GHK–Copper Peptide in Skin Remodeling and Anti-Aging

**Introduction**

Since the dawn of time, people have tried to discover the secret of eternal youth and find a way to reverse aging. Among the most popular ingredients of the elusive elixir of youth sought by ancient mages and alchemists were herbs, powdered gems, gold, silver and mercury as well as animal parts and extracts. The most common belief was that the «essence of youth» can be transferred from young to old people – often in a form of blood or other body fluids. Today it is known that plasma and other body fluids (such as saliva) indeed contain a multitude of soluble growth factors that can enhance reparative and renewing activity of various cells and tissues (1). Of course neither blood nor saliva or other fluids produced by the human body can be used in cosmetics. Therefore the biological regulators with proven scientific activity that can be purified or produced by chemical synthesis are of particular interest for the cosmetic industry.

One of the anti-aging activities that is present in human plasma and saliva is a human tri-peptide with an amino acid sequence glycyl-L-histidyl-L-lysine and high affinity for copper (II) (Fig. 1), isolated in 1973 by Dr. L. Pickart. First discovered as an activity in human plasma albumin that causes old human liver tissue to synthesize proteins like younger tissue, today GHK-Cu is widely used as a cosmetic ingredient with confirmed anti-aging and reparative properties (2).

**GHK-Cu in Skin Remodeling**

Although copper-binding tri-peptide glycy-l-L-histidyl-L-lysine (GHK-Cu) was first discovered as an activity in human plasma albumin that causes old liver cells behave like young cells, the following studies soon began to focus on its wound healing and skin remodeling activity. These studies provided valuable insight into GHK-Cu’s molecular actions and revealed many mechanisms by which GHK-Cu exhibits anti-aging and tissue renewing properties. In particular it has been established that GHK-Cu can increase production of key skin proteins such as collagen as well as other important components of dermal matrix. In addition it has been proven that GHK-Cu modulates the skin remodeling process, being able to stimulate both breakdown and synthesis of dermal matrix components. While GHK-Cu is used in most studies, GHK without copper has been used in a few instances. But all the available evidence is that GHK exerts its biological effects as its copper complex. In 1988, Maquart et al. from Université de Reims Champagne-Ardenne (France) found that GHK-Cu at concentrations of 10^{-12} and 10^{-11} M, maximized at 10^{-9}M was able to stimulate the synthesis of collagen in fibroblasts. The authors noted that GHK sequence is present in the alpha 2(I) chain of type I collagen and suggested that in the organism GHK might be liberated by proteases at the site of a wound healing (3).

![Fig. 1 The molecular structure of the tri-peptide GHK-Copper](image-url)
In 1992 Maquart et al. used the method of wound chambers to determine and quantify components of extracellular matrix (ECM) produced during wound healing in rats. GHK increased an accumulation of total ECM proteins, collagen and glycosaminoglycans as well as increased DNA synthesis. At the same time, another tri-peptide L-glutamyl-L-histidyl-L-proline had no significant effect, which confirmed that the observed stimulation of skin proteins production was a result of a specific action of GHK-Cu (4).

P. Ehrlich first observed that GHK simultaneously increases both the synthesis of new type I collagen and the breakdown of existing collagens in wounds (5). In 1999 Maquart and co-authors demonstrated for the first time that GHK-Cu regulated skin remodeling process in rats by modulating activity of different metalloproteinases – enzymes that facilitate breakdown of proteins of extracellular matrix (6).

In the next series of experiments, it was shown that GHK-Cu not only modulates activity of different matrix metalloproteinases, but also stimulates anti-proteases, maintaining balance between matrix breakdown and synthesis. The effect was reproduced by the addition of copper ions, but not by the GHK alone, which suggests that copper binding activity of GHK is more likely the key mechanism in its tissue remodeling activity (7). The ability of GHK-Cu to stimulate both proteases and anti-proteases indicates that it can balance the breakdown of ECM proteins, preventing excessive skin damage.

Maquart et al. also demonstrated that injection of GHK-Cu (2 mg per injection) into the rat experimental wounds resulted in the increased production of collagen I and glycosaminoglycans (dermatan sulfate and chondroitin sulfate). Northern blot analysis also showed increased production of two small proteoglycans of the dermis – biglycan and decorin. According to the mRNA analysis, the level of decorin continued to increase until the end of the GHK treatment (day 22th). The ability of GHK-Cu to stimulate decorin production was also confirmed in fibroblast culture (8).

It was particularly interesting that GHK-Cu increased decorin, since this proteoglycan functions by regulating assembly of collagen fibrils, preventing scar formation and decreasing the level of TGF-beta, which is known to increase scarring (9–10). In addition, GHK-Cu has been shown to decrease TGF-beta production in serum-free fibroblast culture (human cells obtained during facial surgery) (11). This may be explained through the increased decorin level, however it is also possible that GHK-Cu itself inhibits TGF-beta.

Also, GHK-Cu was found to attract immune and endothelial cells to the site of injury, which is another explanation of its wound healing promoting activity. The most recent study (2007) of wound healing activity of GHK-Cu was conducted in Taiwan. Huang et al. evaluated the effect of GHK alone or in combination with LED irradiation (light emitting diode irradiation, 625–635 nm) on human fibroblasts. Combined GHK and LED treatment resulted in 12.5-fold increase in cell viability, 230% increase in basic fibroblast growth factor (bFGF) production, and 70% increase in collagen I mRNA production compared with the LED irradiation alone (13). Since fibroblasts are the key cells in skin reparative and renewal processes, the ability of GHK-Cu to support these cells increasing their functional activity may in part explain its wound healing and skin rejuvenating activity.

GHK peptide belongs to a family of biologically active regulators that are naturally present in extracellular matrix (ECM) and released from it after injury. These «first emergency response» molecules are called matrikines (14). In addition to being a part of collagen molecule, GHK sequence is also found in a glycoprotein SPARC that is produced by endothelial cells in the site of injury. After an injury GHK released from a SPARC molecule by proteolysis (15). Therefore GHK-Cu is not only naturally present in skin, but it is also liberated from skin’s proteins after stress or injury. Considering its ability to stimulate the synthesis of key skin proteins as well as to increase functionality of skin fibroblasts, it should be quite effective as a wound healing agent. And indeed, animal experiments fully support this statement.

GHK Improves Wound Healing in Animals

A series of in vivo experiments confirmed wound healing activity of GHK-copper. In rabbits, GHK facilitated wound healing, causing better wound contraction, faster development of granular tissue and improved vessel growth (16). Also GHK alone and in combination with high dose helium neon laser stimulated granulation, increased the formation of new blood vessels and elevated the level of antioxidant enzymes in the dermal wounds in rabbits (17).

GHK-Cu was used to create a novel wound dressing – collagen membrane incorporated biotinylated GHK. This material has been shown to significantly improve wound healing processes in rats when compared to non-treated wounds and to wounds treated with collagen matrix alone. Collagen dressing with incorporated GHK stimulated wound contraction and cell proliferation, as well as increased expression of antioxidant enzymes (18).

The most important finding was GHK’s ability to improve wound healing in difficult to heal wounds, such as diabetic wounds in rats. GHK-Cu treatment resulted in faster wound contraction and epithelization, higher level of antioxidants such as glutathione and ascorbic acid, increased synthesis of collagen, and activation of fibroblasts and mast cells (19). Also GHK-Cu improved healing of ischemic open wounds in rats. Wounds displayed faster healing, decreased concentration of metalloproteinases 2 and 9 as well as of TNF-alpha (a major inflammatory cytokine) compared with vehicle alone or with untreated wounds (20). GHK-Cu triggered an accelerated healing of the injury after mild thermal burns to the backs of pigs. The rate of the burn wound healing gave a linear, dose-response up to 1% ionic copper in the cream. In pigs, punch biopsies were used to create wounds. The defects were filled with graded amounts of GHK-Cu in a cream or control substances. In a dose dependent manner, GHK-Cu stimulated wound healing and collagen synthesis. The effect was highly localized to the immediate skin area that was treated (21).

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Animal experiments confirmed that GHK-Cu has great potential to improve skin conditions. It speeds up skin repair, improves circulation, strengthens antioxidant defense, and increases production of skin proteins. All these qualities are highly desirable in cosmetics. The ability of GHK-Cu to improve difficult-to-heal wounds can be helpful in managing skin healing after plastic surgery, especially in advanced age patients and in patients with underlying health conditions.

- Cosmetic Use of GHK-Cu

Today peptide-based cosmetic products continue gaining popularity. However, not all peptides that are used in today’s cosmetic products have enough scientific data confirming their efficacy. Also, the main problem with peptide-based cosmetics is that most peptides cannot pass through stratum corneum and therefore cannot reach viable layers of epidermis. Since peptides are used for their cell regulatory activity, inability to permeate stratum corneum is a serious handicap.

Mazurowska et al. demonstrated that GHK-Cu is able to pass through the lipid barrier of the stratum corneum and to reach epidermal cells (22). Moreover, GHK-Cu complex appears to be the only peptide-copper complex that can penetrate stratum corneum membranes (23). At present, GHK-Cu undoubtedly has more scientific support data than any peptide used in today’s cosmetic practice (24). Its efficacy was confirmed in several placebo-controlled independent trials. A study of 20 women compared the skin’s production of collagen after applying creams containing GHK-Cu, vitamin C, or retinoic acid to thighs daily for twelve weeks with mild to advanced photodamage, as compared to a placebo control and an eye cream containing vitamin K. The GHK-Cu cream performed better than both controls in terms of reducing lines and wrinkles, improving overall appearance, and increasing skin density and thickness (27).

In another 12-week facial study of 67 women between 50-59 years with mild to advanced photodamage, a GHK-Cu cream was applied twice daily and improved skin laxity, clarity, firmness and appearance, reduced fine lines, coarse wrinkles and mottled hyperpigmentation, and increased skin density and thickness. The result was assessed visually by a trained technician (wrinkles, pigmentation, laxity, roughness, overall appearance) as well as using ballistometer (firmness of the skin) and ultrasound (skin density). The GHK-Cu cream also strongly stimulated dermal keratinocyte proliferation as determined by histological analysis of biopsies. At the same time, GHK-containing cream has proved to be very safe. GHK-Cu complex at 20x use level was proved to be non-allergenic. It also did not produce eye irritation (28).

- Stimulation of Hair Growth

From the very first experiments with GHK-Cu, we observed exceptionally large hair follicles developing at the periphery of wounds treated with GHK-Cu. Today it is known that hair follicles are the important source of stem cells that actively participate in skin repair and are capable of differentiating into many skin cell lineages (29).

The hair growth stimulating effect of GHK-Cu was confirmed in the following experiment. A 25-day-old C3H mouse was shaved and injected in three spots with GHK-Cu. According to the radioisotope analysis, GHK-Cu was present at the injection site for about 30 sec, before clearing from the area. However, this brief exposure was enough to stimulate hair growth. In 12 days there was strong hair growth stimulation at the injection site (Fig. 2) (30).

In fuzzy rats a copper binding peptide PC1031, a structural analog of GHK (ghkfv-copper complex) affected hair growth with its effect comparable to that of minoxidil. Both 5% minoxidil and 5% PC1031 almost doubled follicle size after 3-4 months of treatment, and caused 80% increase in the number of anagen hair follicles. An increased DNA synthesis and cell proliferation was confirmed in the enlarged hair follicles (31).
The mechanism of GHK-Cu induced hair growth was investigated in experiments with GHK-Cu analog – AHK (L-alanyl-L-histidyl-L-lysine-Cu2+). It was found that AHK-Cu stimulated dermal papilla cells (DPCs) – specialized fibroblasts important in the morphogenesis and growth of hair follicles. AHK caused elongation of hair follicles, stimulated proliferation of DPCs and prevented their apoptosis.

Today, analogs of GHK-Cu with hydrophobic amino-acids are used to enhance hair growth in the commercial product Tricomin (Procyte Corp). Also a product GraftCyte (Procyte Corp) is used to increase success of hair transplants with clinically confirmed efficiency (33).

**GHK’s Effect on Skin Stem Cells**

The ability of GHK-Cu to rejuvenate aging skin and to stimulate hair growth indicates its important role in skin and hair renewal process. Recent studies revealed one possible mechanism of this rejuvenating action. It has been discovered that GHK is able to restore proliferative potential of adult stem cells residing in skin. In 2009, a group of researchers from the Seoul National University (Republic of Korea) used multilayered skin equivalent (SE) model to demonstrate that GHK-copper is able to increase proliferative potential of basal human keratinocytes as well as expression of epidermal stem cell markers.

Epidermal stem cells are slow cycling cells in a basal layer that maintain non-differentiated state through the entire life time of an organism. They give rise to transient stem cells that in turn differentiate into keratinocytes. In case of an injury, epidermal stem cells are able to differentiate into all types of cells found in the skin, including fibroblasts, melanocytes, endothelial cells etc. Although not all characteristics of epidermal stem cells are known, their main features are believed to be a cuboidal shape, high integrin expression and an expression of p63 protein – a member of p53 family – that is often used as a stem cell marker. Loss of proliferative potential of skin stem cells is associated with flattened shape and decreased expression both integrins and p63. Integrins are surface proteins that play an important role in maintaining an attachment of stem cells to the basement membrane. It is believed that such attachment is required for maintaining immature, non-committed state of stem cells – their «stemness».

In cell cultures GHK copper (0.1-10 microM) stimulated proliferation of keratinocytes in a dose dependent manner and was not toxic in these concentrations. An addition of GHK-copper resulted in noticeable changes in epidermal basal cells. Their integrins and p63 expression markedly increased and their shape became more cuboidal, as is characteristic for stem cells. The authors concluded that GHK-copper is able to maintain «stemness» of basal keratinocytes as well as to revive their proliferative potential (34).

This discovery is especially important in the light of the recent findings that suggest that age-related decline of the pro-

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**Fig. 3 GHK-Cu effects on aged skin**
liferative capacity of stem cells may be due to disrupted regulation from the aged environment rather than to stem cells senescence. In contrast to the prevalent theory, according to which all human cells have limited proliferative capacity of approximately 50 divisions (so called Hayflick limit), in some renewing tissues stem cells have been shown to undergo more than 1000 divisions without showing signs of senescence (35). New findings suggest that epidermal stem cells may be intrinsically aging resistant and that local microenvironment, rather than cellular senescence, is responsible for their functional decline (36). GHK’s ability to revive proliferative potential of epidermal stem cells makes it a powerful player in skin repair and rejuvenation process (Fig 3).

**GHK-Cu Restores Function in Damaged Cells**

A recent study showed GHK-Cu’s ability to restore function of irradiated fibroblasts to that of intact cells. The researchers used cultured human fibroblasts obtained from cervical skin that was either intact or exposed to radioactive treatment (5000 rad). GHK copper (10⁻⁹ M) was added in a serum free medium directly to the cell culture. An equivalent amount of plain serum-free medium was added to control cells (37). Although irradiated fibroblasts survived and replicated in the cell culture, their growth dynamics were markedly different from that of intact cells. The growth of the irradiated cells was especially delayed at 24 and 48 hour measurements. However, the irradiated fibroblasts treated with GHK showed much faster growth, similar to the normal (non-irradiated control cells). In addition GHK-treated irradiated fibroblasts showed increased production of growth factors bFGF and VEGF, which was significantly higher than that of irradiated and intact control cells (37).

Fibroblasts are central cells in both wound healing and tissue renewal processes. They not only synthesize different components of ECM, but also produce a number of growth factors that regulate cell migration and proliferation, angiogenesis, epithelialization etc. However, fibroblasts are sensitive cells that can be damaged by many factors. The fact that GHK-Cu restored damaged fibroblasts’ activity up to a level to that of normal, non-irradiated fibroblasts, opens new possibilities in enhancing fibroblasts’ function in aged skin.

**GHK-Cu Suppresses Cancer**

This area of GHK-copper’s activity may belong to medicine rather than cosmetology, however it sheds an important light on the protective and reparative role of GHK-Cu in the organism. In the study published in 2010 by Hong Y. et al. (Department of Colorectal Surgery, Singapore General Hospital, Singapore) GHK-Cu was selected out of 1309 biologically active substances as an activity that was able to reverse expression of certain genes involved in metastatic spreading of colon cancer. Another substance that produced such effect was plant alkaloid securinine. GHK-Cu was not toxic and produced effect at a very low concentration - 1mkM (securinine was active at 18 microM) (38).

This research is the first that reports direct anti-cancer activity of GHK-copper. However, there is also indirect evidence suggesting an important role of GHK-copper in preventing cancer. First of all, GHK-Cu can exhibit anti-tumor activity by increasing decorin (10). It has been shown that stimulation of decorin expression leads to the regression of certain tumors, such as gliomas. Decorin also was found to inhibit growth of various tumors and to prevent metastasis of breast cancer (39). One of the important qualities of decorin is its ability to inactivate TGF-beta. It is known that TGF-beta helps the tumor to escape the action of the host’s immune system by suppressing immune response, causing apoptosis of immune cells and modulating the activity of growth factors (40).

GHK-Cu is also proved to decrease TGF-beta, although this effect may be secondary – due to the increased decorin level.

Another contribution of GHK-copper into anti-tumor defense of an organism as well as into prevention of other age-related degenerative conditions is its antioxidant and anti-inflammatory action.

**Anti-inflammatory and Antioxidant Action**

It is known that inflammation is always accompanied by increased oxidative damage. One reason is that immune cells use reactive oxygen species (ROS) and other powerful oxidants (such as hypochlorite and peroxinitrite) to combat the bacteria. Since free radicals play an important role in the aging process, antioxidant and anti-inflammatory ingredients are often included in anti-aging cosmetic formulations (41).

It was found that GHK-Cu suppresses inflammation by lowering the level of acute phase inflammatory cytokines such as TGF-beta and TNF-alpha (42). GHK also reduces oxidative damage by modulating iron levels (43) and by quenching toxic by-products of lipid peroxidation (44-45). Since lipid peroxidation plays an important role in skin aging and UV-induced skin damage, the ability of GHK to form complexes and sequester toxic by-products of lipid peroxidation has a protective effect against skin aging and photo-damage. One of the possible application of GHK-Cu is sun-protective cosmetics. It is known that many synthetic UV-filters can generate free radicals and increase oxidative damage to the skin. An addition of GHK-Cu to sun-protective cosmetics may decrease the level of harmful by-products of lipid peroxidation, reduce inflammation and skin damage.

**How Does GHK-Cu Work?**

GHK tri-peptide has a strong affinity for copper and more often exists in a form of GHK-Cu. On the basis of available data it was proposed that GHK-Cu might function by modulating copper intake into cells (46). It is now well established that the biological significance of GHK-Cu primarily results from its unique relationship with copper. Since copper ions are used by more than a dozen enzymes involved in different biological processes in the cell,
supplying the tissues with copper can improve many aspects of tissue metabolism including antioxidant defense, tissue repair, oxygenation etc. Among enzymes that use copper are an antioxidant enzyme superoxide dismutase (SOD), lysyl oxidase is required for connective tissue formation, tyrosinase responsible for melanin synthesis and many others (47).

Another important function of copper is signaling. For example, low tissue copper prompts proliferation of stem cells, while sufficient tissue copper is required for stem cell differentiation into cells needed for tissue repair (48). An inadequate level of copper can prevent stem cells from differentiation and hamper tissue repair (49). By modulating copper level GHK-Cu may act as a »switch« that curbs inflammation and prompts skin healing and remodeling process (Fig. 4). Small size and mobility allow GHK-copper easy access to molecular receptors and speedy travel in the extracellular space.
However, it would be a mistake to view GHK-Cu simply as a copper transporting protein. As it has been discovered recently, its biological activity goes well beyond copper binding.

A number of studies show that tripeptide is able to bind not only with copper, but also with a variety of biological molecules as well as promote cell attachment to different substrates. Rabenstein et al. demonstrated that GHK is able to form complexes with heparin (50). It is known that heparin shares common characteristics with heparin sulfate – one of the main GAGs of the epidermal basement membrane (51). The ability of GHK to bind with heparin indicates its ability to bind also with heparin sulfate, and therefore – with basement membrane and ECM. Since GHK also can bind with cellular receptor, its ability to bind with ECM allows it to facilitate cell adhesion to ECM structures, stimulating their migrating and differentiating (52) (Fig. 5).

By modulating copper level and facilitating cellular interaction with extracellular matrix, GHK-Cu is able to exhibit broad reparative, protective and rejuvenating activity acting as a natural skin wellness and age reversal agent.

**Conclusion**

Human tripeptide GHK-Cu is a natural skin wellness molecule that exhibits a broad range of reparative, anti-aging, and protective actions. By regulating processes of skin renewal and repair, it ensures not only fast healing of dermal wounds, but also exhibits rejuvenating action by increasing water-holding molecules in dermal matrix, stimulating collagen synthesis, balancing the action of skin proteases and reducing oxidative damage. Particularly interesting is its ability to maintain epidermal stem cells as well as to restore viability of damaged skin cells such as irradiated fibroblasts. Since fibroblasts and adult stem cells play a crucial role in skin renewal and wound healing process, GHK-Cu presents itself as a natural skin renewing, repairing and rejuvenating ingredient. Possible applications of cosmetics containing GHK-copper include:

1. Anti-aging topical formulations – slowing down aging, reducing wrinkles, improving skin complexion, reducing unwanted pigmentation, improving skin structure.

2. Cosmetic formulations intended to be used before and after aggressive cosmetic procedures and plastic surgery – speeding up skin healing, reducing the risk of side effect, improving outcome. Such formulations would be especially valuable for patients of advanced age or with underlying health conditions.

3. Topical formulations for reducing inflammation and redness associated with various cosmetic manipulations.


**References**


(10) Zhang L, Li XJ, Zhang X, Li YY, Xu WS. Re- combinant human decorin inhibits cell proliferation and downregulates TGF-beta production in hypertrophic scar fibroblasts. Burns 2003; 35(5);634-641


(15) Lane TF, Iruela-Arispe ML, Johnson RS, Sage EH. SPARC is a source of copper-binding peptides that stimulate angiogenesis. J Cell Biol. 1994 May;125(4):929-43


GHK-COPPER PEPTIDE

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