Elasticity  Firmness  Cellular adhesion

plant-EGF
The importance of growth factors in the skin

Growth factors are naturally occurring proteins that stimulate cellular growth, proliferation and differentiation. They play an important role in maintaining a healthy skin structure, mediating communication between epidermal and dermal cells by binding to specific receptors on the surface of the target cell. Well known to play an important role in reversing the effects of skin aging, topical application of human-like growth factors has been shown to act locally stimulating cellular renewal, reducing wrinkles and boosting collagen synthesis.

**EGF**

Epidermal Growth Factor is a 7 kDa protein that stimulates cell growth, proliferation, and differentiation by binding with high affinity to its receptor on the surface of the target cells. This binding initiates the MAPK/ERK (protein-tyrosine kinase) pathway and ends with nuclear DNA expressing proteins.

EGF is naturally found in our skin but its presence diminishes notably with age (over 50% after the age of 30) and UV light exposure and the renewal of epidermal cells noticeably slows down. The deficiency of EGF results in skin aging, in formation of wrinkles, freckles and age marks.

EGF works very efficiently even at very low concentrations. Topically applied, and has been found to accelerate the rate of skin renewal and re-epithelialization, to stimulate collagen production and to help preventing photodamage and fine lines and wrinkles. EGF shows a good complementary effect with humectants such as hyaluronic acid.

**plant-EGF**

*plant-EGF (Nicotiana benthamiana Hexapeptide-40 sh-Oligopeptide-1)* is synthetic bioengineered highly purified Epidermal Growth Factor produced in plants.

It is a biotech ingredient for the prevention of skin aging. Its ability to induce collagen and fibronectin biosynthesis helps improving the appearance of wrinkles and the elasticity of the skin.

***In vitro efficacy tests***

*All assays were third party performed*

**Wound Healing Assay**

Human keratinocytes (HaCaT cells) serum starved were seeded in 24-well plates and grown up to 90% confluence. Then 1 mm scratch was made with a pipet tip and pictures were taken immediately. Cells were allowed to close the damage when kept at 37°C.

Migration of HaCaT cells in a dose-dependent manner highlights the regenerative properties of *plant-EGF*, based on its promoting effect on skin cell migration, proliferation and survival. Wound healing assay demonstrated the potential to significantly stimulate and promote skin cell regeneration by 100% in 78 hours under pro-aging conditions. *plant-EGF* high purity guarantees activity at very low concentrations.

CN: Untreated cells; (1): 1 ng/ml *plant-EGF*; (5) 5 ng/ml *plant-EGF*; (10) 10 ng/ml *plant-EGF*.

1 mm wounds were closed at 78 hours after treatment under pro-aging conditions.
Human keratinocytes cell proliferation

The aim of this assay is to assess quantitatively the effects of plant-EGF on the stimulation of the cell cycle and viability of keratinocytes, the predominant cell type in the epidermis.

Cell viability of HaCaT cell extracts from the wound healing assay was assessed by MTT. Untreated cells were used as negative control. plant-EGF promotes proliferation of HaCaT cells in a dose dependent manner, supporting the regenerating and re-epithelializing performance of the wound healing assay.

Proliferation increased by 152% at 78 hours after treatment under pro-aging conditions

Human fibroblast cell proliferation

Dermal fibroblasts are the cells that synthesize collagen and the extracellular matrix to maintain the skin’s structure. As a result of the process of aging turnover of new fibroblast decreases, collagen production slows and the skin becomes thinner and wrinkled.

Primary dermal fibroblasts were seeded in 24-well plates, grown up to 40% confluence and treated with different concentrations of plant-EGF for 24 hours. Cell viability was assessed by MTT assay and showed significant stimulation of fibroblasts proliferation at 1 ng/ml versus the untreated control.

Fibroblast proliferation boosts the skin repairing process stimulating collagen synthesis and the formation of connective tissue.

Proliferation increased by 95% at 24 hours after treatment

Type I Collagen synthesis

Collagen is one of the main structural proteins of human skin, over 90% of the skin’s total proteins, and provides structural support to cells and tissues.

Collagen I expression profile was performed by indirect immunofluorescence on primary dermal fibroblasts grown up to 60-70% confluence, showing a remarkable intracellular increment of collagen versus the untreated fibroblasts 24 hours after treatment with 5 ng/ml of plant-EGF.

Type I Collagen expression profile after treatment with plant-EGF at 5ng/ml. Scale bar 20 µm.
Fibronectin synthesis

Fibronectin together with collagen and proteoglycans are integral components of the ECM, and plays an important role in cell adherence.

Fibronectin expression profile was performed by indirect immunofluorescence on primary dermal fibroblasts grown up to 60-70% confluence, **showing an extracellular increment of the protein 24 hours after treatment, and a better organization** versus the untreated fibroblast after treatment with different concentrations of plant-EGF.

**Fibronectin expression profile** (red fluorescence) after treatment with **plant-EGF** at 1ng/ml and 5 ng/ml. Scale bar 50 µm

Elastin synthesis

Elastin gives the skin with the ability to stretch and recoil and plays a critical role in supporting and maintaining strong skin cells. Even being the longest lasting protein in the body, elastin damage caused by sun overexposure and aging may be sufficiently repaired and the skin gradually loses its elasticity.

Elastin expression profile was performed by indirect immunofluorescence on primary dermal fibroblasts grown up to 60-70% confluence, **showing an intracellular increment of the protein 24 hours after treatment**. An intense Elastin fluorescence can be noticed in the plant-EGF treated cells.

**Elastin expression profile** (green fluorescence) after treatment with **plant-EGF** at 1ng/ml and 5 ng/ml. Scale bar 50 µm

Uses

Improving the skin appearance particularly after age 30: anti-aging, anti-wrinkle, anti-sagging, improving aspect of scars and marks, minimizing pores.

Bibliography

**DESCRIPTION:** **plant-EGF** is a single chain recombinant human peptide produced by transient expression in *Nicotiana benthamiana* plants. The starting gene is a synthesized copy of the human gene which codes for Epidermal Growth Factor, used as such or adapted to the production host. It contains a maximum of 54 amino acids which may contain disulfide bonds and/or glycosylation. The protein consists of the proper sequence of the 20 standard amino acids.

**SUGGESTED INCI:** Nicotiana benthamiana Hexapeptide-40 sh-Oligopeptide-1

**IECIC 2014:** OLIGOPEPTIDE-1

**CAS NUMBER:** 62229-50-9

**ORIGIN:** Biotechnology.

**CONCENTRATION:** ≤5%

**PROTEIN PURITY:** 97%, purified by sequential chromatography (Affinity and Anionic exchange-FPLC).

**APPEARANCE:** Lyophilized white fine powder.

**RECOMMENDED DOSE:** 0.01 ppm - 0.02ppm.

**SOLUBILITY:** Soluble in water.

**RECONSTITUTION:** Reconstitute with deionized water and prevent from microbial contamination. Trace dilution method is suggested to dissolve the highly pure, frozen dried product. Add to the cosmetic formula during the final phase, preferably below 40°C.

- 100 µg vials: add 5 ml of water and use it in 5 - 10 liters/kg of final formula.
- 250 µg vials: add 5 ml of water and use it in 12 - 25 liters/kg of final formula.
- 500 µg vials: add 5 ml of water and use it in 25 - 50 liters/kg of final formula.

**STERILE FILTRATION:** 0.22 µm

**ENDOTOXIN LEVEL:** Less than 0.04EU/µg as measured by LAL method.

**RECOMMENDED pH:** 5-8.

**PROTEASE ACTIVITY:** Very low to zero protease activity.

**STORAGE:** This lyophilized powder is stable at room temperature. We recommend being stored at 2-8°C. Use immediately after reconstitution, or keep aliquots at -20°C. Keep away from direct sunlight.

**TOXICOLOGY:** The toxicological profile of the product for cosmetic purposes was assessed *in vitro*.

**Cytotoxicity on human dermal fibroblasts:** No cytotoxic.

**Cytotoxicity on human epidermal keratinocytes:** No cytotoxic.

**Genotoxicity:** No genotoxic. Ames test (OECD 471).

**Ocular Irritation:** Not irritating for the eyes (NRU - Neutral Red Uptake test).

**Skin irritation:** Not irritating for the skin (OECD 439).

**Pro-sensitizing potential:** Potentially not sensitizing for the skin. (Monocytes cell line THP-1).
The development of this product had financial support under R+D Programs of biotechnology, research, and plant care. Regeneration, beauty, and skincare are key aspects of the product. The laboratory at Plantaderma, Madrid Scientific Park, is located at c/ Santiago Grisolía 2, D132, 28760 Tres Cantos, SPAIN. The contact information is T +34 916 331 340, F +34 916 333 197, info@plantaderma.es, www.plantaderma.es.