**Understanding cosmeceutical peptides**

**KEYWORDS:** peptides; cosmeceuticals; palmitoyl tripeptide-38; palmitoyl tripeptide-5; wrinkling; laxity; photodamage; textural variances; hyperpigmentation

**Abstract** The development in recent years of novel synthetic cosmeceutical peptides has led to significant advances in the clinical management of aging skin. Thanks to the outstanding collagen building capacity of some of these peptides, this category of compounds is rapidly becoming the cornerstone of the skin care industry, enabling the formulation of high-quality topical products that diminish lines and wrinkles, smooth texture, attenuate laxity, and reduce discoloration. Two agents, in particular, have revolutionized anti-aging skin care: palmitoyl tripeptide-38 and palmitoyl tripeptide-5. This article reviews the science behind peptides, the research supporting their use, and key factors to ensure the efficacy of peptide-based formulations and achieve optimal results.

**INTRODUCTION**

With age, the skin undergoes changes that affect the integrity of its support structure and the ability of its cells to communicate, transfer nutrients, regenerate, and repair. These changes include progressive thinning of the epithelium, a more compact stratum corneum, and reduced granular layer thickness (1, 2). The dermoepidermal junction flattens, due to the retraction of the epidermal papillae as well as microprojections of basal cells into the dermis (3, 2).

The production of collagen gradually diminishes (4). As a result of these changes, the skin becomes thinner, lax, and less elastic, which leads to the development of bags, lines, wrinkles, and hyperpigmentation.

Facial wrinkles are typically the first visible signs of aging—although factors other than senescence contribute to their appearance, including sun damage and muscle movement. As the aging population is growing and set to rise in the coming decades, the demand for products proven to attenuate and prevent these and other visible manifestations of aging is steadily climbing.

A testament to this phenomenon is the consistent increase in revenue performed by premium anti-aging products. In 2011 this market grew, despite the recession, by an impressive 14 percent, reaching $3.3 billion (5). Such increased demand and market growth have driven manufacturers in the skin care industry to develop new advanced formulations and ingredients, within a group of topical products collectively known as cosmeceuticals.

**COSMECEUTICALS**

The term “cosmeceutical” defines any bioactive agent that exerts both cosmetic and pharmaceutical therapeutic effects on the skin (6). Cosmeceuticals have been associated with many benefits: they help prevent ultraviolet (UV) damage, due their ability to reduce free radical formation; and they can improve the functioning of the lipid barrier, reduce pore size, smooth texture, and brighten skin tone (7). For all these reasons, cosmeceuticals have a wide range of applications in skin care, and are largely used in formulations aimed at reducing the visible signs of aging—lines, wrinkles, laxity, coarsening, and discoloration (7).

There are six main categories of cosmeceuticals: antioxidants, growth factors, anti-inflammatory agents, polycarboxylic acids, pigment-lightening agents and peptides (6, 8). Antioxidants protect against free radical-induced oxidative stress, which is a major cause of photodamage, inflammation and carcinogenesis (9, 10). Other benefits associated with their use include improved skin fibroblast synthesis, water retention and cell renewal. Some of the most effective antioxidants are retinol (vitamin A), niacinamide (vitamin B₃), ascorbic acid (vitamin C), tocopherol (vitamin E), ubiquinone and flavonoids (e.g., grape seed and green tea extracts) (6). Growth factors have an active role in dermal matrix wound healing. Additionally, findings from clinical studies indicate that some of these compounds may also have the ability to stimulate collagen production (6). Anti-inflammatory agents inhibit the release of proinflammatory cytokines by blocking the activity of the enzymes cyclo-oxygenase and lipo-oxygenase. Among others, they include icodocalcione A, derived from liquorice, and silymarin, found in milk thistle (6). Polysaccharides (e.g., glycolic acid, lactic acid, gluconolactone) are known for their humectant, moisturizing and exfoliating properties (6). Pigment-lightening agents aid in fading hyperpigmentation and unifying skin tone. Among the most frequently used is hydroquinone, which inhibits melanin synthesis by blocking the activity of the enzyme tyrosinase (6). Finally, we have peptides which consist of short amino acid sequences that are subunits of larger protein molecules. They are designed to interfere with some of the most important processes responsible for skin aging and its visible manifestations. For example, they can increase collagen production, inhibit repeated muscle movements, and stimulate wound repair (6).
PEPTIDE CHARACTERISTICS AND TYPES

Similar to proteins, peptides are organic compounds made of chains of amino acids connected by peptide bonds. However, they differ from proteins in that they are markedly smaller. They consist of two, three, a few (3 to 10) or many amino acids, and are referred to as dipeptides, tripeptides, oligopeptides and polypeptides, respectively [11]. Proteins are typically made of one or more polypeptides. There is a characteristic resemblance between the amino acid sequences of peptides and those contained in larger proteins like collagen. This has led to the idea that peptides could increase the production of this particular component of the extracellular matrix (ECM) and, consequently, mitigate the visible signs of aging associated with collagen depletion [12]. Indeed, studies have confirmed that peptides have excellent collagen building properties [13], which places them among the most groundbreaking anti-aging ingredients available. Although they are not tested in the same way as most mainstream FDA-regulated compounds [14], the evidence supporting their benefits is increasing, making their use in skin care extremely desirable. Three main types of cosmeceutical peptides are available: carrier peptides, neurotransmitter-affecting peptides, and signal peptides [14].

Carrier Peptides

Carrier peptides play a key role in delivering essential metals to the skin, particularly copper, an important cofactor in the biosynthesis of collagen and elastin via activation of lysyl oxidase, downregulation of matrix metalloproteinases (MMPs) and anti-collagenase activity [6, 14]. The tripeptide glycy-l-histidy-l-lysine (GHK) spontaneously binds copper to form a copper-tripeptide complex, which facilitates the uptake of the metal at the cellular level. This, in turn, has been shown to stimulate collagen synthesis, resulting in reduced wrinkles, increased skin firmness, and improved texture [14, 15]. Notably, copper tripeptide also increases levels of MMP-2 and MMP-2 messenger RNA, as well as the secretion of the metalloproteinase inhibitors TIMP-1 and TIMP-2, which suggests a role in collagen remodeling [14, 16]. Another carrier peptide with MMP synthesis suppressing activity used in skin care formulations is dipalmitoyl hydroxyproline (17).

Neurotransmitter-Affecting Peptides

The hexapeptide sequence acetyl-glutamyl-glutamyl-methoxyalanyl-glutamyl-arginy1-arginylamide (acetyl-hexapeptide-3 or Argireline) is a well-known member of the neurotransmitter-affecting group. Its main clinical effect is to reduce the appearance of lines and wrinkles caused by the repeated movement of facial muscles over time [14]. There is evidence that the mechanism of action is related to the inhibition of calcium-dependent release of acetylcholine at the neuromuscular junction, via interference with the soluble N-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) complex [18, 19].

Signal Peptides

Signal peptides are involved in the repair mechanisms of human dermal fibroblasts following skin injury. One such compound is the elastin-derived palmitoyl oligopeptide. A combination of palmitic acid and the hexapeptide valine-glycine-valine-alanine-proline-glycine (VGVAPG), palmitoyl oligopeptide has demonstrated the ability to stimulate fibroblast synthesis and proliferation [21]. The pentapeptide sequence lysine-therine-arginine-asparagine (PHSRN) sequence contained in this therine-lysine-serine (KTTPS) increases the production of collagen and fibronectin [22]. Further, in combination with palmitic acid, it has recently been reported to attenuate facial wrinkles, in both in vitro skin models and clinical patient cohorts [23]. Available signal peptides also include palmitoyl tetrapeptide-7, dipeptide-2, and the complex palmitoyl oligopeptide-palmioty oligopeptide tetrapeptide-7 (Matrixyl 3000), which have been associated with improved skin firmness and elasticity, enhanced toxin elimination, and increased ECM synthesis, respectively [24]. Some signal peptides have demonstrated anti-collagenase activity and, consequently, have the potential to reduce collagen breakdown [25]. Finally, palmitoyl tripeptide-38 and palmitoyl tripeptide-5 have been shown to be highly effective at stimulating the production of collagen and hyaluronic acid, and promoting dermal thickening [24].

NEXT-GENERATION AGENTS

While cosmeceutical peptides in general are considered a major advance in the clinical management of aging skin, arguably the most revolutionary are the newest synthetic signal sequences palmitoyl tripeptide-38 and palmitoyl tripeptide-5. The research to date suggests that these compounds are among the most scientifically advanced ingredients for use in high-performance skin care formulations that aim at promoting the key processes that lead to healthier, younger-looking skin.

Palmitoyl Tripeptide-38

Palmitoyl tripeptide-38 (Matrixyl synthe’6) is a matrikine-mimetic compound with significant ECM building and strengthening properties, that regulates cell activity, wound repair, and collagen tissue remodeling [26]. As a matrikine mimic, palmitoyl tripeptide-38 activates signaling pathways that initiate the cascade of biochemical processes involved in the synthesis of the following key constituents of the ECM [26, 13]:

- Together with type I and III, this protein accounts for 80 to 90 percent of the total amount of collagen in the human body. It is the major component of the dermis and is typically found in the skin, tendons, and bone ligaments, among other tissues [27].
- Often referred to as “collagen of youth” because of its ability to enhance skin smoothness and elasticity, this type of collagen is produced by immature mesenchymal cells in the earliest phases of wound healing, before type I collagen synthesis initiation [28]. It is mostly found in elastic tissues, including the skin, muscles, and blood vessels [27]. The amount of type III collagen in the skin diminishes with age in favor of collagen I [29].

- Collagen IV. This is the primary component of the extracellular basement membrane that separates epithelial and endothelial cells, and of the dermoepidermal junction [30]. It has important wound healing stimulation properties [30]. These result from its capacity to interact with keratinocytes, which induce the secretion of granulocyte-macrophage colony stimulating factor (GM-CSF) and, consequently, epidermal cell proliferation immediately after a skin injury [31].
- Fibronectin. A non-collagenous glycoprotein relatively abundant in the ECM [32]. Fibronectin has been shown to increase in vivo activity levels of bone morphogenetic protein-1-like proteinases, which play a critical role in ECM production [32]. The proline-histidine-serine-arginine-asparagine (PHSRN) sequence contained in this
glycoprotein regulates fibroblast adherence, migration, growth, and differentiation (33). It also appears to have a role in the stimulation and acceleration of in vivo tissue repair (34).

- **Hyaluronic Acid.** Also referred to as hyaluronan or HA, hyaluronic acid is a highly versatile component of the ECM with excellent bio-compatibility, non-immunogenicity, biodegradability, and viscoelasticity (35). Known to facilitate wound repair, hyaluronic acid is thought to improve skin moisture and elasticity. Additionally, it has been associated with long-lasting smoothing effects and facial wrinkle reduction due to its capacity to attract and retain water and fill space (17, 35). Hyaluronic acid has a demonstrated ability to facilitate the delivery of active ingredients to the inner layers of the skin, enabling them to exert their beneficial effects from the inside (35). Its free radical scavenging properties indicate that it may also have a role in UV protection (35).

- **Laminin-5.** Alongside collagen and proteoglycans, laminins are important components of the basement membrane. Laminin-5 is an adhesion glycoprotein specific to epithelial tissues where it complexes with heparansulfomucos, thus contributing to the mechanical processes that anchor the epithelium to the dermis (36, 37). It also regulates keratinocyte migration, facilitating the healing of injured epidermis (38).

- **Heat shock protein 47 (HSP47).** One of the glycoproteins produced in the endoplasmic reticulum and Golgi apparatus of collagen-producing cells (39), the expression of HSP47 is upregulated in stress conditions such as exposure to extreme temperatures, heavy metals and oxidative stress (39). HSP47 is an essential player in the biosynthesis of collagen (40). It has a well-documented role of “molecular chaperone” in the dissociation of newly-produced procollagen molecules (41) and their subsequent folding and assembling after injury (42).

Quantitative measures of the stimulatory activity of palmitoyl tripeptide-38 on the above ECM components – type I, III, and IV collagen; fibronectin; hyaluronic acid; laminin-5; and HSP47 – have been performed in vitro tests (13) that examined the effects of twice daily applications of a formulation containing two percent Matrixyl synthe’6 on skin explants for five days. They found that type I, III, and IV collagen significantly increased (p<0.01) by 105, 104, and 42 percent, respectively (13). Hyaluronic acid, HSP47, laminin-5, and fibronectin also increased significantly (p<0.01), by 174, 123, 75, and 59 percent, respectively. Other in vitro analyses found significant increases (p<0.01) of 20 and 37.5 percent in type I collagen synthesis in non-aged skin explants and experimentally aged ones (29).

Importantly, the above effects translate into visible improvements on the skin’s surface. Of particular significance in this regard are the results of a placebo-controlled clinical study of 25 women aged 42 to 70 years. In this study, a formulation containing two percent palmitoyl tripeptide-38 was applied twice daily for two months on two facial skin areas especially prone to wrinkling: the forehead and the crown of the head. In the former, wrinkle volume and depth decreased by 31 and 16.3 percent, respectively, whereas lifting improved by 28 percent. In the latter, wrinkle surface, volume, and maximum depth diminished by 28.5, 21.1, and 15 percent, respectively; lifting improved by 12.6 percent. It is worth noting that the reductions in wrinkle depth observed in both facial skin areas are especially important, given that this parameter is the most significant factor influencing the visual perception of wrinkles (13).

**Palmitoyl Tripeptide-5**

Palmitoyl tripeptide-5 mimics the action of thrombospondin 1 (TSP-1), a multifunctional adhesive glycoprotein of the ECM (43), that stimulates the production of type I and II collagens, and fibronectin via the activation of tissue growth factor β (TGF-β) (44, 4). For this reason, palmitoyl tripeptide-5 is considered a key collagen building ingredient for anti-aging skin formulations aiming at attenuating lines and wrinkles, and correcting laxity. The collagen building properties of palmitoyl tripeptide-5 were originally observed in in vitro studies of human fibroblasts. These findings that fibroblast cultures treated with the novel tripeptide showed an increase in collagen synthesis of 119 percent, compared with cultures treated with standard TGF-β (4). In a clinical study, 60 volunteers received twice daily applications of a product containing 2.5 percent palmitoyl tripeptide-5. After 84 days, the study participants showed significant visible reductions in average skin roughness and wrinkle depth (45). The skin smoothing effects of the tripeptide were confirmed in another clinical study that included 37 volunteers with mild to moderate fine and coarse periorcular and perioral wrinkles (17). Twice daily applications of a palmitoyl tripeptide-5-containing formulation on the affected areas significantly reduced (p<0.003) mean wrinkle severity by more than 50 percent. Visible improvements were observed within as little as 15 minutes from the first application and peaked at three months – the duration of the treatment. In both clinical studies, palmitoyl tripeptide-5 was found to be safe and well tolerated, with no major side effects (17, 45).

**ACHIEVING OPTIMAL PERFORMANCE**

While strong collagen building properties make newer peptides essential ingredients of modern anti-aging skin care formulations, a number of key factors need to be considered to ensure maximum efficacy and safety. Using the correct amounts of peptide is one such factor. For example, the recommended use level is 10 percent for Argireline (24), two percent for palmitoyl tripeptide-38, and one to three percent for palmitoyl tripeptide-5 (13, 45). Practicing dermatologists should proactively seek this information from manufacturers, or refer to the product monograph of the specific peptide they are interested in using. A further important factor to consider is that, although peptides have intrinsic efficacy, this can be enhanced to achieve optimal performance by adding other cosmeceuticals to formulations; antioxidants are especially important in this regard. As described earlier, antioxidants can attenuate and prevent the negative effects of oxidative stress (9, 10), which is not only a cause of premature skin aging and disease, it can also damage the active ingredients that may be present in a formulation. Hence, the addition of antioxidants helps ensure that the peptide will actually work, while minimizing the risk of photodamage to the skin. Examples of compounds with these properties are grape seed extract and epigallocatechin gallate (EGCG), a key polyphenol contained in green tea leaves. Both have demonstrated potent protective effects against oxidative stress (46, 47). In addition, there is some evidence that EGCG can increase dermal thickness by inducing keratinocyte proliferation, whereas grape seed extract can promote dermal wound repair via the stimulation of vascular endothelial growth factor expression (6).

Several other antioxidants are considered important co-ingredients of peptide-containing formulations. For example, retinal has been associated with improved water retention,
cell renewal, and fibroblast synthesis, as well as reduced collagen breakdown, resulting in fewer lines and wrinkles [6]. The antioxidant niacinamide reduces transepidermal water loss (TEWL), increases collagen production, and inhibits oxidative glycation of proteins. Overall, these effects contribute to improved skin tone and texture, reduced lines and wrinkles, and diminished yellowing of the skin (Maillard reaction) [6]. Ascorbic acid promotes wound repair and fibroblast regeneration, prevents inflammation, and is thought to have skin lightening properties [6]. Tocopherol provides photoprotective effects and acts as a humectant [6].

Another key ingredient is calcium hydroxymethionine (hydroxymethionine-Ca). A natural analogue of methionine, this compound can chelate calcium, acquiring the ability to stimulate keratinocyte proliferation, which plays a key role in maintaining epidermal cell cohesion [48]. Since the proliferation and turnover of keratinocytes naturally diminishes with age [49], hydroxymethionine-Ca represents a desirable addition to anti-aging products, increasing firmness and consequently the appearance of loose skin.

CONCLUSION

Cosmeceutical peptides have become increasingly popular in dermatology in recent years. They combine strong collagen building properties with the ability to reduce repeated muscle movements and promote wound repair, conferring excellent performance to skin formulations aimed at mitigating the visible signs of aging. Two newer peptides in particular have been associated with significant benefits in studies: palmitoyl tripeptide-38 and palmitoyl tripeptide-5. Both are considered essential agents for the production of collagen, a key component of the ECM. As such, they can assist in maintaining and improving the natural elasticity, thickness, and smoothness of the skin; reduce the appearance of lines and wrinkles; and promote wound repair and regeneration after injury. These effects are enhanced by the remarkable ability of palmitoyl tripeptide-38 to stimulate the production of other major constituents of the ECM, including fibronectin, hyaluronic acid, laminin-5, and HSP47. To ensure efficacy and safety, it is important to verify that formulations contain the amount of a particular peptide recommended by the manufacturer. Optimal product performance can be achieved by using peptides in combination with other cosmeceuticals, such as certain antioxidants (e.g., grape seed extract, epigallocatechin gallate, retinol, and niacinamide) and hydroxymethionine-Ca, among others.

REFERENCES AND NOTES