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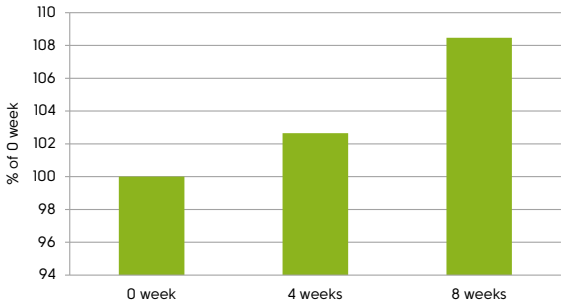
HYALURONIC ACID

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 hydrating 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% HySilk (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 25 to 66 years, showed an average increase in skin hydration by 8 per cent.

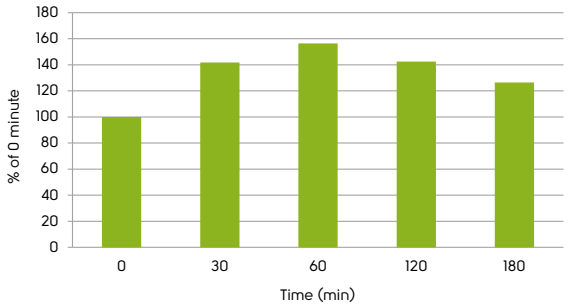
Mechanisms of action

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 epidermis and dermis. Therefore environmental influence 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, $p \leq 0.05$, 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 solution
Clarity of 1% aqueous solution (660 nm, 1 cm)	< 0.010
Loss on drying (%)	≤ 10.0
Molecular weight (MDa)	1.30 – 2.2
Kinematic viscosity (0.05% solution) (cSt)	1.75 – 2.7
pH of 0.5% aqueous solution	5.0 – 8.0
Protein (%)*	≤ 0.1
Microbial contamination (CFU/g)	< 100
Sodium hyaluronate (%)*	> 93.0
Uronic acids (%)*	> 45.0
Ash (%)*	< 10.0

* calc. on dry basis

SOURCE

- fermentation, Streptococcus equi, susp. 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 butylene 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 substances, 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." SOFT Journal 4: 2-8

Version: E-01/2014

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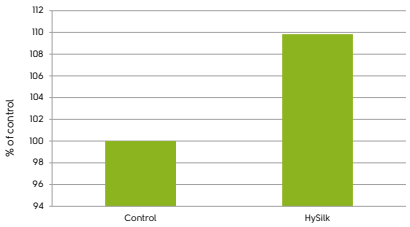
HYSILK®

HySilk is hyaluronic acid a low molecular weight and has a very broad range of activity in the skin. It works both to change the physical properties of the epidermis, especially due to its ability to immobilize a large volume of water in its structures, and is also active in relation to cells, so it is capable of acting positively on intercellular communication.

Support of epidermal hydration by improving intercellular communication

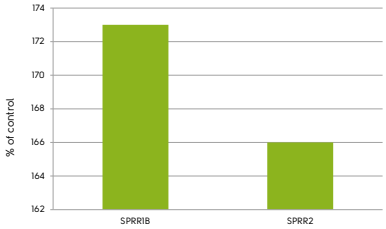


Hydration promotion after HySilk treatment, 8 volunteers treated with 0.005% HySilk (24-43 years) + 29 volunteers control group, Daily application for 4 weeks, $p \leq 0.05$, Measured by MPA 580, Corneometry

During the *in-vivo* study, HySilk's positive effect on the hydration of the upper layers of the epidermis was observed, manifested by lower transepidermal water loss (TEWL) and higher water content.

HySilk's ability to partially penetrate into the skin has been demonstrated, in view of its moisturizing qualities, to make a major contribution to increased water content in the stratum corneum. Compared with the control group, hydration rose by 10%.

Mechanisms of action



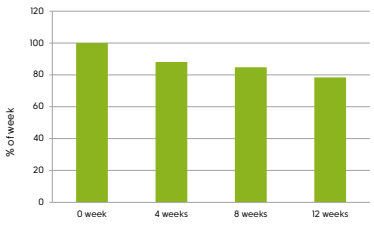
Changes in gene expression in HaCaT keratinocytes after 0.1% HySilk 48 hours treatment, n=1, Measured by DNA array

HySilk's basic mechanism of action is the improved barrier function of the stratum corneum. HySilk's molecular weight enables it to penetrate into the deep layers of the stratum corneum in just a matter of hours, where it operates on several levels. First, it has the capacity to protect phospholipids against peroxidation by UV radiation and free radicals. These phospholipids are part of the natural moisturizing factor (NMF), the presence of which is essential for the proper barrier function of the skin.

Secondly, HySilk has a positive effect on the activity of beta-glucocerebrosidase, an enzyme responsible for the release of ceramides from their glucosyl precursors. Ceramides are then incorporated into the lipid bilayer of corneocytes, and the barrier function is enhanced.[2]

The last positive mechanism is the natural ability of hyaluronic acid to immobilize a large quantity of water in its structures, which helps to restore proper NMF functioning in the epidermis.

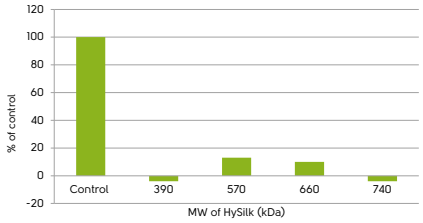
Reduced oiliness by controlling the immune response



Oiliness reduction after HySilk treatment, 8 volunteers treated with 0.005% HySilk (24-43 years) + 29 volunteers control group, Daily application for 12 weeks, $p \leq 0.05$, Measured by MPA 580, Glossymetry

Although an oily film on the surface of the skin helps to reduce transepidermal water loss (TEWL), excessively oily and very shiny skin is not aesthetically pleasing. HySilk has shown an ability to reduce the oiliness of skin. During an *in vivo* study on volunteers treated with 0.005% HySilk cream, more than 20% reduction of oiliness was seen in 12 weeks.

Mechanisms of action



TNF-alpha production from keratinocyte primoculture 24 hours after UV radiation, 5 mJ/cm² UVB, 0.05% HySilk treatment, n=1, Measured by ELISA

The oily film on the skin's surface is formed by sebocytes in in skin glands; one job is to help reduce TEWL. The barrier function and adequate hydration may, however, replace the function of oily film to some extent, rendering its excessive production unnecessary.

It is known that proinflammatory cytokines, particularly tumor necrosis factor TNF-alpha and prostaglandin E2 (PGE2), trigger the production of sebum in sebocytes[1]. A state of chronic inflammation, a deviation of the immune system characterized by the increased production of these substances, is common in premature aging skin.[3]

HySilk influences the TNF-alpha production, helping to stabilize the immune system in the skin.[4] Decreased TNF-alpha production thus has a positive effect by reducing sebum production in sebocytes.

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: **HySilk®**, powder

Origin	biotechnological processing
Appearance	white to slightly yellow powder or granules
Appearance of 0.5% aqueous solution	clear to slightly opalescent, colourless solution
Clarity of 1% aqueous solution (660 nm, 1 cm)	< 0.010
Loss on drying (%)	≤ 10.0
Molecular weight (SEC-MALS)	150 kDa – 1.3 MDa
pH of 0.5% aqueous solution	5.0 – 8.0
Protein (%)*	< 0.20
Microbial contamination (CFU/g)	< 100
Sodium hyaluronate (%)*	> 93.0
Uronic acids (%)*	> 45.0
Ash (%)*	< 10.0

* calc. on dry basis

SOURCE

- by fermentation produced Hyaluronic acid with standard molecular weight, additionally split by a controlled combination of different physical methods to desired molecular weight
- non-GMO
- non-animal materials used during the manufacturing process

SOLUBILITY

- fully soluble in water. Speed of dissolving depends on molecular weight, HySilk with lower molecular weight dissolves quicker.
- soluble in a mixture of ethylalcohol and isopropylalcohol with water. Solubility depends on molecular weight and concentration – the lower molecular weight, the better solubility.
- soluble in mixture of propylene glycol and butylene glycol with water (1:1)
- insoluble in non-water miscible solvents

COMPATIBILITY AND PROCESSING

- sensitive to heat and extreme pH values. Stability increases with decreasing molecular weight.
- very sensitive to free radicals
- incompatible with cationic substances, e.g. quarternized polymers and proteins (Quarterniums, Polyquarterniums, etc.)

TOXICOLOGY

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

Literature:

[1] Choi, J. J., M. Y. Park, et al. (2012). "TNF-alpha increases lipogenesis via JNK and PI3K/Akt pathways in SZ95 human sebocytes." J Dermatol Sci 65(3): 179-88

[2] Redoules, D., R. Tarroux, et al. (1998). "Epidermal enzymes: their role in homeostasis and their relationships with dermatoses." Skin Pharmacol Appl Skin Physiol 11(4-5): 183-92

[3] Thornfeldt, C. R. (2008). "Chronic inflammation is etiology of extrinsic aging." J Cosmet Dermatol 7(1): 78-82

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

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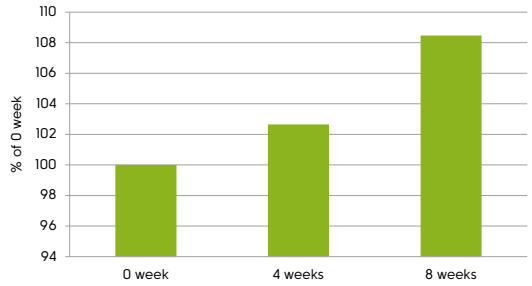
HYACTIVE®

HyActive's low molecular weight enables it to penetrate into the skin, where it interacts with skin cells and extracellular matrix components.

In particular, it enhances skin texture and reduces wrinkles, as has been demonstrated in a battery of *in vivo* tests.

Smoothing of the skin texture due to the influence on epidermal structure

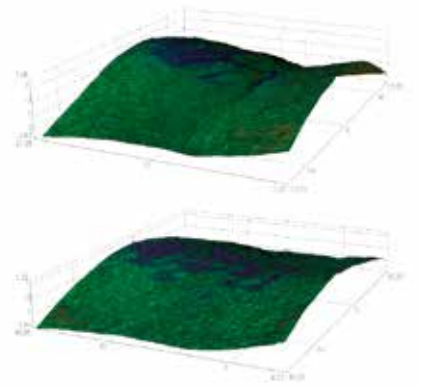


Average pore size area after 0.005% HyActive treatment, 8 volunteers (24-45 years) + 29 volunteers control group, Daily application for 8 weeks, $p \leq 0.05$, Measured by VisioFace

Treatment with 0.005% HyActive significantly improves skin texture due to pore size reduction.

In the test subjects, compared with the control, a nearly 50% reduction in pore size relative to the baseline measurements was identified after eight weeks of application. An improvement in skin texture was also evident on the LifeViz 3D image.

Wrinkle reduction due to the positive impact on extracellular matrix

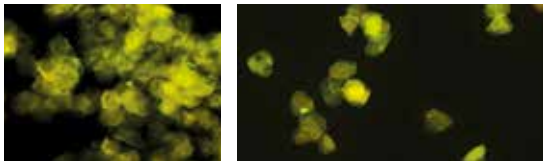


In vivo effect of 0.005% HyActive application on wrinkles; Measured by 3D Life Viz.

After the 12-week application of cream containing 0.005% HyActive, wrinkle reduction was demonstrated. 3D LifeViz pictures showed a decrease in both the scale and depth of wrinkles.

Mechanisms of action

In-vivo tests demonstrated the accelerated recovery of the stratum corneum, a reduction in the size of desquamated keratinocyte aggregates and improvements in the structure of the deeper layers of the stratum corneum in terms of the content of ECM proteins.

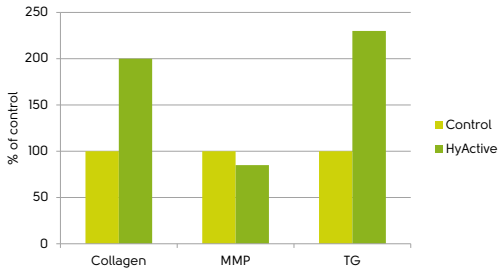


Ilustrative picture of large (left) and normalized (right) keratinocyte aggregates

Explanation of these positive mechanisms is the activation of proteases disrupting intercellular connections in the upper layers of the stratum corneum, thus ensuring its enhanced quality and uniform desquamation.[1]

A complementary effect is the stimulation of the endogenous production of hyaluronic acid, leading to augmentation of the epidermis.

Mechanisms of action



Relative changes in production of collagen, MMP's and transglutaminase after HyActive treatment, Measured by DNA-array (collagen and MMP, 0.1%, n=1) and enzyme activity from strips (TG, 1%, n = 6, $p \leq 0.05$)

In vitro testing showed the stimulation of collagen synthesis, activation of the enzyme transglutaminase (TG) and reduced matrix metalloproteinase (MMP) activity.

Together, these processes help to restore the extracellular matrix, increasing its volume and its stabilization. These synergistic processes augment and subsequently visibly reduce wrinkles in the treated skin.

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: **HyActive®**, powder

Origin	biotechnological / chemical processing
Appearance	white to creamy fine powder
Appearance of 1% aqueous solution	clear to slightly opalescent, colourless solution
Clarity of 1% aqueous solution (660 nm, 1 cm)	≤ 0.010
Loss on drying (%)	≤ 10.0
Molecular weight (SEC-MALS) (kDa)	10 – 150
pH of 0.5% aqueous solution	5.0 – 8.0
Protein (%)*	≤ 0.20
Microbial contamination (CFU/g)	≤ 100
Sodium hyaluronate (%)*	≥ 93.0
Uronic acids (%)*	≥ 45.0
Ash (%)*	≤ 10.0

* calc. on dry basis

SOURCE

- by fermentation produced Hyaluronic acid with standard molecular weight, additionally split by a controlled combination of different physical methods to desired molecular weight
- non-GMO
- non-animal materials used during the manufacturing process

SOLUBILITY

- dissolves rapidly in water
- soluble in a mixture of water with ethylalcohol, isopropylalcohol, propylene glycol and butylene glycol at the concentration of organic solvent up to 70%
- insoluble in non-water miscible solvents

COMPATIBILITY AND PROCESSING

- HyActive solution is relatively stable. Only small changes in molecular weight occur while heating at extreme pH values.
- very sensitive to free radicals
- incompatible with cationic substances, e.g. quarternized polymers or proteins (Quarterniums, Polyquarterniums, etc.)

TOXICOLOGY

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

Literature

[1] Marks, R. (2004). "The stratum corneum barrier: the final frontier." J Nutr 134(8 Suppl): 2017S-2021S

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