

Bioactive peptides: signaling the future

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Summary

Natural processes within the body are modulated almost exclusively by the interaction of specific amino acid sequences, either as peptides or as subsections of proteins. With respect to skin, proteins and peptides are involved in the modulation of cell proliferation, cell migration, inflammation, angiogenesis, melanogenesis, and protein synthesis and regulation. The creation of therapeutic or bioactive peptide analogs of specific interactive sequences has opened the door to a diverse new field of pharmaceutical and active cosmetic ingredients for the skincare industry. Here, we describe the origin of such sequences, their role in nature, their application to dermatology, as well as the advantages and challenges posed by this new technology.

Keywords: bioactive peptides, active ingredient, acne, antiaging, anti-inflammatory and wound healing

Introduction

The current understanding of peptides in both the consumer skincare and cosmetic dermatology marketplaces is generally tied to applications for collagen stimulation and “Botox-like” wrinkle-smoothing effects. This limited perspective is often based upon the widespread use and promotion of products containing commercial ingredients such as Matrixyl™ (Sederma) and Argireline® (Lipotec). However, as the critical and ubiquitous involvement of peptides in all aspects of skin homeostasis becomes better defined and appreciated, we should anticipate significant

expansion in the application of bioactive peptides in the field of dermatology.

As the majority of all natural processes within the body are signaled or modulated exclusively by the interaction of specific amino acid sequences, either as peptides or as fragments of proteins, peptides hold future promise for a wide range of therapeutic applications. For use in mimetic therapies, peptides can be readily and almost infinitely modified through amino acid substitution and modification. This attribute separates peptide technology from virtually all other ingredients and therapeutics for managing challenges relating to potency, solubility, toxicity, specificity, formulation, skin penetration, and cost. Conversely, strategic manipulation of other small molecules and natural products usually requires significant chemistry and is, more often than not, impractical. As a result, bioactive peptides provide not only an extraordinarily wide range of active ingredients, but a technology platform capable of being developed and targeted to form-fit specific skin types, skin colors, skin ages, and skin conditions. Here, we discuss the potential of this technology platform and the array of sources within skin biology that are being, and can be, accessed and exploited for application to skin care.

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Cited companies

Allergan, Inc. (Irvine, CA)

Atrium Biotechnologies (Quebec City, Canada)

Grant Industries (Elmwood, NJ)

Lipotec (Barcelona, Spain)

Pentapharm (Basel, Switzerland)

Sederma (Le Perray en Yvelines, France)

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Wound healing peptides for treatment of the aging face

The basement membrane that separates the epidermis and the dermis is rich in extracellular matrix (ECM) proteins including collagens, epiligrin, laminin, fibronectin, elastins, and heparin sulfate proteoglycans.¹ In addition to anchoring cells and organs, the ECM serves as a mediator of receptor-induced interactions between cells, guiding growth, and differentiation. Damage to the matrix causes repair to be initiated through processes such as protein synthesis and cell differentiation and proliferation. Most of these functions are related to signaling by peptides that are released from the ECM to cells through cell membrane receptors. Chronologically, aged skin shows decreased production of new collagen and increased proteolytic activity resulting in increased collagen degradation. Senescent fibroblasts show decreased synthesis of type I collagen and proliferate at a much slower rate than fibroblasts in young skin. Therefore, peptides modeled on repair signaling sequences such as palmitoyl pentapeptide-3 (Sederma, Le Perray en Yvelines, France) and palmitoyl oligopeptide (Sederma) have been developed as cosmetic ingredients that enhance skin rejuvenation.

Palmitoyl pentapeptide-3 originates from pro-collagen and stimulates production of collagens I and III in addition to fibronectin.^{2–4} Palmitoyl oligopeptide is an elastin sequence and stimulates the growth of fibroblasts and accelerates angiogenesis.⁵ The next generation of such signal inducing peptides has been designed to amplify such signals and direct those signals to multiple ECM components producing a larger and more homogeneous response of repair. These peptides have been termed Replikines™ and Combikines™.⁶

Peptides involved in the body's system of innate immunity respond to physical damage and initiate and modulate the restoration of barrier function. For example, an endogenous peptide of the innate immune system termed LL-37 stimulates wound vascularization and re-epithelialization of healing skin in an animal model.⁷ Recently, specific peptide subunits of LL-37 that induce growth factor-mediated keratinocyte proliferation and migration have been identified.^{7,8} Acceleration of wound healing has also been demonstrated with other innate immunity peptides such as a derivative of Cecropin B.⁹ A peptide, palmitoyl hexapeptide-14 (Grant Industries, Elmwood Park, NJ), designed using an innate immunity peptide template has been shown to stimulate cell migration, collagen synthesis, and fibroblast proliferation and scaffolding. The multifunctional nature of innate immunity peptides and fragments thereof hold potential for a wide range of applications and have already been

clinically demonstrated to show improvements in signs of aging such as fine lines and wrinkles and skin firmness.

It is generally assumed by the cosmetics industry that inflammation hastens skin aging.¹⁰ Dehydroepiandrosterone (DHEA), a secretory product of the human adrenal gland, has been characterized as exhibiting a wide array of therapeutic benefits including slowing of the aging process¹¹ accelerating wound healing, and reducing inflammation via the interleukin-6 (IL-6) pathway. Although DHEA levels decline with age, the benefits of supplementation of DHEA for combating the effects of aging have yet to be fully proven in humans. However, a group of peptides derived from DHEA called riginins have been shown to modulate cytokine levels and to down-regulate IL-6.¹² One such peptide, palmitoyl tetrapeptide-7, has been developed as an active ingredient by Sederma marketed as Rigin™. The ability of Rigin™ to down-regulate IL-6 was compared to DHEA *in vitro* in both resting and inflamed cells and the two actives were comparable. Marketing materials related to Rigin™ indicate that this reduction in IL-6 can produce increased skin firmness, smoothness, and elasticity.

Peptide mimetics: a proposed alternative to Botulinum neurotoxin

Botulinum neurotoxins (Botox®, Allergan, Irvine, CA) cause muscle paralysis by blocking acetylcholine release at nerve-muscle junctions through a very specific and exclusive endopeptidase activity in the presynaptic exocytosis machinery. In search of a topical alternative to botulinum neurotoxin, synthetic peptides that emulate the amino acid sequence of the synaptic protein SNAP-25 were shown to be specific inhibitors of neurosecretion at micromolar concentrations. To avoid the need for injection of material and facilitate membrane permeability, new sequences that are shorter while preserving a biological activity have been pursued. A six-amino-acid peptide derived from SNAP-25 has been shown to produce the desired interference with the neurosecretion.¹³ This hexapeptide (acetyl hexapeptide-3) is marketed under the name Argireline® (Lipotec, Barcelona, Spain). A clinical study published in the *International Journal of Cosmetic Science* reported that acetyl hexapeptide-3 at a 10% concentration reduces the depth of wrinkles up to 30% after 30 days of use.¹⁴ These findings suggest that the hexapeptide is a bio-safe cosmetic alternative to reduce facial wrinkles and it is reasonable to expect that other more potent peptide-based mimetics of toxin effects will be identified in the future.

Another source of such bioactive peptides is based upon the observation that many venomous organisms

produce toxins that disrupt neuromuscular communication in order to paralyze their prey. Waglerin-1 from the venom of Wagler's pit viper, *Tropidolaemus wagleri*, is a 22-amino-acid peptide that causes paralysis by competitively antagonizing muscle acetylcholine receptors.¹⁵ A synthetic tripeptide that mimics the effect of Waglerin-1 has recently been marketed as Syn-ake® (Pentapharm, Basel, Switzerland) for reducing wrinkles by inhibiting muscle contractions. Acting at the postsynaptic membrane, Syn-ake® is a reversible antagonist of the muscular acetylcholine receptor.

Peptides of the innate immune system for treatment of acne and dermatoses

The aforementioned peptides of the innate immune system also play a significant role in protection against pathogens and toxins. Peptide-induced activities include modulation of inflammation, binding of toxins, and neutralization of bacteria and fungi. Inflammation associated with skin conditions that have bacterial involvement is often, partially, due to lipopolysaccharide (LPS) released from the outer membrane of Gram-negative bacteria and lipoteichoic acid (LTA) released from Gram-positive bacteria. Innate immunity peptides such as defensins and LL-37 are well known for binding and neutralizing bacterial debris including LPS and LTA, resulting in down-regulation of pro-inflammatory cytokines.¹⁶ Another example comes from granulysin-derived peptides that suppress *Propionibacterium acnes*-stimulated cytokine release.¹⁷ A synthetic peptide designed to bind LTA, oligopeptide-10 (Grant Industries), has been developed for inclusion in topical anti-acne treatments. Oligopeptide-10 has also shown potential in mitigating symptoms associated with yeast and fungi colonization, including dandruff and seborrheic dermatitis and tinea pedis.¹⁸

As technology evolves, we should anticipate opportunities to combine bioactive peptides to more comprehensively address specific skin conditions. For example the combination of oligopeptide-10 with a peptide capable of down-regulating cytokine-mediated responses involving IL-6 and IL-8 may have multiple applications, particularly for sensitive skin. Such a product would combine the benefit of binding pro-inflammatory toxins with a reduction of redness that has already been initiated.

Recent findings also suggest that lack of modulation of peptides of the innate system may contribute to a sundry of skin conditions.¹⁹ Notably, there is mounting evidence that overexpression of a pro-inflammatory component of LL-37 may be an important contributor to rosacea.²⁰ Thus, identification of therapeutic modalities that reduce

the activity of such pro-inflammatory peptides on skin may hold future therapeutic promise.

Potential application of peptides in pigmentation modulation

Ultraviolet (UV) radiation stimulates melanogenesis by human epidermal melanocytes, both in the skin and in cultured cells. Increased production of epidermal melanin can lead to mottled and spotty hyperpigmentation and a more aged appearance. Conversely, lack of pigment can result in UV damage of the skin, in some cases leading to melanoma. There is evidence that the melanocortin-1 receptor (MC1-R) is a key control point for skin pigmentation.²¹ Using peptide analogs of α -MSH, acting as MC1-R agonists, certain peptides have been shown to be more potent than α -MSH in stimulating melanogenesis, reducing apoptosis and release of hydrogen peroxide and enhancing repair of DNA photoproducts in melanocytes exposed to UV radiation.²² Skin keratinocytes also express factors that are involved in melanogenesis. Keratinocyte protease-activated receptor 2 (PAR-2) has been shown to affect melanosome transfer from melanocyte to keratinocyte. PAR-2 activating peptide, SLIGRL, enhances melanosome ingestion by keratinocytes, thus increasing pigmentation.²³

In contrast, the human homolog of agouti-signaling protein (ASIP) blocks the binding of α -MSH to the MC1-R and inhibits the effects of α -MSH on human melanocytes.²⁴ Treatment of human melanocytes with recombinant human ASIP blocks the stimulatory effects of α -MSH on cAMP accumulation, tyrosinase activity, and cell proliferation. Direct tyrosinase inhibition has been identified in select peptide sequences such as the cyclic peptide, cyclo(Pro-Val-Pro-Tyr). Although an effective tyrosinase inhibitor, this has not been pursued for clinical study due to the difficulty in large-scale synthesis.

For inhibition of melanogenesis, peptide conjugates have also been studied for their ability to potentiate the activity of nonpeptide molecules. For example, the addition of a tripeptide to kojic acid exhibited 100-fold inhibitory activity of tyrosinase compared with kojic acid alone. In addition, the storage stability was improved approximately 15-fold and toxicity reduced over the parent kojic acid molecule.²⁵

Discussion

The requirements for an effective and safe dermatological therapeutic or active ingredient are the same no matter the origin of the molecule or its intended indication. These requirements are as follows:

- 1 The molecule exhibits a proven specific beneficial bioactivity that would lead to a rational demonstrable effect.
- 2 The bioactivity does not have a negative consequence either theoretically or experimentally due to its mechanism of action.
- 3 The molecule does not exhibit toxicity such as cytotoxicity, irritation, immunogenicity, or mutagenicity.
- 4 The molecule is capable of reaching its desired target intact and in its active form.
- 5 The molecule can be formulated in such a way as to be stable, compatible with other components, and be delivered effectively to the skin.

Collectively, these are not easily achieved criteria. For a new technology paradigm to emerge, these criteria not only have to be met but be applicable across the wide range of product-acceptable bioactivities.

Peptides have significant advantages over many other technologies in addressing these criteria primarily based upon their chemistry. This is not to say that traditional technologies are inferior, but the potential for peptides to significantly add to what is currently available is immense.

Peptides consist of chains of amino acids which can be modified in innumerable ways to increase receptor binding, increase specificity, decrease toxicity, and increase skin penetration, stability, and solubility. In this way, the field of bioactive peptides for dermatological applications has changed significantly in recent years. From humble beginnings of a single peptide capable of stimulating collagen, technological advances have created newer peptides capable of targeting most aspects of dermal health. These advances include neutralizing

toxins, stimulating fibroblast scaffolding, reducing inflammation, and other desirable effects.

As an aging population is seeking an ever-increasing breadth of bioactivity and increased potency from the ingredients in skin care products, consumers demand more explanation of cosmetic science and underlying technology. This has led to an emphasis on a science-based focus in functional cosmetic ingredients. Activities that result in diminished lines and wrinkles, smoother skin texture, and reduced redness and skin discoloration are the key target endpoints. Providing this functionality are antioxidants, growth factors, peptides, anti-inflammatories, polysaccharides, and pigment-lightening agents. As described above, the role of peptides in a wide range of processes is becoming more fully understood and the potential application of such activities more fully appreciated. However, to maintain that activity in a product and subsequently translate that activity into a beneficial effect for the consumer creates issues that also need to be addressed. Peptide concentration should be supported by clinical studies and product-specific studies.

The compatibility and stability of a bioactive peptide within a cosmetic formulation can provide significant clues as to whether it can be delivered in an active form. Binding, particularly of charged peptides, by other ingredients may prevent release from the formulation or prevent the peptide from being released in an active form. If the desired bioactivity can be demonstrated by the formulated peptide or, by the use of a Franz Cell, be demonstrated to be released from the formulation in an active form, a level of comfort can be achieved. For example, Fig. 1 (a) shows different peptides released from the same

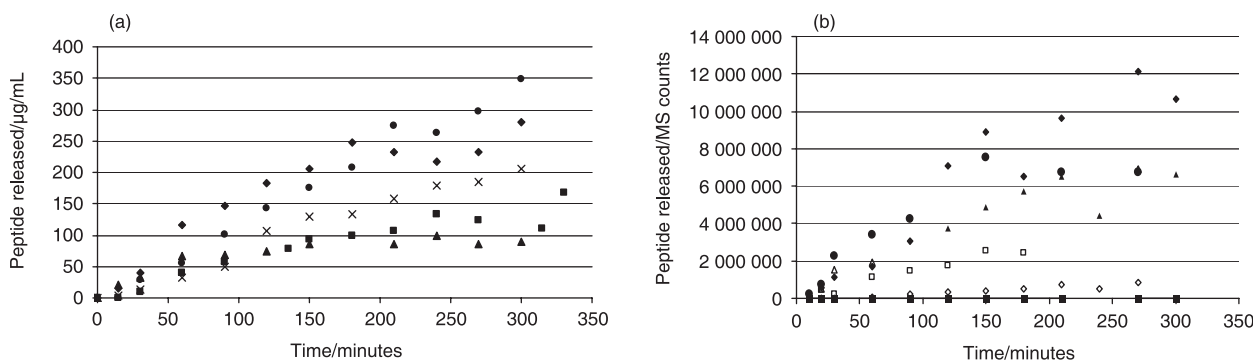


Figure 1 (a) Release of a range of distinct peptides from a single formulation as determined using standard Franz Cell and mass spectroscopy (MS) protocols. Key: X 17 residue + 4 charge linear peptide; ◆ 15 residue + 6 charge linear peptide; ▲ 6 residue + 2 charge linear peptide; ● 6 residue + 2 charge lipidated peptide; ■ 6 residue + 2 charge hydrophobic linear peptide. (b) Release of a single peptide from a range of chemically distinct formulations as determined using standard Franz Cell and MS protocols. Key: Neutral aqueous gel ◆; negatively charged aqueous gel ■; emulsion ▲; emulsion ●; cream ◇ and moisturizer □.

formulation, and Fig. 1(b) shows release data for the same peptide released from a range of different formulations. These data demonstrate that the delivery of peptide must be tested on a case by case basis.

Because human skin functions as a physicochemical barrier, it has been historically assumed that molecules over 500 MW are unable to traverse the stratum corneum (SC).²⁶ More recent studies have demonstrated that this paradigm does not hold true, particularly in the case of dry or aged skin.^{27,28} In addition, with the advent of newer and more potent penetration enhancers, either peptide or chemical in origin, larger and larger molecules are being transported.^{29,30} Magainin, an antimicrobial peptide over 10 amino acids in length, actually facilitates the uptake of molecules into and through the stratum corneum.²⁵ With the advent of more and more sophisticated software, there is considerably more predictability in estimating a compound's behavior with respect to skin penetration. Ham *et al.*³¹ demonstrated that, by simple amino acid substitution, skin penetration of peptides may be significantly increased. Stability of small bioactive peptides can be increased by protection against exoproteases. The cost of synthesis of natural sequences can be reduced by conservative substitutions with less expensive amino acids. The charge, hydrophobicity, and amphipathicity of peptides can all be modified by sequence changes to facilitate addition to specific formulations based upon pH requirements or reactivity with other components.

Finally, the location of the site of action is a key driver in the development of bioactive peptides. The two extremes are exemplified by oligopeptide-10, a peptide designed to bind LTA on the surface of the skin and in pores. At the other extreme, there is acetyl hexapeptide-3, the bioactive peptide from Argireline®, the target of which is SNARE complex formation, which takes place in the motor neuron. Clearly, the latter has a greater challenge in reaching the target.

The application of bioactive peptides to the cosmetics industry holds great promise due to a wide range of activities, chemistries and indications that can be developed. The cost to benefit ratio will depend on ability to optimize the amino acid sequences for maximal bioactivity and targeted benefits.

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