Chapter 2

Anti-Aging Skin Care Ingredient Technologies

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Core Messages

- The impact of bioactive skin care ingredient technology, pharmaceutical methods, and drug delivery systems have resulted in the development of cosmoleculars and the advancement of cosmeceuticals™ in anti-aging skin care ingredient technology.
- Anti-aging skin care ingredients are assessed: antioxidants, hydroxy acids, beta glucans, minerals, peptides, and growth factors.
- Topical antioxidants have both protective and rejuvenation benefits. Currently under research and development are spin traps (phenyl butyl nitrone).

Contents

2.1 Introduction

The past decade has witnessed the progression of the field of cosmeceuticals moving toward one of cosmoleculars™. The impact of advanced technologies as well as pharmaceutical methods and drug delivery systems has resulted in the field of cosmetic dermatology. This chapter will attempt to give the practitioner a base of current knowledge in the field of cosmetic dermatology. The skin care consumer has been faced with a literal flood of products into the marketplace designed to address various cosmetic concerns. As research and development of new bioactive ingredients and knowledge of existing ingredients continue to grow, and new technologies reflect increased stability and delivery of these ingredients to the skin, this trend will only continue to grow.

2.2 Reassessing the Skin Care Regimen

The cosmetic and beauty industry is one of the world's oldest professions, dating as far back as 1000 B.C. to the Picts, a tribe in Scotland. The use of ointments and oils was recorded on papyrus by the Ancient Egyptians, and cold cream is said to have been invented by the ancient Greek physician Galen. The quest for beautiful skin will bring many patients seeking expert advice to the dermatologist's office. The aim of this section is to try and simplify a topic that is constantly changing. Technologic advances of the past several decades have provided a great deal of information about skin structure and function as well as cellular and molecular mechanisms of aging.

The skin's appearance is dependent on many factors, including brightness and the way it reflects light. Healthy looking skin and how it reflects light is as important to younger-looking skin as is diminishing wrinkles. Lack of proper skin care can accelerate the aging process. It is therefore worthwhile to include a review of basic skin care, which comes down to cleansing and moisturizing.

The stratum corneum (SC) is a highly specialized structure whose brick and mortar composition is made up of terminally differentiated corneocytes (brick) intertwined within a specialized lipid matrix (mortar), which forms the skin's protective moisture barrier [1]. The SC is made up of dead corneocytes that are formed following apoptosis or planned death of migrating keratinocytes. The ability of the SC to retain moisture is through a variety of small-molecular-weight compounds collectively called the natural moisturizing factor (NMF) [2, 3]. The NMF functions as a humectant and consists of many compounds, including lactic acid, urea, and amino acids, which are breakdown products of filaggrin and cis-urocanic acid whose role is not clear but is believed to have a free-radical-scavenging role [4, 5]. The highest levels of NMF are found in the lowest regions of the SC where the greatest amount of moisture is retained.

The lipid matrix of the SC is made up of bipolar lipids in alternating hydrophilic and hydrophobic rows. The lipids consist of fatty acids, ceramides, and cholesterol, which form the SC mortar by surrounding the NMF thereby preventing moisture loss known as TEWL (transepidermal moisture loss.) Without this lipid bilayer, the hydrophilic NMF would evaporate and the resultant TEWL would clinically result in dry and aged-looking skin.

Cleansing is necessary in order to remove environmental dirt, microorganisms, makeup, and metabolic byproducts that can otherwise be damaging to the skin. Finding a cleanser appropriate for skin type that will not harm the moisture barrier while ensuring that a moisturizer is used to replenish and protect the moisture barrier is as important as any anti-aging ingredient.

2.3 Aging Skin

How skin ages depends on a number of factors. The primary factor that determines the way a person ages is underlying genetics. Other internal influences include diet, lifestyle, drug, and alcohol history. Smoking, a cause of premature aging of the skin, has been directly linked with elevations in matrix metalloproteinase-1 (MMP-1), which is a zinc-dependent protease responsible for degradation of dermal collagen [6]. Environmental exposures, including weather changes and pollutants, have a direct impact on skin aging, with the most profound degradative changes caused by chronic UV exposure with resultant photoaging.

Chronically aged skin that loses the scaffolding of the dermal structural proteins elastin and collagen in addition to epidermal thinning appears loose and wrinkled. There is atrophy of adnexal structures with a decrease of oil-secreting glands and the skin's moisture retaining ability, resulting in dryness and scaling. Continued loss of elasticity results in sagging, jowliness, and deep furrows. Photoaging compounds the structural changes by accelerating aging with even more pronounced wrinkling. There are more epidermal changes with pigmentary alterations of mottling and wrinkling than seen in chronologically aged skin alone.

The question of how and why we age has been the subject of much thought and discussion. As we learn more about aging and cell-signaling pathways, the approach to aging evolves. If humans are built with internal repair mechanisms, why do we age with degenerative changes? Many scientists are now starting to view physical aging as a disease process. The cellular and molecular mechanisms involved in aging reveal an intricate series of signals, markers, and pathways, all of which are programmed to monitor and control the lifespan of a cell as it ages. By studying these molecular events and pathways, the field of anti-aging will be furthered by the use of cosmoleculars™.

2.4 Antioxidants

The use of antioxidants in any anti-aging skin care regimen is essential in order to combat and prevent further damage. Vitamins have been used to combat free radical damage for many years. Unfortunately, they get used up rather quickly since it takes one vitamin to neutralize one free radical. Enzymes are more efficient free radical scavengers; however, they depend on the presence of a healthy cellular environment and certain trace minerals to synthesize them. There is growing evidence of the synergy that exists in using combinations of antioxidants along with sunscreens. Some antioxidants have protective benefits while others work as protectants in addition to stimulating age-reversal changes.

2.4.1 Spin Traps–Phenyl Butyl Nitrone

We are familiar with free radical damage that occurs with oxidative stress by sun, environmental pollutants, and cigarette smoking. However, free radicals are formed as result of normal oxygen metabolism and therefore are a byproduct of normal physiologic function. Damaging free radicals are created when an aberrant electron “spins” out of its orbit leaving a highly unstable molecule. The very newest antioxidants, which are known as “spin traps,” have the ability to catch or trap the aberrant electron as it starts to spin out of control and return it to its orbit before it can do any damage. Although the use of spin traps in dermatology is in its infancy, these compounds show a great deal of promise.

Spin traps were originally used as a way to measure free radical activity both in vivo and in vitro through their ability to form stable complexes [7, 8]. Their uses in degenerative diseases associated with aging have been a subject of study due to their ability to trap and neutralize free radicals. The most well-known spin trap is phenyl butyl nitrone (PBN) [9]. Numerous studies by Dr. J. Carney and his associates have been performed that have demonstrated the anti-inflammatory, neuroprotective, age-reversing effects of PBN. Interestingly, it is not so much their capacity to neutralize free radicals that is responsible for the protective behavior of spin traps but, rather, their ability to modulate proinflammatory cytokines [10].

2.4.2 Vitamin E

Topically applied vitamin E plays an enormous role in protecting the skin from free radical damage. Vitamin E is the most abundant antioxidant found in skin, and it is produced in human sebaceous glands in its alpha- and gamma-tocopherol forms. These tocopherols are part of a natural protective mantle that has been described and is, in fact, the first line of protection against environmental stress. As the vitamin E levels of the skin diminish, the production of alpha- and gamma-tocopherols occurs in the sebaceous glands and is delivered to the skin’s surface via sebum [11]. Oxidative damage occurs when the rate of depletion of vitamin E exceeds the rate of production. The important role of sebaceous glands and sebum in the production and delivery of vitamin E to the skin may explain the often-made observation that oily skin tends to age more slowly than drier skin. Perhaps those with oily skin have a higher vitamin E level and therefore more natural protection than those with dry skin.

The very properties that make alpha-tocopherol such a powerful antioxidant causes it to break down in the presence of oxygen or upon exposure to light. For that reason, alpha-tocopherol acetate, which is the more stable esterified form, is used in cosmetics. Since alpha-tocopherol acetate is not an antioxidant and has no antioxidant activity, it must first convert to its active alpha-tocopherol form. Years of debate questioned the ability of alpha-tocopherol acetate to be delivered to the skin and bioconverted to an active form. Finally, in 1990, the bioconversion of alpha-tocopherol acetate to free alpha-tocopherol was able to be demonstrated [12]. In addition, formulation enhancement using certain delivery systems has demonstrated the ability to deliver significant levels to the skin followed by bioconversion once in the skin.

The use of vitamin E in skin care has anti-aging benefits based on its moisturization properties but mostly on its protective capabilities. Dr. Lester Packer documented the depletion of vitamin E levels in skin following UV radiation [13]. In addition, he was able to document significantly higher levels in the skin following the application of a cream containing 5% tocopherol acetate over 10 days. He also demonstrated the antioxidant role of vitamin E against the oxidative stress caused by ozone [14, 15].

The protective role of vitamin E extends to skin care preparations by enhancing their stability and shelf life. A change in color or texture is a sure sign that a cosmetic product is oxidizing and should not be used. Patients ought to be told that the breakdown of the product will continue as it is applied to their skin. When cosmetic products contain ingredients that are easily oxidized, such as vitamins or natural extracts, the use of alpha-tocopherol in conjunction with ascorbyl palmitate acts as a powerful antioxidant system preventing oxidation. In addition, the combination of alpha-tocopherol and ascorbyl palmitate can prevent the formation of carcinogenic nitrosamines [16].

The enhanced ability of vitamin E as a moisturizer with its added benefits of skin smoothness and softness is attributed partly to its ability to penetrate the skin and provide cumulative benefits [16]. Vitamin E enhances the photoprotective effects of sunscreen, and when combined with vitamin C, the two are even stronger as photoprotectants [17, 18].

2.4.3 Vitamin C

Vitamin C is a major water soluble antioxidant that plays a vital role in photoprotection as well as in collagen synthesis. The body reservoir of vitamin C decreases with age, and habits such as smoking decrease reserves even more. Vitamin C is not produced in the body and must be consumed entirely through diet and oral supplementation. Likewise, in the skin where vitamin C plays a vital role in photoprotection and aging, it must be topically supplemented since, unlike vitamin E, it is not produced in the skin.

The role of vitamin C in photoprotection has been demonstrated by the dramatic reduction of vitamin C in skin following UV radiation. In addition, a combination of both vitamins E and C work synergistically to enhance their photoprotective effects. This reinforces the benefit derived from enhancing photoprotection by combining antioxidants with sun-protection products [17, 18].

Vitamin C is an essential cofactor for the hydroxylation of proline and lysine, a necessary step in collagen synthesis. In fact, fibroblasts in cell culture will selectively secrete collagen when vitamin C is added in a dose-dependent fashion. Its role in collagen synthesis is probably responsible for the wrinkle-reducing and skin-firming effects that vitamin C has on aged skin [19, 20, 21]. Vitamin C also appears to reduce signs of photoaging. In addition, topical vitamin C increases levels of tissue inhibitors of collagen-degrading matrix metalloproteinase1 (MMP-1) [22].

The ability of topical vitamin C to reduce hyperpigmentation has been demonstrated and has found its way into various skin-lightening products. Vitamin C is able to lighten hyperpigmented skin through the inhibition of the enzyme tyrosinase [23].

Many forms of vitamin C have been used in various topical formulations in efforts to stabilize this highly unstable molecule. However, any form of vitamin C that is applied to the skin must convert to the L-ascorbic acid form in order to be recognized by the body and deliver a benefit. Stabilizing vitamin C was an impossibility until 1988 when Dr. Sheldon Pinnell from Duke University, one of the pioneers of topical vitamin C research, was able to stabilize ascorbic acid in solution. The same study also showed the presence of ascorbic acid in the skin days after the application with an increase in the level of collagen as well, proving the penetration and benefit of the topically applied vitamin C [24, 25].

There are so many different vitamin C variations used in skin care that rather than naming each one, the process can be simplified by dividing them in either water soluble (L-ascorbic acid and magnesium or sodium ascorbyl phosphate), fat-soluble esters (ascorbyl palmitate, ascorbyl tetra-isopalmitate), or anhydrous systems. Unfortunately, the long-term stability of topical vitamin C preparations remains a concern. The most stable vitamin C preparations remain anhydrous or completely water free.

2.4.4 Coenzyme Q10

Coenzyme Q10 (CoQ10) is a powerful free radical inhibitor that inhibits lipid peroxides from forming in plasma membranes. CoQ10 plays a very important role in cellular energy production and works in the mitochondrial adenosine triphosphate (ATP) energy-producing pathway of the cell [26]. The presence of CoQ10 in the mitochondria may play a role in preventing oxidative stress induced cellular apoptosis since it is in the mitochondria where the final apoptotic signal is dispatched.

As we age, CoQ10 levels diminish, as does cellular energy production, which may improve by adding CoQ10. The vast majority of information about CoQ10 is based on its oral use. Topically, it has demonstrated antioxidant activity as well as inhibition of collagenase expression in UV-irradiated human fibroblasts. Topical application of CoQ10 has been reported to show a reduction in wrinkles; however, more studies need to be done.

2.4.5 Idebenone

Idebenone is a powerful synthetic analog of CoQ10, which shows a great deal of promise. In a study comparing the photoprotective properties of topical idebenone to those of vitamin E, kinetin, CoQ10, vitamin C, and lipoic acid, idebenone consistently demonstrated the highest level of antioxidation and photoprotection [27]. At the present time while this chapter is being written, idebenone is not yet available for topical use. However, it will soon be available under the brand name Prevage (Allergan, Irvine, CA, USA).

2.4.6 Lipoic Acid

Lipoic acid is a very powerful antioxidant that has the unusual advantage of being both water and fat soluble and is an important cofactor in mitochondrial dehydrogenases. It has a great deal of anti-inflammatory activity, which is one of the reasons that Dr. Nicholas Perricone has credited lipoic acid as one of the major antioxidants in skin care. Studies have shown the ease with which lipoic acid is able to penetrate the skin, after which it converts into its active by-product dihydrolipoic acid (DHLA) [28, 29].

Topical application of 3% lipoic acid has demonstrated its ability to decrease UVB-induced erythema, which demonstrates its photoprotective and anti-inflammatory properties. Also, a 12 -week study demonstrated that using a topical cream containing 5% alpha-lipoic acid was quite effective in treating signs of photoaging [30, 31].

2.4.7 Polyphenols

Green tea polyphenols have been included in a growing number of skin care products for their antioxidant and anti-inflammatory effects [32]. These polyphenolic compounds are called epicatechins, and the most powerful member of this group is called epigallocatechin-3-gallate (EGCG). Studies have demonstrated the ability of EGCG pretreated skin to inhibit erythema, myeloperoxidase activity, and inflammation following UVB irradiation [33, 34]. Studies have also demonstrated UV-radiated skin pretreated with green tea polyphenols shows a histologic decrease in sunburn cells [35, 36]. In addition, pretreated skin had less DNA damage as evidenced by fewer UV-induced DNA pyrimidine dimers formed than in untreated skin.

Another polyphenol that differs from those found in green tea are the procyanidins. Procyanidins are powerful free radical scavengers whose richest source is from the seeds of red grapes. Grapeseed extract is rich in polyphenols, and studies have reported it to have higher antioxidant activity than both vitamins C and E [37, 38]. In fact, in mice, grapeseed polyphenols have demonstrated greater inhibition of lipid peroxidation than green tea polyphenols [39]. The role of polyphenols, whether from green tea or grapeseed extract, has a great deal of potential as part of a growing natural anti-aging skin care market. With all natural ingredients, however, it is important to standardize extraction methods as well as assays for their activity.

2.4.8 Selenium

Selenium is an essential trace element with antioxidant, anti-inflammatory, and anticarcinogenic activities. As an anti-aging skin care ingredient, selenium's protective ability lies in its essential role as a cofactor in the formation of the important protective enzyme glutathione peroxidase [40]. Selenium's anti-inflammatory properties have been demonstrated by its ability to inhibit skin damaging UV-induced inflammatory cytokines [41]. Selenium as a topical ingredient does not penetrate skin well and must be used in its selenomethionine form in order to be bioavailable.

2.4.9 Carotenoids

Carotenoids are dietary antioxidants, which include lycopene, lutein, and beta-carotene. The sources of these natural free-radical-scavenging compounds include leafy green vegetables, carrots, and tomatoes. Carotenoids have free-radical-scavenging properties and inhibit lipid peroxidation as well [42]. Most studies associated with carotenoids have used them in their oral form. However, there have been reports of the photoprotective effects by topically applied carotenoids [42]. In cell culture study on human skin fibroblasts, there was a decrease in the level of UVB-induced thiobarbituric acid-reactive substances by pretreatment with carotenoids. In the study, carotenoids were delivered to fibroblasts through liposomes 20 min prior to UV radiation, and measurements were taken 1 h later [42]. Although carotenoids work best synergistically, by themselves, lycopene was the strongest photoprotectant followed by lutein then beta-carotene [43].

Formulating with these compounds has been tricky since they are pigments and influence the color of the cosmetic in the jar as well as on the skin. However, newer technologies are being developed that are resulting in colorless carotenoids.

2.5 Vitamin A–Retinoids

The essential role of retinoids in the normal development and keratinization of skin dates back to 1925 when Wolbach and Howe described abnormal keratinization in vitamin-A-deficient enzymes. The topical use of retinoids has been more extensively studied than any other compound in dermatology. Retinoids play an important role in skin development and regulate the growth and differentiation of keratinocytes [44]. The ability of topical retinoids to reverse photoaging as well as chronologic aging makes the use of a retinoid a staple in any cosmetic regimen [45, 46].

Topical vitamin A has the ability to diminish the signs of aging by decreasing fine lines and wrinkling. In addition, there is a normalization and enhancement of elasticity [47]. Improvement of skin tone and texture is a benefit of vitamin A, which enhances skin lightening when used in conjunction with skin lighteners.

Vitamin A is a lipid-soluble molecule whose structure has been amenable to formulation variability resulting in several structurally different forms. The first generation, or nonaromatic form, includes all-trans retinoic acid (tretinoin) and its 9-cis-isomer and 13-cis-isomer (isotretinoin) forms [44, 48]. The other topical forms are polyaromatic isomers of retinoic acid, or arotinoids, which include adapalene and tazarotene [49]. Retinoic acid regulates the growth, differentiation, and normalization of skin by recognizing and binding to specific retinoic acid receptors (RARs) and retinoic X receptors (RXRs), which are ligand-activated transcription factors. These receptors bind to regulatory regions of DNA where they activate gene transcription. Within each receptor family there exist subtypes and multiple isoforms belonging to each subtype.

Vitamin A derivatives used cosmetically in nonprescription products include retinol, retinaldehyde, retinyl palmitate, and retinyl esters. Retinol and retinaldehyde, an intermediate product in the conversion of retinol to all-trans retinoic acid, are natural forms of vitamin A [44]. The most common nonprescription forms used are retinol and retinyl palmitate. In order to be of any benefit, each must convert to the all-trans retinoic acid form in order to bind ligands. Retinol has been shown to penetrate the skin more effectively than retinaldehyde, retinyl palmitate, and even retinoic acid [50]. This was determined by measuring 4-hydroxylase activity, which is directly related to the level of all-trans retinoic acid in the skin [51]. Since each of the vitamin A derivatives must bioconvert to all-trans retinoic acid, the 4-hydroxylase level has become a marker of the bioavailability and effectiveness of these ingredients.

Retinol is the most abundant form of vitamin A in the skin; however, it is extremely sensitive to light and air during formulation in skin care. If retinol is not handled in the correct conditions, it will quickly oxidize, and an irritating, harmful product will result.

2.6 B Vitamins

Panthenol (provitamin B5) is the stable analog of pantothenic acid that has been used in skin care, nail products, and most especially in hair products through its enhanced moisturization benefits. Pantothenic acid enhances wound healing through cell proliferation and protein synthesis and quickly penetrates the skin. Known for its moisturizing and soothing effects, it can play a role in anti-aging skin care through its enhanced cell proliferation and healing abilities [52, 53].

Nicotinamide (niacinamide) is another B vitamin that has entered the skin care arena. Most studies of this vitamin have focused on the anti-inflammatory effects of nicotinamide and its benefit in acne treatment. The role of nicotinamide as a potential anti-aging ingredient has yet to be explored; however, one possible role is in the biosynthesis of ceramides and other stratum corneum lipids [54].

2.7 Alpha-Hydroxy Acids (AHAs)

About 25 years before glycolic acid made its dramatic entry into the cosmetic skin care market in the early 1990s, lactic acid had been described as part of the skin's NMF and was used with great success in skin moisturizers. In addition, Drs. Van Scott and Yu described the effectiveness of lactic acid in treating ichthyosis and disorders of keratinization. The combined use of topical retinoids as well as glycolic acid as an ingredient in cosmetic skin care products and in in-office peeling products has revolutionized the anti-aging skin care market [55].

The effects of alpha-hydroxy acids (AHAs) are determined by their pH and concentration levels. Although these naturally occurring organic acids are often referred to as fruit acids because they are found in many common fruits such as citrus fruits (citric acid), apples (malic acid), and grapes (tartaric acid), the two most widely used AHAs are not components of fruit. Glycolic acid is a sugar cane derivative, and lactic acid is derived from milk.

There have been a number of beauty benefits associated with the use of AHAs in facial skin care, and they have the ability to reduce the cohesion of dead corneocytes to the skin, giving the skin a smoother, less wrinkled, and less mottled appearance. It is ideal to couple these products with topical retinoids and lightening agents to enhance these effects. The effectiveness of AHAs in reversing the signs of aging were also coupled with problems of stinging, burning, and irritation, which were usually associated with a pH less than 3.5.

2.8 Polyhydroxy Acids (PHAs)

The polyhydroxy acids (PHAs) are the next generation of AHAs. They provide the anti-aging, skin-smoothing benefits of the AHAs without the potentially irritating side effects of burning and stinging. PHAs include gluconolactone and lactobionic acid, which are structurally larger molecules than AHAs allowing for slower skin penetration and thus fewer side effects [56, 57].

In addition to the exfoliative benefits of AHAs, PHAs provide additional benefits of enhanced stratum corneum barrier function and moisturization with humectant properties. This makes for enhanced skin compatibility and use for most skin types, including sensitive skins. PHAs are also protective since most of them contain antioxidant properties.

2.9 Beta-Hydroxy Acids (BHAs)

The most frequently used beta-hydroxy acid is salicylic acid. It is found in most over-the-counter (OTC) products and has been used primarily in the treatment of acne. Part of its effectiveness as an acne treatment may stem from its lipid solubility and ability to penetrate sebum [58]. More recently, salicylic acid has been used in the treatment of photoaging with in-office peels of 20–30%. These can be quite helpful in patients who are unable to tolerate alpha-hydroxy acids since irritancy levels tend to be less with salicylic acid. In addition, it can be quite useful to combine or alternate both AHAs and BHAs since their mechanisms of action differ, and using both may be quite beneficial.

2.10 Beta-Glucan

Beta-glucans were first described in 1941 and belong to a class of compounds known as biological response modifiers. Although isolated from different sources, including oat, barley, and reishi mushrooms, the most biologically active are isolated from cell membranes of baker's yeast (*Saccharomyces cerevisiae*) [59]. The ability of Beta-glucans to stimulate and activate macrophages has resulted in multiple applications, including wound healing, infectious disease, oncology, and dermatology [60].

In the epidermis, where macrophage-derived cells include both keratinocytes and Langerhans cells, beta-glucans act to stimulate the protective qualities of these cells as our first line of defense. Topical beta-glucans can accelerate wound healing and increase resistance to infection by enhancing macrophage-mediated phagocytosis [61]. Studies have also demonstrated that beta-glucans have photoprotective properties similar to those of vitamin E by their ability to sustain levels of reduced glutathione in the skin following UV radiation [62]. Beta-glucans are extremely soothing and calming to the skin through their reinforcement of skin macrophages, which have implications in minimizing irritancy potential of products.

The potential uses of beta-glucans in dermatology are numerous. In personal-care products for shaving, where nicks and cuts, razor burn, irritation and folliculitis are problematic, the protective, wound-healing, anti-irritating effects of beta-glucans can be quite helpful. The photoprotective effects of beta-glucans as well as their ability to soothe, moisturize, and protect the skin from potential irritation that can occur with other treatment products, makes them quite useful in anti-aging skin regimens [63].

2.11 Skin Respiratory Factors

Skin respiratory factors (SRF), also called tissue respiratory factors (TRF), have been used in cosmetics for their ability to renew and revitalize the skin. These ingredients revitalize cellular metabolism through the stimulation of cell respiration. The ability of an ingredient to stimulate cell respiration and cellular metabolism can be determined by Warburg assay, which measures oxygen uptake in living cell homogenates. As cell respiration and metabolism increase, cell energy increases, as evidenced by increased cellular ATP levels measured in the cell suspension [64].

Although a number of botanical ingredients with the ability to enhance cell respiration have been isolated, the most abundant source is baker's yeast [65]. However, unlike beta-glucans, which are isolated from the cell walls of baker's yeast, compounds that stimulate cellular respiration are extracted from the cytoplasm. These cytoplasmic elements generally contain mitochondrial components of the cell, which can enhance cellular energy [66].

As skin ages, it exhibits a certain amount of physiologic fatigue, which is compounded by oxidative stress and environmental factors. This fatigue, which increases with age, is paralleled by the progressive decrease in cellular energy and metabolism as well as diminished cellular function. The addition of ingredients to anti-aging skin care that contain mitochondrial cytoplasmic yeast extracts can result in the stimulation of cellular respiration followed by enhanced cellular metabolism, vitality, and increased cell renewal.

2.12 Copper

Products containing copper have gained increasing popularity in the anti-aging skin care market over the past several years. In humans, copper binds to the high-affinity tripeptide glycyl-l-histidyl-l-lysine (GHK) to form a copper-GHK complex [67]. This copper-GHK complex plays a vital role in human tissue repair, and its ability to accelerate wound healing has been demonstrated both in vitro and in vivo. In addition, copper is a vital cofactor in the activation of the powerful antioxidant enzyme superoxide dismutase [68, 69].

The ability of the copper-GHK complex to stimulate the production of both collagen and glycosaminoglycans in a dose-dependent fashion has been demonstrated in cell-culture studies of human fibroblasts. Other observations include the fact that the copper-GHK complex may also play a role in angiogenesis during wound healing. The role of copper-GHK as an anti-aging ingredient may be explained by its role in wound healing and its ability to stimulate extracellular matrix proteins.

A number of clinical studies have been performed using copper-GHK-containing products. Significant clinical improvements in photoaged skin were demonstrated in patients treated with facial and eye creams containing the copper-GHK complex [70, 71]. These improvements include a decrease in skin wrinkling, laxity, and roughness. These changes, as well as the lack of irritancy, give copper an important role in the anti-aging skin care market.

2.13 Peptides

Initially, peptides were derived from much larger molecules, which were enzymatically cleaved in order to isolate active fragments for use in skin care. Proteolytically cleaved peptides are still relatively large molecules. Advances in peptide chemistry were made with the advent of molecular biology. Molecular biology has enabled us to learn the exact amino acid sequences of molecules such as the matrix proteins type IV collagen and laminin. Knowing the amino acid sequence of these molecules enables the production of peptides that are five to ten amino acids in size.

The advantages of using tiny peptide fragments is in their specificity. In fact, much of the future of medicine including dermatology is in the use of peptides that will be able to stimulate or inhibit certain processes through receptor recognition. Currently, two of the most well-known peptides being used in skin care are palmitoyl pentapeptides, also known as Matrixyl; and acetyl hexapeptide-3, also known as Argirelene.

Matrixyl is a pentapeptide that has been used as a procollagen analog to stimulate collagen production in skin. This procollagen pentapeptide sequence was first described in 1993 as being able to promote synthesis of types I and III collagen and fibronectin when added to fibroblast cell cultures [72]. The sequence Lys-Thr-Thr-Lys-Ser (KTTKS) has a fatty acid moiety called palmitoyl added to it in order to enhance its penetration in to the skin. Sederma (Le Perray en Yvelines, France), the company that holds the patents to pal-KTTKS (Matrixyl), sponsored a study that was presented as a poster at the 2002 World Congress of Dermatology in Paris, France. In this 4-month study, pal-KTTKS was able to decrease skin roughness by 27%, wrinkle volume by 36%, and wrinkle depth by 27%. Skin biopsies demonstrated increased density and thickness of elastin fibers in the dermis with improvement in type IV collagen. Studies performed by Sederma over 6 months using a cream containing 4% Matrixyl were impressive. Wrinkle depth decreased by 68% over 6 months, and wrinkle density decreased by 28%, 31%, and 47% over 2, 4, and 6 months, respectively. According to testing, in order to be effective at wrinkle reduction, Matrixyl must be used at a minimum concentration of 2% and ideally between 4 and 8%.

Argirelene has been marketed as having a relaxing effect on muscles and has therefore been touted as an alternative to Botox. Argirelene's mechanism of action has been studied in vitro and appears to inhibit vesicle docking by inhibiting formation of the soluble N-ethylmaleimide-sensitive fusion attachment protein receptor complex (SNAP) [73, 74]. By inhibiting SNAP formation, Argirelene inhibits the release of catecholamines, including epinephrine and norepinephrine, in vitro. Clinical studies are rather limited at this time, and penetration in vivo has yet to be determined. One study using Argirelene around the eye area found a 17% improvement in periorbital rhytides after 15 days and a 27% improvement after 30 days. According to studies from the company, Argirelene should be used at a 10% concentration for optimal results.

2.14 Conclusion

The field of anti-aging cosmetic ingredients has progressed from that of cosmeceuticals to cosmoleculars™. Much work still needs to be done, including developing assays in vitro that emulate actual chronologically aged skin. Thus far, all aged skin that is studied in cell culture is aged by UV irradiation, which is a photoaging model. In addition, when looking at collagen-promoting behaviors, fibroblast cell cultures do not emulate skin; skin is not in a state of active wound healing and fibroblast expansion under normal circumstances.

The use of growth factors in skin care, though sold in many products, is still quite early in its development. There is only one growth factor that is FDA approved for clinical use in wound healing, and that is platelet-derived growth factor (PDGF). It took approximately 35 years for the approval of PDGF, and yet many other growth factors are being used in skin care. Far more work needs to be done to prove they penetrate the skin as they are quite large. After proving skin penetration, safety must be of primary concern since there are multiple receptors that are up- and down-regulated. We do know what some of these receptors are; however, there are many more that remain unknown. Therefore, much more work needs to be done in this area.

As our knowledge and technology continue to grow, so will our use of peptide fragments and DNA oligopeptides. This is the cosmolecular™ connection of skin care still in its infancy but which points to an exciting future.

References

1. Elias PM (1983) Epidermal lipids, barrier function and desquamation. J Invest Dermatol 80 (Suppl 1): 44–49
2. Jacobi O (1959) About the mechanism of moisture regulation in the horny layer of the skin. Proc Scient Sect Toil Goods Assoc 31: 22–26
3. Blank IH (1952) Factors which influence the water content of the SC. J Invest Dermatol 18: 430–433
4. Angelin JH (1976) Urocanic acid a natural sunscreen. Cosmet Toil 91: 47–49

5. Baden HP, Pathak MA (1967) The metabolism and function of urocanic acid in the skin. J Invest Dermatol 48 : 11–17
6. Lahmann C et al (2001) Matrix metalloproteinase-1 and skin ageing in smokers. Lancet 24 : 935–936
7. Floyd RA (2000) Nitrone inhibition of age associated oxidative damage. Ann NY Acad Sci 899 : 222–237
8. Floyd RA et al (2002) Nitrones, their value as therapeutics and probes to understanding aging. Mech Ageing Dev 123(8) : 1021–1031
9. Carney and Floyd (1991) Protection against oxidative damage to CNS by alpha-phenyl-tert-butyl nitrone (PBN) and other spin-trapping agents: A novel series of nonlipid free radical scavengers. J Molec Neurosci 3(1) : 47–57
10. Pogrebniak HW (1992) Spin trap salvage from endotoxemia: the role of cytokine down-regulation. Surgery 112(2) : 130–139
11. Jens J et al (1999) Sebaceous gland secretion is a major physiologic route of vitamin E delivery to skin. J Invest Dermatol 113 : 1006–1010
12. Norkus, Edward P (1990) Bioconversion of vitamin E acetate to free tocopherol, New York Medical College
13. Packer Lester (1991) Protective effects of Vitamin E acetate against UVB/UVA radiation, University of California
14. Trevithick J R(1993) Reduction of sunburn damage to skin by topical application of vitamin E acetate, Scanning Microsc 7(4) : 1269–1281
15. Thiele JJ et al (1997) Ozone-exposure depletes vitamin E and induces lipid peroxidation in murine stratum corneum. J Investig Dermatol 108 : 753–757
16. Djerassi D (2001) The role of vitamins in high performance cosmetics. Cosmetics and Toiletries Manufacturers Worldwide
17. Darr D et al (1996). Effectiveness of antioxidants (vitamin C and E) with and without sunscreens as topical photoprotectants. Acta Derm Venereol 76 : 264–268
18. Chan AC (1993) Partners in defense, vitamin E and vitamin C. Can J Physiol Pharmacol 71 : 725–731.
19. Phillips CL et al (1992) Ascorbic acid and transforming growth factor-ß1 increase collagen biosynthesis via different mechanisms: coordinate regulation of pro-alpha 1(I) and pro-alpha 1(III) collagens. Arch Biochem Biophys 295 : 397–403.
20. Geesin JC et al (1988) Ascorbic acid specifically increases type I and type III procollagen messenger RNA levels in human skin fibroblast. J Invest Dermatol 90 : 420–424.
21. Phillips CL et al (1994). Effects of ascorbic acid on proliferation and collagen synthesis in relation to the donor age of human dermal fibroblasts. J Invest Dermatol 103 : 228–232.
22. Nusgens BV et al (2001) Topically applied vitamin C enhances the mRNA level of collagens I and III, their processing enzymes and tissue inhibitor of matrix metalloproteinase 1 in the human dermis. J Invest Dermatol 116: 853–859.
23. Takashima H et al (1971) Ascorbic acid esters and skin pigmentation. Am Perfum Cosmet 86 : 29–36.
24. Pinnell SR (2001) Topical l-ascorbic acid: Percutaneous absorption studies. Dermatol Surg 27: 137–142
25. Pinnell, Sheldon R (1988) New stabilized ascorbic acid solution–percutaneous absorption and effect on relative collagen synthesis. Journal of Cutaneous Aging & Cosmetic Dermatology 1(2)
26. Hoppe U, et al (1999) Coenzyme Q10, a cutaneous antioxidant and energizer. Biofactors 9 : 37
27. DiNardo J et al (2004) Antioxidants compared in a new protocol to measure protective capacity against oxidative stress–part II, American Academy of Dermatology, Washington D.C.
28. Moini H et al (2002) Antioxidant and prooxidant activities of alpha-lipoic acid and dihydrolipoic acid. Toxicol Appl Pharmacol 182(1)84–90
29. Podda M (2001) Activity of alpha-lipoic acid in the protection against oxidative stress in skin. Curr Prol Dermatol 29 : 43–51
30. Rijnkels JM et al (2003) Photoprotection by antioxidants against UVB-radiation-induced damage in pig skin organ culture. Radiat Res 159(2) : 210–217
31. Beitner H (2003) Randomized, placebo-controlled, double blind study on the clinical efficacy of a cream containing 5% alpha-lipoic acid related to photoageing of facial skin. Br J Dermatol 149(4) : 841–849
32. Katiyar SK et al (2000) Green tea and skin. Arch Dermatol 136 : 989–989
33. Katiyar SK et al (1999) Prevention of UVB-induced immunosuppression in mice by the green tea polyphenol (-)-epigallocatechin-3-gallate may be associated with alterations in IL-10 and IL-12 production. Carcinogenesis 20 : 2117–2124
34. Zhao JF et al (1999) Green tea protects against psoralen plus UVA-induced photochemical damage to skin. J Invest Dermatol 113 : 1070–1075
35. Katiyar SK, Elmets CA (2001) Green tea polyphenolic antioxidants and skin photoprotection. Int J Oncol 18 : 1307–13013
36. Katiyar SK (2001) Green tea polyphenol (–)-epigallocatechin-3-gallatetreatment to mouse skin prevents UVB-induced infiltration of leukocytes, depletion of antigen-presenting cells, and oxidative stress. J Leukoc Biol 69 : 719–726
37. Bagchi D et al (2000) Free radicals and grape seed proanthocyanidin extract: importance in human health and disease prevention. Toxicology 148: 187–197
38. Bagchi D et al (1997) Oxygen free radical scavenging abilities of vitamins C and E, and a grape seed proanthocyanidin extract in vitro. Res Commun Mol Pathol Pharmacol 95 : 179–189
39. Zhao J et al (1999). Anti-tumor-promoting activity of a polyphenolic fraction isolated from grape seeds in the mouse skin two-stage initiation-promotion protocol and identification of procyanidin B5–3'-gallate as the most effective antioxidant constituent. Carcinogenesis 20 : 1737–1745

40. Serwin AB, Chodynicka B (2001) The role of selenium in skin. Wiad Lek (Poland) 54(3–4):202–207
41. Greul AK et al (2002) Photoprotection of UV-irradiated human skin: an antioxidative combination of vitamins E and C, carotenoids, selenium and proanthocyanidins. Skin Pharmacol Appl Skin Physiol 15(5):307–15
42. Cesarini JP et al (2002) Immediate effects of UV radiation on the skin: modification by an antioxidant complex containing carotenoids. Photodermatol Photoimmunol Photomed 19(4):182–189
43. Eichler O et al (2002) Divergent optimum levels of lycopene, beta-carotene and lutein protecting against UVB irradiation in human fibroblasts. Photochem Photobiol 75(5):503–506
44. Roos TC et al (1998) Retinoid metabolism in the skin. Pharmacol Rev 50(20):315–333
45. Kligman AM et al (1986) Topical tretinoin for photoaged skin. J Am Acad Dermatol 15:836–859
46. Varani J et al (2000) Vitamin A antagonizes decreased cell growth and elevated collagen-degrading matrix metalloproteinases and stimulates collagen accumulation in naturally aged human skin. J Invest Dermatol 114:480–486
47. Djerassi D (2001) The role of vitamins in high performance cosmetics. Cosmetics and Toiletries Manufacturers Worldwide
48. Fisher GJ et al (1998) Retinoic acid receptor-gamma in human epidermis preferentially traps all-trans retinoic acid as its ligand rather than 9-cis retinoic acid. J Investig Dermatol 110:297–300
49. Prystowsky JH (2001) Topical retinoids. In: Wolverton, SE (ed) Comprehensive dermatologic drug therapy. Saunders, Philadelphia pp 578–594
50. Duell EA et al (1997) Unoccluded retinol penetrates human skin in vivo more effectively than unoccluded retinyl palmitate or retinoic acid. J Investig Dermatol 109:301–305
51. Duell EA (1996) Retinoic acid isomers applied to human skin in vivo each induce a 4-hydroxylase that inactivates only trans-retinoic acid. J Investig Dermatol (106):316–320
52. Weiser H (1987) Wound healing effects of topical application of panthenol on mammalian skin. Hoffmann-La Roche
53. Lacroix B (1988) Effect of pantothenic acid on fibroblast cell cultures. Res Exp Med 188:391–396
54. Tanno O et al (2000) Nicotinamide increases biosynthesis of ceramides as well as other stratum corneum lipids to improve the epidermal permeability barrier. Br J Dermatol 143:524–531
55. Johnson AW (2002) Hydroxy acids. Skin moisturization. Cosmetic Science and Technology Series 25:323–352
56. Grimes P et al (2004) The use of polyhydroxy acids (PHAs) in photoaged skin. Cutis 73 (Suppl 2):3–13
57. Edison BL et al (2004) A polyhydroxy acid skin care regimen provides antiaging effects comparable to an alpha-hydroxy acid regimen. Cutis 73 (Suppl 2):14–17
58. Leveque JL, Saint-Leger D (2002) Salicylic acid and derivatives. Skin moisturization. Cosmetic Science and Technology Series 25:353–363
59. Zulli, F.et al (1998) Improving skin function with CM-glucan, a biological response modifier from yeast. Int J Cosmet Sci 20(2):79–86
60. Leibovich SJ, Danon D (1980) Promotion of wound repair in mice by application of glucan. J Reticuloendothel Soc 27:1–11
61. Czop JK, Austen KF (1985) A beta-glucan inhibitable receptor on human monocytes: its identity with the phagocytic receptor for particulate activators of the alternative complement pathway. J Immunol 134:2588–2593
62. Zulli F et al (1995) Photoprotective effects of CM-glucan on cultured human cells. Euro Cosmetics 11:46–50
63. Elmets CA (1992) Photoprotective effects of sunscreens in cosmetics on sunburn and Langerhans cell photodamage. Photodermatol Photoimmunol Photomed 9:113
64. Hersh T (2003) Reparatives for chemosurgery and laser therapy. United States Patent: 6,630,442
65. Keller SJ et al (1991) Isolation and characterization of a tissue respiratory factor from bakers yeast. J Cell Biol 304:21
66. Bentley JP et al (1990) Peptides from live yeast cell derivative stimulate wound healing. Arch Surg 125:641–646
67. Pickart L, Lovejoy S (1987) Biological effects and the mechanism of action of the plasma copper-binding growth factor glycyl-l-histidyl-l-lysine. Methods Enzymol 147:314–328
68. Harris ED (1992) Copper as a cofactor and regulator of copper, zinc superoxide dismutase. J Nutr 122:636–640)
69. Pickart L et al (1986) Gly-l-his-l-lys: copper (II) – A human plasma factor with superoxide dismutase-like and wound-healing properties, In: Rolitio (ed) Superoxide and superoxide dismutase in chemistry, biology and medicine. Elsevier, Amsterdam pp 555–558
70. Leyden JJ et al (2002) Skin care benefits of copper peptide containing facial creams. Amer Acad Derm Poster Abstract
71. Leydon JJ et al (2002) Skin benefits of copper-peptide-containing eye creams. Amer Acad Derm Poster Abstract
72. Katayama K et al (1993) A pentapeptide from type I procollagen promotes extracellular matrix production J Biol Chem 268:9941–9944
73. Gutierrez LM et al (1997) A peptide that mimics the c-terminal sequence of SNAP-25 inhibits secretory vesicle docking in chromaffin cells. J Biol Chem 272:2634–2639
74. Blanes-Mira C et al (2004) Small peptides patterned after the N-terminus domain of SNAP25 inhibit SNARE complex assembly and regulated exocytosis. J Neurochem 88(1):124–135