# Neova® Refining Eye Lift Reduces the Appearance of Periorbital Wrinkles with DNA Repair and Copper Peptide Technologies

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**Abstract**
Neova® Refining Eye Lift treatment combines the advanced DNA repair of Mitosomes, the restorative action of copper peptide and the biological activity of Argireline to reduce the appearance of fine lines and wrinkles around the eye. New to the formula is the Mitosome DNA repair enzyme, encapsulated in liposomes, to target damaged DNA (within cells in the upper layer of skin surrounding the eye) for protection from continuing photodamage that results from UV irradiation. Copper peptides have been shown to enhance the deposition of new extracellular matrix. Argireline is known as a needle-free alternative to Botox® because of its effect to inhibit muscle contractions which form deep lines of expression. These functions are supplemented by enhanced moisturization action provided by Sodium hyaluronate and the tissue rebuilding and restorative action of a specialized Saccharomyces Lysate ingredient.

## Introduction

The natural aging process normally begins in the mid-20s. In the skin, the synthesis of collagen and other components of the extracellular matrix are slower and elastin, the substance that enables skin to be flexible, is less functional. The dead skin cells at the surface do not shed as quickly and the production of new skin cells decreases.

One of the main causes of the visible signs of aging skin is sun exposure. Freckles, age spots, spider veins on the face, rough and leathery skin, fine wrinkles that disappear when stretched, loose and dry skin, a blotchy complexion, actinic keratoses and skin cancer can all be attributed to sun exposure.

Photoaging also occurs over a period of years, especially around the eyes. With repeated exposure to the sun, the skin loses the ability to repair itself. Studies have shown that repeated ultraviolet (UV) exposure breaks down collagen and other matrix components and impairs the synthesis of new collagen while DNA damage accumulates leading to increasing incidences of skin cancer and lower rates of cell renewal. The resulting accumulation of fine lines, wrinkles, sagging skin, dark circles and puffiness can add up to an increased appearance of age.

## Copper Peptide Complex®

Copper is essential to vital cellular and enzyme processes required for human health, and is the third most abundant trace metal in the body, after iron and zinc. Since the 1830s, copper has been known to be an essential nutrient. Copper plays a key role in several of the body's essential enzyme systems needed for tissue repair and other biological responses. These copper-based enzyme systems allow tissue to repair itself, blood vessels to form, wounds to close and inflammation to decrease. Copper is now known to be critical to the normal repair and healing process in all tissue, including connective tissues that comprise human skin, internal organs and bones.

Skin health, dermal wound healing, and general soft tissue repair requires many of the same biological processes such as reconstitution of an extracellular matrix and increased blood flow (angiogenesis). Copper is utilized by essentially every cell and organ; resulting in the formation of important copper-dependent enzymes - including cytochrome C oxidase (energy production), superoxide dismutase (antioxidation) and lysyl oxidase (cross-linking of elastin and collagen in skin)[1-2].

In numerous studies, copper peptides have been shown to promote new blood vessel growth, enhance the expression of growth factors, activate matrix metalloproteases, and stimulate the formation of new collagen, elastin, and glycosaminoglycan components of tissue to accelerate the repair process[3-9].

Refining Eye Lift contains the copper peptide L-Alanine-L-Histidyl-L-Lysine Copper (INCI Alanine/Histidine/Lysine Polypeptide Copper HCl) or AHK-Cu. This is a multifunctional copper peptide which both increases dermal cell proliferation and viability while increasing the deposition of collagen to renew the extracellular matrix.

Dermal fibroblasts are the main cells responsible for the production of collagen and other proteins essential for healthy skin. The response of human dermal fibroblasts to AHK-Cu has been investigated using cultured cells. The results show that AHK-Cu increases the growth and viability of dermal fibroblasts while stimulating the production of collagen[10].

Normal human dermal fibroblasts were seeded into a 96-well cell culture plate and grown to confluence in high glucose media supplemented with 10% fetal calf serum for three days. They were then placed in low glucose media with 2% serum and various amounts of AHK-Cu complex. After an additional three days, the cells were incubated with neutral red to assess their viability and stained with sulforhodamin B and counted to determine the number of cells.

The results in Figures 1 and 2, show that the addition of AHK-Cu complex stimulated the proliferation of the normal human dermal fibroblasts and increased their viability.

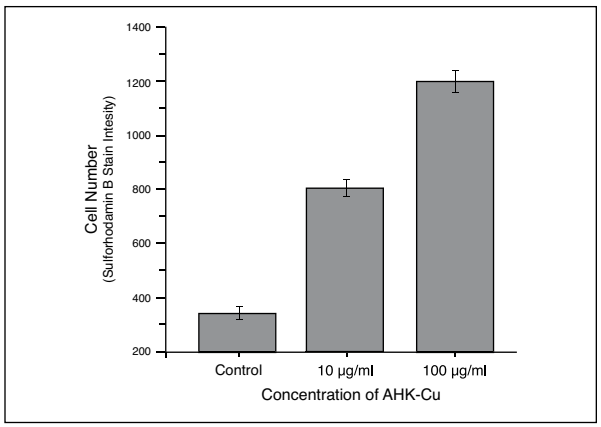


**Figure 1.** Effect of AHK-Cu on Dermal Cell Proliferation.

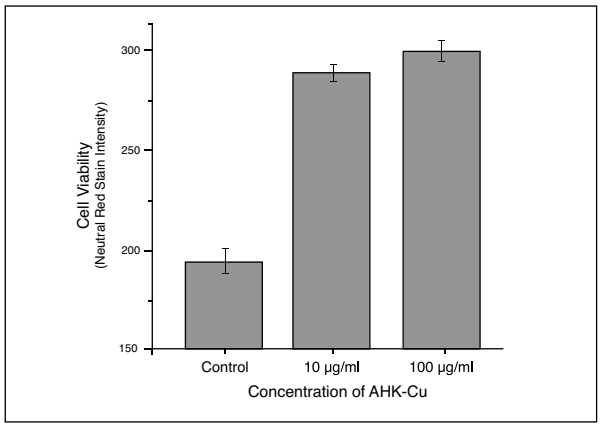


**Figure 2.** Effect of AHK-Cu on Dermal Cell Viability.

The effect of the AHK-Cu copper peptide complexes on collagen formation was discovered by determining the amount of collagen type I produced by human fibroblasts in cell culture. After 3 days incubation with increasing amounts of AHK-Cu, the culture media was collected and analyzed for collagen type I content by a sandwich ELISA assay using purified antibody to collagen type I.

The results below in Figure 3 show that the addition of AHK-Cu complex stimulates the formation of collagen type I by 300% over the control.

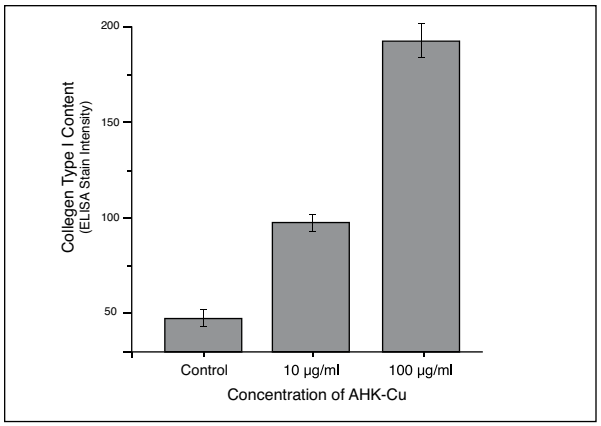


**Figure 3.** Effect of AHK-Cu on Dermal Cell Collagen Production.

## Mitosomes

Mitosomes contain a DNA repair enzyme that recognizes the most common form of oxidative damage to DNA and initiates the repair process. Mitosomes contain an enzyme (OGG1 for 8-oxoguanine DNA glycosylase 1) produced from the common plant Arabidopsis that recognizes this damaged base (8-oxo-guanine) and initiates its removal[11-12].

These radicals damage DNA by oxidizing its nucleotide bases from to form 8-oxo-guanine. Mitosomes shorten the time for nucleus DNA repair from 24 hours to 2 hours, Figure 4 Mitosomes tested at 0.3% repair 75% of the oxidative damage on DNA. In vitro testing at 0.5% shows that Mitosomes repair DNA in the mitochondria.

In vitro testing has shown that 1% Mitosomes completely repaired 8-oxo-guanine in cells in 2 hours, while 0.3% completed repair in about 3 hours[11].

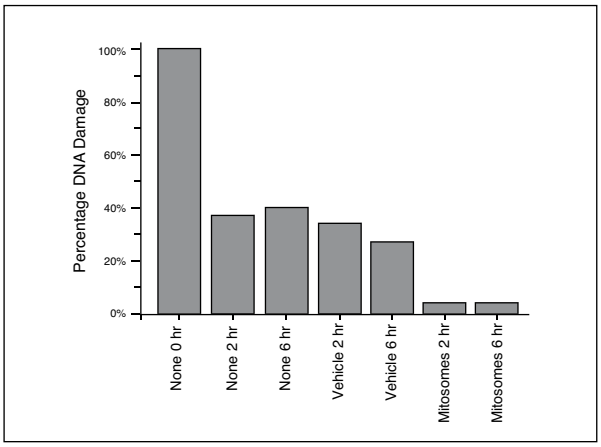


**Figure 4.** Inhibition of Glutamate Release in Neuron Cell Culture.

Mitochondrial DNA protection is also important to anti-aging activity. Damage from oxygen radicals is a main factor in aging. These reactive oxygen species come from pollutants in the environment, from UV-A induced reactions in skin, and from the body's own stress responses. In addition, oxygen radicals are the inevitable side-effect of energy mitochondria. The accumulation of damage to mitochondria is considered an important element of aging.

A vital feature of the Mitosomes enzyme is that it also protects mitochondria. The enzyme has a "mitochondrial localization signal" (MLS), which is recognized by the cells transport system, and guides it into mitochondria. This ability allows Mitosomes to repair the DNA in mitochondria after oxidative damage which occurs during normal cellular respiration.

## Argireline®

Argireline (Hexapeptide-8 and formerly known as Acetyl Hexapeptide-3) is a six (hexa-) amino acid chain linked to acetyl fatty acid as a penetration enhancer. The sequence of amino acids is EEMQRR (Glutamic Acid, Glutamic Acid, Methionine, Glutamine, Arginine, Arginine). Its structure is based on the cleavage product, SNAP-25, involved in the mechanism of action of Botulinun neurotoxin type A (Botox)[13-16].

Argireline is known as a needle-free alternative to Botox because its effect is to inhibit muscle contractions which form deep lines of expression. Acetylcholine is the neurotransmitter responsible for sending the message signaling a muscle to contract. Botox prevents muscle contraction by paralyzing the muscles: the acetylcholine sends its message but the muscle cannot respond. Argireline works by blocking the acetylcholine from reaching the muscles: the acetylcholine sends its message but the muscle does not receive it as endocytosis is blocked[13-14]. (Figure 5.)

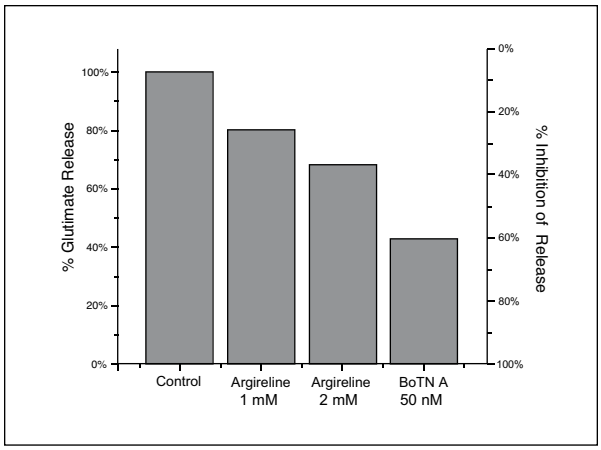


**Figure 5.** Inhibition of Glutamate Release in Neuron Cell Culture

In performance evaluations, Argireline has proven active against skin wrinkling. In a study with 10 volunteers, application of Argireline applied twice a day for 30 days resulted in an average of a 17% reduction in the depth of wrinkles. (Figure 6.)

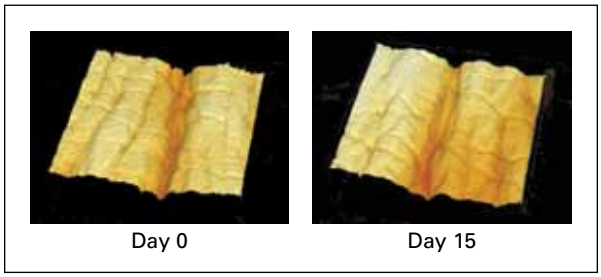


**Figure 6.**

In a similar study with 14 volunteers for 28 days, skin silicon imprints combined with topography analysis showed a reduced wrinkle depth up to 30%. (Figure 7.)

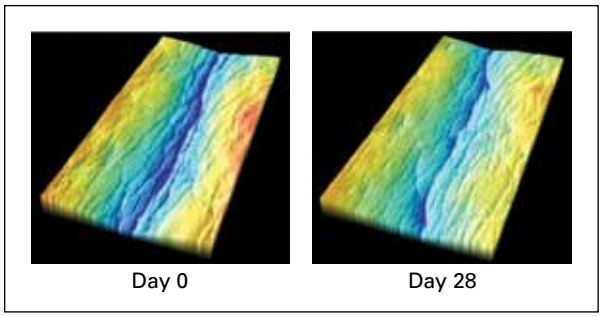


**Figure 7.**

## Sodium Hyaluronate

Sodium hyaluronate is the sodium the salt of hyaluronic acid. Hyaluronic Acid (HA) is the naturally occurring and widespread component found within the extra-cellular matrix within bodily tissues, especially those of the face. Its water-binding and water-attracting attributes fill up the spaces between the connective fibers collagen and elastin in the dermis.

Sodium hyaluronate has a smaller molecular size than HA (making it especially penetrative), and is able to hold more water than any other natural substance—up to 1,000 times its weight in water.

## Saccharomyces Lysate

Saccharomyces Lysate is extracted from a type of yeast that ferment sugars and is a source of beta-glucan, which is a good antioxidant. The type of Saccharomyces Lysate used in the Refining Eye Lift is also termed Tissue Respiratory Factor that has been shown to promote tissue regeneration, increase oxygen consumption (Figure 8) by cultured human fibroblasts, and inhibits inflammation[17].

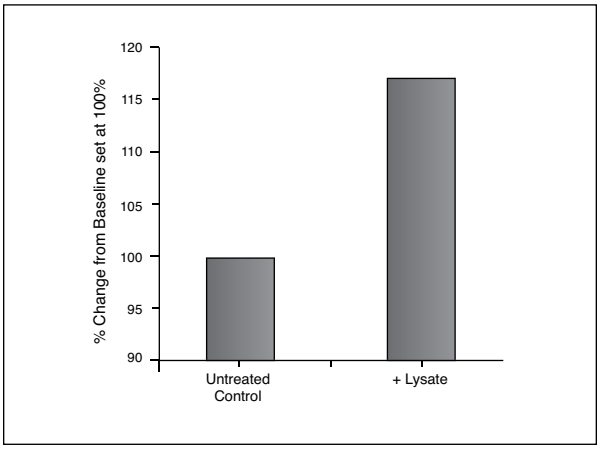


**Figure 8.** Percent Change in Respiration in Human Fibroblasts.

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Botox is a registered trademark of Allergan, Inc.

Neova and Copper Peptide Complex are registered trademarks of Procyte Corporation.

## References

1. Jackson EM. The Importance of Copper in Tissue Regulation and Repair: A Review. Cosmetic Dermatology 1997;10 (10):35-6.

2. Milne DB. Copper in clinical practice. Clin Lab News 1993;19:80-1.

3. Buffoni F, Pino R, Dal Pozzo A. Effect of tripeptide-copper complexes on the process of skin wound healing and on cultured fibroblasts. Archives Internationales de Pharmacodynamie et de Therapie 1995;Vol.:-60.

4. Huang PJ, Huang YC, Su MF, Yang TY, Huang JR, Jiang CP. In Vitro Observations on the Influence of Copper Peptide Aids for the LED Photoirradiation of Fibroblast Collagen Synthesis. Photomed Laser Surg 2007;25:183-90.

5. Maquart FX, Bellon G, Chaqour B, Wegrowski J, Patt LM, Trachy RE et al. In vivo stimulation of connective tissue accumulation by the tripeptide-copper complex glycyl-L-histidyl-L-lysine-Cu2+ in rat experimental wounds. J Clin Invest 1993;92:2368-76.

6 Maquart FX, Pickart L, Laurent M, Gillery P, Monboisse JC, Borel JP. Stimulation of collagen synthesis in fibroblast cultures by the tripeptide-copper complex glycyl-L-histidyl-L-lysine-copper(2+). FEBS Lett 1988;238:343-6.

7 McCormack MC, Nowak KC, Koch RJ. The effect of copper tripeptide and tretinoin on growth factor production in a serum-free fibroblast model. Arch Facial Plast Surg 2001;3:28-32.

8. Oddos T, Jumeau-Lafond A, Ries G. Requirement Of Copper And Tripeptide Glycyl-L-Histidyl-L-Lysine-Cu (GHK) Complex Formation For Collagen Synthesis Activity In Normal Human Dermal Fibroblasts. American Academy of Dermatology 60th Annual Meeting February 22-27, 2002 New Orleans, LA . 2002.

9 Pollard JD, Quan S, Kang T, Koch RJ. Effects of copper tripeptide on the growth and expression of growth factors by normal and irradiated fibroblasts. Arch Facial Plast Surg 2005;7:27-31.

10. Data on File, ProCyte Corporation. 2003.

11. Barnett Products Corporation Brochure. Roxisomes™, DNA Protection from Oxygen Radicals. 2009.

12. Wulff BC, Schick JS, Thomas-Ahner JM, Kusewitt DF, Yarosh DB, Oberyszyn TM. Topical treatment with OGG1 enzyme affects UVB-induced skin carcinogenesis. Photochemistry and photobiology 2008;84:317-21.

13. Lipotech. Argireline. www.the-argireline-site.com . 2009.

14. Lipotech. Argireline - Product Brochure. 2009. Lipotech.

15. Ruiz M A, Clares B, Morales M E, Cazalla S, Gallardo V. Preparation and stability of cosmetic formulations with an anti-aging peptide. Journal of cosmetic science 58[2], 157-171. 2007.

16. Blanes-Mira C, Clemente J, Jodas G, Gil A, Fernandez-Ballester G, Ponsati B et al. A synthetic hexapeptide (Argireline) with antiwrinkle activity. International Journal of Cosmetic Science 24[5], 303-310. 2002.

17. Arch Personal Care Products. Biodynes TRF Data Sheet. 2009.