



SERIES IN  
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# COSMECEUTICAL SCIENCE IN CLINICAL PRACTICE



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## 2 Proteins and Cytokines Used for Rejuvenation

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### INTRODUCTION

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

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

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

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

### PEPTIDES AS STIMULANTS FOR REJUVENATION

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

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

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

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

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

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

### PEPTIDES INVOLVED IN RETINOID EFFECTS

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

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

#### TOPICAL GROWTH FACTORS

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

#### MECHANISM OF ACTION OF LASER/HEAT SHOCK PROTEINS

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

#### PEPTIDES AS ANALOGS OF NEUROMUSCULAR INHIBITORY MOLECULES

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

#### PEPTIDES AS REPLACEMENT MOLECULES FOR COLLAGEN AND ELASTIN

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

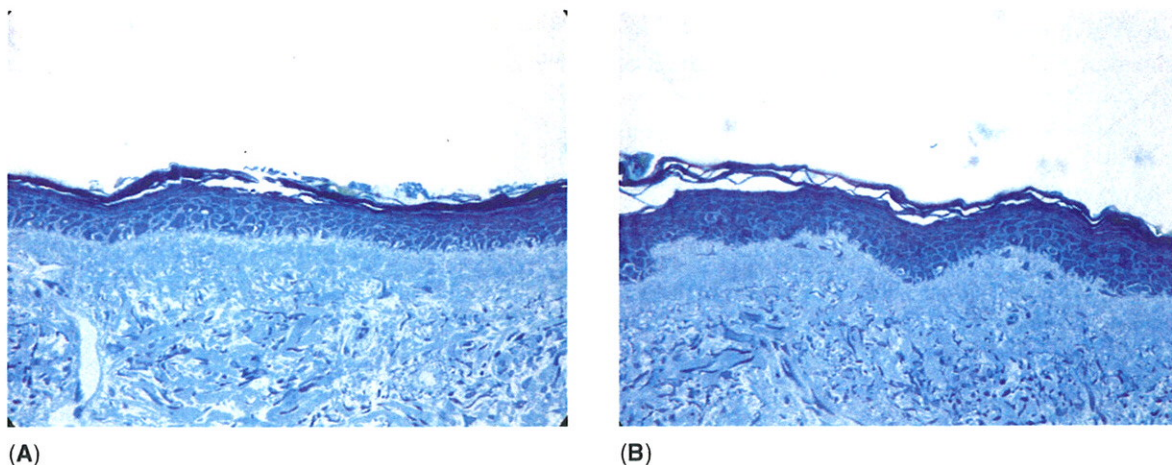


Figure 2.1 (A, B) Examples of histology obtained by light microscopy indicating a thickened epidermis, a slightly improved dermal-epidermal junction as well as a significantly increased cellularity, particularly in superficial dermis, reflecting new collagen synthesis at the end of the six-month treatment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

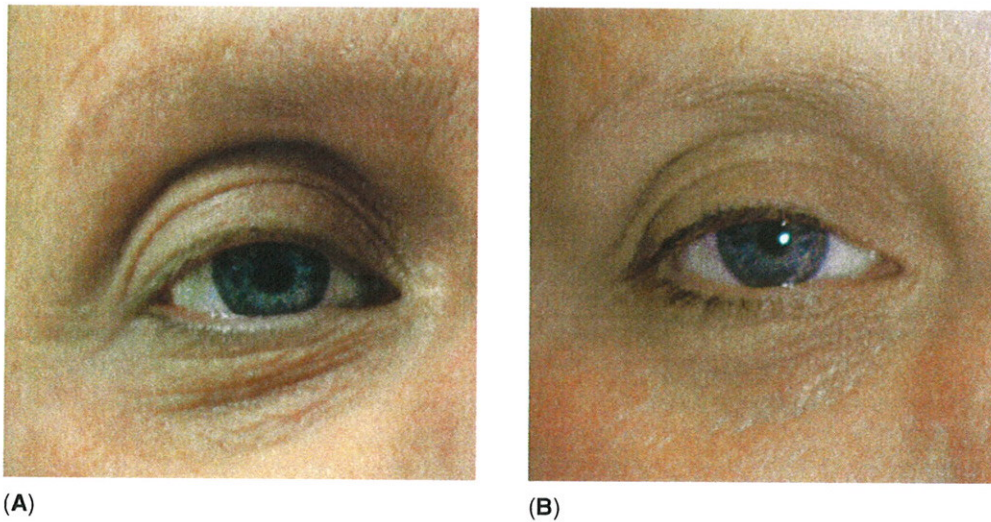


Figure 2.2 Photographs taken (A) before and (B) after six consecutive weeks of twice-daily application of the human growth factor and cytokine containing eye cream demonstrating reduced dark circles after the treatment period. Source: From Ref. 8. Courtesy of Dr. Mary L. Lupo, Dr. Joel L. Cohen, Dr.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

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

#### CYTOKINES AND SIGNAL PEPTIDES AS STIMULANTS OF MORE YOUTHFUL CELLULAR MACHINERY

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

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

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

#### CONCLUSION

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

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