

## The use of synthetic polypeptides in cosmetics

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### Abstract

*Cosmetic peptides are one of the active components of modern day cosmetic preparation. Peptides are short chains sequences of amino acids. Amino acids are the basic building blocks of proteins and many other different types of organic molecules. Many skincare products use peptides to treat wrinkles. There are three main groups of anti wrinkle peptides: signal peptides, neurotransmitter-affecting peptides and carrier peptides. This article reviews the most popular peptides used in cosmetics. According to them established own sequences of peptides which were synthesized and used in the subsequent studies.*

### 1. Introduction

On the cosmetics market appeared peptides, which have to replace the popular hydrates and hydrolysed collagen and elastin. Peptides are transmitters of information in the tissues, which makes it a great demand for this type of ingredients used in cosmetology and pharmacy. Peptides in cosmetics are usually called biomimetic peptides, which means substances that mimic the mechanism of action and the effects exerted by their natural counterparts. Introduction to cosmetic products of peptides is the result of research conducted *in-vitro* and *in-vivo*, confirming the therapeutic action of these substances in many biological processes.

### 2. Background

As far back as the 1930s, yeast extracts have been used in medications for their enhancement of wound healing [1]. As technology progressed, this allowed a protein fraction to be recovered from the extract of fermented yeast. Through studies of this protein extracts, a beneficial effect on wound healing and improved collagen

synthesis has been demonstrated [2, 3]. To date, more than 500 assorted proteins have been identified from the yeast *Sachcaromyces cerevisiae* [4]. Through these research it was noted that peptides, which have low molar mass, are the growth of the yeast enzyme factories. The peptides are theorized to up-regulate cellular growth factors leading to skin healing as a result of the simulation of angiogenesis and granulation tissue and new collagen synthesis [5]. From this early events, peptides began to existing as ingredients in cosmetics.

### Peptides in cosmetics

Peptides as components of cosmetics are small particles consisting of 6-7 amino acids, but there are also exceptions to this rule (8- and 20- peptides have been found). Peptides are characterized by small molecular weights (500-1000 Da). Short molecules more easily penetrate the epidermis. Long linear molecules, more or less branched, more difficult to penetrate the cells of the stratum corneum. Peptides, depending on the form of the cosmetic product can penetrate directly through the epidermal cells or between cells on a curvy road lipid intercellular cement. Finally, locate in the dermis, and the speed of this process depends mainly on the chemical nature of the amino acid and unnatural hydration of the stratum corneum, for example through dressing occlusion. At that time, hydrophilic substances have the greatest ability to penetrate and remain in the aquatic ground.

Lipophilic substances penetrate and remain in the intercellular cement. The easiest way to penetrate have the particles with the amphiphilic properties.

Some peptides are able to regulate the components produced by fibroblasts, and after the introduction into the dermis, we can observed significantly improve the appearance of the skin profile [6]. In addition, peptides have capabilities of binding water in their structure, they are also excellent humidifiers. This property is used in the preparations, which improving hydration and barrier function or even relaxing mimetic wrinkles. All through primary timbers of peptides – amino acids, which are a component of the natural factor dampening NMF [7]. Therefore concluded that the peptides are active in both the epidermis and dermis (fig. 1-3).

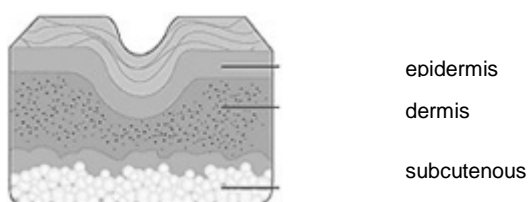


Fig. 1. Construction of human skin [8]

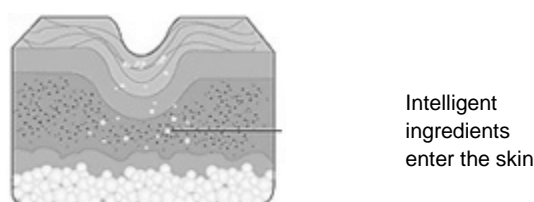


Fig. 2. Peptides pass through the skin layers [8]

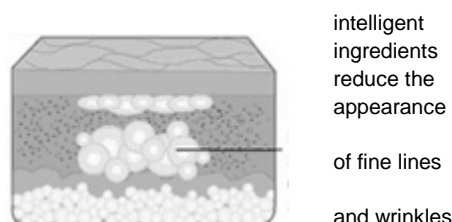


Fig. 3. The effect of active peptides [8]

During studies the role of peptides in the body and with the development of these studies, there was an opportunity to use

peptides as a selectively acting biologically active compounds. Today it is known that the peptides are in many cases the information relays, whose presence in the cell determines its specific activity. Knowing the amino acid sequences, it is possible to create a peptide selectively stimulating the process exactly where we want. It was found that the mechanism of action of peptides depending on their sequence, based on 3 core processes. One of them is to reduce muscle contractions, in effect prevents the creation of facial wrinkles. These peptides act on the skin as soft drugs diastolic. The second process is the regeneration and restoration of damaged tissue, and subsequent stimulation of dermis cells, responsible for the synthesis of collagen and elastin. Peptides are able to turn on and turn off complex regulatory processes. In practice, they are not miraculous elixir of youth, which will eliminate wrinkles, but the effect their actions will certainly be noticeable – the skin younger, more elastic and wrinkle clearly shallower [8].

Many research *in-vitro* using peptides and include them as components for products to protect the skin. Dermatology practice suggests that the results are not always possible to translate the reactions *in-vivo*. Each active ingredient must be absorbed in a stable form, capable of living and likely to accomplish its functions in the dermis [9]. The advantage of peptides is a small particle size, which helps them overcome the barriers of skin and there is no need to close them in the media patches. Cosmetic peptides stimulate the natural physiological processes in the skin. They are active in low concentrations and meet the global requirements of components for cosmetics, such as excellent chemical and microbiological purity and guaranteed stability [10]. Cosmeceutical peptides can cause real benefit to their patients, if they know how and when they should apply them. Confirmed the clinical benefit is improved skin hydration and consequently improve the appearance. This suggests that

the cosmetic industry got a tool that with surgical precision affects the condition of our skin. Looking optimistically to the future with the amino acids can make a lot of combinations with anti-wrinkle properties [9].

### 3.1. Signal peptides

Skin is made up mostly of collagen which is the foundation that gives skin its support and thickness. Young people have lots of collagen and taut, smooth skin. In contrast, older people have much less collagen and thin, wrinkled skin. Collagen is protein and is made up long chains of amino acids strung together. When it is broken down, short segments of 3–5 amino acids form, called peptides. Peptides are not just junk collagen, but these “mini proteins” are active molecules using as ingredients in lots of creams and lotions designed to improve the appearance of aging skin.

Signal peptides with the ability to increase fibroblast production of collagen can decrease collagenase breakdown of existing collagen [11]. When we age collagen is destroyed but not replaced. As a result young, smooth skin becomes thin and wrinkled over time.

Signal peptides are stimulating peptides, which are applied to the reconstruction of elastic layers of the skin. In fact, their mechanism of action is based on the cellular functions of fibroblasts. Over time, fibroblast activity decreases, the consequence is a slowing down of protein synthesis which support for the skin. This is one of the factors associated with biological aging, which leads to the formation of wrinkles and loss of firmness. Introduced from outside the molecule peptides of appropriate sequence of amino acids are able to stimulate fibroblasts. Stimulate the production of skin proteins that are degraded with age. There are also a signal for fibroblasts to increased production of proteins, according to their name. Stimulate also peptidoglycans, fibronectin, glycosaminoglycans [8].

As mentioned earlier biologically active peptides are small molecules, consisting of few amino acids. However, because of their specific structure, the cuticle is a barrier for them not to move. This problem was resolved by connecting the peptide chain fatty acids, whose presence in the structure allows a good penetration through the skin and easier access to the destination [12]. The process of obtaining such peptides IQ rely on cut the chains of amino acids which have sufficient sequence from a much larger protein molecules. Then join them to the chain fatty acids. This occurs in the process lipofilization of peptides (fig. 4).

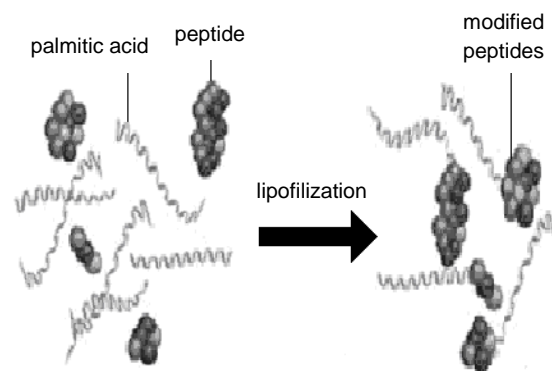


Fig. 4. Lipofilization of peptide [12]

Through this process, peptides can penetrate the precision of skin cells, which stimulate the production of new collagen by activating the appropriate genes. This allows you to rebuild the destroyed structures of the skin and smooth wrinkles. Whereas the process of regeneration of damaged fibers in the skin. In addition, collagen causes marked increase in skin firmness and elasticity, and halting the processes of its flaccidity, and biological aging [12].

The research of *in-vitro* and *in-vivo* demonstrated a beneficial effect cosmecutical peptides, both on the skin covered by chronological aging and the skin affected photoageing. Reducing degradation of collagen fibers, improving the compactness of the skin, improve skin appearance of the profile, and faster wound

healing effects are confirmed in the *in-vitro* research. By contrast, research conducted in living organisms show shallow and reduce the length of wrinkles and improve hydration of tissues, as well as smoothing the skin. Signal peptides found in cosmetics are fighting the aging of the skin.

Nowadays there are several known bioactive amino acid sequences which are commonly included in cosmetic formulas. The most popular signal peptide for cosmetic use is the sequence lysine-threonine-threonine-lysine-serine (KTTKS) found on type I procollagen. It is currently marketed under the name of palmitoyl pentapeptide-3 or Matrixyl® (Sederma) [13]. This pentapeptide has been demonstrated to stimulate feedback regulation of new collagen synthesis and to result in an increased production of extracellular matrix proteins such as types I and II collagen and fibronectin [14]. KTTKS is linked to palmitic acid in order to enhance delivery through the epidermis for action in the dermis. It is also considered to be a synthetic isomer (an isomer is a molecule that has an identical structure to another molecule but different atomic components). Matrixyl® stimulates the lower layers of the skin to heal themselves, thus accelerating the healing of wounds. Cells called fibroblasts are responsible for knitting together wounds of the skin. As you may know, as one ages wounds take longer to heal. This is partially because fibroblasts slowly lose the capacity for collagen production. Matrixyl® stimulates the "matrix" layers in the skin - primarily collagen and fibronectin. When stimulated, the skin produces more collagen. Loss of collagen is what leads to thinning skin and the wrinkling of newly inelastic skin. Matrixyl helps to counteract this natural part of the aging process.

Another signal peptide is tripeptide, lysyl-valine-lysine, has been shown to have similar effects on the up-regulation of transforming growth factor-beta when combined with palmitic acid and bistrifluoroacetic acid. This peptide is

currently marketed under the name of palmitoyl-tripeptide-3/5 or Syn®-Coll [15].

Applying signal peptides directly to skin is a way to replace lost collagen and treat aging skin. When collagen breaks down, it forms specific peptides. These peptides act as a signal to tell your skin it was damaged. Introduced from the outside active molecule of peptides stimulate skin cells and make new collagen. As a result, loss of collagen is reconstructed.

### 3.2. Neurotransmitters-affecting peptides

Neurotransmitters-affecting peptides are compounds called relaxant peptides due to the function they perform. Their specific activity rely on the relaxation muscles responsible for the formation of the mimic wrinkles. Peptides of this group work by blocking the flow of nerve impulses from cells located in the skin to the muscles. Therefore, compounds acting by this mechanism, referred to as neuropeptides. Their activity is similar to the mechanism of botulinum toxin injection. The effect of this treatment is almost immediate smoothing wrinkles. Action of neuropeptides is not as strong as the effect of botulinum toxin. However, regular use of products containing this compounds give excellent results. In addition, the advantage of therapy neuropeptides is that they are not invasive and do not cause complications. These peptides have a beneficial effect on the state of the skin: moisturize it, liquor, firm and reduce the roughness. These features make the preparations of peptides can also be used after treatments with botulinum toxin for the fixation of their performance [12].

The idea of applications neuropeptides in cosmetic was due to previous use of botulinum neurotoxin (Botox) in aesthetic medicine to reduce facial wrinkles. Scientists have long worked on the syntheses of this compound counterpart, indicating a much lower toxicity and similar therapeutic effects. Studies on peptides helped to create oligopeptides with similar mechanism of the action which has botulinum toxin. Peptides,

which slow down transmission of nerve impulses function as cosmeceuticals minimize facial muscle contractions, resulting in the reduction of wrinkles. Most of them occur on the cosmetics market in the name of patented, because of the therapeutic effect [11].

Acetyl hexapeptide-3 (Ac-Gly-Glu-Met-Gln-Arg-Arg-NH<sub>2</sub>) is a synthetic peptide patterned from the N-terminal end of the protein SNAP-25 that inhibits SNARE complex formation and catecholamine release. This peptide is currently marketed as Argireline® (McEit [Tianjin] International TradeCo., Ltd.) [16]. Acetyl hexapeptide-3 was found to inhibit vesicle docking by preventing formation of the ternary SNARE complex, which is involved in synaptic vesicle exocytosis [17, 18]. The neuropeptide reduces the intensity of muscle tension, which promotes ease and reduce facial wrinkles, induced by continuous facial movement.

Argireline® is the active and safe ingredient, non-toxic, topical alternative to Botox (Botulinum toxin type A), and has been scientifically proven effective in reducing wrinkles and preventing the formation of new lines in a safe and natural manner. The discovery of this hexapeptide (six naturally occurring amino acids in combination) was an exciting find, as Argireline® has proven to inhibit two internal processes that strongly contribute to the formation of wrinkles. The first is: the SNARE complex, a natural formation of proteins in aging skin that stimulates neurotransmitters, provoking facial muscle contractions, thereby causing wrinkles and lines. The second is: overproduction and release of catecholamine-hormones, such as adrenaline, which affect the sympathetic nervous system and cause wrinkles through repetitive facial movements. Modulation of catecholamine secretion presented below (fig. 5-8).

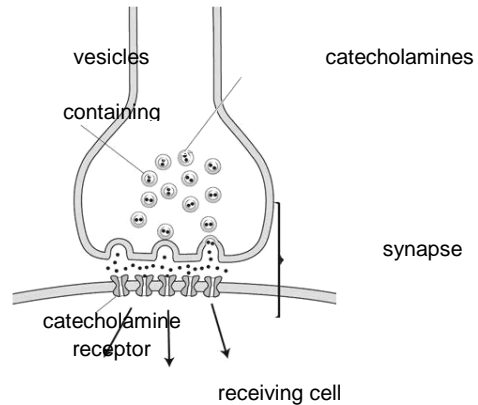


Fig. 5. Normal synapse [16]

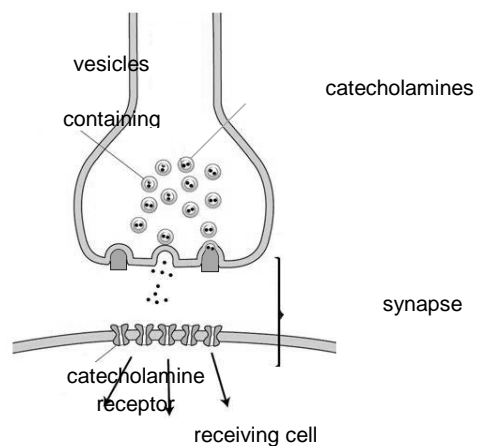


Fig. 6. Partial secretion blocking [16]

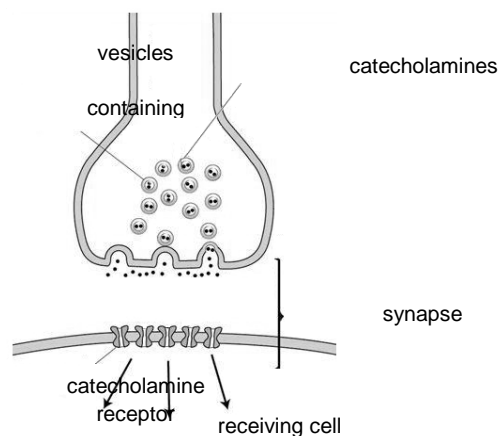


Fig. 7. Synaptic distance increase [16]

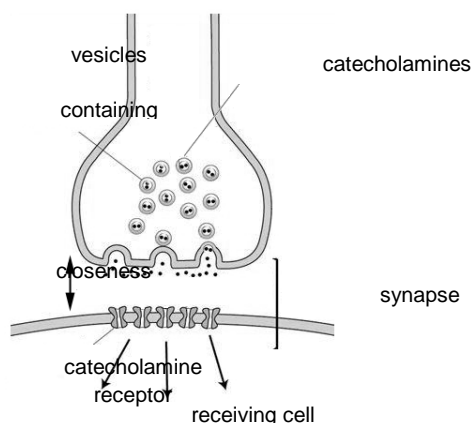


Fig. 8. Reversible in 6 months [16]

Argireline® works by inhibiting both the SNARE complex and overproduction of catecholamine, skin smoothes out. Also reduces the intensity and frequency of neurotransmitters that cause muscle contractions.

### 3.3. Carrier peptides

Carrier peptides are a small family of peptides that include a copper molecule in their structure. These peptides have been studied due to their ability to encourage the skin to heal wounds. Indeed, carrier peptides certainly have a salutary effect on various tissue types: the stomach lining, bones, and intestines as well as skin. Peptides are small fragments of proteins, but proteins are the key building blocks of nearly all living tissues. Certain kinds of peptides have an avid affinity for copper atoms, to which they bind themselves very tightly. The resulting compound consisting of a peptide and a copper atom are known as copper peptides or carrier peptides.

In the 1970s, researchers discovered the healing properties of copper peptides. Put simply, copper peptides seem to encourage the body's own healing mechanisms. Dr. Loren Pickart discovered that a variety of copper peptides have the ability to aid in tissue regeneration. Copper peptides boosted the body's healing abilities. Dr. Pickart patented a number of specific copper peptides. They seemed to be particularly

effective in healing wounds, skin lesions and even gastrointestinal ulcers. In addition, copper peptides help wounds heal. Copper peptides break down a specific type of tissue that forms scars (known as extra-large collagen aggregates). When these aggregates are broken down, wounds heal with far less scar tissue. Copper peptides also offer a significant anti-inflammatory benefit. In short, they help wounds heal faster, better and with less scarring [19].

A popular carrier peptide is tripeptid Gly-His-Lys, which forms a complex with copper ions and has an important function in our skin. This peptide sequence is consistent with that found in the extracellular matrix proteins as the  $\alpha$ -chain fragment of collagen and is released from collagen during wound healing or inflammation. Peptide GHK shows a wide range of biological activities such as stimulation of hair growth, collagen synthesis, superoxide dismutase - like activity, wound healing, tissue repair and angiogenesis [19, 20]. Tripeptide also possesses a high affinity for  $\text{Cu}^{2+}$  ions and spontaneously builds a complex (GHK-Cu). GHK-Cu also plays a key role in mammalian organism because it directly participates in wound healing and tissue repair [20-22].

For cosmetic purposes, GHK-Cu complex was created synthetically, starting from the isolation from human plasma. Copper peptide was developed to improve the transfer capability of copper in the tissues, increase flexibility and strengthen the skin of fibers. This combination of metal and peptide enhances the level of MMP/TIMPs and helps restore the youthful appearance of skin tissue. Additionally, it causes the synthesis of type I collagen in fibroblasts and synthesis dermatan sulfate, heparin sulfate, cytochrome-C oxidase and tyrosinase. Dermatan sulfate and heparin sulfate are substances from the group of glycosaminoglycans, or mucopolysaccharides hygroscopic properties, to ensure constant concentration of water in the dermis. Peptide complexes with copper

improves the appearance by stimulating the regeneration of damaged skin. The complex, on the one hand stimulates the activity of metalloproteinases I and II (remove damaged collagen and elastin of the extracellular matrix), on the other hand stimulates the synthesis of fibroblasts [23, 24].

Both tripeptide GHK and complex GHK-Cu cause the beneficial effects by stimulating collagen. The combination of carrier peptide with copper increases the level of matrix metalloproteinases MMP-1 and MMP-2 mRNA as well as TIMP-1 and TIMP-2, which restores skin firmness [11]. The studies have shown that this modified peptide is not only working to accelerate the process of regeneration, but also to rebuild damaged tissue.

Peptides containing copper can not be an effective ingredients for wrinkles, because wrinkles are not wounds. Wrinkles are simply folds in the epidermis, the main action of copper peptides is not effective on wrinkles. Copper peptides are sometimes used by doctors to help skin heal after cosmetic surgical procedures or severe chemical resurfacing. One possible application of copper peptide is to use it after cosmetic or dermatological treatments, such as dermabrasion, chemical peeling, and laser skin rejuvenation. Copper peptides can be used to counteract some of the more serious irritations caused by such treatment with a derivative of vitamin A (Retin-A). Application of peptide within two hours after surgery to avoid local irritation, redness and scaling associated. Copper peptides are readily used by doctors than vanity, it helps heal the skin after surgical or chemical. There is not currently sufficient research to prove that copper peptide is an effective anti wrinkle ingredient.

#### 4. Conclusion

Peptides in cosmetics are one of the new, popular options to treat aging skin. Most studies used to justify the incorporation of these ingredients into skin care products. As

practicing dermatologists show, use of cosmetic peptides increases from year to year. Through *in-vitro* studies the following results have been noted: improved skin hydration, improved appearance of the skin, increase in skin density and thickness. These results do not always translate into *in-vivo* actions. For any active ingredient to work, it must be absorbed in the stable form into the viable dermis. It is not an easy task to penetrate the barrier of the skin. Therefore, included palmitic acid helps peptides to go through the skin barrier. There is, however, soft clinical data and evidence that peptides are able to overcome skin barrier and produce beneficial effects for their patients.

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