and thickness.[25, 38] Indeed, a double-blind, randomized clinical trial of 75 volunteers found that daily use of GA 5% for a period of 3 months led to a statistically significant improvement in skin texture in addition to the noted reduction in discoloration. At least one grade of improvement was reported by 76% of patients using the cream compared to only 13% in the control group.[39] Furthermore, a study of 10 patients applying GA 20% combined with either RA 0.05% or vitamin C 10%, zinc sulfate 2% and tyrosine 0.05% showed up to a 68% clinical improvement of stretch marks.[40]

GA has been frequently prescribed to treat various skin pigment disorders such as PIH and melasma.[41, 42] GA may enhance the penetration of hydroquinone (the standard treatment for PIH), allowing for greater reduction in melanocyte activity.[43] In one double-blind, randomized study of 65 patients comparing hydroquinone 4% with azelaic acid 20% cream and GA 15% or 20%, one quarter of patients in both groups experienced significant improvements in PIH after 24 weeks. However, the azelaic acid/GA cream caused more irritation in the form of redness or dryness, prompting the researchers to suggest that azelaic acid/GA cream should be used preferentially for patients with multiple conditions, such as acne vulgaris or rosacea where the therapeutic benefit would outweigh the side effects of this treatment.[41] Melanin pigment was less prominent in skin treated with glycolic or lactic acid plus acetic or citric acid.[33] These findings extend to patients with darker skin types as demonstrated by a double-blind study of 39 Hispanic women suffering from melasma. Study patients underwent twice-daily facial application of hydroquinone 4%, GA 10%, vitamins C and E plus sunscreen versus plain sunscreen. After 12 weeks, 75% of women using the study cream experienced a decrease in facial pigmentation compared to only 13% using sunscreen alone.[44]

GA and retinaldehyde (RAL) have distinct and complementary activities in skin care, but when

utilized together cause increased irritation. However, when formulated together as RALGA, increased skin delivery of RA into the skin is achieved without the typical side effects commonly associated with high concentrations of topical RA.[45] Also, α-hydroxy acids including GA have been shown to reduce oxidative damage after UV exposure.[46] GA skin peels have become a common practice to treat various dermatological problems.[27] Skin takes 1–4 days to recover from such a peel, thus spacing successive skin peels 2 weeks apart is recommended.[23] In the author's experience, GA is a more effective agent for the treatment of dyschromia and has demonstrated little benefit when used alone for the treatment of mild acne. Furthermore, in the absence of an appropriate vehicle or adjunctive emollient, GA at higher concentrations (>10%) consistently causes transient skin irritation and scaling. The author recommends cautious use of GA in patients with neutral to oily skin and performing a patch test in patients with Fitzpatrick skin types III–VI.

GA has demonstrated some promise as a wound healing agent. GA creams have been shown to reduce corneocyte cohesion, decrease the thickness of the stratum corneum while increasing viable epidermal thickness, and increase glycosaminoglycan and collagen production.[47] GA 25% for 6 months demonstrated a 25% increase in skin thickness over baseline; this was confirmed histologically.[40] Researchers attributed this improvement to an increase in mast cell degranulation with subsequent increased expression of factor XIIIa transglutaminase, an enzyme normally present in healing wounds.[40]

Depigmenting Agents

Arbutin

Tyrosinase is the enzyme for conversion of the substrate tyrosine to melanin in melanocytes, providing pigmentation to skin. Various compounds that bind to the active site of tyrosinase to inhibit its activity have been developed as agents to lighten skin and ameliorate unwanted pigmentation. These agents include hydroquinone, kojic acid, and arbutin, amongst others.

Arbutin, the β-D-glucopyranoside derivative of hydroquinone, is a naturally occurring chemical derived from the extract of bearberry plant and is commonly used to treat PIH.[48] Alpha-arbutin, also known as 4-hydroxyphenyl-alpha-D-glucopyranoside, is a 20-fold more potent form of arbutin.[49] The mechanism of action for arbutin is also competitive inhibition of the substrate binding site of tyrosinase.[50] Microemulsion preparations of arbutin resist hydrolysis better than aqueous formulations, where stability is markedly diminished.[51]

The clinical effects of arbutin as a skin lightener remain unclear. A human skin model treated with 250 μg of α-arbutin reduced melanin synthesis by 40% without affecting cell viability.[52] In contrast, arbutin was also shown to increase overall melanin content through a tyrosinase-independent fashion.[53] A recent study demonstrated arbutin inhibited dermal pigmentation induced by UV radiation by 43.5% alone and 63.3% when combined with aloesin, a chromone derivative in aloe vera.[54] More recently, deoxyarbutin, a derivative of arbutin, has shown promise as a skin lightener. Although hydroquinone has been the gold standard depigmenting agent, it can be very irritating and causing scarring with long-term use.[55] In addition, animal studies have demonstrated that HQ is possibly carcinogenic, an effect attributed to benzene metabolites.[56] This has led to safety concerns by the FDA, who is now considering joining the worldwide ban of HQ. Similarly, there is some evidence suggesting that kojic acid can be mutagenic, causing countries like Japan to prohibit its use.[57] Therefore, there is some excitement about deoxyarbutin as a potentially safe and less irritating alternative to HQ and kojic acid. One study demonstrated effective inhibition of mushroom tyrosinase in vitro by deoxyarbutin at a 10-fold lower Ki than HQ and 350-fold lower than arbutin. This was partially validated by a clinical study showing topical treatment with deoxyarbutin for 12 weeks resulted in a significant or slight reduction in overall skin lightness and improvement of solar lentigines (Table 3).[58]

However, the author remains skeptical for several reasons. First, most post-inflammatory pigmentation can be thought of as essentially a melanin tattoo located in the papillary dermis. Second, assuming the primary skin insult has subsided, there is no evidence that tryosinase blockade plays a role in the improvement of PIH. Indeed, this argument also holds true for conditions such as melasma, which often is recalcitrant to tyrosinase inhibitors. The author concedes that depigmenting agents possess a role in pigmentary

Table 3. Inhibition of tyrosinase by selected depigmenting agents.

Compund	Ki (μM)
Deoxyarbutin	0.05
Hydroquinone	0.54
Kojic Acid	7.7
Arbutin	17.6

dermatoses where the primary location of the unwanted melanin is epidermal and a result of ongoing inflammation driving excessive tyrosinase activity. Third, many cosmeceuticals contain miniscule quantities of active depigmenting agents, which although relevant for in vitro experimentation, remain inadequate in the face of a lipophilic barrier such as the stratum corneum. These products represent a significant expense when one factors their extended use (6 to 12 mo). Since the author has yet to encounter a patient that has not been disappointed after purchasing these products at the cosmetic counter or drugstore, one may wish to consider the cost-benefit ratio of this treatment approach. Alternative regimens include treatment with a FDA-approved laser indicated for improvement of dyschromia, hyperpigmentation, or melasma. In all cases, physicians should emphasize at minimum appropriate sun avoidance measures such as daily use of sunblock (≥SPF30) and protective clothing.

We found no published studies on the effect of arbutin on wound healing. Since arbutin appears to directly modulate melanocyte activity, it seems unlikely that it would significantly impact the wound healing process. Nevertheless, a role in reducing PIH should not be overlooked.

Barrier Enhancing Agents

Ceramide

Ceramides are a family of lipid molecules composed of sphingosine and a fatty acid. Ceramides are found in the lipid bilayer of the cell membrane composing about 30% of stratum corneum lipids, where they promote water retention and barrier function.[59] Recent studies have proposed that ceramide deficiency leads to atopic dermatitis, a condition characterized by diminished skin barrier function. Specifically, ceramides 1 and 3 were found to be reduced, while levels of cholesterol were found to be increased in skin from patients with atopic dermatitis. One enzyme that appears to be overactive is glucosylceramide deacylase, which degrades acyl glucosylceramides to glucosylsphingosine. Lack of substrate leads to a deficiency in acyl ceramides which are required to maintain epidermal barrier function.[60] This led researchers to postulate that topical emollients formulated with lipid compositions identical to those naturally found in skin may restore barrier function in atopic patients, effectively reducing dependence on topical steroids and immunomodulators.[60, 61]

Indeed, a recent study using a topical formulation composed of ceramide 2.1%, free fatty acids 0.8% and cholesterol 0.8% in an oil-and-water vehicle found that twice daily application for 3 weeks resulted in a significant improvement in transepidermal water loss measurements in 22 of 24 patients who previously failed standard therapy.[59] Electron microscopy revealed a significant increase in the lamellar membrane thickness, indicating restoration of the stratum corneum layer. These findings have been extended to eyelid skin where application of ceramide gel 2–5 times per day for 4 weeks was shown to increase water content by 10.6% in patients with atopic blepharitis.[62] In the author's short period of exposure to recently commercially available formulations of ceramide-based emollients, patients have reported significant improvement in skin dryness with reduced outbreaks of atopic dermatitis. The author has used the same formulation on himself and found similar benefit on skin dryness, but further studies comparing its use to other classical emollients such as white petrolatum should be performed to avoid reaching anecdotal conclusions.

In terms of wound healing, speculation exists that ceramide may promote activation of enzymes involved in skin regeneration. A recent study found that ceramide derived from acid sphingomyelinase activates cathepsin D, an aspartate protease that regulates epidermal differentiation.[63] The effect of ceramides alone on preventing transepidermal water loss was found to be less effective than a 3:1:1:1 ratio of stratum corneum lipid layer components cholesterol, ceramide, palmitate, and linoleate.[64] Taken together, these data suggest that topical application of ceramide may be used to promote barrier enhancement. Direct studies must be undertaken to further explore the relationship between ceramide and wound healing.

Phosphatidylcholine

Phosphatidylcholine (PC), a lecithin-derived phospholipid, constitutes a major component of cell membranes and is a precursor to acetylcholine. As an injectable agent, PC has been shown to be an efficacious lipolytic agent in recent open-labeled, peer-reviewed clinical studies.[65, 66] Although the mechanism underlying this benefit remains unclear, previous work has linked PC to increased insulin sensitivity, thereby tipping the metabolic balance in fat tissue towards lipolysis.[66] Despite the presence of open-label studies, no double-blind, placebo-controlled studies to confirm these results have been conducted. PC is normally administered dissolved in sodium deoxycholate, a bile salt, and recent studies have not shown that deoxycholate alone can reduce lipomas and cause necrosis of adipose tissue, bringing into question the actual role of PC.[67]

As a cosmeceutical agent, PC may also serve as a penetration enhancer in topical formulations, as it was recently shown to promote caffeine absorption in a Franz chamber model.[68] However, most research has been on injectable formulations, and further study is clearly warranted on cosmeceutical applications of PC. Currently, neither PC nor sodium deoxycholate are FDA-approved alone or in combination as lipolytic injectable agents.

Anti-Oxidants

Vitamin C

L-ascorbic acid, commonly known as vitamin C, is the most abundant endogenous aqueous phase reductant in the human body.[69, 70] Ascorbic acid is a low molecular weight antioxidant that scavenges UV light-induced free radicals capable of damaging cell membranes and DNA. Topical use of vitamin C has been limited by poor skin penetration and chemical instability, as it is easily oxidized in air, heat, or under alkaline conditions.[71, 72] L-ascorbate undergoes reversible oxidation to dehydroascorbic acid. This metabolite regains its anti-oxidant properties after reduction by glutaredoxin. D-isoascorbic acid, also known as erythorbic acid or D-araboascorbic acid, maintains similar redox potential as L-ascorbate but is not as efficiently stored within tissue.[71]

While oral supplementation of L-ascorbate to augment cutaneous levels has not been show to be helpful, efficacious levels of L-ascorbate in the skin can be achieved after topical application of L-ascorbate 15% solution. Maximum levels were found 3 days post-application with a half-life of 4 days.[73] Improved stability of L-ascorbate in a topical formulation has also been achieved by supplementing with tyrosine, zinc, resveratrol, grape seed extract, and L-ergothioneine, leading to a 20-fold increase in skin levels.[74–76] On a molar basis, L-ascorbic acid is the predominant antioxidant in skin, found at levels 15-fold greater than glutathione, 200-fold greater than vitamin E, and a 1000-fold greater than ubiquinol/ubiquinone.[77] It is found in a 6-fold greater concentration in the epidermis versus the dermis.[78] L-ascorbate is much less sensitive than ubiquinol, glutathione, or vitamin E to photo-induced degradation and decreased levels were found in the plasma of patients with solar keratosis and basal cell carcinoma.[79, 80] One of the primary roles of L-ascorbate as an antioxidant is to facilitate the reduction of oxidized precursors to α-tocopherol, the most potent inhibitor of lipid peroxidation.[81–86] Lipid peroxidation is known to cause cell death and induce apoptosis, leading to carcinogenesis, amongst other effects. In the presence of reactive oxygen species such as after sun exposure, α-tocopherol is converted to tocopheroxyl radical, a form lacking antioxidant activity. However, L-ascorbate is able to reduce tocopheroxyl radical in order to restore its anti-oxidant activity. Thus it is clear that the presence of L-ascorbate in the epidermis is necessary for vitamin E regeneration and subsequent maintenance of its important antioxidant effects.

L-ascorbate has many functions and is the primary vitamin deficient in scurvy. This disease is characterized by worsening acne, woody inflammatory and painful edema, perifollicular petechial hemorrhages, and hyperpigmentation of facial skin.[87–89] Knowledge of these cutaneous manifestations has led to the use of L-ascorbate for the treatment of pigmentary disorders.[90] Vitamin C is known to both inhibit melanin synthesis and reduce oxidized melanin.[91] In a multi-center double-blind study, L-ascorbate was shown to reduce chloasma and pigmentation following contact dermatitis.[92] Another double-blind, randomized split-face study of 16 patients with melasma underwent either ascorbic acid 5% or hydroquinone 4% topical treatment for 16 weeks Subjective improvement of good or excellent was reported in 63% and 93% of patients, respectively.

Objective assessment using colorimetric measurements, however, detected no statistical difference between the two arms. Investigators concluded that although hydroquinone demonstrated better subjective scores, ascorbic acid may prove beneficial in the therapy of melasma because it is almost completely free of side effects.[93] As mentioned above, the FDA has recently considered removal of hydroquinone from all OTC cosmetic products due to safety concerns including cancer risk with long-term exposure. Notwithstanding, the author has not seen sufficient benefit to warrant use of vitamin C as a skin lightener in his ethnically diverse patient population. Although vitamin C is especially popular in Asia, stability issues in topical formulations continue to hamper its potential use. Some manufacturers have turned to liposomal encapsulation of vitamin C to reduce the risk of oxidation during shelf storage and home use; however, to the best of the author's knowledge, no published research studies have quantified the efficiency of the liposomal encapsulation process. In fact, the author has evaluated several liposomal formulations and has found all preparations turn from an opaque color to a shade of brown upon exposure to air, suggesting that a significant quantity of vitamin C remains extra-liposomal, or in the vehicle. These issues must be more definitively resolved in order to substantiate the widespread use of vitamin C containing cosmeceuticals.

L-ascorbate has long been known to promote wound healing; an effect resulting from its ability to promote collagen synthesis.[70] It acts as a cofactor for prolyl and lysyl hydroxylases and stimulates transcriptional regulation of collagen synthesis.[94] It also inhibits elastin biosynthesis, thereby reducing photo-induced elastosis.[95] Addition of L-ascorbate 50 μg/ml to serum culture media was also shown to improve epidermal barrier function by increasing sphingolipid production.[96–99] A double-blind split-face study of ten patients compared application of a complex containing ascorbic acid 10% (water soluble) and tetrahexyldecyl ascorbate 7% (lipid soluble) in an anhydrous polysilicone gel base to the vehicle alone.[100] After 12 weeks, 40% of patients reported unilateral improvement of the ascorbic acid-treated side with respect to wrinkling, pigmentation, inflammation and hydration; no improvement was reported for the side treated with vehicle alone. Furthermore, type I collagen mRNA increased and biopsies showed more collagen on the treated side.

In another double-blind, randomized, split-face study, 19 subjects were treated with ascorbic acid 10% or the vehicle for 3 months. Subjective reports grading wrinkling, tactile roughness, coarse rhytides, skin laxity and yellowing showed an 84% global improvement relative to the vehicle-treated side. The side effects reported were transient stinging (55%), erythema (24%), and dry skin (<1%).[75] A significant improvement in the appearance of abdominal striae was found after 12 weeks of once-daily application of L-ascorbic acid 10% combined with GA 20%.[40] Taken together, these data suggest that topical application of L-ascorbate can reduce the appearance of rhytides and striae by promoting neocollagenesis. However, the author reiterates the same cautions about vitamin C stability in topical formulations cited above.

Other clinically notable functions include anti-inflammatory effects in psoriasis,[101] decreased erythema and sunburn cell formation after UVB/UVA phototoxicity,[102] protection against UVB-induced immunosuppression[103] and photo-induced aggravation of Darier's disease,[104] a photosensitive dermatosis. In the latter study, topical application of L-ascorbate was found to be as effective as topical sunscreens. Consistent with this, another study showed that L-ascorbate was able to prevent tanning in humans.[105] Skin cancer is the most common malignancy in the United States and its incidence has been recently rising due to increased sun exposure. Topical application of L-ascorbate 15% in combination with α-tocopherol 1% afforded a 4-fold protection against UV-induced erythema and thymine dimer formation suggesting utility for skin cancer prevention.[106] In addition, when mice were injected with ascorbic acid intraperitoneally, higher doses of ionizing radiation were required to induce skin desquamation and death when compared to placebo, suggesting a possible anti-carcinogenic effect.[72]

Both physicians and patients have generally come to accept L-ascorbate as a wound-healing agent. Ascorbic acid and its more stable derivative 2-O-alpha-D-glucopyranosyl-L-ascorbic acid have both been shown to significantly stimulate the production of hepatocyte growth factor, a cytokine linked to wound healing and organ regeneration.[107] Thus, researchers may have found a tangible link between wound healing and vitamin C, in addition to the more established role as an antioxidant. In another study, mice were given either double-distilled

water or different doses of ascorbic acid prior to exposure to gamma radiation, and the resultant wound was monitored by videography. The pretreated mice showed improved survival compared to the control group, and also demonstrated a dose-dependent increase in the rate of wound contraction.[108] To further enhance efficacy, investigators suggested increasing absorption of vitamin C using skin electroporation.[109] Not surprisingly, ablative lasers and microdermabrasion devices were shown to enhance the topical uptake of ascorbic acid.[110] Both, however, involved disruption of the stratum corneum at the risk of infection. More recently, a nonablative laser device known as Fraxel™ was shown to enhance topical uptake of ascorbic acid in the absence of stratum corneum disruption, suggesting a mechanism involving ultrastructural modification of the lipid layer.[111]

Vitamin E

Vitamin E is a lipid-soluble nutrient that is derived from dietary intake of leafy vegetables, plant oils, whole-wheat flour, milk, eggs, meat, and soy nuts.[112] Vitamin E has been found to be essential in fetal development.[113] Its primary role in human skin appears to be in functioning as an antioxidant.[114] Through its action as a free radical scavenger, vitamin E serves as the most potent inhibitor of lipid peroxidation.[81–86] By damaging cell membranes, lipid peroxidation functions as an important promoter of cell death and apoptosis, leading to detrimental effects such as carcinogenesis, amongst others. There are at least 8 known naturally occurring tocopherols with vitamin E activity. In this regard, the α-tocopherol form maintains the greatest biological activity in addition to comprising approximately 90% of the tocopherols in animal tissue. It is an optical isomer with the "D" form possessing greater activity than the "L" form.[115] Vitamin E shows reduced stability when exposed to air or UV light, and its levels are decreased in individuals with increased dietary consumption of polyunsaturated fatty acids.[116] In the skin, it is the predominant fat-soluble vitamin and its activity is replenished with the help of vitamin C and thiols.[86]

It is clear that deficiency of vitamin E leads to physiological consequences. In a controlled study, investigators fed rats a diet deficient in vitamin E for 3–6 months. After one month, lipid peroxide levels (a risk factor for carcinogenesis) and insoluble collagen content (a sign of photodamage) were increased in vitamin E deficient rats but not in controls.[117] Although readily available in oral form as a vitamin supplement, numerous studies indicate that this route of administration may be ineffective in preventing photoaging and skin cancer. For example, in one study, mice fed oral vitamin E supplements were not protected against UV-induced erythema.[118] These findings seem to parallel similar studies performed in humans. Volunteers were fed vitamin E 400 IU per day for 6 months. This dose was approximately 10-fold greater than the recommended daily allowance.[115] Despite this treatment, patients showed no change in the minimal erythema dose or histological changes on skin biopsy when compared to control patients.[119] These results were similar to another study, which showed that oral tocopherol supplementation had no effect on UV-induced erythema, although they did protect against cellular damage in skin.[120] The latter finding indicates that oral vitamin E was absorbed, suggesting route of administration did not affect plasma levels and subsequent skin delivery. All in all, these studies indicate that oral supplementation is inadequate in protecting skin against photodamage and the risk of carcinogenesis.

However, it is known that topical administration of α-tocopherol leads to percutaneous absorption through either the epidermis or hair follicle.[121] Topical application of vitamin E to hairless mice reduced UVB-induced erythema by 40%–55% and edema by 26%–61%.[121, 122] Similarly, topical application of α-tocopherol to guinea pig skin inhibited UVB-induced sunburn.[123] These effects may be due to a vitamin E-dependent reduction in UV-induced free radical formation, as one study showed a 60% reduction in free radicals after exposure to either UVB or UVA.[124] These findings have been extended to humans. Patients suffering from actinic reticuloid show an increased sensitivity of dermal fibroblasts to UVA.[125] Topical application of α-tocopherol corrected this abnormal sensitivity. Additional studies have demonstrated that treatment of human fibroblasts with α-tocopherol led to a reduction of lipid peroxidation levels.[126] Similarly, an increased survival of human keratinocytes exposed to solar simulators was demonstrated.[127] This effect may be due to an enhanced induction of heat shock protein 70, a protective stress response protein. Biopsies of UV-exposed human skin pretreated

with topical vitamin E demonstrated a significant reduction in the number of damaged sunburn cells.[128, 129] In fact, combination of vitamin E with ascorbic acid and glutathione increased the minimal erythema dose by 50%, indicating that the efficacy of topical α-tocopherol may be enhanced by other antioxidants.[118] Recently, investigators have also shown that the vitamin E supplementation was able to abrogate the side effects induced by oral retinoid therapy.[130]

More recent studies in mice have shown that topical vitamin E application can block photocarcinogenesis.[131, 132] This may be related to its ability to block UV-induced cylcopyrimidine dimer formation in the epidermal p53 gene, a known tumor suppressor involved in repair of UV damage.[133] In addition, recent evidence shows that patients with basal cell carcinoma and actinic keratosis, the most common forms of skin cancer and pre-cancer worldwide, respectively, have reduced levels of vitamin E compared to controls.[80] Finally, α-tocopherol has also been shown to inhibit pigment production by human melanoma cells by blocking the activity of tyrosinase and by acting as a direct UV blocker with peak absorption at 290 nm.[134, 135]

Recently, scientists have begun substituting the more stable α-tocopherol with its γ form, which has a number of advantages that have been previously overlooked. For example, γ-tocopherol is a better trapper of lipophilic electrophiles and oral supplementation of γ-tocopherol increases not only γ-tocopherol tissue levels but also those of α-tocopherol. Additionally, γ-tocopherol has exhibited suppression of cycloxygenase-2 activity in macrophages and epithelial cells, suggesting an anti-inflammatory role. This effect was not observed with similar concentrations of α-tocopherol.[113]

However, there remains some debate as to the exact role of vitamin E in wound healing.[136, 137] A study that used topical vitamin E cream to dress burn wounds found no significant difference in range of motion, scar thickness or cosmetic appearance of the wounds compared to the vehicle alone. Furthermore, a high incidence of contact allergy was observed in the vitamin E arm.[138] In another study, vitamin E was shown to decrease the tensile strength and hydroxyproline content of healing incisional wounds.[47] Studies comparing palm vitamin E with α-tocopherol concluded the former enhanced wound repair in rats, as assessed by wound contraction rates and protein content.[139] This is consistent with a study performed on Yorkshire pigs that found significantly decreased healing times with vitamin E treatment either before or after laser injury.[140] The investigators concluded that vitamin E may reduce the negative consequence of inflammation by preventing peroxide accumulation and subsequent lysosomal and cell membrane instability. Although promising, further clarification of vitamin E's mechanism of action appears warranted. The author has discovered a not insignificant number of patients whose contact dermatitis etiology could be attributed to use of vitamin E containing moisturizers. Since vitamin E is a naturally occurring hormone normally present in the skin, physicians should instruct patients to bring their products in to the office for an examination of the ingredient list. The author has found modified forms of vitamin E or other preservatives are often the underlying cause of the contact dermatitis. Nevertheless, patients often refuse to discontinue vitamin E use citing failure of a plethora of other emollients to provide the same benefit they perceive.

Plant phenols

Plant phenols, such as green tea and pomegranate extract, have emerged as an especially popular subset of cosmeceutical agents due to a trend toward "natural" products. However, their effectiveness and therapeutic utility remain relatively unknown to dermatologists and other physicians.

Green tea comes from the bud of the tea plant *Camellia sinensis*. Green tea extract contains substantial amounts of polyphenols including epicatechin, epigallocatechin, epicatechin-3-gallate, and epigallocatechin-3-gallate (EGCG). EGCG, in particular, has been shown to provide anti-inflammatory and antioxidant capabilities.[141] EGCG also appears to be involved in the response to UV radiation and regulates the expression of proteins involved in skin damage. Topical application of 0.5% green tea polyphenols significantly reduced signs of epidermal pathology in flaky skin mice, a model of psoriasis; this effect was attributed to efficient caspase 14 processing and reduction in proliferating cell nuclear antigen levels.[142] In another rat model of UV photodamage, 2% topical EGCG applied 30 minutes prior to UVA exposure was shown to significantly decrease sunburn cells and dermo-epidermal activation, while topical EGCG applied after UVA

exposure showed no significant change from control.[143] These and other reports support the notion that EGCG protects against UV damage when applied topically before sun exposure. In a double-blinded, placebo-controlled trial of green tea extracts, combination treatment with topical 10% green tea cream and 300 mg twice-daily oral supplementation of green tea showed no histological or clinical improvement of photoaged skin. However, there was significant increase in the elastic tissue content of the treatment group.[144] Although data from animal models appeared promising, there remains little evidence for the use of green tea as a cosmeceutical in humans.

Like green tea extracts, pomegranate extracts have been lauded by the cosmeceutical industry for their antioxidant and anti-tyrosinase effects. A recent study using isolates of a polysaccharide fraction from pomegranate at 4 μg/ml demonstrated an inhibition of 1,1-diphenyl-2-picrylhydrazyl and 2,2'-Azinobis[3-ethylbenzothiazoline-6-sulfonate] free radical activities by 69% and 88%, respectively, attesting to its antioxidant properties. Interestingly, 10 μg/ml pomegranate extract also showed inhibition of mushroom tyrosinase by 43%, suggesting potential use as a skin whitener.[145] In a study on immortalized human keratinocytes, one group showed that pretreatment with pomegranate extracts (10–40 μg/ml) inhibited: UVB-mediated cell death, decreases in intracellular glutathione content, and increases in lipid peroxidation. Additionally, pomegranate extracts were shown to inhibit UVB-mediated upregulation of matrix metalloproteases, as well as phosphorylation of MAPK and c-Jun, two pathways well-known to be altered in photo- and oxidative damage. These results suggest that pomegranate extracts protect against UVB-induced oxidative stress, although further studies in humans are required to justify use in skin care products.[146] To date, there are no clinical trials evaluating the effects of topical pomegranate extract either as a sunscreen agent or as a therapeutic agent to improve the appearance of photodamaged skin. Despite the popularity of such products on the cosmeceutical market and the encouraging but limited basic science evidence, more clinical evaluation is needed before these plant extracts are to be accepted as efficacious cosmeceuticals.

Vitamin K

Vitamin K1, also known as phytonadione or phylloquinone, is 2-methyl-3-phytyl-1, 4-napthoquinone. This vitamin is not synthesized by humans; however, it is found in green leafy vegetables and is produced by bacteria resident to the human intestinal tract.[7, 147] Phytonadione is a lipid-soluble vitamin necessary for the hepatic synthesis of clotting factors II, VII, IX, and X. In the absence of vitamin K, these proteins are found as inactive precursors. In the presence of vitamin K, these precursors are activated by a hepatic enzyme known as γ-glutamyl-carboxylase. This enzyme converts several glutamate residues near the N-terminus of each precursor to γ-carboxyglutamyl. The negative charge on these residues allows the clotting factors to bind ionic calcium, facilitating interaction with phospholipid surfaces and initiation of clot formation.[148] The observation that vitamin K deficiency causes problems with bleeding and decreases clot formation led to its speculative role in preventing or correcting sun-induced vascular manifestations such as actinic purpura.

Not much is known about the benefits of topical application of vitamin K. Although numerous manufacturers market that topical application of vitamin K creams can prevent bruising by pretreatment prior to procedures, clinical trial data has yet to establish this definitively. In one study involving prevention and healing of laser-induced purpura, 22 patients were divided into 2 groups: one group to be treated prior to and the other subsequent to laser treatment. Participants applied a vitamin K cream to one side of the face twice daily for 2 weeks either before or after laser treatment. No difference between vitamin K and control groups was observed when applied prior to laser treatment.[149, 150] However, vitamin K application post-laser therapy significantly reduced bruising relative to placebo. A similar study of 20 subjects treated with an acrylate copolymer cream containing vitamin K 1% plus retinol 0.3% for 14 days after laser treatment found decreased average purpuric discoloration scores relative to controls; this difference became significant by 3 days.[150] In a separate study of actinic purpura, healing time was significantly shortened from 8 to 2.5 days after application of vitamin K 1% cream for 5 to 8 days. Similar results were achieved for trauma-induced purpura.[151]

Since topical application of vitamin K in these settings does not lead to a significant effect on plasma levels of vitamin K, the exact mechanism of the improved healing of bruises remains unknown. However, the vitamin K-dependent enzymatic activation of clotting factors may not be

restricted to the liver, as γ-glutamyl-carboxylase has also been shown to be present in the epidermis.[152] This may explain why topical application of vitamin K may exert local effects on bruising. The author has had a significant number of patients utilize vitamin K containing topical formulations for the treatment of lower eyelid dyschromia. To date, no patient has reported any significant improvement in the appearance of this condition. The author believes that infraorbital dyschromia is likely caused by age-related fat atrophy with subsequent vascular show (mainly venous blood). Therefore, the author does not recommend its use for this condition although a potential role for post-laser surgery cannot be discounted since this involves active microscopic bleeding from papillary dermal capillaries and not a vascular show phenomenon. Currently, there has been little research performed on the effects of vitamin K on skin regeneration and wound repair.

Skin Renewal Agents

Vitamin A

Vitamin A is an important nutrient obtained exclusively from the environment. After ingestion of dietary foods containing food containing all-trans-retinol, its esters or certain carotenoids, the gastrointestinal tract is able to deliver this vitamin to the liver, where it is stored. The liver is then able to monitor plasma levels and adjust the amount of retinol delivered to the skin. However, numerous studies have found that plasma levels often do not correlate well with those found in the skin. Therefore, it may be important for certain patients to supplement their store with topical application of vitamin A. Retinoic acid (tretinoin), its 13-cis isomer isotretinoin, as well as various synthetic retinoids are used for therapeutic purposes, whereas retinaldehyde, retinol, and retinyl esters, because of their controlled conversion to RA or their direct receptor-independent biologic action, can be used as cosmeceuticals. Numerous studies have indicated that all-trans-retinol and its aldehyde retinal have the greatest biological potency *in vivo*. Hydrogenation of retinol destroys its biological activity. In fact, the 3-dehydroretinol form retains only 40% activity.[115]

All-trans-retinoic acid, more commonly known as tretinoin, represents the active form of retinol. Many products designed to treat skin aging contain RA in varying concentrations. The irritant effects of RA can be circumvented by substituting retinol in its place. In addition, only 1%–2% of topically delivered RA is absorbed by skin under normal conditions compared to 31% in dermatitic skin.[112] One reason for this may be the lack of RA-specific transport machinery available in the plasma and skin, which instead possess binding proteins specific for all-trans-retinol.[112] Furthermore, excess retinol can be stored in the skin in its ester form. This is achieved through two unique enzymes, acyl CoA: retinol acyl transferase and lecithin:retinol acyltransferase, This storage mechanism is unique to retinol and helps reduce side effects clinically. On the other hand, RA cannot be stored in ester form, and excess levels in the skin lead to the quite commonly encountered side effects of irritation, erythema, peeling, acne exacerbation, and photosensitivity. Finally, although no studies have shown the teratogenecity of topical RA as it does not correlate to serum levels, use of RA is discouraged by many providers for any potentially child-bearing woman.

Thus, some researchers have turned to topical retinol for treatment of certain dermatological conditions including acne, solar keratosis, photoaging, hyperpigmentation, and dysplastic nevi. Investigators confirmed that RAL is just as effective as RA but lacks many of its side effects.[153] One animal study that compared topical RAL with RA found both to possess equivalent comedolytic potential.[154] These benefits are achieved through epidermal thickening of atrophic skin, dermal regeneration, and diminishing pigmentation and desquamation of hypertrophic skin. These studies support RAL as a key molecule in the metabolism of vitamin A by keratinocytes.[154]

A study on acne treatment instructed 1,709 patients to use RAL 0.1% and GA 6% in combination with their normal treatments, excluding retinoids. After 90 days, the study reported "very good" or "good" tolerance of the treatment and global efficacy in 97% and 61% of all participants, respectively.[155] In a similar 3-month study, 50% of patients reported "important/very important" global improvement versus 26.3% for vehicle at 2 months, and 86.1% versus 58.8% at 3 months, respectively. The treatment was well-tolerated in all of the patients.[156] Histologic studies have confirmed these clinical studies, with topical RAL and GA decreasing melanin content visualized by Fontana-Masson staining.[157] Debate over RA

versus RAL continues to this day. Many dermatologists believe that skin irritation is a sign of efficacy and lack of erythema is a strong indicator of expected clinical benefit. The author has observed a large volume of patients using over-the-counter cosmetic preparations containing RAL. In this cohort, the anti-aging effects of RAL have been modest at best, and can likely be attributed to the consistent use of a well formulated emollient. On the other hand, those patients utilizing prescription strength formulations containing RA uniformly present with noticeable improvement of fine lines, pigmentation, oiliness, and acneiform lesions albeit with a predictable level of erythema and scaling. Thus, in the best estimate of the author, there has been no clear demonstration that the efficacy of retinoids can be uncoupled from its side effects for these clinical endpoints.

Not surprisingly, vitamin A seems to have the greatest potential for applications in wound healing of all the vitamins. One *in vitro* porcine study found that vitamin A activates the transforming growth factor (TGF)-β1 pathway. Injured and non-injured full-thickness ileum explants were harvested from piglets and cultured in serum-free medium supplemented with all-trans retinol for 24 or 48 hours. All concentrations of the retinol accelerated recovery of the wounded ileal wall, and active TGF-β1 was found only in the explants supplemented with retinol. The study concluded that the increased activation of latent TGF-β1, a form that appears to increase healing rates, may indeed be due to the presence of vitamin A.[158] A number of experiments confirm RA's beneficial effects. Guinea pigs pretreated with topical RA healed more quickly than the control group.[159] Another study suggested a role of RA in epidermal regeneration by induction of a heparin-binding epidermal growth factor (EGF)-like growth factor in keratinocytes.[160]

Endogenous growth factors and oligopeptides

Growth factors are large proteins that are synthesized by a variety of cells in the body and play an important role in the regulation of immunity, cell division, wound healing, and tissue regeneration. In the skin, several relevant growth factors have been discovered. These include TGF-β, EGF, platelet-derived growth factor (PDGF), fibroblast growth factor (FGF), and keratinocyte growth factor (KGF). In general, each growth factor binds its specific receptor with resulting stimulation or inhibition of cell division and multiplication. These substances have garnered much attention due to their mode of action and effects, making them candidates for a variety of therapeutic arenas, including anti-aging of skin and wound repair.[9]

For example, after injury to the skin, TGF-β is secreted, resulting in a signal inducing fibroblasts to produce more collagen and elastin, components necessary for the normal appearance and integrity of skin.[161] In response to TGF-β, tenascin-C expression is upregulated spatially and thought to present pro-migratory tracks during skin repair.[162] PDGF is involved in would healing and promotes the generation of granulation tissue, new blood vessels, and extracellular matrix. Specifically, PDGF, as well as TGF-β, can also stimulate fibroblasts to produced glycosaminoglycans and modulate the proliferation of smooth muscle cells.[163] Similar to PDGF, FGF has been shown to stimulate collagen synthesis, new blood vessel formation, and promote wound healing.[164] EGF, on the other hand, induces matrix metalloproteinase activity, enzymes capable of degrading the extracellular matrix.[165] While this allows for skin cell migration during wound healing, the matrix metalloproteinase response of EGF stimulation has also been linked to the ability of cancer cells to invade tissue leading to metastasis. The most recent peptide active ingredient to be included in cosmeceutical compounds is KGF, although its effects on skin remain poorly understood.

Because of their benefits on wound healing, a number of growth factors have been incorporated into topical serums and cream formulations (Table 4). These growth factors require extensive purification methods and are therefore relatively expensive priced up to $600 per ounce. Despite their hefty price tag, growth factor efficacy when delivered via a topical formulation remains unclear. Since these proteins are quite large and carry a charge, it is not clear that they are capable of penetrating skin. Furthermore, with growth factors capable of promoting both positive effects such as wound healing and negative effects such as tumor invasion, only physicians with extensive knowledge of their effects should dispense topical medications containing growth factors.

TNS Recovery Complex is a commercial product containing various growth factors like vascular endothelial growth factor and TGF-β, as well as

Table 4. Growth factor products.

Growth factor	Product	Manufacturer
Multiple (TGF-β)	TNS Recovery Complex Gel	SkinMedica Inc
EGF	Révive Sensitif Cellular Repair Cream	Bays Brown Laboratories
KGF	Révive Intensité Volumizing Serum	Bays Brown Laboratories
Recombinant TGF-β1	Transformation Line	Jan Marini Skin Research Inc
Placental Extract, Skin Growth Factor	Facial Day Cream	Natural Bissé U.S.A.
TGF-β1	Citrix CRS Serum	Topix Pharmaceuticals

natural antioxidants, soluble collagens and matrix proteins. In studies conducted using tissue culture models, TNS Recovery complex was shown to promote a number of effects including collagen synthesis, blood vessel formation, and fibroblast and keratinocyte proliferation. One study (sponsored by the manufacturing company) claims that in trials of more than 90 volunteers, there was up to an 80% reduction in wrinkles, a 44% reduction in wrinkle depth, a 62% reduction in the appearance of fine lines and a measurable improvement in skin texture over a period of 45 days. The company claims that some of the compounds in their product have been shown to heal burns and wounds. These studies are interesting; however, no control with vehicle alone was performed, mitigating a more definitive conclusion regarding efficacy of the product.

A recent randomized study involving CRS cream, a commercially available formulation consisting of TGF-β1, L-ascorbic acid and *Cimicifuga racemosa* extract was conducted on 32 subjects, who were instructed to apply either a combination of CRS or vitamin C plus the extract to one half of their faces twice daily for 3 months. Physicians reported significant improvements in wrinkles for the CRS-treated side of the face in 27 out of 31 patients. Of those 27 patients, there was a 21.7% improvement in physician-rated wrinkle scores. The mean improvement in the CRS arm as a whole was 12% versus 7% for the vitamin C plus extract group.[166] Investigators concluded that TGF-β1 was responsible for this difference in efficacy, although not found to be statistically significant. The authors postulated that TGF-β1, when formulated in liposomes rather than as an aqueous solution, was not degraded and could therefore be delivered to the dermis. Although theoretically interesting, no evidence to support this conclusion was provided. Since TGF-β1 is not a small molecule at 25 kDa, more definitive experiments must be undertaken to substantiate the modest yet quite expensive contributions of TGF-β1, which could just as easily be dismissed to chance alone based on their statistical analysis.

The use of growth factors as topical treatments is limited due to size of the protein as well as charge. These two factors represent barriers to ease of penetration across the stratum corneum. To overcome this problem, researchers have begun designing short sequence peptides, also known as oligopeptides, based on the notion that a similar activity to their larger growth factor counterparts can be achieved by retaining the receptor binding motif. In an NIH-sponsored study, the procollagen fragment lysine-threonine-threonine-lysine-serine, also called KTTKS, was found to promote the synthesis of collagen types I and III and fibronectin in a tissue culture model. This pentapeptide was shown to be the minimum fragment necessary for stimulation of collagen and fibronectin production.[167] Unfortunately, this fragment faced the same challenge as growth factors because it was charged and therefore unable to penetrate the skin.

This was overcome by adding palmitate (Pal), a long chain fatty acid tail, to the peptide, allowing it to more easily penetrate the skin. In a double-blind, vehicle-controlled study of 49 women treated with 3 parts per million of Pal-KTTKS twice a day for 4 months, a 13% reduction in skin roughness and 27% decrease in wrinkle depth was noted. Overall wrinkle volume was diminished by 36%. Histological analysis of 6 of these women at 2-month intervals demonstrated increased elastin thickness and density in addition to augmentation of type IV collagen at the dermal-epidermal junction.[168] These studies suggest that Pal-KTTKS was an effective topical treatment for fine wrinkles. Another study of 92 women using Pal-KTTKS

twice daily for 3 months showed significant improvement of facial lines and wrinkles as assessed by digital photos graded in a blinded fashion by expert analysts.[168] This was consistent with reports indicating equal efficacy of Pal-KTTKS to retinol in a split-face study conducted over 4 months. However, Pal-KTTKS offered the significant advantage of avoiding retinoid side effects such as redness and irritation.

Although more rigorous studies with Pal-KTTKS have been performed than with other peptide ingredients, several key questions remain: 1) What is the evidence that Pal-KTTKS does not simply get stuck in the stratum corneum? 2) What is the evidence showing that the palmitate tail confers sufficient lipophilicity to allow KTTKS to travel to the papillary dermis, where it can induce neocollagenesis? and 3) If palmitate allows KTTKS to penetrate through the stratum corneum, what is the evidence that it is not immediately degraded during its diffusion pathway from the granular layer past the dermal-epidermal junction into the papillary dermis? A simple fluorescent labeling experiment would answer all of the above questions. The absence of such an experiment in the published literature leads the author to conclude that it is highly unlikely that 3 parts per million of Pal-KTTKS is responsible for the effects reported in the above studies. Instead, the author speculates these effects are more reasonably attributed to the chronic use of a high quality moisturizer.

Neither Pal-KTTKS nor short chain peptides have been evaluated for possible applications to wound healing, although there remains continued investigation for cosmetic use. Its potential seems significant, based on the above clinical studies, making Pal-KTTKS a natural candidate for further clinical investigation as a wound healing agent.

Conclusion

The burgeoning therapeutic options represented by cosmeceuticals encompass a wide range of compounds of various biochemical functions. Clinically, several have become accepted for the treatment of hyperpigmentation, wrinkles, and other skin blemishes. Recent scientific studies have led to encouraging results in their cross-application for wound healing. This can be attributed to their pleiotropic biological activity ranging from promoting neocollagenesis and synthesis of other extracellular matrix components to their ability to modulate the inflammatory response. There is scientific data supporting a select number of cosmeceutical agents such as glycolic acid, ceramide, vitamin A, vitamin C, and growth factors as promoters of wound healing promoters in humans. Amongst the emerging peptide/growth factor class, clearly Pal-KTTKS enjoys much commercial success as a cosmeceutical active ingredient despite the critical absence of key scientific studies. The aim of this review is to educate physicians who regularly interface with aesthetically oriented patients about the current body of basic science and clinical literature so that they may more widely disseminate accurate information based on valid interpretation of these studies. This process should allow all interested practitioners to make evidence-based decisions about cosmeceuticals while providing greater clarity between the line separating hype and optimism. Although further studies are clearly warranted, awareness of the effects of cosmeceutical agents will provide yet another tool in the treatment armamentarium of physicians actively seeking to improve patient care and outcomes.

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