Technical Report

Product

ARGIRELINE® *peptide*

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Revision

27

Lipotec

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Contents

A main concern: the expression lines

One of the main concerns of a high percentage of population is avoiding the first and visible signs of aging. Individuals wish to maintain a **young appearance as long as possible**.

The appearance of **frown and wrinkle lines** in the forehead, glabella, lateral periorbital area (of a relative intensity) as well as chin, upper lip wrinkling, nasolabial folds and platysma neck bands are among the most **striking signs** that betray an **aged skin** [1]. These changes may **arise naturally along with the years** being the result of certain biochemical, histological and physiological changes which are usually **enhanced by environmental exposure**.

There are also other secondary factors that may help in the wrinkles formation and even originate them themselves. They include the constant pull of **gravity**, frequent and constant **positional pressure** of facial skin (during sleeping for instance) or **repeated facial movements** caused by the contraction of the muscles of facial expression causing characteristic folds, furrows and creases [1, 2].

The excessive stimulation of the muscle fibres in the face (which pulls the skin inwards) increases the well-known expression lines, which can become evident at the age of 30. **Attenuating the muscle contraction can be a useful strategy** to remove or delay the appearance of these undesirable lines as much as possible.

A cosmetic treatment targeting the right mechanism implied in muscle contraction can help to minimise these undesirable lines.

The SNARE complex in muscle contraction

Muscle contraction is a process where both the nerve and the muscle are involved in a synapse known as the Neuromuscular Junction (NMJ). **Muscles are contracted when they receive a neurotransmitter released from inside a vesicle of the motor neuron**. This neurotransmitter release is a highly regulated cascade that proceeds through an orchestrated sequence of protein-protein interactions that culminate in the fusion of the neurotransmitter-loaded vesicles with the neuron membrane [3].

A localised **change in membrane potential** (action potential) is transmitted along the neuron to the terminal where it triggers the **entry of calcium ions into the neuron** [3]. When these ions enter the pre-synaptic terminal, the vesicles containing the Acetylcholine (**ACh**) neurotransmitter are induced to join other elements of the neuron in order to release ACh outwards.

This is a complicated process **mediated by** the so-called SNAP Receptors (**SNARE**) proteins, which include the Vesicle-Associated Membrane Protein (**VAMP**) and the membrane-associated protein **syntaxin** and SyNaptosomal Associated Protein (**SNAP-25**) [3]. Such proteins directly govern vesicle docking and fusion through the formation of a ternary structure known as the **SNARE complex**, which is like a cellular hook that captures vesicles and fuses them with the membrane [3, 4].

Once the fusion of these vesicles occurs, **ACh is liberated** into the synapse between nerve and muscle cells. There, ACh binds to Acetylcholine Receptors (**AChR**) located on the surface of muscle cells, finally leading to **muscle contraction**.

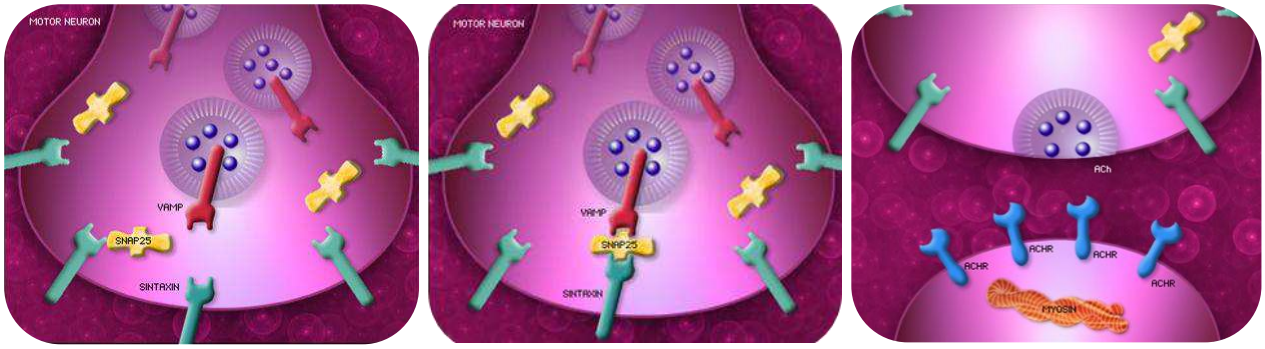


Fig. 1. ACh release and muscle contraction.

The SNARE complex is essential for ACh release at the neuromuscular synapse, which causes muscle contraction.

SNARE proteins are special targets in cosmetics

Structurally, the SNARE complex is a parallel four-helix bundle formed by the coiled-coil arrangement of two helices from SNAP-25 and one each from syntaxin and VAMP [3]. The centre of the complex shows the presence of leucine-zipper layers interspersed with an ionic layer and surrounded by a hydrophobic core.

The SNARE complex is the target of Botulinum Neurotoxins (**BoNTs**), a family of naturally occurring **neurotoxins that potently block neurosecretion.** These BoNTs cause a specific proteolysis of SNAP-25 that avoids this complex assembly and the fusion of ACh vesicles onto the nerve membrane. Therefore, they **impede the exocytosis of the neurotransmitter** and its release into the synaptic cleft, making the affected nerve terminals incapable of stimulating **muscle contraction**, which ends up in **muscle paralysis**.

Although BoNTs and specially type A were a revolution in cosmetics and have been extensively used to attenuate expression wrinkles, they have certain limitations, as they need strict medical control (highly toxic) and require regular intervals of application [5].

Thus, topical **cosmetics** causing the same effects but safely are an **interesting alternative** or even an additional and **complementary treatment** between BoNTs sessions to diminish expression lines.

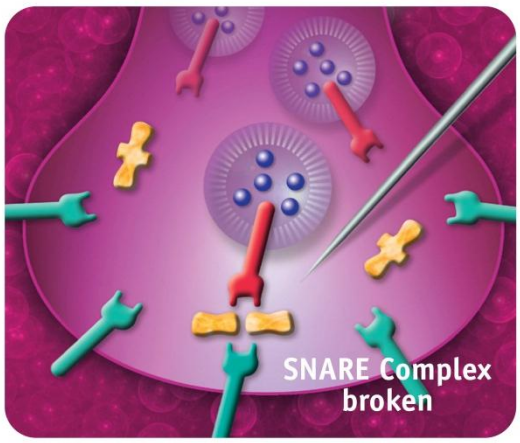


Fig. 2. SNARE complex inhibition by BoNT.

There is a need for topical cosmetic alternatives to reduce expression lines safely.

ARGIRELINE® *peptide*, the revolution for expression wrinkles

ARGIRELINE® *peptide* was designed to act in the pre-synaptic mechanism to reduce the contraction that leads to the wrinkle formation.

ARGIRELINE® *peptide* is a **replica of the N-terminal end of SNAP-25**, which competes with this natural protein for a position in the SNARE complex, **destabilizing its formation**, **without breaking** any of its components. If the SNARE complex is slightly destabilized, the vesicle cannot release neurotransmitters efficiently. Consequently, muscle is **relaxed rather than paralysed**, decreasing the formation of lines and wrinkles.

ARGIRELINE® *peptide* targets the same protein complex as Botulinum Toxin A, modulating muscle contraction. Thus, this ingredient is a safer active with proved efficacy in **destabilizing the SNARE complex**, **reducing wrinkle formation** and **minimising the depth, volume and length of existing wrinkles** and expression lines *in vivo,* as well as skin roughness.

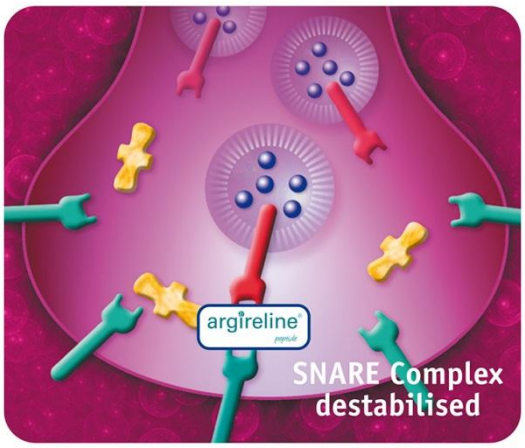


Fig .3. SNARE complex modulation by ARGIRELINE® *peptide*.

ARGIRELINE® *peptide* is an excellent anti-wrinkle ingredient that reduces muscle contraction and its expression lines.

In vitro efficacy

MODULATION OF SNARE COMPLEX FORMATION

This test evaluates the antagonistic competitive efficacy of ARGIRELINE® *peptide* with the wild type SNAP-25 in assembling with syntaxin and synaptobrevin to form the SNARE complex.

SNARE complex was reconstituted using recombinant VAMP and syntaxin proteins and *in vitro* transcribed and translated [^{35}S]-SNAP-25. Equimolar amounts of VAMP and syntaxin were incubated in the absence or presence of ARGIRELINE® *peptide* for 2 h at 4 °C. Then, [^{35}S]-SNAP-25 was added and the mixture was further incubated at 4 °C during 12 h.

The SNARE complex assembly was stopped by addition of Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE) buffer, and the samples were analysed by SDS-PAGE on 12% gels, followed by fluorographic detection on Kodak X-Omat AR X-ray films. Heat disassembles the SNARE complex, thus it was used as positive control.

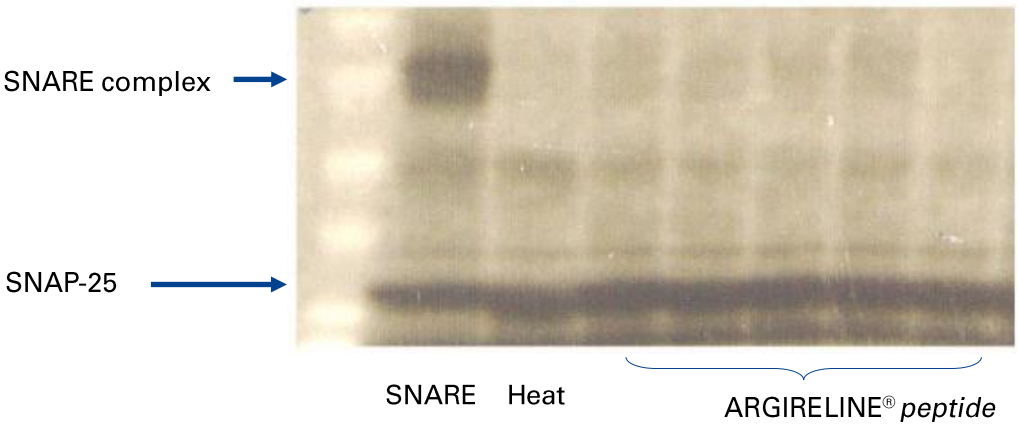


Fig. 4. Modulation of the SNARE complex formation by ARGIRELINE® *peptide*.

The top band corresponds to the SNARE complex and the bottom band shows SNAP-25 presence. The first lane on the left was a control with intact SNARE complex while the second lane showed SNARE complex disassembled by heat, where only the SNAP-25 band was visible. The other lanes showed how **the number of SNARE complexes was lower** when the hexapeptide was present.

ARGIRELINE® *peptide* destabilizes the SNARE complex formation.

INHIBITION OF GLUTAMATE RELEASE

Inhibition of glutamate release by depolarised neuron cells is a validated cell assay for measuring the potential activity of compounds on the inhibition of neuronal exocytosis. The K^+-induced depolarisation of hippocampal cultures in the presence of extracellular calcium ions results in the release of glutamate, which is the most abundant excitatory neurotransmitter in the nervous system.

Primary neuron cells were loaded with L-[^{3}H]-glutamate. Afterwards, they were incubated with ARGIRELINE® *peptide* for 1 h. The release of L-[^{3}H]-glutamate was carried out by depolarisation in physiologic buffer.

The culture media was collected and the quantity of L-[^{3}H]-glutamate was determined by a scintillation counter. The results were normalised regarding the release of L-[^{3}H]-glutamate in absence of the test item (control) and they were corrected from the basal release in absence of calcium.

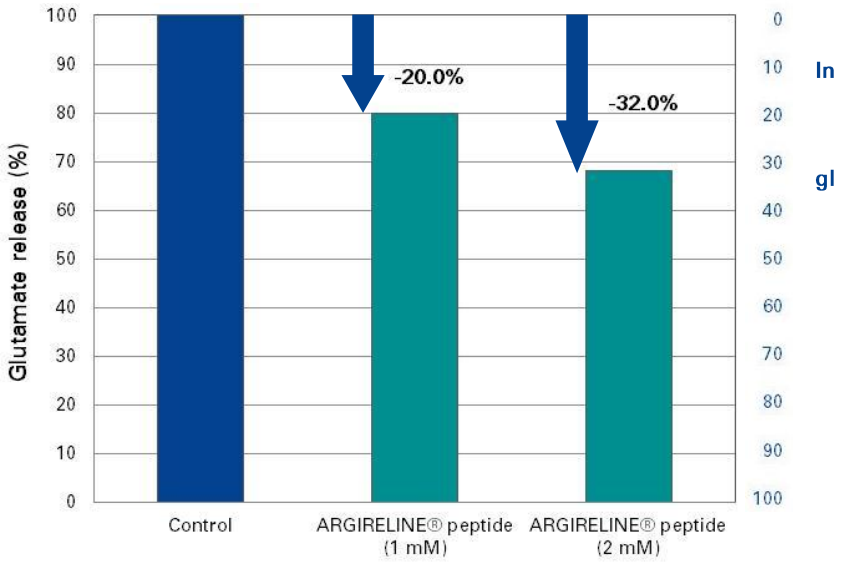


Fig. 5. Inhibition of glutamate release.

The active peptide **decreased glutamate release in a dose-dependent manner**, up to 32.0% at 2 mM, implying a reduction of neuronal exocytosis.

ARGIRELINE® *peptide* effectively modulates glutamate release, indicative of anti-expression wrinkle activity.

In vivo efficacy

REDUCTION OF WRINKLE DEPTH

The anti-wrinkle power of a cream with ARGIRELINE® *peptide solution* was evaluated *in vivo*.

A panel of 10 female volunteers (average age: 44) applied a cream containing 10% ARGIRELINE® *peptide solution* around one eye and a placebo cream around the other twice a day for 30 days.

The silicone imprints of the treated areas were obtained before the treatment and after 15 and 30 days. Then, they were analysed by confocal microscopy.

The depth-coded image generated from section series is a real map of the surface structure of the sample.

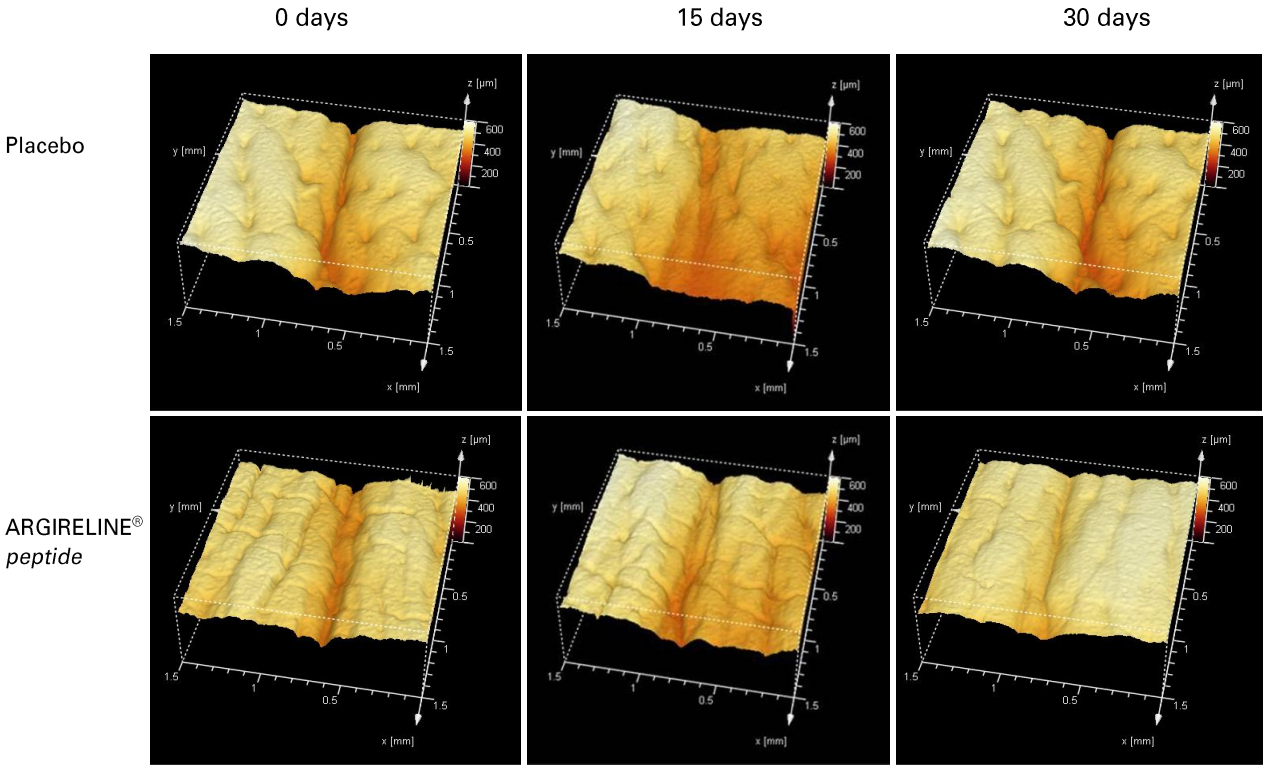


Fig. 6. Images belonging to imprints of the treated areas at different times.

The active peptide **decreased wrinkle depth** by an average of **16.9% and 27.0%** after 15 and 30 days respectively.

ARGIRELINE® *peptide* noticeably diminished wrinkle depth.

ROUGHNESS DIMINUTION IN WRINKLES

To assess the effect of ARGIRELINE® *peptide solution C* in reducing skin roughness, 18 female volunteers between 35-55 years old presenting wrinkles around the eyes were chosen.

Volunteers applied a cream containing 5% ARGIRELINE® *peptide solution C* on the face, including the crow's feet area, twice a day for 28 consecutive days. Skin replicas were taken of the eye crow's feet area and analysed with the PRIMOS technique before and after the 28 days. Macrophotographies of the face were also taken and examined, before and after the treatment.

The evolution of the arithmetic roughness average (Ra) evolution, understood as the average of all heights and depths to the reference plane, was represented.

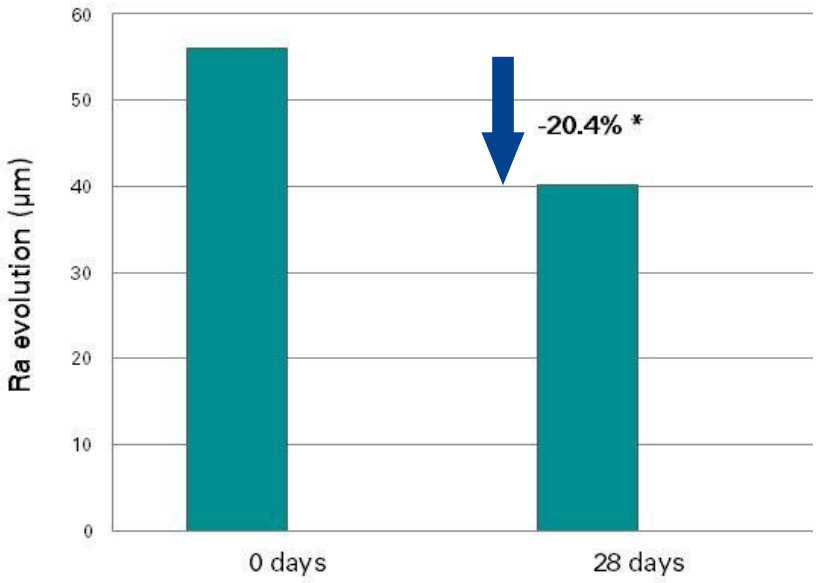


Fig. 7. Mean results of the roughness average before and after the treatment (*p<0.05).

The active peptide **reduced Ra** by **20.4%** after 28 days (statistically significant value).

Skin roughness was clearly decreased by ARGIRELINE® *peptide*.

0 days 28 days

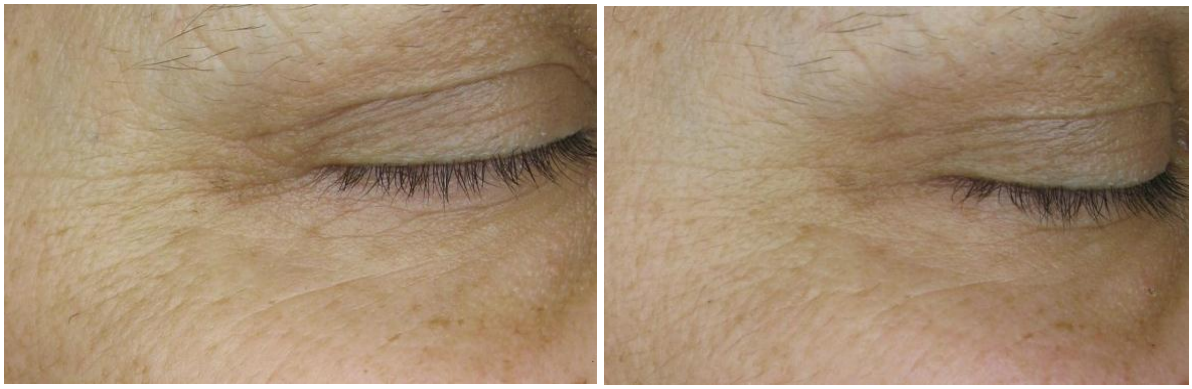


Fig. 8. Images of a volunteer before and after the treatment.

0 days 28 days

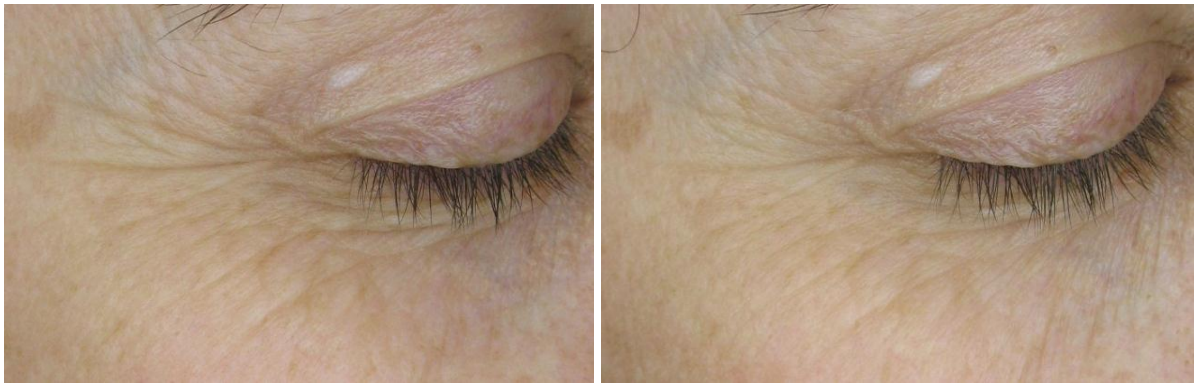


Fig. 9. Images of another volunteer before and after the treatment.

ARGIRELINE® *peptide* improves the wrinkles of the crow's feet area.

EFFECT ON WRINKLE VOLUME AND LENGTH

Another study was carried out to evaluate the efficacy of a cream containing ARGIRELINE® *peptide solution C* in decreasing wrinkles, using the Fast Optical *In vivo* Topometry of human Skin (FOITS) technique.

A panel of 24 female volunteers (between 35-45 years old) was selected to apply a cream containing 2% ARGIRELINE® *peptide solution C* on half of the face, focusing on the crow's feet area, and a placebo cream on the other half twice a day for 7 days.

Pictures were taken and skin topography was quantitatively evaluated, after FOITS measurements (wrinkle volume and length) were recorded at the beginning and end of the treatment.

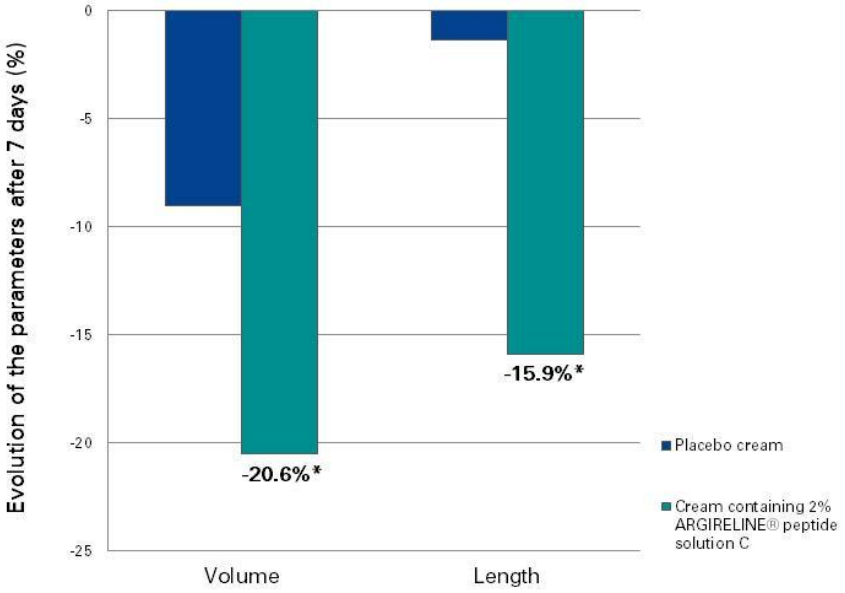


Fig. 10. Evolution of the wrinkle parameters after 7 days (*p<0.05).

The peptide **diminished** the **average volume and length** of the **wrinkles** by **20.6% and 15.9%** respectively, after 7 days. Both values were statistically significant.

ARGIRELINE® *peptide* reduced wrinkle volume and length after 7 days.

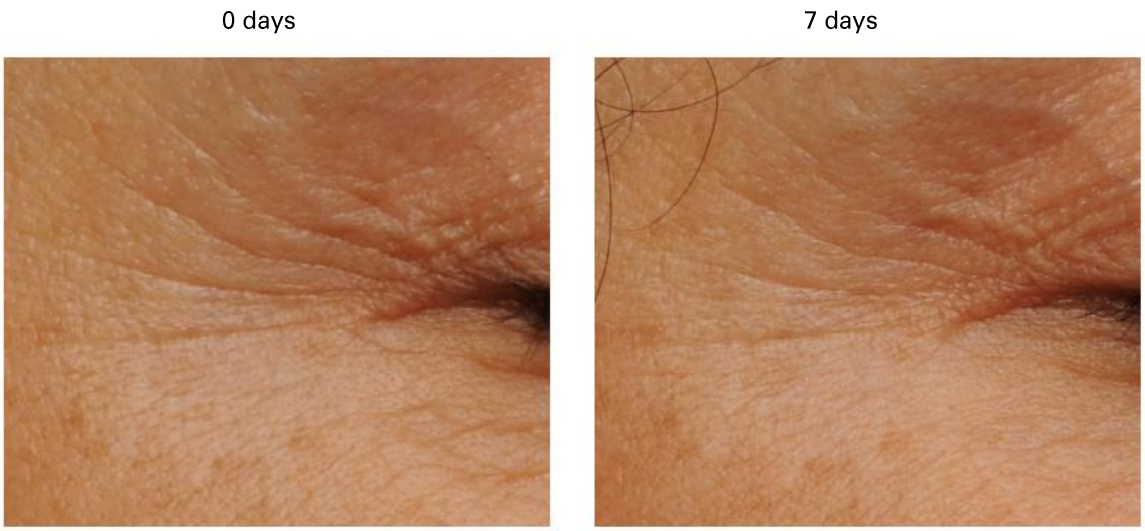


Fig. 11. Images of a volunteer before and after the active treatment.

ARGIRELINE® *peptide* visibly ameliorated wrinkle appearance after just 1 week.

Cosmetic properties

ARGIRELINE® *peptide*:

- is an anti-wrinkle hexapeptide that **destabilizes the SNARE complex arrangement**, reducing the formation of expression lines (induced by muscle contraction) on the skin surface.
- decreased **glutamate release** (up to **32.0%** at 2 mM) in a dose-dependent manner, implying a noticeable reduction of **neuronal exocytosis**.
- is an excellent and effective active ingredient against wrinkles, which offered an average **wrinkle depth decrease** of **16.9%** and **27.0%** after 15 and 30 days of *in vivo* treatment respectively (10% *peptide solution*).
- **reduced the average roughness** (Ra) of skin in volunteers, by **20.4%** after 28 days (5% *peptide solution C*).
- **ameliorates wrinkle appearance**, proven by the decrease of wrinkle **volume** by **20.6%** and wrinkle **length** by **15.9%** after just **7 days** (2% *peptide solution C*).

Cosmetic applications

ARGIRELINE® *peptide* can be incorporated into formulations where the **removal of deep lines or wrinkles** on the forehead or around the eyes area is desired. It especially targets the appearance of the expression lines and delays their formation.

Thus, it is perfect to add into facial creams, sera and treatments intended to fight the first expression wrinkles and to incorporate into anti-aging products.

Technical data

INCI NAME OF THE ACTIVE INGREDIENT

Active ingredient	INCI name
ARGIRELINE® *peptide*	Acetyl Hexapeptide-8

PRESENTATION AND PRESERVATIVE

Solution containing 0.05% of active ingredient.

Code	Product presentation	Preservative
P06-PD010	ARGIRELINE® *peptide solution C*	Preservative free

Other versions available. Please contact your sales representative for further information.

Application data

PROCESSING

ARGIRELINE® *peptide solution C* can be formulated in the aqueous phase in the final step of the manufacturing process. In case of preparing an emulsion, it should be added once the emulsion is formed. In both cases, it should always be provided that the temperature is below 40 °C although it can exceptionally remain stable for 2 h at 80 °C.

ARGIRELINE® *peptide* is stable at a pH range between 3.0 and 8.0.

INCOMPATIBILITIES

Oxidants, electrophiles and tannins.

SOLUBILITY

Soluble in water.

DOSAGE

A dosage of 2-10% of ARGIRELINE® *peptide solution C* is recommended in final cosmetic formulations.

References

1. Benedetto AV. Environmental and skin aging. *Clin Derm*. 16:129:139, 1998.

2. Stegman SJ, Tromovitch TA, Clogau RG. The skin of the aging face in cosmetic dermatologic surgery. 2nd edn. Mosby year book. St Louis. MO, pp:5-15, 1990.

3. Blanes-Mira C, Merino JM, Valera E, *et al*. Small peptides patterned after the N-terminus domain of SNAP25 inhibit SNARE complex assembly and regulated exocytosis. *J Neurochem*. 88:124-135, 2004.

4. Chen YA, Scheller RH. SNARE-mediated membrane fusion. Nature Reviews Molecular Cell Biology 2: 98-106, 2001.

5. Blanes-Mira C, Clemente J, Jodas G, *et al*. A synthetic hexapeptide (Argireline) with antiwrinkle activity. *J Cosm Sci.* 24:303-310, 2002.

HYALURONIC ACID, Sodium salt

Standard & high molecular weight sodium salt of hyaluronic acid

An active ingredient of biotechnological origin for the cosmetic industry

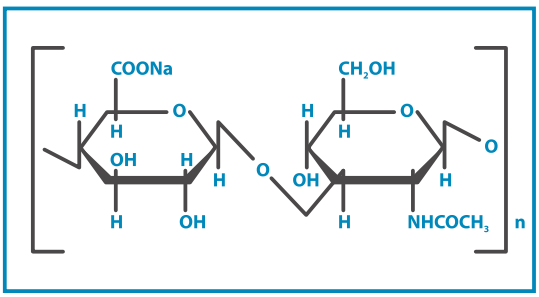
CPN

PRODUCT DESCRIPTION

Hyaluronic acid (HA) is a linear polysaccharide built from regularly alternating monosaccharides (glucuronic acid and N-acetylglucosamine) that form a basic disaccharide unit. The degree of polymerisation is in the order of 10^4 units. This implies that the molecular weight is in the range of millions of Daltons.
Hyaluronic acid is the most hydrated biopolymer known. In living tissue it serves as a connective tissue organiser and water holding substance. In water milieu, HA molecules give rise to spherically coiled structures consisting of about 99 % of immobilised water. Being a part of animal connective tissue, HA is free of immunogenic activity, it is a non-toxic and non-irritating substance.

Trade name:	**Hyaluronic acid**, sodium salt
Chemical name:	poly(sodium-β-D-glucuronate-[1-3]-β-N-acetyl-D-glucosamine-[1-4])
Other names:	sodium hyaluronate, hyaluronan, **Hyaluronic acid**
CAS No:	9067-32-7
INCI name:	Sodium Hyaluronate
EINECS/ELINCS:	232-678-0

Structural formula:



Source: **Hyaluronic acid** is produced by fermentation of a selected streptococcus sp. bacterial strain. The ability of HA production can be positively affected by selecting a special production medium. As biotechnological methods are used to produce HA, the product is free of contaminants such as glycosaminoglycans and proteins of animal origin. During the production process GMOs are not used.

Solubility:

- fully soluble in water. Speed of dissolving depends on molecular weight.
- soluble in a mixture of ethylalcohol, isopropylalcohol, propylene glycol and butylene glycol with water up to ratio (1:1)
- insoluble in non-water miscible solvents

Compatibility and processing:

Hyaluronic acid solution is

- sensitive to heat. Heating to 90°C for 45 min. can lead to the molecular weight decrease up to 20 %.
- sensitive to low and high pH. Extreme values lead to molecular weight decrease, which is further enhanced by product heating.
- incompatible with cationic substance, e.g surfactants or polymeres (Polyquaternium-4, Polyquaternium-10, etc.)

Toxicological data:

- non-irritating
- non-cytotoxic
- non-phototoxic

Supplied forms: powder, solution 1 %

Specification:	**Hyaluronic acid**, powder	
	Origin:	biotechnological processing
	Appearance:	white powder or granules
	Appearance of 0.5 % aqueous solution:	clear to slightly opalescent, colourless, viscous solution
	Clarity of 1 % aqueous solution (660 nm, 1 cm):	< 0.010
	Residue on drying (%):	> 90.0
	Molecular weight (MDa):	1.3 - 1.8
	Kinematic viscosity (0.05 % solution) (cSt):	1.75 - 2.46
	pH of 0.5 % aqueous solution:	5.0 - 8.0
	Protein content (%)*:	< 0.10
	Total microbial count (CFU/g):	< 100
	Uronic acid - UA (%)*:	> 45.0
	Sodium hyaluronate - UA x 2.067 (%)*:	> 93.0
	Ash (%)*:	< 10.0
	Preservative:	none

**calc. on dry basis*

Hyaluronic acid can be supplied with any molecular weight in the range of 1.3 - 2.5 MDa.

Maximum batch size (powder form):	100 kgs
Storage:	store in originally sealed packaging at 2 - 25°C; protect from sunlight
Shelf-Life:	24 months

USE IN COSMETICS

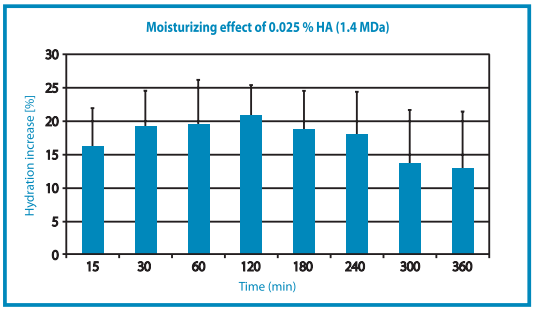
Daily skin care:	0.02 - 0.10 %
Night and reparative preparations:	0.01 - 0.05 %
After sun:	0.02 - 0.10 %
Decorative cosmetics:	0.005 - 0.02 %
Pre-shaves/After shaves:	0.02 - 0.10 %
Cleansers:	0.005 - 0.6 %

EFFICACY DATA

MOISTURIZING EFFECT

Certain level of stratum corneum hydration is essential for skin suppleness and elasticity, as well as for enzyme reactions facilitating stratum corneum maturation events, regular corneodesmolysis and ultimately desquamation. **Hyaluronic acid** only partially penetrates into the skin. It is a substance forming a film on the surface and so hydrating the topical layers of stratum corneum and reducing irritation and the influence of environment on the skin.

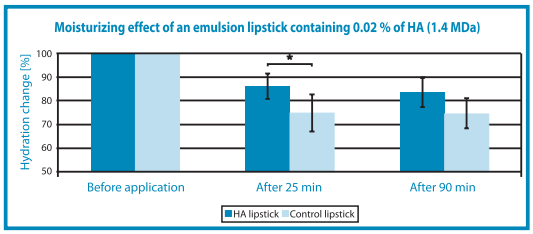
Skin moisturizing

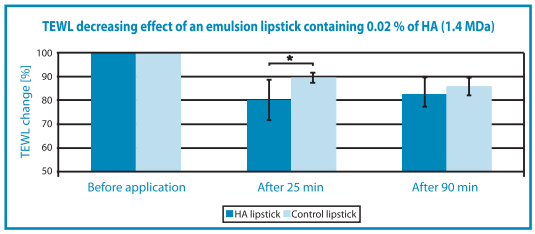


The measurement of cutaneous moisturizing (stratum corneum hydratation) was carried out in a group of 8 volunteers aged between 32 and 47 using Corneometer CM 820 PC. The results are expressed as a percentage increase of hydration reached by emulsion with 0.025 % **Hyaluronic acid** compared to control emulsion.

Lips moisturizing

For **Hyaluronic acid** delivery to the lips, emulsion-based lipstick was developed. Its effectiveness was estimated by TEWL and stratum corneum hydratation measurements.





Immediately after application of commonly used lipsticks, the decrease of SC hydration is recorded which is inherent to method based on a capacitance measurement. In comparison to control, the emulsion lipstick with **Hyaluronic acid** is able to increase the water content within superficial layers in the reach of corneometric measurement significantly, as well as to decrease TEWL at the same time.

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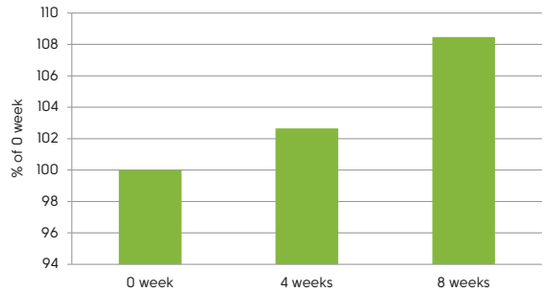
Version: E-01/2009

CONTIPRO
BIOTECH

HYALURONIC ACID

Hyaluronic acid is a linear polysaccharide of high molecular weight, naturally occurring in the human body. It is applied here as a connective tissue organizer and hydrating substance on the basis of its status as the most hydrated polymer known. The enormous water-binding capacity of hyaluronan is an essential characteristic influencing its biological effects, and, as it is a naturally occurring substance, Hyaluronic acid is free of immunogenic activity, and is a non-toxic and non-irritating substance.

Hydration improvement by affecting the skin's epidermal structure



Effect of Hyaluronic acid on skin water content; 8 volunteers treated with 0.005% Hyaluronic acid (23-46 years) + 29 volunteers control group, Daily application for 8 weeks, Measured by MPA 580; Corneometry

As discovered in an *in vivo* study, the natural ability of hyaluronic acid to bind water was reflected, as expected, in an increase in skin hydration in the volunteers monitored. The study, conducted on a group of volunteers aged 23 to 46 years, showed an average increase in skin hydration by 8 per cent.

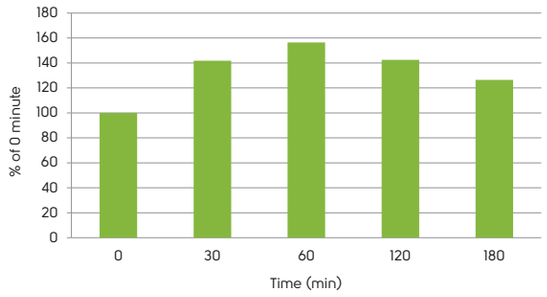
Mechanisms of action

Skin hydration is the cornerstone of the anti-aging concept for the simple reason that adequate water content is essential for the proper functioning of all of the human body's structures. With a certain degree of exaggeration, ensuring hydration is a basic condition for the normalization of all the internal mechanisms affecting skin aging.

Hyaluronic acid influences internal processes taking place in the skin in two ways. The first mechanism is the formation of a film on the skin's surface, a product of the physico-chemical properties of hyaluronan. This film hydrates the surface of the skin and prevents water loss.

Optimal hydration helps to maintain the epidermal barrier function and thereby contributes to suitable conditions for the cells of the epidermis and dermis. Adverse environmental influences and, consequently, cell stress are reduced.
Due to these effects, hyaluronic acid also has soothing effects on the skin, prompted by the creation of a cool surface film and by the modulation of the environment for skin cells.

Improving skin texture by influencing intercellular communication



Moisturizing effect of 0.1% HA, 6 volunteers (27-33 years), Immediate response for 180 minutes, p≤0.05, Measured by MPA 580, Corneometry

The *in vivo* study showed that the increased hydration was accompanied by the very positive effect of hyaluronic acid on TEWL reduction and the quality of skin texture.[1] This intensely perceived touch-related issue, in comparison with the control, positively influenced all persons treated with 0.005% hyaluronic acid from the group of 37 volunteers monitored in the study.

Mechanisms of action

Hyaluronic acid can have a bearing on the texture of the skin mainly because of its ability to form a hydrating surface film on the skin, which reaffirms the strong links between the individual signs of aging.

This moisturizing film essentially macerates the stratum corneum and thus indirectly affects the disruption of intercellular structures in the stratum corneum and the integrity of the epidermis as a whole. The battery of tests indicated appropriate maceration, regulating the desquamation process, accompanied by a beneficial effect on the quality of the epidermis. This normalizes desquamation and improves the mechanical properties of the epidermis, manifested by even desquamation, the augmentation of inequalities and, therefore, better skin texture.

All data were obtained in the relevant *in vivo* and *in vitro* measurements and, subject to registration, can be accessed at www.contipro.com/anti-aging

SPECIFICATION: **Hyaluronic acid**, powder

Origin	biotechnological processing
Appearance	white powder or granules
Appearance of 0.5% aqueous solution	clear to slightly opalescent, colourless, viscous solution
Absorbance of 1% aqueous solution (660 nm, 1 cm)	< 0.010
Residue on drying (%)	> 90.0
Molecular weight (MDa)	1.30 – 1.80
Kinematic viscosity (0.05% solution) (cSt)	1.75 – 2.46
pH of 0.5% aqueous solution	5.0 – 8.0
Protein (%)*	≤ 0.1
Microbial contamination (CFU/g)	< 100
Uronic acid – UA (%)*	> 45.0
Sodium hyaluronate – UA x 2.067 (%)*	> 93.0
Ash (%)*	< 10.0
Preservative	none

* calc. on dry basis

SOURCE

- fermentation, *Streptococcus equi,* subsp. zooepidemicus bacterial strain
- non-GMO
- non-animal materials used during the manufacturing process

SOLUBILITY

- fully soluble in water. Speed of disolving depends on molecular weight.
- soluble in a mixture of ethylalcohol, isopropylalcohol propylene glycol and butylen glycol with water up to ratio 1:1
- insoluble in non-water miscible solvents

COMPATIBILITY AND PROCESSING

- solution is sensitive to heat. Heating to 90 °C for 45 min. can lead to the molecular weight decrease up to 20%
- sensitive to low and high pH. Extreme values lead to molecular weight decrease, which is further enhanced by product heating.
- incompatible with cationic substance, e.g. surfactants or polymers (Polyquarternium-4, Polyquarternium-10, etc.)

TOXICOLOGY

- non-irritating
- non-cytotoxic
- non-phototoxic

Literature

[1] T.Muthny, M. Moravcova (2013). "Skin aging in the context of sun damage and immune response alterations." SOFW Journal 4: 2-8

Version: P-02/2013

CONTIPRO
BIOTECH

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EUROPEAN UNION
EUROPEAN REGIONAL DEVELOPMENT FUND
INVESTMENT IN YOUR FUTURE

Hyaluronic acid

Product dossier

CONTIPRO
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INDEX

Section I.: Marketing

every face has a story

Contipro Anti-aging concept

Product portfolio of Contipro Biotech is focused on the development and production of active substances intended both for preventing skin aging and treatment of skin already showing signs of aging (matured skin). Due to the complex character of this problem Contipro introduced the concept covering both laic understanding of these issues through „*in vivo* signs of aging", as well as a scientific approach that is necessary to respect the complexity of the aging process.

In our concept there can be found both *in vivo* proof of effectiveness of our products (horizontal axis) and the scientific suggestion of their specific mechanism of action (vertical axis) based on *in vitro* tests. This approach allows selecting products that meet your requirements the best for the efficiency of the final cosmetic applications.

As individual aging processes are very closely connected to each other, tested products showed a generally positive effect on various signs of *in vivo* aging. However, to maintain clarity and the relevance of our products in particular, we present maximally two major effects of each product in the first table (see Figure 1).

Anti-aging chart

	hydration	oiliness	texture	wrinkles	elasticity	colour
energy	SCHIZOPHYLLAN				OLIGOHYAFERRE	
imunity		HYSILK	CARBOXYMETHYLGLUCAN	OLIGOHYAFERRE	CARBOXYMETHYLGLUCAN	TANACTINE TENNELIDERM
communication	HYSILK	TENNELIDERM	HYALURONIC ACID	SCHIZOPHYLLAN		TANACTINE
epidermal structure	HYALURONIC ACID CELLCON		HYACTIVE			
dermal structure				HYACTIVE GLUTAPROL	ELASELF GLUTAPROL	

The following text focuses on the effects declared in the previous chart and is only introductory. You will find detailed information related to the effects of other materials in the section dedicated to *in vivo*.

Product concept

Hyaluronic acid (HA) is a linear polysaccharide built from regularly alternating units of D-glucuronic acid and N-acetylglucosamine.

HA is the most hydrating biopolymer known. In living tissues it works as a water reservoir. In water solutions, HA molecules give rise to spherically coiled structures nesting about 99% of immobilized water.

Thanks to the fact, that HA is of a natural character, it finds the utilization in a broad range of cosmetic applications. Its additinal benefit is also a fact, that hyaluronic acid, as a part of animal connective tissue, is free of immunogenic activity, it is a non-toxic and non-iritating substance.

In case of Contipro´HA substance, these crucial aspects are secured by special manufacturing process in our modern production facilities.

Source of our HA is a selected strain of Streptococcus equi sp., known for its potential to produce high molecular weight product, free of any irritating contaminants.

Additional purification process developed specifically by Contipro leads to a product, which provides you with a lot of benefits such as safety of the product, its easy proccessability and high efficiency proven in vivo.

Raw material function

Major claims of Hyaluronic acid

Contipro´s Hyaluronic acid has demonstrated positive effects on several signs of aging during *in vivo* testing. The highest efficiency presented in Contipro conception chart is in hydration and improvement in skin texture.

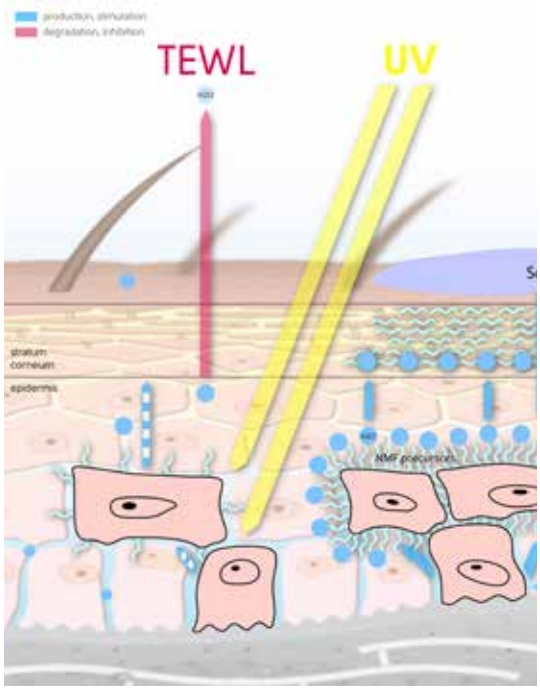
Mechanism of hydration by Hyaluronic acid

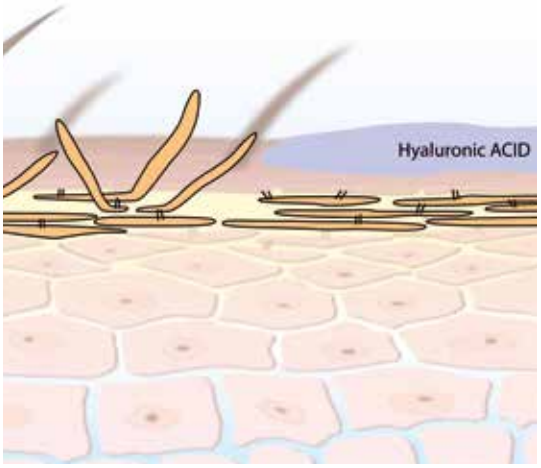
No genuine solution to any of the external signs of aging is inherently possible unless a suitable environment for skin cells is created. By ensuring adequate hydration, we provide the skin with the ammunition to start the physiological reparative processes that slow down aging.

One reason for the loss of water from the skin is its evaporation through the stratum corneum if the barrier function is weakened. Hyaluronic acid creates a film on the skin's surface as a product of its physico-chemical properties. This film hydrates the surface of the skin and prevents water loss. Optimal hydration helps to maintain the barrier function of the epidermis and thereby contributes to conditions conducive to the cells of the epidermis and dermis. Regarding that, the negative environmental influences are suppressed and, consequently, so is cell stress.

Mechanism of texture improvement by Hyaluronic acid

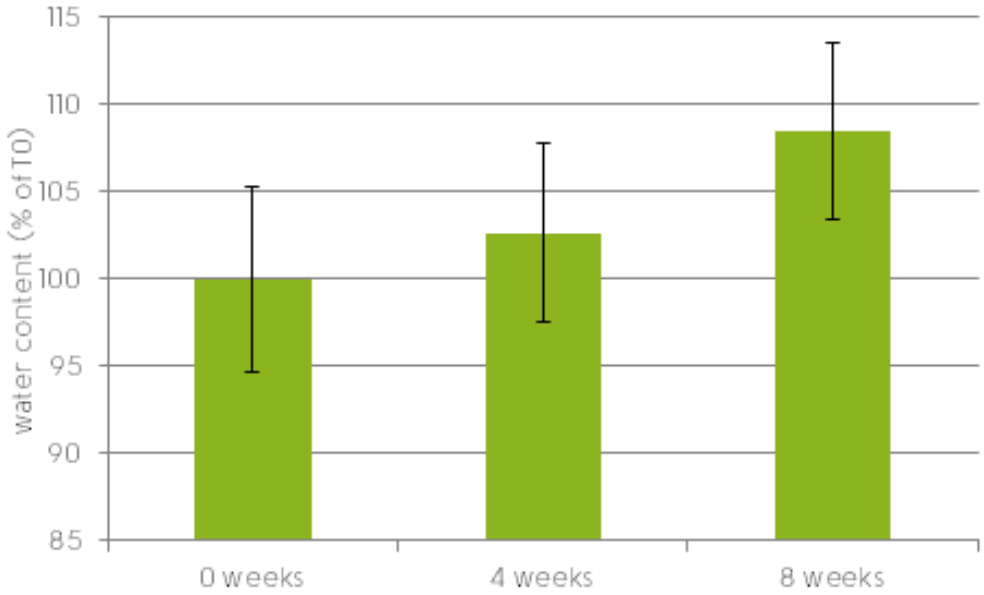
The texture of the skin is often subject to adverse processes occurring as the skin ages. In particular, dry skin is very quickly manifested as a sign of worsening of the skin texture. Dry skin does not desquamate so well, it tends to develop micro-fractures and other inequalities, and it generally gives a visibly damaged impression. Hyaluronic acid has a bearing on the texture of the skin mainly because of its ability to form a hydrating film on the skin surface. This moisturizing film essentially softens the stratum corneum and thus indirectly affects the disruption of intercellular structures in the stratum corneum and the integrity of the epidermis as a whole. This slows down and normalizes desquamation and improves the mechanical properties of the epidermis, resulting in better skin texture.

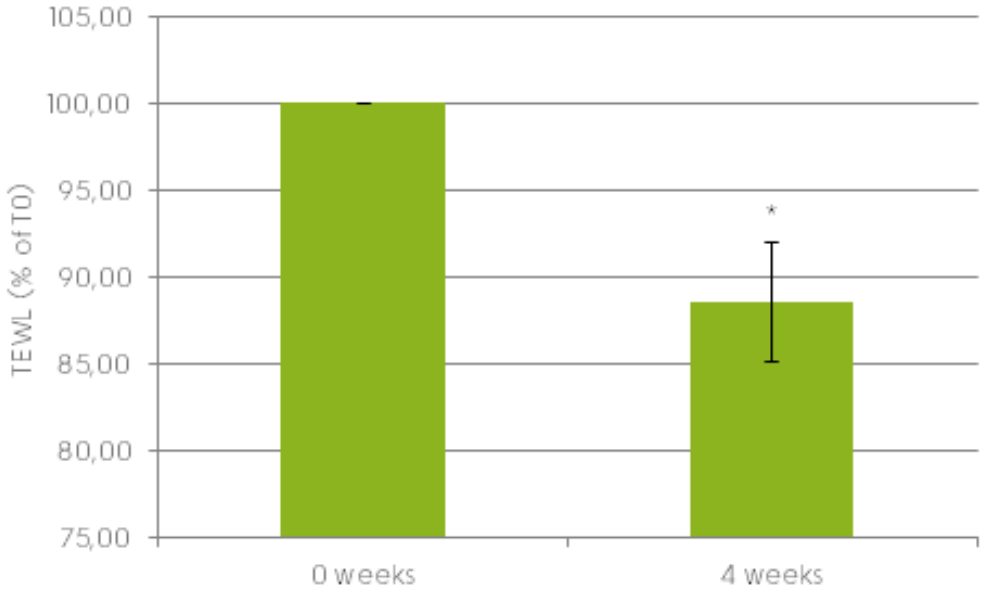




Effect of Hyaluronic acid in vivo

Efect of Hyaluronic acid (0.005%) after 4 (8) weeks of daily treatment on skin hydration and TEWL.





Raw material general properties and characteristics

Hyaluronic acid (HA) is a linear polysaccharide built from regularly alternating monosaccharides (D- glucuronic acid and N-acetylglucosamine) that form a basic disaccharide unit. The degree of polymerization is in the order from 104 units. This implies that the molecular weight is in the range of millions of Daltons.

Hyaluronic acid is the most hydrating biopolymer known. In living tissues it works as a connective tissue organizer and water holding substance. In water milieu, HA molecules give rise to spherically coiled strucures consisting of about 99% of immobilized water.

Due to the polyanionic nature of HA, its properties are very sensitive to pH and to the ionic strength of the solution.

Since HA is normally found in the body, it is free of immunogenic activity. HA forms cellular coats and it is a normal component of blood lymph and connective tissue.

HA of different molecular weight has a very diverse biological activity and the penetration properties. Topical application of the substance has different effects depending on MW[1].

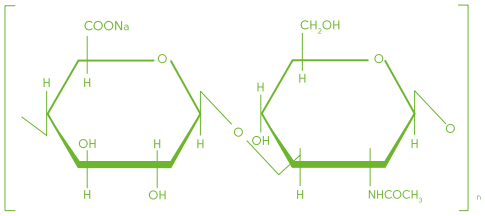
[1]Efficacy of cream-based novel formulations of hyaluronic acid of different molecular weights in anti-wrinkle treatment. Pavicic T, Gauglitz GG, Lersch P, Schwach-Abdellaoui K, Malle B, Korting HC, Farwick M. J Drugs Dermatol. 2011 Sep; 10(9):990-1000.

Section II.: Technical data

Raw material description

Structural formula

$[C_{14}H_{20}NNaO_{11}]_n$



Sodium hyaluronate is a widespread naturally occurring polymer, which is found in many types of body tissues.

Molecular weight of the Hyaluronic acid ranges between 1300 - 1800 kDa and is without any chemical modification. For product specification see the section Enclosures.

Raw material production

General description of manufacturing process

Hyaluronic acid is a high molecular weight sodium salt of hyaluronic acid.

Hyaluronic acid is obtained by fermentation of a non-haemolytic microbial strain Streptococcus equi and after a certain adjustment to the medium it is obtained by filtration. It is then repeatedly precipitated and washed until completely clean. After that, it is dried.

The process consists of steps that are performed in the designated areas. Production unit (factory buildings and equipment) are intended solely for the production of sodium hyaluronate and fraction of industrial medium are subject to strict selection.

Raw material source

Hyaluronic acid is a product of fermentation of micro-organisms of the family Streptococcaceae, specifically the type of Streptococcus equi, ssp zooepidemicus.

Streptococci are micro-organisms forming an oval or round cells with a diameter less than 2.0 µm. They are a facultative anaerobic organisms growing at temperatures of optimum around 37 °C, which is due to the fact that most species are associated with higher mammals. Some species form the housing.

S. equi is capable of producing endogenous HA, which is then transported to the surface of the microorganism where protective casing is formed. Since S. equi is commensal of horses, produced hyaluronic acid is not immunogenic. As the hyaluronic acid is imposed on the cell surface, HA is not extracted from the microorganism but only from the medium.

As the biotechnological method is used to produce Hyaluronic acid, the product is free of contaminants such as glycosaminoglycans and proteins of animal origin. GMOs are not used during the production process (see Section III).

Specific substances used during the preparation or in relation to the shelf-life

During Hyaluronic acid production, only purified water and isopropyl alcohol as solvents are used.

For pH adjustement and cleaning procedures during the production process acetic acid and NaOH are used.

As the product is supplied in powder form, no preservatives are used for storage.

Packaging, shelf-life, storage conditions

Hyaluronic acid is supplied in powder form, sealed in polyethylene (PE) bag, put into a three layer aluminium foil.

Store in a dry place in originally sealed packaging.
Storage temperature 2–25 °C.
Keep away from sunlight.

Shelf-Life: 24 months

Formulation

Recommended dosage

Recommended concentration in final products is 0.01 – 0.1%.

Critical factors

Temperature:

Hyaluronic acid solution is sensitive to heat. Heating to 90°C for 45 min. can lead to the molecular weight decrease up to 20%.

pH:

Hyaluronic acid solution is sensitive to low and high pH values. Extreme values lead to molecular weight decrease, which is further enhanced by product heating.

Recommended range of pH for formulations is 5.5-8.

Incompatibility:

Hyaluronic acid is incompatible with cationic substances, e.g surfactants or polymers (Polyquaternium-4, Polyquaternium-10, etc.).

Processing

Dissolution:

Hyaluronic acid is fully soluble in water. Speed of disolving depends on molecular weight. It is also soluble in a mixture of ethylalcohol, isopropylalcohol propylene glycol and butylene glycol with water up to ratio 1:1.

Stirring, emulsions processing:

Hyaluronic acid is added into formulations in the form of solution. It is possible to prepare solution in the concentration up to 2% depending on the molecular weight and required volume and viscosity.

In the case of O/W emulsions, it is recommended to add Hyaluronic acid solution into emulsion at the temperature lower than 70°C, preferably during phase of emulsion cooling. If it is necessary to minimize volume of added solutions during cooling phase (e.g. because of technological reasons) it is possible to add Hyaluronic acid solution into water phase before emulsion processing. The reduction of molecular weight can occur therefore it is recommended to consider using product with higher molecular weight.

In the case of W/O emulsions, solution of Hyaluronic acid is added into water phase before emulsion processing. It is heated again, therefore the risk of molecular weight reduction can appear.

Preservation

Hyaluronic acid solutions must be used up to 12 hours from the preparation or must be preserved to avoid contamination and microbial growing.

It is possible to preserve Hyaluronic acid solutions by common preservatives, e.g. phenoxyethanol, phenonip, benzylalcohol etc.

Recommended applications

Hyaluronic acid is suitable for wide spectrum of formulations. You can use it in following final products and concentrations:

DAY CREAM 0.02–0.10%

NIGHT CREAM 0.01–0.05%

EYE CREAM 0.01–0.05%

SERUM 0.05–0.1%

DECORATIVE COSMETIC 0.005–0.1%

TONIC 0.01–0.05%

MAKEUP REMOVER 0.01–0.1%

AFTER SUN LOTION 0.02–0.1%

AFTERSHAVE 0.02–0.1%

From the point of final products function, Hyaluronic acid is suitable for following types of products:

MOISTURIZING

ANTI WRINKLE

REVITALIZING

PROTECTING

Formulation example

See model formulation for Gel Eye Make-up Remover in section V. Enclosures.

Section III.: Certification & Registration

Chemical info

CAS, INCI, EINECS

Chemical name:

Poly (sodium-β-D-glucuronate-[1-3]-β-N-acetyl-D -glucosamine-[1-4])

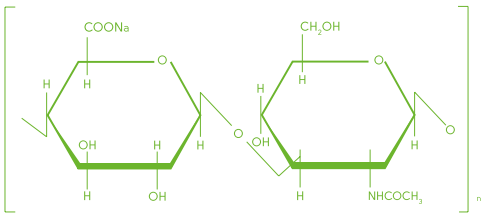
CAS No: 9067-32-7

INCI name: Sodium Hyaluronate

EINECS/ELINCS: 232-678-0

Other names: Hyaluronic acid, sodium salt

Structural formula:



Nature of special risks attributed to dangerous substances and preparations

(R-sentences / H-sentences)

Hyaluronic acid is not-classified as a dangerous substance or mixture according to the CLP/EU-GHS.

Standard instructions for safe handling and storage of chemical substances and preparations

(S-sentences / P- sentences)

S47: Keep at temperature not exceeding 2–25 °C.

S49: Keep only in the original container.

P234: Keep only in original container.

P410+411: Protect from sunlight. Store at temperatures not exceeding 2–25 °C.

Cytotoxicity

0.2 ml of Hyaluronic acid, sodium salt (concentrations 100 – 10 000 µg/ml) was added to 3T3 fibroblasts and treated for 24 hours. Evaluation was performed by means of fluorimetry (due to the viscosity of material with the highest concentration, evaluation was performed microscopically in that case). Hyaluronic acid, sodium salt was not cytotoxic up to concentration 10 000 µg/ml.

Phototoxicity

Test was performed according to OECD TG 432. Eight different concentrations (21.5 – 4 640 µg/ml) of Hyaluronic acid, sodium salt were added to 3T3 fibroblasts and incubated for 1 hour. They were theyn exposed to UV light for 50 min. Evaluation was performed by means of fluorimetry. Hyaluronic acid, sodium salt did not have phototoxic potential.

Acute skin irritation/corrosion

35 µl of 1% water solution of Hyaluronic acid, sodium salt per patch field was applied for 24 hours. Evaluation was performed visually 20 minutes – 48 hours after patch removal. Hyaluronic acid, sodium salt did not irritate the skin at concentrations below 1 percent.

Embrytoxicity

Embryotoxicity of Hyaluronic acid, sodium salt was tested by means of the methods CHEST I AND II on chicken embryos of early developmental stage. Evaluation was performed visually. In the CHEST I, Hyaluronic acid, sodium salt (HA) in doses of 6 – 100 µg/embryo had no influence on mean lengths of new-formed caudal extremities of the chick embryos after 24 hours of incubation. In the CHEST II test, HA in doses of 6 – 100 µg/embryo had no influence on chick embryos. HA in doses of 6 – 100 µg had no adverse embryotoxic or teratogenic effects on 2, 3 and 4 days old chick embryos. None of the used doses (even in the maximum one) had a disadvantageous influence on the embryonal development.

Irritation of chorioallantoic membrane

Hyaluronic acid, sodium salt was administered to chorioallantoic membrane of the chick embryo in concentrations of 0.06 – 1%. Evaluation was performed visually. HA in tested doses of 6 – 100 µg/embryo had no irritant effect on the chorioallantoic membrane

over the whole time interval from 20 seconds to 24 hours.

Cytotoxicity

Hyaluronic acid, sodium salt in concentration 0.01, 0.02 and 0.05% in a suspension culture of the human lymphocytes had no effect on liberation of cytosolic LDH (liberated lactate dehydrogenase) into the media in the course of 1 – 3 hours. HA had no cytotoxic effect on the cells of the human lymphocytes.

Haemolytic activity

A haemolytic effectiveness of Hyaluronic acid, sodium salt was tested for 1.0, 0.5, 0.2 and 0.1% HA solution in saline. 5 ml of the HA solution with named concentrations were added to 1 ml of the human red cell suspensions and incubated for 20 minutes. Evaluation was performed by spectrophotometer. HA in the concentration 1.0 – 0.1% revealed no haemolytic effect on human erythrocytes.

Mutagenicity

1% solution of Hyaluronic acid, sodium salt underwent testing on gene mutations in genome of Salmonella typhimurium strains according to procedure OECD 471. The sample was added in volumes 100 – 500 µl per dish. HA had no mutagenic effectiveness for all indicator strains used regardless of metabolic activation.

Genotoxicity

Genotoxicity of Hyaluronic acid, sodium salt was determined by means of induction of non-programmed synthesis of DNA (UDS) in fibroblasts from human embryonal lungs. 100 µl of HA were added to cells in concentrations 0.006 – 0.1% and no significant extent of reparative DNA synthesis was detected, thus HA had no genotoxic properties.

Toxicity

Test	Model	Performed	Result
Cytotoxicity	*in vitro*, 3T3 NR uptake	2006	non-cytotoxic
Phototoxicity	*in vitro*, 3T3 NR uptake	2006	non-phototoxic
Acute skin irritation/corrosion	*in vivo*, human forearm	2000	non-irritant
Embrytoxicity	chick embryo	1993	non-embryotoxic
Irritation of chorioallantoic membrane	HET-CAM	1993	non-irritant
Cytotoxicity	human lymphocytes	1993	non-toxic
Haemolytic activity	human erythrocytes	1993	non-haemolytic
Mutagenicity	BRMT (S.typhimurium)	1997	non-mutagenic
Genotoxicity	human lung fibroblasts	1997	non-genotoxic

Heavy metals

The content of heavy metals in our product is not monitored on a regular basis; determination of the heavy metals content is performed upon a customer's request.

Microbial count

Microbial contamination of this product is < 100 CFU/g.

Stability data

The long-term stability testing runs under following conditions: 25 °C ±2 °C/ 60% RH ±5% R

PARAMETER	SPECIFICATION	RESULT					
		month					
		0	6	12	18	24	36
Loss on drying (%)	≤ 10.0	7.3	7.2	4.8	7.3	7.3	7.4
Kinematic viscosity (cSt)	monitoring	2.10	2.07	2.02	2.01	1.95	1.95
Molecular weight (SEC-MALS) (MDa)	≥ 1,3	1.59	1.58	1.54	1.54	1.49	1.49

The available data show that all parameters are within acceptance limit after 36 months of the study.

Registration

Product certification

ECOCERT/COSMOS:

COSMOS certificate has been granted according to the Standards for Ecological and Organic Cosmetics (see the section Enclosures).

REACH

Hyaluronic acid (sodium salt) is exempted from a scope of Regulation (EC) No 1907/2006 (REACH) as amended as a non-toxic polysaccharide of nature origin.

The product is not classified as a SVHC substance according to Annex XIV to REACH Regulation or candidate substance as well.

Company certification

Contipro Biotech s.r.o. meets all requirements for good manufacturing practises and for the biotechnological production of sodium hyaluronate according to ISO standards. Contipro Biotech s.r.o. has been awarded ISO 22716:2007 and ISO 9001:2000 certificates (see the section Enclosures).

GMO-free statement

No genetically modified material or material derived from genetically modified organisms are used in the manufacturing process.

Animal-sources-free statement

No material of animal origin is used during the manufacturing process..

Legal status in key territories

To the best of our knowledge there are not any any restrictions on use of sodium hyaluronate in cosmetic products according to legislation in force and lists of prohibited cosmetic ingredients (EU Cosmetic Regulation, CIR, Canadian Hot List, TSCA).

Section IV.: Scientific support

In vivo study

Two *in vivo* studies have been conducted in Contipro Holding to provide efficacy evidence of our raw materials. In 2012, the study was focused to observe long term effect of very low doses. In 2013, the aim was to obtain results from shorter time period using higher levels.

Proband group characteristics

Study 2012: men (cca 25%) and women (cca 75%), control group – 29, age 24-66 years (31 in average), HA group 8, age 23-46 years (30 in average).

Study 2013: men (cca 25%) and women (cca 75%), control group – 15, age 24-63 years (32 in average), HA group 7, age 20-49 years (31.6 in average).

Study design

Study 2012: 84 days, daily application (15,6 ug of HA/day in average) on one half of the face.

Study 2013: 42 days, daily application (380 ug of HA/day in average) on one half of the face.

Volunteers were instructed to habit as usual including using their usual cosmetics. To start with using any NEW cosmetics during the study was not allowed.

Single test methodics

Study 2012: Measurement period – start of the study, 28 days, 56 days, 84 days, measured areas - forehead, periorbital area, cheek.

Study 2013: Measurement period – start of the study, 14 days, 28 days, 42 days, measured areas - forehead, periorbital area, cheek.

TEWLmetry: Transepidermal water loss as a marker of skin barrier function was measured by standard probe by Courage and Khazaka. The method is based on diffusion law.

Corneometry: Hydration of upper layers of epidermis was measured by standard probe by Courage and Khazaka. The principle of the method is measurement of electrical capacitance.

Study 2012: Measurement period – start of the study, 28 days, 56 days, 84 days, measured areas - forehead, periorbital area, cheek.

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Corneometry: Hydration of upper layers of epidermis was measured by standard probe by Courage and Khazaka. The principle of the method is measurement of electrical capacitance.

Detailed description of used raw material

O/W emulsion was used as tested formulation. The concentration of active ingredient (HA) was 50 ug/g (0.005%) in Study 2012 and 1000 ug/g (0,1%) in Study 2013. The same formulation without HA was used for control (placebo) group. The total consumption of formulation per study was aprox. 20 g per person both in Study 2012 and 2013.

Formulation content

Formulation content:

Triglycerides - 12%

Mixture of glyceryl stearate + PEG 100 stearate - 5%

Mixture of sodium acrylate + acryloildimethyl taurate copolymer + isohexadekane + polysorbate 80 – 2%

Vitamin E acetate – 1%

Demineralized water – 75% (placebo), 74,5 (study 2012), 65% (study 2013)

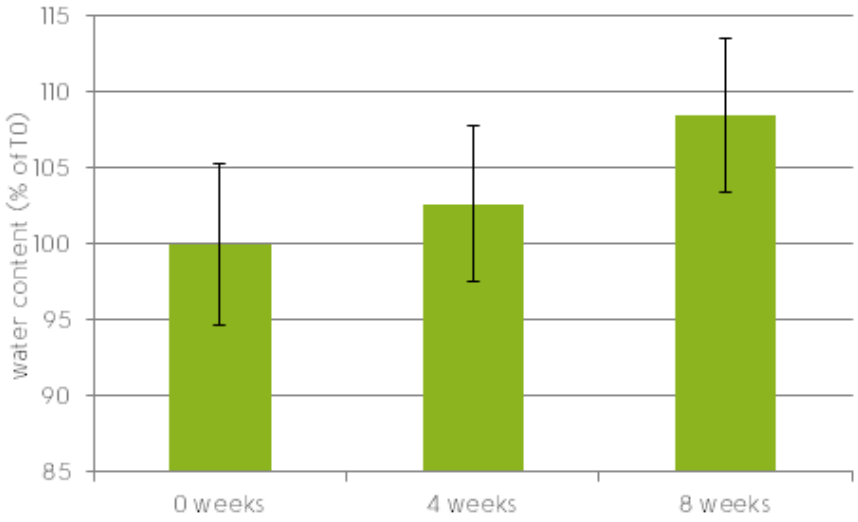
Glycerine – 4%

1% solution of HA – 0% (placebo), 0,5% (study 2012), 10% (study 2013)

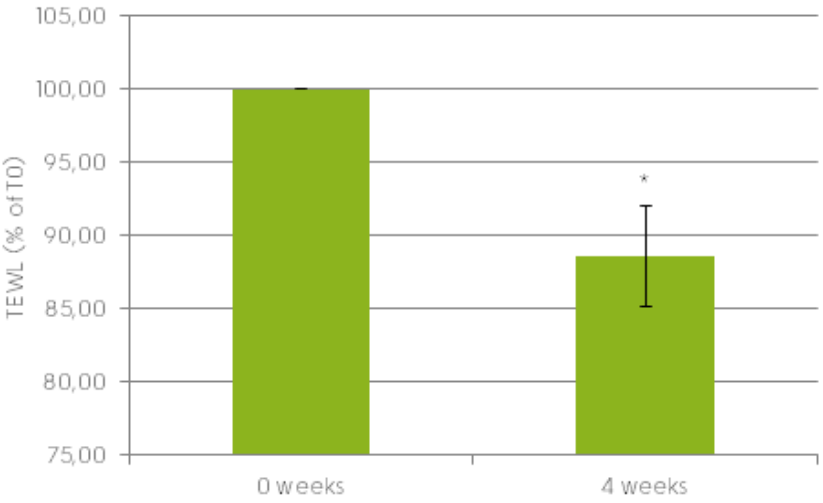
Benzylalcohol – 0.8%

Fragrance – 0.2%

Statistical significance of the results 2012



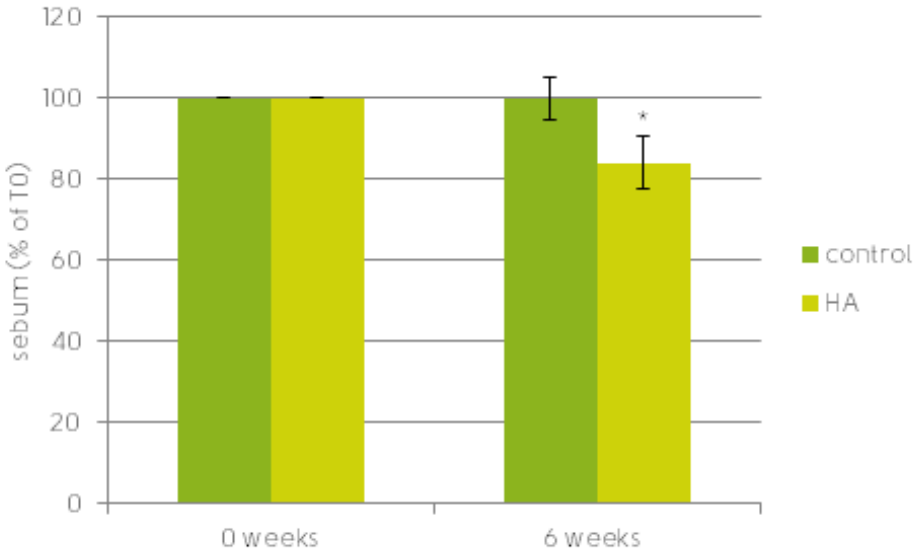
Skin water content (%) cheek, MPA 580 Corneometer CM825, trend.



Trans-epidermal water loss (%), whole face, MPA 580 Tewlmeter TM300, *p≤0.05 [1,2].

Study 2013

There were similar results obtained in 2012 and 2013 considering skin hydration and texture. Moreover, using higher levels in 2013 caused decrease of sebum production. This effect might correspond with higher hydration and better barrier function of the skin. – see below.



Sebum production (%), whole face, Sebumeter, p = 0.02 (HA T0 week vs HA T6 weeks)

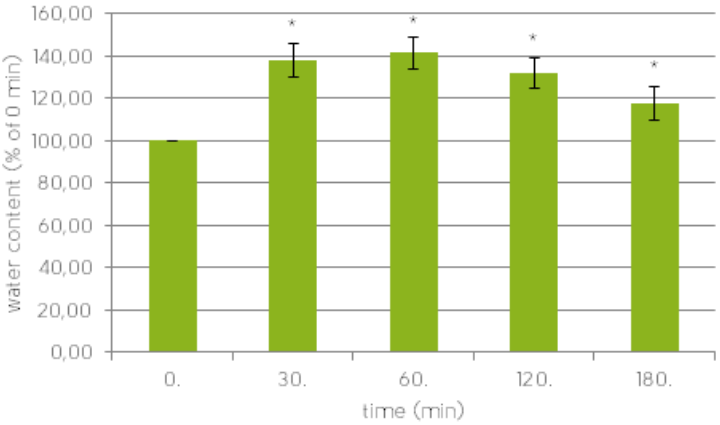
Additional *in vivo* and *in vitro* tests

Test 1: Immediate hydration

Material specification: Hyaluronic acid 1.66 MDa

Theory: High molecular weight Hyaluronic acid does not penetrate in the skin but it creates a protective film on skin surface. Thanks to its ability to bind big amount of water, this film is able to immediatelly increase the water content in stratum corneum which improves conditions in lower layers of skin.

Methodology of test: This test was performed on 6 volunteers in age 27-33 years. 0.1% HA in emulsion was applicated on inner forearm and hydration of stratum corneum and TEWL were measured 30, 60, 120 and 180 min. after application by standard probes by Courage and Khazaka. Data were normalized to the values measured right before the application of HA.



n = 6, MPA 580 Corneometer CM825, mean ± SEM,
p < 0,05 compared to T0

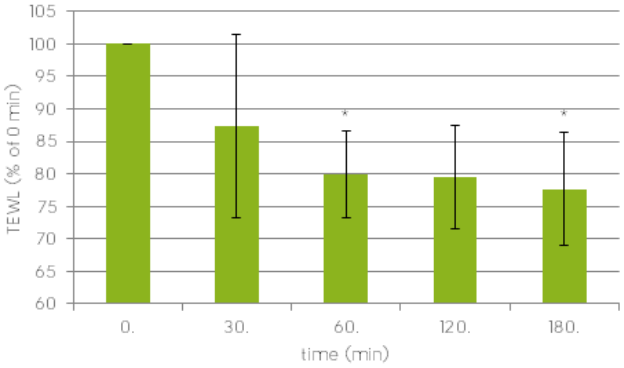
Test 2: Immediate trans-epidermal water loss

Material specification: Hyaluronic acid 1.66 MDa

Theory: High molecular weight Hyaluronic acid does not penetrate into the skin but it creates a protective film on skin surface. This film not only increases the hydration of the stratum corneum, but also decreases immediate loss of water. This effect helps to maintain barrier function of the skin.

Methodology of test: See Test 1

n = 6, MPA 580 Tewlmeter TM300, mean ± SEM,
$p < 0.05$ compared to T0



Related literature

Muthný, T. & Moravcová, M. Skin aging in the context of sun damage and immune response alterations. Sofw J. 2–8 (2013).

Muthný, T. & Papež, T. The many faces of hyaluronan. Househ. Pers. Care Today 26–28 (2012).

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Section V.: Enclosures

Safety data sheet

HYALURONIC ACID, SODIUM SALT

SAFETY DATA SHEET

ISSUE DATE: 29 July 2010
REVISION DATE: 1 December 2011

1. IDENTIFICATION OF THE SUBSTANCE AND OF THE COMPANY

1.1 PRODUCT IDENTIFIER
1.1.1 Trade name: Hyaluronic acid, sodium salt - powder
1.1.2 INCI name: Sodium hyaluronate

1.2 RELEVANT IDENTIFIED USES OF THE SUBSTANCE AND USES ADVISED AGAINST
For use in cosmetics

1.3 DETAILS OF THE SUPPLIER OF THE SAFETY DATA SHEET
Address: Contipro Biotech s.r.o., Dolní Dobrouč 401, 561 02 Dolní Dobrouč, Czech Republic
Telephone: +420 465 520 035
E-mail: sales@contipro.com

1.4 EMERGENCY TELEPHONE NUMBER
Manufacturer (Reception): +420 465 519 530

2. HAZARDS IDENTIFICATION

2.1 CLASSIFICATION OF THE SUBSTANCE
Not classified as dangerous according to the Regulation (EC) No 1272/2008

2.2 LABEL ELEMENTS: Not relevant

2.3 OTHER HAZARDS: None

Not identified as PBT or vPvB according to the Annex XIII of the Regulation (EC) No 1907/2006 as amended

3. COMPOSITION / INFORMATION ON INGREDIENTS

3.1 SUBSTANCE
Hyaluronic acid, sodium salt (polysaccharides)
CAS number: 9067-32-7

3.2 MIXTURE: Not applicable

4. FIRST AID MEASURES

4.1 DESCRIPTION OF FIRST AID MEASURES
4.1.1 Inhalation: If breathed in, move person to the fresh air. Seek medical attention.
Skin: After contact with skin wash with water and soap.
Eye: In case of contact with eyes, rinse immediately with plenty of water.
Ingestion: Seek medical attention in case of any sickness.
4.1.2 Not applicable

4.2 MOST IMPORTANT SYMPTOMS AND EFFECTS
No adverse effects supposed when in contact with skin, eyes or mucous membranes.

4.3 IDENTIFICATION OF ANY IMMEDIATE MEDICAL ATTENTION AND SPECIAL TREATMENT NEEDED: Not applicable

5. FIREFIGHTING MEASURES

5.1 EXTINGUISHING MEDIA: Water foam, water spray, dry chemicals, carbon dioxide

5.2 SPECIAL HAZARDS ARISING FROM THE MIXTURE: Combustion products include CO, CO_2

5.3 ADVICE FOR FIREFIGHTERS: Gas mask (CO)

6. ACCIDENTAL RELEASE MEASURES

6.1 PERSONAL PRECAUTIONS, PROTECTIVE EQUIPMENT AND EMERGENCY PROCEDURES: Avoid from contact with skin/eyes

6.2 ENVIRONMENTAL PRECAUTIONS: None

6.3 METHODS AND MATERIALS FOR CONTAINMENT AND CLEANING UP: After spillage wash with 50 % ethanol or propanol

7. HANDLING AND STORAGE

7.1 PRECAUTIONS FOR SAFE HANDLING: Adhere to common safety measures

7.2 CONDITIONS FOR SAFE STORAGE, INCLUDING ANY INCOMPATIBILIES
Store in a dry place in originally sealed packaging. Storage temperature 2-25 °C. Recommended to store at the temperature of 2-8°C after opening. Keep away from sunlight.

7.3 SPECIFIC END USE: Cosmetic ingredient

8. EXPOSURE CONTROL / PERSONAL PROTECTION

8.1 CONTROL PARAMETERS: No exposure limits

8.2 EXPOSURE CONTROLS
8.2.1 Not applicable
8.2.2 Individual protection measures, such as personal protective equipment:
Hygiene measures: Good industrial practice. Do not drink, eat or smoke while working
Eye/face protection: Use chemical safety goggles when necessary
Hand protection: Wear laboratory gloves
Skin protection: Wear casual working clothes
Respiratory protection: Not required
Thermal hazard: Not applicable
8.2.3 Environmental exposure controls: No special environmental precautions

9. PHYSICAL AND CHEMICAL PROPERTIES

9.1 INFORMATION ON BASIC PHYSICAL AND CHEMICAL PROPERTIES

Appearance:	white to slightly yellow powder or granules
Odour:	odourless
Boiling point:	not applicable
pH:	5.0 - 8.5 for 0.5 % aqueous solution
Melting point/freezing point:	data not available
Flash point:	not applicable
Evaporation rate:	not applicable
Flammability (solid, gas):	hygroscopic, poorly flammable
Explosive limits:	none
Vapour pressure:	not applicable
Relative density:	not applicable
Solubility:	soluble in water and in a mixture of water and water-miscible organic solvents (e.g. ethanol, propanol) insoluble in fats and anhydrous organic solvents
Partition coefficient (n-octanol/water):	data not available
Auto-ignition temperature:	data not available
Decomposition temperature:	data not available - polysaccharide, decomposition rate increases with temperature significantly above 50 °C
Viscosity:	data not available
Explosive properties:	none
Oxidizing properties:	none

9.2 OTHER INFORMATION
Hygroscopic polysaccharide

10. STABILITY AND REACTIVITY

10.1	REACTIVITY: No hazardous reactions
10.2	CHEMICAL STABILITY: The product is stable under the appropriate storage conditions
10.3	POSSIBILITY OF HAZARDOUS CHEMICAL REACTION: Not known
10.4	CONDITIONS TO AVOID: Direct sunlight, temperatures above 50 °C
10.5	INCOMPATIBLE MATERIALS: Oxidising agents
10.6	HAZARDOUS DECOMPOSITION PRODUCTS: Unidentified organic compounds

11. TOXICOLOGICAL INFORMATION

11.1 INFORMATION ON TOXICOLOGICAL EFFECTS

11.1.1 SUBSTANCES

Acute toxicity:	non-toxic
Skin irritation:	non-irritating
Eye irritation:	non-irritating
Respiratory or skin sensitisation:	non-sensitising
Germ cell mutagenity:	non-mutagenic
Carcinogenicity:	data not available
Reproductive toxicity:	non-toxic for reproduction
STOT-SE/STOT-RE:	data not available
Aspiration hazard:	not applicable

IARC: Not identified as carcinogenic.

POTENTIAL HEALTH EFFECTS: No toxic properties have been detected

12. ECOLOGICAL INFORMATION

12.1	TOXICITY: No negative effect
12.2	PERSISTENCE AND DEGRADABILITY: Fully biodegradable
12.3	BIOACCUMULATIVE POTENTIAL: Does not accumulate in living organisms
12.4	MOBILITY IN SOIL: Not applicable
12.5	RESULTS OF PBT AND VPVB ASSESSMENT: Not PBT or vPvB
12.6	OTHER ADVERSE EFFECTS: None

13. DISPOSAL CONSIDERATION

13.1 WASTE TREATMENT METHODS

Waste must be disposed of in accordance with the local, regional and national environmental regulations.
Not dangerous waste

14. TRANSPORT INFORMATION

Not dangerous goods

15. REGULATORY INFORMATION

15.1 SAFETY, HEALTH AND ENVIRONMENTAL REGULATIONS/LEGISLATION SPECIFIC FOR THE SUBSTANCE OR MIXTURE

Regulation (EC) No 1907/2006	(REACH)
Regulation (EC) No 1272/2008	(CLP/EU-GHS)
Regulation (EC) No 1223/2009	(Cosmetic Regulation)

15.2 CHEMICAL SAFETY ASSESSMENT

Not classified as a dangerous substance or mixture according to the CLP/EU-GHS.

16. OTHER INFORMATION

16.1 EXPLANATORY NOTES

STOT	specific target organ toxicity
SE	single exposure
RE	repeated exposure
IARC	International Agency for Research on Cancer
INCI	International Nomenclature of Cosmetics Ingredients
PBT	persistent, bio-accumulative, toxic for reproduction
vPvB	very persistent, very bio-accumulative

16.2 REVISIONS

Revision 01: structure modified according to the requirements of the Regulation (EC) No 1907/2006
Revision 02: editorial changes, change in the name of the manufacturer, design changes

General specification

Product Name: Hyaluronic acid, powder
INCI name: Sodium Hyaluronate
Quality Class: Cosmetic
Manufacturer: Contipro Biotech s.r.o.
Shelf Life: 2 years

METHOD	UNIT	SPECIFICATION LIMITS	METHOD REF.
Origin	---	biotechnological processing	
Appearance	---	white powder or granules	206.1.1-012
Appearance of 0.5% aqueous solution	---	clear to slightly opalescent colourless solution	206.1.1-012
Clarity of 1% aqueous solution (660nm, 1 cm)	---	< 0.010	206.1.1-008
Loss on drying	%	≤ 10.0	206.1.1-002
Molecular weight	MDa	1.30 - 1.80	206.1.1-013
Kinematic viscosity (0.05% solution)	cSt	1.75 - 2.46	206.1.1-003
pH of 0.5% aqueous solution	CFU/g	5.0 - 8.0	206.1.1-220
Protein*	%	≤ 0.01	206.1.1-227
Microbial contamination	CFU/g	< 100	206.1.1-800
Sodium hyaluronate *	%	> 93.0	206.1.1-359
Uronic acids *	%	> 45.0	206.1.1-359
Ash *	%	< 10.0	206.1.1-014

* calc. on dry basis

LRQA
Business Assurance

CERTIFICATE OF APPROVAL

This is to certify that:

Contipro Biotech s.r.o.
Dolní Dobrouč 401
561 02 Dolní Dobrouč
Czech Republic

has been approved by Lloyd's Register Quality Assurance
to the following Good Manufacturing Practices standard:

ISO 22716:2007

For the following scope:

Production of active cosmetic ingredients.

This certificate forms part of the approval identified by certificate number PRA 0004196.

Approval
Certificate No: PRA 0004196/E

Original Approval: 19 November 2010

Current Certificate: 10 August 2013

Certificate Expiry: 9 August 2016

Issued by: Lloyd´s Register EMEA, Prague office,
for and on behalf of Lloyd´s Register Quality Assurance Limited

This document is subject to the provision on the reverse.
Táborská 31, 140 00 Prague 4, Czech Republic
For and on behalf of 71 Fenchurch Street, London EC3M 4BS, United Kingdom
This approval is carried out in accordance with the LRQA assessment and certification procedures and monitored by LRQA

LRQA
Business Assurance

CERTIFICATE OF APPROVAL

This is to certify that the Quality Management System of:

Contipro Biotech s.r.o.
Dolní Dobrouč 401
561 02 Dolní Dobrouč
Czech Republic

has been approved by Lloyd's Register Quality Assurance
to the following Quality Management System Standards:

ISO 9001:2008

The Quality Management System is applicable to:

Research, development and production of biopolymers for cosmetics, nutrition. Research and development of pharmaceutical substances and pharmaceutical preparation based on biopolymers.

This certificate forms part of the approval identified by certificate number PRA 0004196

Approval
Certificate No: PRA 0004196/D

Original Approval: 14 January 2003

Current Certificate: 10 August 2013

Certificate Expiry: 9 August 2016

Issued by: Lloyd's Register EMEA, Prague office,
for and on behalf of Lloyd's Register Quality Assurance Limited

UKAS MANAGEMENT SYSTEMS
001

This document is subject to the provision on the reverse.
Táborská 31, 140 00 Prague 4, Czech Republic
For and on behalf of 71 Fenchurch Street, London EC3M 4BS, United Kingdom
This approval is carried out in accordance with the LRQA assessment and certification procedures and monitored by LRQA.
The use of the UKAS Accreditation Mark indicates Accreditation in respect of those activities covered by the Accreditation Certificate Number 001.
Macro Revision 13

F363(GL)v04

VERIFICATION OF THE RAW MATERIALS CONFORMITY TO THE ECOCERT NATURAL AND ORGANIC COSMETIC STANDARD

THIS DOCUMENT IS NOT AN ORGANIC CERTIFICATE

Company: **Contipro Biotech s.r.o.** Attestation n° 4011

HyActive* Function: **Active ingredient**

INCI: Sodium Hyaluronate

Conformity*: YES **100** % of natural origin (**0** % of physically processed vegetal ingredients) **0** % synthetic

Comments:

*to the requirements of the standard related to the raw materials.

Hyaluronic Acid, Sodium Salt* Function: **Active ingredient**

INCI: Sodium Hyaluronate

Conformity*: YES **100** % of natural origin (**0** % of physically processed vegetal ingredients) **0** % synthetic

Comments:

*to the requirements of the standard related to the raw materials.

Drawn up in l'Isle Jourdain, valid from 01/01/2014
until 31/12/2014

Matthieu Bouffartigue
Raw Materials Service Manager

WARNING: The approval of the raw material(s) listed above is PERSONAL to the beneficiary named herein, and the BUYERS of the raw material(s) ARE IN NO EVENT AUTHORIZED TO MAKE REFERENCE TO THE APPROVAL BY ECOCERT GREENLIFE OR TO USE AN ECOCERT LOGO, whether in its communication or on the packaging or labeling of the raw material(s) or of a finished cosmetic product.

ECOCERT GREENLIFE S.A.S. - Capital 50 000 € – Lieudit Lamothe Ouest – 32600 L'Isle Jourdain - France
Tél. + 33 (0)5 62 07 51 09 - Fax : +33 (0)5 62 07 74 96 – www.ecocert.com
TVA Intracommunautaire n° FR 55 509 534 095
CREDIT MUTUEL 2200 20246201 29 - SIREN 509 534 095 RCS AUCH - APE 7120B

Raw material supplier statement

We, Contipro Biotech s.r.o., hereby confirm, that our product:

HYALURONIC ACID, SODIUM SALT, powder

INCI name: Sodium Hyaluronate (CAS number 9067-32-7)

Is obtained by fermentation of a non-haemolytic microbial strain, Streptococcus equi and it is produced in the Czech Republic.

The product does not contain any potentially allergenic substance listed in Annexes of Regulation (EC) 1223/2009.

Hyaluronic acid (sodium salt) is exempted from a scope of Regulation (EC) No 1907/2006 (REACH) as amended.

The product is not classified as a SVHC substance according to Annex XIV to REACH Regulation or candidate substance as well.

No material of animal origin which could be susceptible to TSE/BSE is used during the manufacturing process; therefore the product is TSE/BSE free.

No genetically modified material or material derived from genetically modified organisms is used in the manufacturing process.

The product does not contain any nanomaterial compounds.

The product has never been tested or re-tested on animals for cosmetic purposes.

The product does not contain any carcinogenic and mutagenic substances or substances toxic for reproduction listed in Annex VI of Regulation (EC) 1272/2008.

The product is free from glycol esther, phthalates, sulphates, parabens, phenoxyethanol and formaldehyde.

The product does not contain pesticides or their residues.

The content of heavy metals in our product is not monitored on a regular basis; determination of heavy metals content is performed upon a customer's request.

Hyaluronic acid formulation example

Non-perfumed make-up remover is designed for everyday cleansing of the skin area. The combination of aloe vera, hyaluronic acid and panthenol provides moisturizing and calming effects whereas vitamin E supports regenerative processes in the skin.

	Ingredients		% W/W	INCI Nomenclature
A	Carbopol 940	[1]	0.20	Carbomer
	Water deionized		10.00	Aqua
B	TEGO SML 20	[2]	2.50	Polysorbate 20
	TEGO SMS 60	[2]	0.50	Polysorbate 60
	Vitamin E Acetate	[3]	0.10	Tocopheryl Acetate
C	Water deionized		58.09	Aqua
	1,3-Butanediol	[4]	3.00	Butylene glycol
	Disodium EDTA		0.05	Disodium EDTA
	D-Panthenol	[3]	0.50	D-Panthenol, water
	HMW Hyaluronic acid Sodium Salt 1%	[5]	20.00	Sodium Hyaluronate
D	Terra-Pure 200X, TN003	[6]	0.20	Aloe Barbadensis Leaf Juice
	Water deionized		2.00	Aqua
E	Sodium hydroxide		0.06	Sodium hydroxide
	Water deionized		2.00	Aqua
F	Benzylalcohol-DHA	[3]	0.80	Benzylalcohol, dehydroacetic acid

Manufacture:

1. Complete A, B and C separately.
2. Add B to C with vigorous stirring.
3. Add A and D to BC while stirring.
4. Add E while stirring.
5. Add F after 10 minutes while stirring.
6. Continue to stir next 15 minutes.

Suppliers

[1] Lubrizol[4] Merck
[2] Evonik
[3] Making Cosmetics
[4] Merck
[5] Contipro Biotech s.r.o.
[6] Terry Laboratories

GfN

Naturally active.

Collaplex 1.0 PHE

Description

Collagen is the most abundant form of protein in our body. It serves primarily a structural role, and in humans it is the principal component of skin, nails, cartilage, tendons and ligaments. It is present in virtually every body organ and it's importance from a biological perspective is reflected in the fact that collagens are quite similar across species.
As a structural protein, collagen is essential to creating the body's physical structure, and as an extracellular matrix it acts as a supporting framework over which our cells are arranged.
Collagen formulations have been used for health purposes for at least 800 years.
Collaplex 1.0 PHE is a ropelike triple helix native collagen for use in high quality cosmetic products. Collaplex 1.0 PHE contains some natural concomitant substances which are bound to the collagen as a result of the mild process of extraction from calves hides originated in Germany. Collaplex 1.0 PHE belongs to high molecular collagen of highest value.

Appearance

opaque, viscous solution

INCI

Aqua, Soluble Collagen

Registration

CAS-No.:
Aqua.....7732-18-5
Soluble Collagen.....9007-34-5

EINECS/ELINCS-No.:
Aqua..... 231-791-2
Soluble Collagen.....232-697-4

Preservatives

Phenoxyethanol.....1.0 %

Stabilizers

Sodium Citrate Buffer.....1.0 %

Efficacy

As we mature, the body's production of collagen slows and this is believed to contribute to an "older" appearance. Oral and topical collagen formulations are thought to help alleviate these age-related skin changes.
Collaplax 1.0 PHE shows an outstanding high water absorption capacity. Over time, the powerful native and natural collagen helps to fill in lines and wrinkles, leaving your skin smooth an plump. In addition this outer film reduces the irritating and negative influences of environment on the skin.
A certain level of stratum corneum hydration is essential for skin smoothness and elasticity;

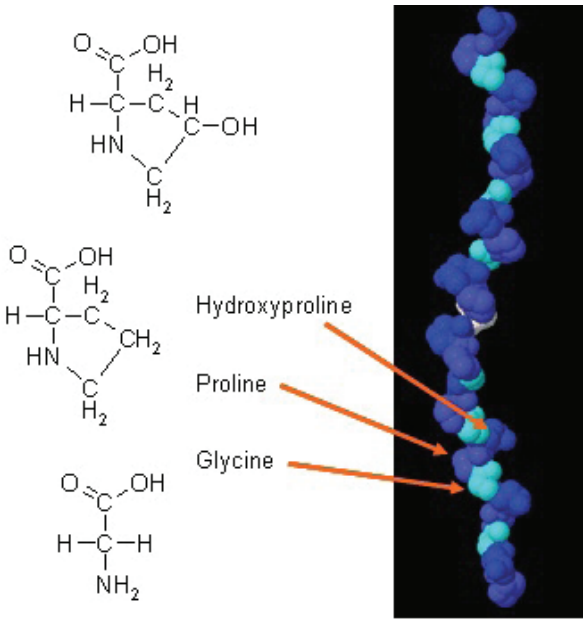
- moisturizing
- film forming
- water-retaining

GfN

Naturally active.

Collaplex 1.0 PHE

Chemical Structure



Characteristics

collagen content.....1.00 - 1.30 %
dry residue.....2.00 - 2.80 %
acid value.....4.50 - 6.50
pH value.....3.50 - 4.00
ash as Na_2SO_4.....0.30 - 0.50 %
HVT-value.....38-49°C
microbiology.....<100 CFU / ml

Application

Collaplex 1.0 PHE is especially suitable and easy to formulate in caring facial cream and skin care formulations.

Application concentration

Skin Care Formulations……..…….....3.0 - 6.0 %
Body Lotions…….....…….....…….0.5 - 3.0 %
After Shave……..…….....…….0.5 - 5.0 %
After Sun…….....…….0.2 - 5.0 %

Incorporation

While all native collagen solutions are temperature sensitive and denature irreversible at temperatures between 38 - 45°C, we necessarily recommend to incorporate Collaplex 1.0 PHE lower than 30°C in the water phase of your formulation.
Collaplex 1.0 PHE existing on acidic pH as a result of added citric buffer.
For using it in After Shaves in combination with Alcohol and water, pH has to be positioned lower than four, otherwise Collaplex 1.0 PHE will precipitate as fibrils.
While using Collaplex 1.0 PHE in water based ampoules or fluids you have to add 2 % Sodium Chloride to slide the isoelectric point to the right direction and to avoid any precipitation after a long term.

Toxicology

non - toxic
non - irritating

Storage & Shelf life

Collaplex 1.0 PHE should be stored in a dry and light protected place between 5 - 20°C.
In closed containers the shelf life is five years.

GfN Herstellung von Naturextrakten GmbH

Straßburg 16
69483 Wald-Michelbach / Germany
Telefon +49 6207 922 80
Telefax +49 6207 922 810
www.gfn-selco.de
info@gfn-selco.de

Leaflet_1015_e
08.03.2018

Fucowhite™

Whitening the skin

MARINE GLYCOBIOLOGY EXPERTISE

LESSONIA
cosmetics + ingredients

Fucowhite™

Whitening the skin

Fucowhite is a clinically validated skin whitening ingredient which significantly decreases skin pigmentation.

Fucowhite is a unique purified fucoidan-phloroglucinol complex, extracted from the brown algae Ascophyllum nodosum by lixiviation in water.

This unique complex of fucoidan with powerful antioxidant polyphenol, makes it an effective and versatile ingredient for whitening application.

Ascophyllum nodosum:

a system to regulate its pigmentation

Ascophyllum nodosum is a brown algae particularly present on the North-Western coasts of Europe. Ascophyllum is very popular amongst the scientific community. It has been claimed to be the most active seaweed on the planet as well as the most researched by the academic community.

Exposed to UV rays on a daily basis, Ascophyllum nodosum has developed a system that regulates its pigmentation. That has inspired LESSONIA in the development of FUCOWHITE to regulate the pigmentation of the skin.

THE UNIQUE COMPOSITION OF Fucowhite™

Fucoidans have diverse bioactivity. They also inhibit UVB-induced MMP expression and are good inhibitors of the dermal remodeling enzymes called matrix metalloproteases. Polyphloroglucinols are the polyphenols found in brown macroalgae. They are powerful antioxydants, inhibiting a variety of free radicals. In Ascophyllum nodosum, the association fucoidan and phloroglucinols plays an important role in the protection from high UV conditions, especially in the intertidal zone.

Fucowhite is not just an association of fucoidans with phloroglucinols. These 2 compounds are linked together to form a complex with a strong tyrosinase inhibition activity.

IN VITRO TESTS

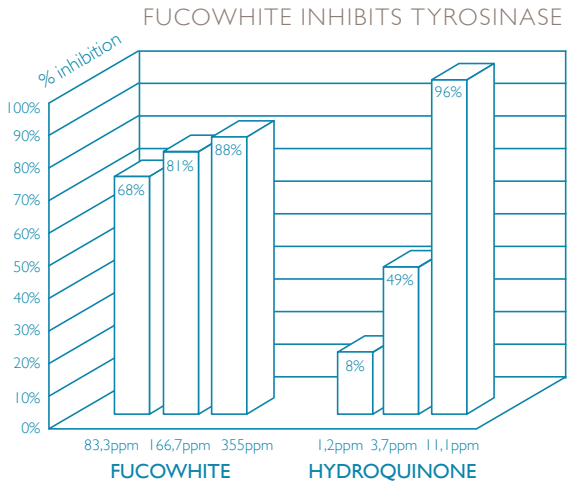
FucoWhite™ mechanism of action

The skin's pigmentation results from the presence of melanin in the epidermis. This pigment is synthesized in vesicles known as melanosomes of specialized cells, melanocytes. The melanin pigments is produced using tyrosine, an essential amino acid that is submitted to a series of reactions, linked to tyrosinase, as the key enzyme in this biosynthesis, to form melanin.

By **inhibiting the tyrosinase activity by 88%** at very low concentration, Fucowhite is an excellent whitening ingredient.

PROTOCOL

The enzyme tyrosinase was incubated with its substrate tyrosine. The product of this reaction (melanin) was analyzed by measuring the optical density at 480 nm.



RESULTS

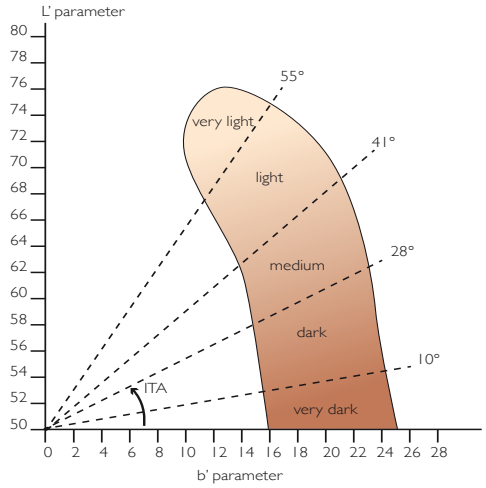
The tyrosinase inhibition of Fucowhite is similar to the activity of hydroquinone, benchmark tested in the same conditions, without causing any skin irritation.

CLINICAL TESTS

Lightening efficacy on Asian skin type

PROTOCOL

- Test realized in Thailand on 24 volunteers.
- Twice a day application of 2% Fucowhite against placebo for 28 days.
- Evaluation of the skin pigmentation (chromametric analysis)



The color of the skin is measured by a chromameter. This appliance makes it possible to define a luminance parameter: L, clarity, from dark to pale and a chrominance ; factor: b* the spectrum from blues to yellows. These parameters have been studied in order to measure the individual typological angle, which defines the degree of pigmentation of an individual's skin.*

RESULTS

FUCOWHITE SIGNIFICANTLY INCREASES THE SKIN CLARITY AND DECREASES THE SKIN PIGMENTATION.

After using 2% Fucowhite twice a day for 28 days:

- **88 % of volunteers** from active group observed an **increase of skin clarity** (L* parameter)
- **63 % of volunteers** from active group observed a **decrease of skin pigmentation** (ITA* parameter); + 3.7 % on average and up to + 36.3% increase in the ITA.

Statistical analysis highlights a significant better effect using the "active cream" on L* parameter and ITA* compared with placebo. It induces a lightening effect characterized by a significant increase in L* and ITA* parameters.

Perfect skin tolerance.

Cosmetic activity

- Whitening ranges for face and body
- Lightening care products
- Anti-age spots creams and serums.
- Protective formulations

Technical data

- INCI name : Glycerin & water & Ascophyllum nodosum extract
- Appearance: brownish liquid
- Solubility : water soluble
- Recommended rate of use: 2.0 %
- Preservative free

Marketing benefits

- A marine skin whitening active ingredient
- Clinical efficacy on Asian skin after only 28 days
- Both antioxidant and whitening benefits
- Available in a preservative-free version
- Available in an organic version

LESSONIA
cosmetics + ingredients

ISO 22716
BUREAU VERITAS
Certification

LESSONIA
Croas ar Neizic
29800 Saint Thonan
FRANCE
Tél. 33 (0)2 98 07 23 65
info@lessonia.com
www.lessonia.com

GfN

Naturally active.

Efficacy Atecoron Plus® PHE 1.2

Main Focus

Within dermis, the interaction of dermal cells and structural proteins is crucial for the integrity of skin flexibility and strength. Fibroblasts synthesize dermal components and remain linked to collagen and elastin fibers via specific anchorage bonds. During the aging process, those dense and homogeneous interactions decrease in number, resulting in loss of skin firmness and plasticity. Visible signs like wrinkles are occuring.

Atelocollagen belongs to this group of fibrous proteins (scleroproteins), that constitute one third of the total protein in mammalian organisms. As an inimitable combination of atelocollagen and sodium hyaluronate (SH) with different molecular weights Atecoron Plus® PHE 1.2 is designed to rebuild the extracellular network and fight effectively and long lasting against visible signs of aging.

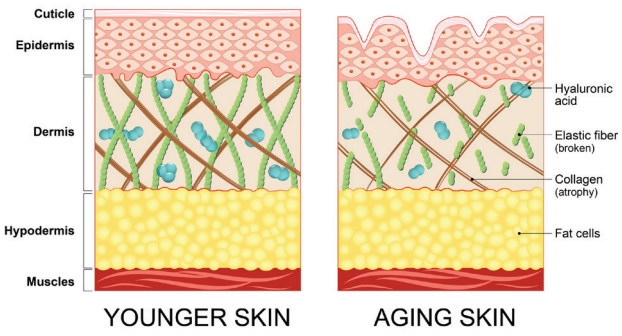


Figure 1: Structure of the extracellular network: A homogeneous density of strong collagen and fibroblast interactions are crucial factors for skin plasticity and firmness.

Mode of action

Due to the unique combination of naturally derived Atelocollagen and 3 types of sodium hyaluronate with different chain length, Atecoron Plus® PHE 1.2 fights signs of aging like wrinkles, in a sustainable mode of action. Atecoron Plus® PHE 1.2 was shown to increase the number of newly synthesized fine collagen / elastic fibers, forming a homogeneous network and improve the inner skin structure.

Nature needs no cosmetics,
but cosmetics need nature.

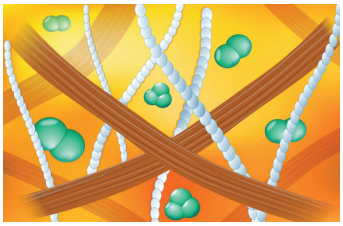


Figure 2: Dermal interactions: Collagen, elastic fibers and fibroblasts connect in the extracellular matrix to form a dense network defining the mechanical properties of the skin.

Study Setup

In vivo studies were performed on 25 female volunteers at the age of 30-60 years with normal skin, Fitzpatrick skin types I and II. Application was twice a day with a hydrogel containing 5 % Atecoron Plus® PHE 1.2 in a split-face double-blind study against placebo over 4 weeks. Parameters were measured by Corneometer CM 825, Primos Lite and VivaScope 3000 before application and after 3h, 2 and 4 weeks.

Test formula:

90.73 % Aqua
5.00 % Atecoron Plus® PHE 1.2
3.00 % Glycerin
1.00 % Phenoxyethanol
0.20 % Xanthan Gum
0.07 % Sodium Hydroxide

Figure 3: Composition of the formulation tested: Placebo formulation was identical but without Atecoron Plus® PHE 1.2.

Skin Hydration

The skin hydration was evaluated by Corneometer measurements. The application of 5 % Atecoron Plus® PHE 1.2 improves skin hydration up to 9.1 % after 28 days.

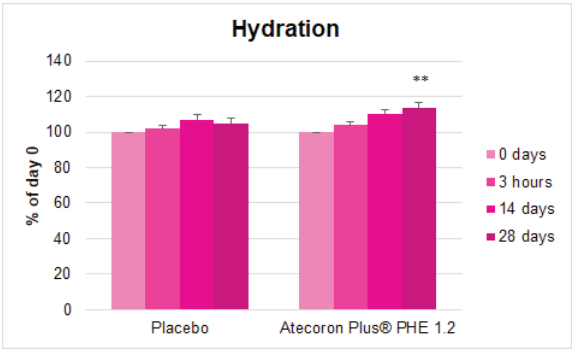


Figure 4: skin hydration: Graphical analysis of the skin hydration determined by Corneometer, compared to placebo. **p < 0.01.

Efficacy Atecoron Plus® PHE 1.2

Wrinkle Depth

The depth of wrinkles was analyzed by Primos Lite. 5 % Atecoron Plus® PHE 1.2 showed a great and significant effect on wrinkle reduction up to 29.1 % compared to starting conditions and placebo.

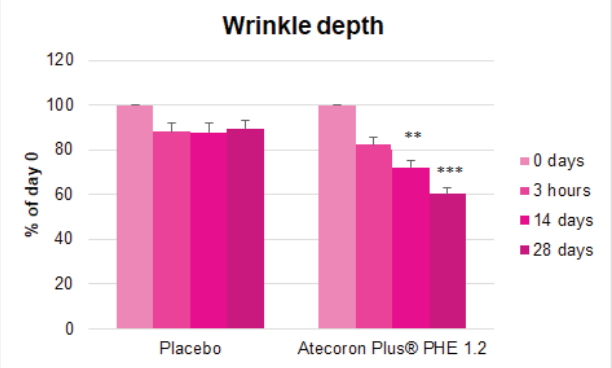


Figure 5: Wrinkle depth: Graphical analysis of the wrinkle depth determined by Primos Lite, compared to placebo. ** $p < 0.01$, *** $p < 0.001$.

Inner skin structure

The alteration of the inner skin structure was determined by analyzing the mean number of collagen / elastic fibers in the papillary dermis by VivaScope. Atecoron Plus® PHE 1.2 improves the inner skin structure by 23.1 %.

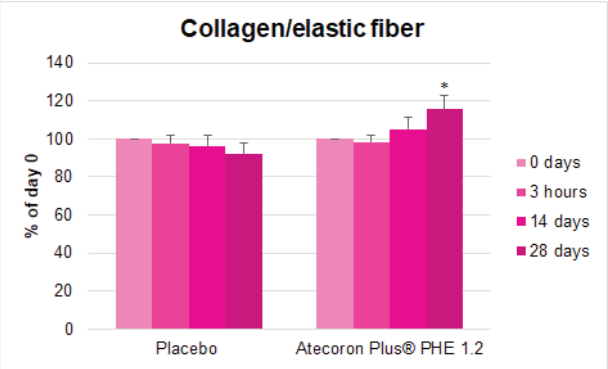


Figure 6: Inner skin structure: Graphical analysis of the inner skin structure determined by VivaScope, compared to placebo. * $p < 0.05$.

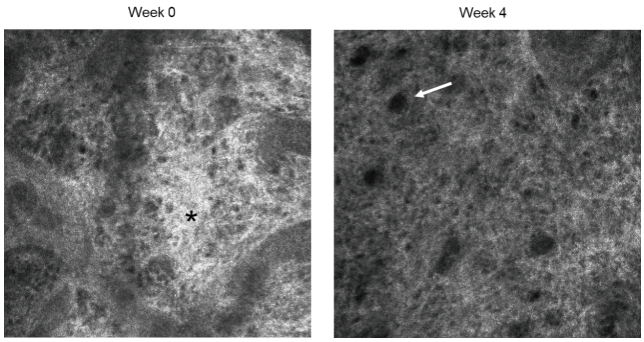


Figure 7: Papillary dermis structure: Representative image of the papillary dermis obtained by VivaScope. At the beginning of the study, collagen / elastic fibers network is visibly damaged, forming thick bundles of clumps, typical for the solar elastosis (*). After 4 weeks, the bright places of amorphous elastotic material disappeared whereas the increased number of the newly produced fine collagen / elastic fibers can be observed, creating a homogeneous network. The picture also shows, that the number and size of dermal papillae increased, although it was not further quantified (white arrow).

Summary

Our in vivo studies proof Atecoron Plus® PHE 1.2 to be a strong enhancer of skin inner structure, resulting in a visibly younger and more hydrated skin.

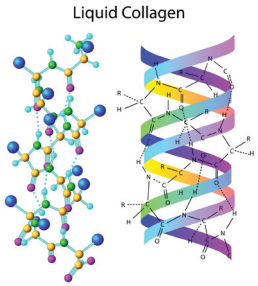


Figure 8: Liquid Collagen: Our Atelocollagen is classified as native collagen and has an intact triple helical structure.

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Leaflet_1072_Efficacy_e
06.02.2019

GfN

Naturally active.

Atecoron Plus® PHE 1.2

Description

Atecoron Plus® PHE 1.2 is an inimitable combination of atelocollagen and different molecular weights of biotechnologically derived Sodium Hyaluronate (SH).
Atelocollagen belongs to the group of scleroproteins, a fibrous protein that constitutes one third of the total protein in mammalian organisms. It is a polypeptide containing three peptide chains that are rich in typical amino acids like proline and hydroxyproline.
Atelocollagen is produced by a mild enzymatic process using pepsin and calfskin. During this process few amino acid sequences, so called telopetides (Greek “telos” means “end”) are split off at the non-helical part of the collagen molecule. This Atelocollagen is classified as native collagen and has an intact triple helical structure.
Hyaluronic Acid is a linear polysaccharide naturally occurring in the human body. The higher the molecular weight of Hyaluronic acid is, the better are the film forming properties. The lower the molecular weight is, the better is the biological activity of this active ingredient. Hyaluronic acid is known to be one of the best moisturizers. The combination of different molecular weights guarantees a perfect hydration of the skin from out- and inside, as well as the improvement of structural integrity of the skin. This product ideally combines Anti-Ageing properties with effective moisturization.

Appearance

Milky strong viscous solution

Nature needs no cosmetics,
but cosmetics need nature.

INCI

Aqua, Glycerin, Sodium Hyaluronate, Atelocollagen

Registration

CAS-No.:
Aqua..... 7732-18-5
Glycerin.....56-81-5
Sodium Hyaluronate.....9067-32-7
Atelocollagen.....9007-34-5
EINECS/ELINCS-No.:
Aqua..... 231-791-2
Glycerin.....200-289-5
Sodium Hyaluronate.....(-)
Atelocollagen.....232-697-4

Preservatives

Phenoxyethanol 1.2 %

Stabilizers

Sodium citrate buffer 0.9 %

Efficacy

Atecoron Plus® PHE 1.2 shows an outstanding high water absorption capacity. The application of 5 % Atecoron Plus® PHE 1.2 improves skin hydration up to 9.1 % compared to starting conditions and placebo after 28 days.
Over time, the powerful native and natural collagen helps to fill in lines and wrinkles, leaving your skin smooth an plump. 5 % Atecoron Plus® PHE 1.2 showed a great and significant effect on wrinkle reduction up to 29.1 % compared to starting conditions and placebo after 28 days.
A certain level of stratum corneum hydration is essential for skin smoothness and elasticity, which is reached by three different molecular weights of sodium hyaluronate in the product.

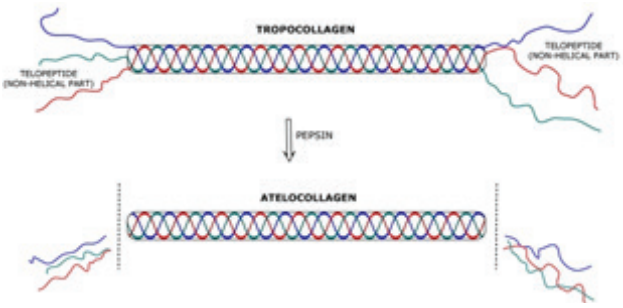
GfN

Naturally active.

Atecoron Plus® PHE 1.2

The alteration of the inner skin structure was determined by analyzing the mean number of collagen / elastic fibers in the papillary dermis by VivaScope. Atecoron Plus® PHE 1.2 improves the inner skin structure by 23.1 % compared to starting conditions and placebo after 28 days.
By the use of this unique combination of actives you will obtain a quick permeating formulation and at the same time a pleasant smooth skin feel will appear.
Please have a look at our Leaflet_1072_Efficacy_e.

Chemical structure



Characteristics

Soluble Collagen	0.50 - 0.65 %
Sodium Hyaluronate (SMW)	0.2 %
Sodium Hyaluronate (LMW) HySilk	0.5 %
Sodium Hyaluronate (VLMW) HyActive	0.3 %
Glycerin	10.0 %
Density	1.00 - 1.10 g / ml
Ash as Na_2SO_4	0.80 - 1.80 %
pH-value	6.0 - 7.0
HVT-value	38 - 49°C
Citrate Buffer	1.0 %
Microbiology	< 100 CFU / ml

Application

Atecoron Plus® PHE 1.2 has a neutral pH and is especially suitable and easy to formulate in hydrogels and water based formulations. Atecoron Plus® PHE 1.2 is also applicable for incorporation into caring facial creams and skin care formulations.

Application concentration

Collagen fluids	5.0 - 12.0 %
Skin Care Formulations	3.0 - 6.0 %
Body Lotions	0.5 - 3.0 %
After Shave	0.5 - 5.0 %
After Sun	0.2 - 5.0 %

Incorporation

Atecoron Plus® PHE 1.2 should be blended into the water phase at max. 30°C. Addition of 1 % Disodium citrate buffer is highly recommended for enhanced stability.

Toxicology

non - irritating to skin and eyes
non - toxic and non - cytotoxic
non - phytotoxic
non - mutagenic

Storage & Shelf life

Atecoron Plus® PHE 1.2 should be stored in a dry and light protected place at 10°C - 25°C.

In closed containers the shelf life is five years.

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06.02.2019

Nature needs no cosmetics,
but cosmetics need nature.

BIOTECHNOLOGY

FINE CHEMISTRY

VEGETAL EXTRACTION

By Solabia

Test it...

...approve it !

Fucogel®

[Soothing and Sensory Moisturizer]

Sirtuins-1 activator

Glycoscience on behalf of epigenetic regulation

Polysaccharide rich in fucose modulating cell communication and epigenetic mechanisms

- Decreasing skin reactivity: for ***sensitive skin***
- Increasing cellular longevity: for ***anti-aging*** applications
- ***Hydration*** & ***sensory***

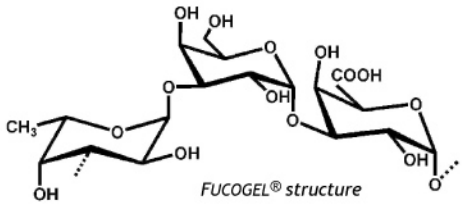
cosmetics Solabia group

Between Nature & Technology

Mode of action

FUCOGEL® is an anionic polysaccharide, at 1% in water, obtained by bacterial fermentation, having a linear structure containing L-fucose, D-galactose and galacturonic acid.

FUCOGEL® could act in the **modulation of the cutaneous sensitivity** thanks to its richness in fucose. It regulates the cellular messages via the membrane receptors of keratinocytes (having a particular affinity for this sugar). Its soothing effect explains its capacity to regulate the epigenetic mechanisms thanks to its **stimulation of sirtuins-1**. This property confers it also anti-aging applications by the increase of cellular longevity.



FUCOGEL® structure

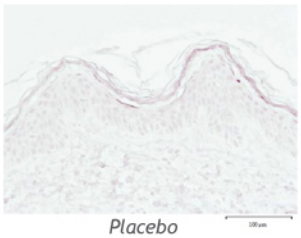
ex Vivo Test

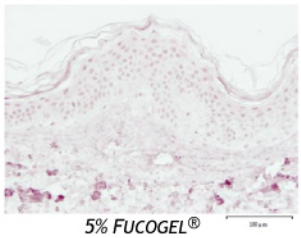
2012 Innovation

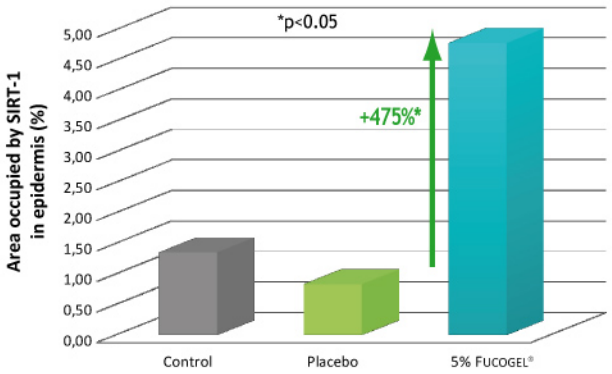
Epidermal sirtuins-1 stimulation

Explants treated **topically** with a formula containing 5% of FUCOGEL® vs Placebo / Quantization of sirtuins-1 by immunolabelling at D8 (in pink)

+475% stimulation of sirtuins-1 with 5% FUCOGEL® vs Placebo


Placebo


5% FUCOGEL®



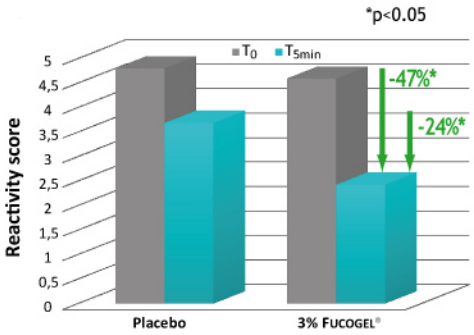
Sirtuins-1 → anti-inflammation
→ anti-aging

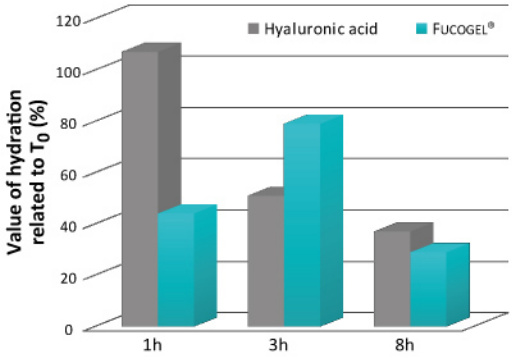
Vivo Tests

Soothing effect

One application of 3% FUCOGEL® vs Placebo on the nasolabial fold of 19 volunteers with sensitive and reactive skin after lactic acid aggression / Soothing effect evaluation after 5 min (stinging test)

With 3% FUCOGEL®
- **-47%** of tingling feelings vs T_0
- **-24%** of tingling feelings vs Placebo





And always...

"Delayed" moisturizing effect

Application of FUCOGEL® vs hyaluronic acid (1% polysaccharide in water) on the forearm of 6 volunteers / Measurement of the hydration by N.M.R. before and after application (1h, 3h, 8h)

FUCOGEL® has a "delay" moisturizing effect increasing gradually the hydration from 1 hour until reaching its maximum 3 hours after. Complementary effect with hyaluronic acid which is a short-term moisturizer (maximum at 1 hour)

to 10%
from 3%

Improvement of « soft touch » perception in formula

Assessment by 12 experts of the effects and texture of a cream containing FUCOGEL® at 3, 5, 7 or 10% vs Placebo

The ⊕
Smoothing effect on damaged hair
(*ex vivo* proven)

Product Characteristics

- INCI/CTFA Name Biosaccharide gum-1
- Preservatives - with phenoxyethanol: FUCOGEL® 1,5P
  - phenoxyethanol free: FUCOCERT® conform to ECOCERT standard
- Recommended dose 1 to 20%

29, rue Delizy - 93698 Pantin Cedex - France - www.solabia.com - Tel. : (+33) 1.48.10.19.40 - Fax : (+33) 1.48.91.18.77

BIOTECHNOLOGY

FINE CHEMISTRY

VEGETAL EXTRACTION

Ω' Ceramide®

By Solabia

Skin restructuring and firming agent

Special Atonic Skin

BIOMIMETIC VECTORIZED OLEIC ACID :

- ANTI-ELASTASE ACTIVITY
- IMPROVEMENT OF THE CUTANEOUS FIRMNESS AND ELASTICITY
- RESTORATION OF SKIN RADIANCE

Ω' ceramide

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cosmetics Solabia group

BIOTECHNOLOGY FINE CHEMISTRY VEGETAL EXTRACTION

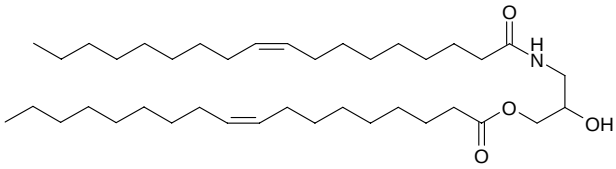
Ω' Ceramide®

By Solabia

Skin restructuring and firming agent
Special Atonic Skin

- **Definition**

OMEGA 9 CERAMIDE® is a ceramide-like molecule obtained by a patented enzymatic solvent-free process, from an olive oil (*Olea Europaea*).



- **CTFA name**

Olive oil aminopropanediol esters

- **Recommended dose**

Until 2%

- **Preservatives**

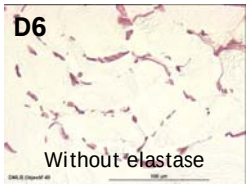
No preservative

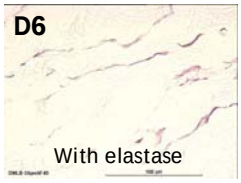
- **Performances** ***(technical file available on request)***

1. *Anti-elastase activity*

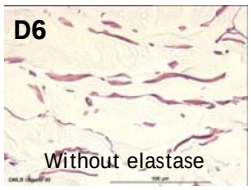
Human skin explants maintained in survival conditions / daily application of a cream containing Omega 9 Ceramide® at 1% / At D5, incubation with elastase / At D6, coloration of fibres matrix of the lower reticular dermis (LRD), microscopic visualization /quantitative measures by image analysis.

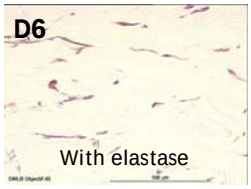
Untreated control



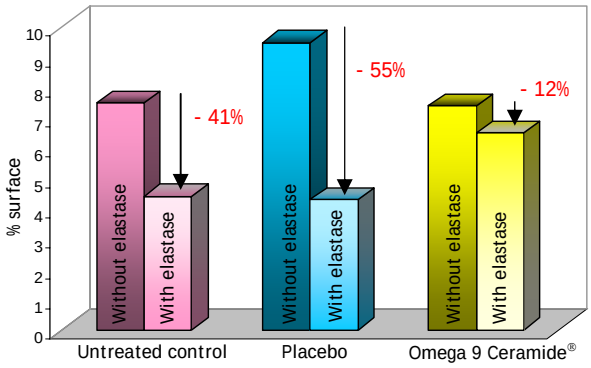


Placebo

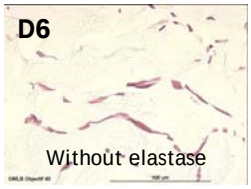


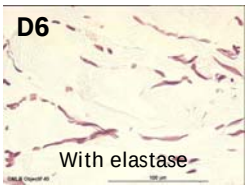


% Surface of elastic fibres in the LRD at D6



Omega 9 Ceramide® at 1%





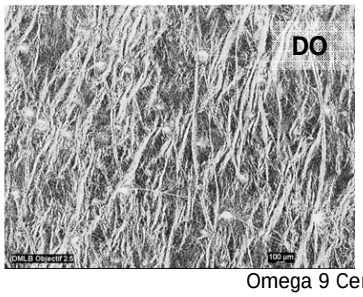
➢ **OMEGA 9 CERAMIDE® at 1% offers 70% of protection against elastase activity vs Untreated control.**

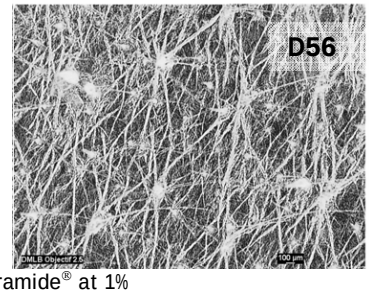
2. *Evaluation of firmness, elasticity and restructuring effect*

In vivo study on 20 volunteers with very dry skin indeed very spoilt / twice-daily application on hemi-face, during 56 days, of a cream containing Omega 9 Ceramide® at 1% vs Placebo / firmness and elasticity evaluation (R5 and R7 parameters) on cheekbones by cutometry / microscopic observation of a *stratum corneum* sample/ image analysis of the cutaneous microrelieve.

After 56 days of use of a cream containing OMEGA 9 CERAMIDE® at 1% vs Placebo :

➢ **Significant improvement of skin firmness and elasticity, expressed by :**
- **increase of 19% of R5 parameter ($p < 0.05$)**
- **increase of 27% of R7 parameter ($p < 0.001$)**





Omega 9 Ceramide® at 1%

➢ **Significant improvement ($p < 0.01$) of microdepression network (+33%)**

➢ **Visible restructuring effect**

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