

# HyaCare® 50

## The Tight Junction strengthening Hyaluronic Acid

### Intended use

Active for skin care

### Benefits at a glance

- 50 kDa hHyaluronic acid
- Supplies hyaluronic acid into the skin
- Increases the content of hyaluronic acid
- Rejuvenates the skin by improving its viscoelastic properties
- Fills wrinkles from inside
- Reduces deep wrinkles significantly
- Reduces crow feet
- Usage concentration: 0.01 – 0.2%

### INCI (PCPC name)

Hydrolyzed Hyaluronic Acid

### Chemical and physical properties (not part of specifications)

Form	Powder
Molecular Weight	20–70 kDa
Solubility in water	10 g/ 100 g

Hyaluronic acid (HA) is a non-sulfated glycosaminoglycan (GAG), naturally distributed in human body. Hyaluronic acid is the major component of the extra cellular matrix. It is found in high quantity in the skin where it is produced by fibroblasts and keratinocytes.

One of its main functions is to hold water in the

intercellular matrix of the connective tissue. This water-binding capacity contributes significantly to the elasticity of the skin. Hyaluronic acid plays the role of water pump maintaining the elasticity by acting as a water reservoir. Hyaluronic acid is also involved in tissue repair. While it is abundant in extracellular matrices, it also contributes to tissue hydrodynamics, movement and proliferation of cells, and participates in a number of cell surface receptor interactions, notably those including its primary receptor, CD44.

When skin is excessively exposed to UVB rays, it becomes inflamed and the cells in the dermis stop producing hyaluronic acid and increase the rate of its degradation. With aging, the quantity of hyaluronic acid and its degree of polymerisation decreases leading to a decrease in the water content hold by the connective tissue and a loss of elasticity that manifests itself in wrinkles.

In normal human skin, hyaluronic acid exists as a polymer of medium molecular weight (600–1,000 kDa). The physiological degradation of hyaluronic acid in the skin is done via internalisation by the keratinocytes and fragmentation by hyaluronidases.

High molecular weight hyaluronic acid has been used for many years as natural moisturizer in the cosmetic industry. In 2003 the FDA has approved hyaluronic acid injections for filling soft tissue defects such as facial wrinkles. Hyaluronic acid injections temporarily smooth wrinkles by adding volume under the skin, with effects typically lasting for six months.

This FDA approval and the significant trend for botox like cosmetics reinforced the demand for hyaluronic acid. Hyaluronic acid is now not only considered a moisturizer but as a relevant anti-aging ingredient. Our in vivo data on our medium molecular weight HyaCare<sup>®</sup> confirmed this statement.

In parallel, intensive developments have been made to check if molecular weight reduction would provide even more skin care benefits. This led to the introduction of HyaCare<sup>®</sup> 50, a new and unique, very low molecular weight hyaluronic acid (50 kDa) produced by fermentation of *Bacillus subtilis* using an environmentally friendly aqueous recovery process.

### Properties

#### • *Ex vivo* permeation study

**Method:** The penetration of different molecular weight fractions has been evaluated using side by side diffusion cells and tritiated hyaluronic acid on dermatomed skin from the porcine ear. The receptor was filled with PBS buffer and the donor with radio labeled HA solution. After incubation for 5 h and 22 h at room temperature, the receptor phase was removed and radioactivity was determined by liquid scintillation counting.

#### Results:

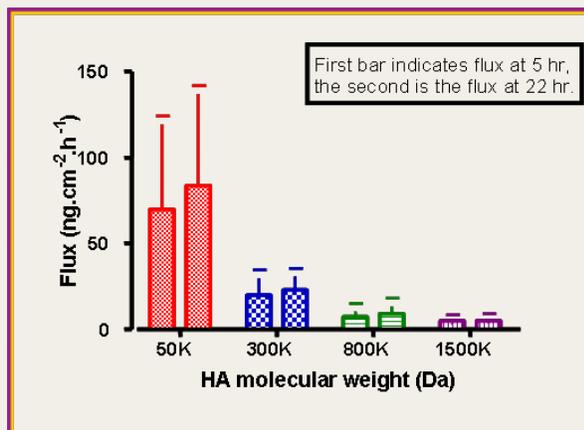


Figure 1 : Influence of hyaluronic acid molecular weight on per cutaneous transport

As seen in figure 1, HyaCare<sup>®</sup> 50 shows a much stronger permeation through the skin in comparison to higher molecular weight products.

#### • *In vitro* gene expression analysis

**Method:** The study was performed at the Department of Clinical Chemistry at the University of Regensburg under supervision of Prof. Gerd Schmitz.

SkinEthic<sup>TM</sup> reconstructed epidermis models were used. A thin, liquid formulation containing 0.5% HyaCare<sup>®</sup> 50, 0.5% HyaCare<sup>®</sup> or no active ingredient (vehicle) were topically applied for 48 hours. After that the skin tissue was harvested, lysed and RNA was isolated. The RNA from the skin models was transcribed into DNA and labelled with fluorescent dyes. Affymetrix<sup>®</sup> HGU133 Gene Chips were used to characterize genome wide expression.

**Results:** It was demonstrated that HyaCare<sup>®</sup> 50 was able to influence gene expression significantly in comparison to HyaCare<sup>®</sup>. More than 40 genes were regulated, including upregulation of junctional control genes. Tight junction and adherens junction proteins are important for the barrier function of the skin and the cellular elasticity.

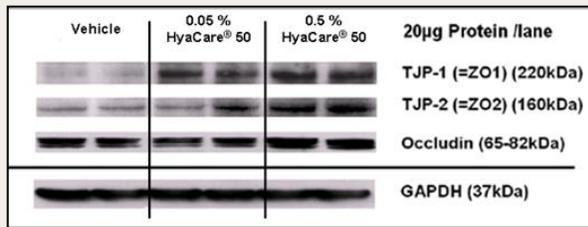
#### • *In vitro* protein expression analysis

In a previous gene expression study an upregulation of junctional genes by HyaCare<sup>®</sup> 50 was observed. Tight and adherens junction proteins connect the cells with each other and are very important for molecule transport, maintenance of ion homeostasis and protection of the viable layers of the epidermis. Therefore, the effect of HyaCare<sup>®</sup> 50 also on the expression of junctional proteins was analyzed.

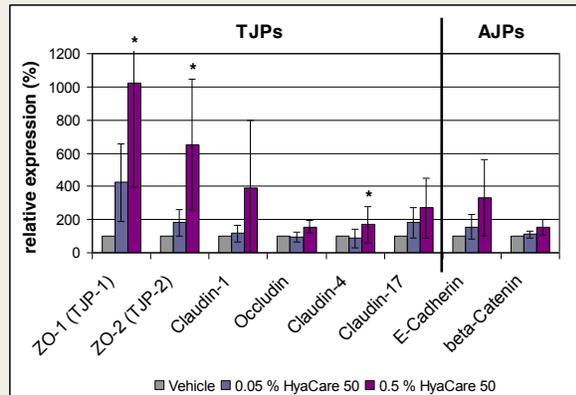
**Method:** The study was performed at the University of Regensburg under supervision of Prof. Gerd Schmitz.

For this study reconstructed human epidermis models (SkinEthic) were topically treated with 0.05% and 0.5% HyaCare<sup>®</sup> 50 for 72 h (n=4). After treatment the proteins were extracted and junctional proteins were analysed and quantified by Western Blotting (Nu-PAGE<sup>®</sup> Novex 4 12% Bis-Tris Gel (Invitrogen) using protein specific antibodies. The resulting intensities were standardized to GAPDH (Glyceraldehyde 3-phosphate dehydrogenase) by LumiAnalyst 3.0 in Biochemical Light Units [BLU].

## Results:



**Figure 2:** Example of Western Blot analysis of significantly induced Tight Junction proteins (TJPs) in SkinEthic models treated with different concentrations of HyaCare® 50



**Figure 3:** Quantification of TJPs and Adherens Junction proteins (AJP) in SkinEthic skin models vs. GAPDH (\* $p < 0.05$ )

The results of this study show that HyaCare®50 induces junctional proteins in human epidermis models. This confirms previous results obtained by gene expression analysis.

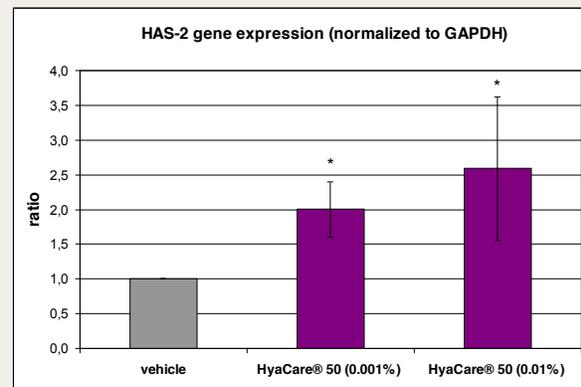
Properly performing Tight Junctions guarantee well balanced ion homeostasis, they protect the viable layers of the skin and finally lead to healthier, younger looking skin.

- **In vitro gene expression study**

In this study the influence of HyaCare® 50 on Hyaluronan synthesis in human dermal fibroblasts was analyzed.

**Method:** The cells were treated with 0.001% and 0.01% HyaCare®50 for 24 h (n=3). After treatment the RNA was extracted and genes of the Hyaluronan synthesis/degradation pathways were analysed by quantitative real time PCR (qRT-PCR). The results were normalized to GAPDH (Glyceraldehyde 3-phosphate dehydrogenase) and compared to vehicle control.

## Results:



**Figure 4:** Expression of HAS-2 gene in human dermal fibroblasts treated with different concentrations of HyaCare® 50 compared to vehicle control and normalized to GAPDH (\* $p < 0.05$ )

HAS-2 is the major Hyaluronan Synthase in fibroblasts and produces high molecular weight hyaluronic acid, which plays a very important role in the formation of the extracellular matrix. The up-regulation of this enzyme by HyaCare® 50 leads to a higher HAS-2 synthesis rate and therefore an induced production of hyaluronic acid in dermal fibroblasts. This leads to an increased content of Hyaluronic Acid in the skin. HyaCare® 50 fills the wrinkles from inside.

- **In vivo efficacy study**

### Long-term evaluation of skin elasticity and roughness

**Method:** The study was performed at ISPE s.r.l., Milan (Italy).

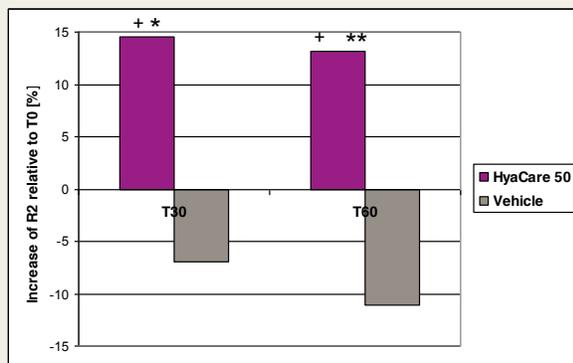
For the study 12 volunteers (female, mean age 51) were recruited. Each volunteer was required not to cleanse or moisture their faces for a minimum of at least 3 hours before the test procedure.

The study was carried out in a climatic room with defined conditions of 24 °C and 50% relative humidity. The assessment of the skin hydration was performed on the peri-ocular area of the face where an O/W cream without (vehicle) and with 0.1% HyaCare® 50 was tested and compared to each other (Half-side test). The application of the two products was randomized among the subject for each area. The volunteers applied the test formulation twice daily for 2 month.

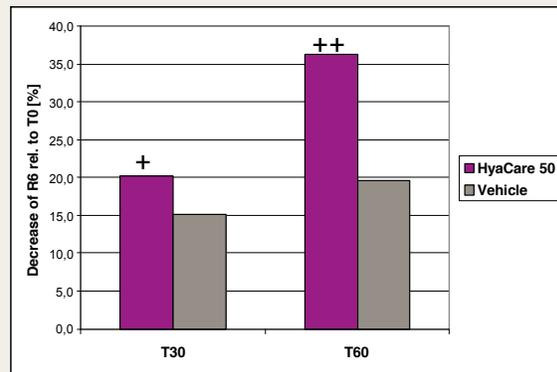
After 4 weeks and at the end of the study skin elasticity and skin roughness was measured. The skin elasticity was assessed with a Cutometer SEM 575 (Courage & Khazaka, Germany) Different parameters describing the skin elasticity were calculated. The parameter R2 describes the overall elasticity ( $R2 = UA/UF$ ) while by R6 the viscoelastic ratio is calculated ( $R6 = UV/UE$ ).

For the measurement of the wrinkle reduction skin replicas were prepared using a fast hardening synthetic polymer (Silflo, Flexico Ltd, UK) and an adhesive disc (3M, 24x40 mm). The replicas were analyzed by special image processing software (Quantilines, Monaderm). This software allows the calculation of Rz, the maximum roughness value. Rz describes especially the deep wrinkles.

**Results:** The area treated with the formulation containing HyaCare® 50 showed a significant increase of the overall elasticity value, equal to 14% after 4 and 8 weeks of application (figure 5).



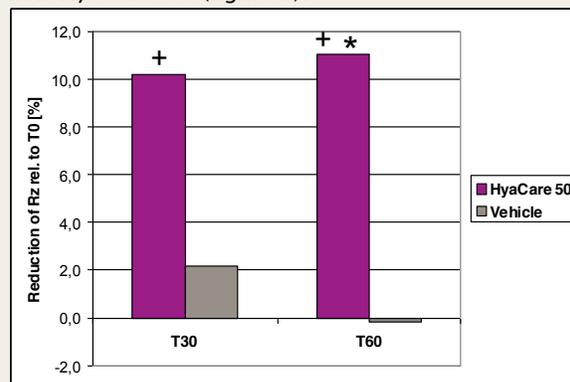
**Figure 5: Overall skin elasticity after 4 and 8 weeks application of test formulations (+ significant compared to start, \* significant compared to vehicle, \*\* highly significant compared to vehicle)**



**Figure 6: Decrease in the skin viscoelastic ratio after 4 and 8 weeks application of test formulations (+ significant compared to start, ++ highly significant compared to start)**

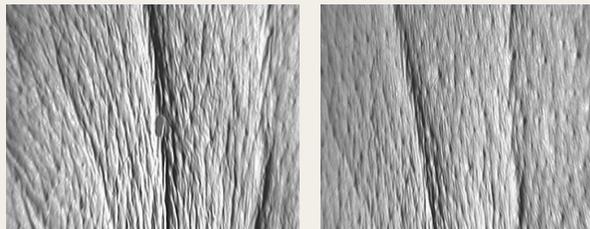
The area treated with HyaCare® 50 showed as well a highly significant decrease in the viscoelastic ratio (R6) equal to 36% after 8 weeks. This reflects the rejuvenation of the skin through the increase of its elastic component (figure 6).

Skin image analysis showed a significant decrease of maximum skin roughness of 10% of the area treated with HyaCare® 50 (figure 7).



**Figure 7: Maximum Roughness ratio after 4 and 8 weeks application of test formulations (+ significant compared to start, \* significant compared to vehicle)**

These data confirmed the anti-wrinkle activity of HyaCare® 50 mainly in terms of decrease of deep wrinkles as illustrated in figures 8 and visible reduction of crow feet as shown in figure 9.



**Figure 8: Skin image analysis before and after 4 weeks of treatment with HyaCare® 50**



**Figure 9: Skin image analysis before, after 4 and 8 weeks of treatment with HyaCare® 50**

### Preparation

HyaCare® 50 is completely soluble in water and can be processed cold. For the preparation of both O/W or W/O emulsions it should be added to the water phase. In O/W emulsions HyaCare® 50 might reduce the viscosity of the emulsion. The desired viscosity of O/W emulsions can be adjusted by increasing the amount of polyacrylates (e.g. TEGO® Carbomer) or by increasing the concentration of consistency enhancers, e.g. TEGO® Alkanol 18 (Stearyl Alcohol), TEGO® Alkanol 1618 (Cetearyl Alcohol), TEGIN® M or TEGIN® 4100 (Glyceryl Stearate). HyaCare® 50 shows good compatibility with other ingredients.

### Recommended usage concentration

0.01 – 0.2% of HyaCare® 50

### Applications

HyaCare® 50 is suitable for O/W and W/O formulations of:

- Anti-wrinkle eye creams
- Anti-wrinkle face cream
- Anti-aging foundations

### Storage

For short term (few weeks), the product can be stored at room temperature. For long term (over several months), the product should be stored in a cool place (4 °C). Avoid freezing.

### Packaging

0.5 kg package

### Hazardous goods classification

Information concerning

- classification and labelling according to regulations for transport and for dangerous substances
- protective measures for storage and handling
- measures in case of accidents and fires
- toxicity and ecological effects

is given in our material safety data sheets.

## Guideline formulations

<b>O/W Anti-Wrinkle Cream – Filler effect</b>	
<b>Mac 534/3/3</b>	
<b>Phase A</b>	
TEGOSOFT® C (Cetearyl Isononanoate)	5.0%
TEGOSOFT® liquid (Cetearyl Ethylhexanoate)	5.0%
TEGOSOFT® DC (Decyl Cocoate)	4.0%
TEGO® Alkanol 1618(Cetearyl Alcohol)	3.0%
TEGIN® 4100 Pellets (Glyceryl Stearate)	1.0%
Stearic Acid	1.0%
Tocopheryl Acetate	2.0%
<b>Phase B</b>	
TEGO® Care CG 90 (Cetearyl Glucoside)	1.0%
SK-Influx® (Ceramide 3; Ceramide 6II; Ceramide 1; Phytosphingosine; Cholesterol; Sodium Lauroyl Lactylate; Carbomer; Xanthan Gum)	5.0%
HyaCare® 50	0.2%
Glycerin	3.0%
Allantoin	0.1%
Water	69.2%
<b>Phase C</b>	
TEGO® Carbomer 134 (Carbomer)	0.1%
Mineral Oil (30 mPa s)	0.4%
<b>Phase D</b>	
Sodium Hydroxide (10% in water)	q.s.
<b>Phase Z</b>	
Preservative, perfume	q.s.

### Preparation:

1. Heat phase A and B separately to approx. 80 °C.
  2. Add phase A to phase B with stirring. <sup>1)</sup>
  3. Homogenize.
  4. Cool with gentle stirring to approx. 60 °C and add phase C.
  5. Homogenize for a short time.
  6. Cool with gentle stirring and add phase D below 40 °C.
- <sup>1)</sup> Important : If phase A has to be charged into the vessel first, phase B must be added **without stirring**.

### W/O Night Cream – Intensive Repair Mac 534/1/2

<b>Phase A</b>	
ABIL® EM 90 (Cetyl PEG/PPG-10/1 Dimethicone)	2.0%
ISOLAN® GI 34 (Polyglyceryl-4 Isostearate)	1.0%
TEGOSOFT® OS (Ethylhexyl Stearate)	5.0%
Paraffinum Perliquidum	12.0%
Microcrystalline Wax (Paracera W 80, Paramelt, B.V)	1.2%
Hydrogenated Castor Oil	0.8%
<b>Phase B</b>	
HyaCare® 50	0.2%
TEGO® Cosmo C 100 (Creatine)	0.5%
Sodium Chloride	0.5%
Water	76.8%
Preservative, perfume	q.s.

### Preparation:

1. Heat phase A to approx. 80 °C.
2. Add phase B (80 °C or room temperature) slowly while stirring.
3. Homogenize for a short time.
4. Cool with gentle stirring below 30 °C and homogenize again.

<b>Hyaluron Complex Anti-Wrinkle Cream</b> Mac 689/3/1	
<b>Phase A</b>	
ABIL® Care XL 80 (Bis-PEG/PPG-20/5 PEG/PPG-20/5 Dimethicone; Methoxy PEG/PPG-25/4 Dimethicone; Caprylic/Capric Triglyceride)	1.0%
TEGO® Care 450 (Polyglyceryl-3 Methylglucose Distearate)	2.0%
TEGIN® M Pellets (Glyceryl Stearate)	3.5%
TEGO® Alkanol 18 (Stearyl Alcohol)	1.5%
TEGOSOFT® DEC (Diethylhexyl Carbonate)	7.5%
TEGOSOFT® OS (Ethylhexyl Stearate)	7.5%
HyaCare® Filler CL (Aqua; Ethylhexyl Stearate; Sodium Hyaluronate Crosspolymer; Polyglyceryl-4 Diisostearate/Polyhydroxystearate/Se bacate; Sodium Isostearate)	2.0%
<b>Phase B</b>	
HyaCare® (Sodium Hyaluronate)	0.1%
HyaCare® 50	0.1%
Glycerin	3.0%
Water	71.8%
<b>Phase Z</b>	
Preservative, perfume	q.s.
<b>Preparation:</b>	
<ol style="list-style-type: none"> <li>Heat phase A and B separately to approx. 70 – 75 °C.</li> <li>Add phase A to phase B with stirring. <sup>1)</sup></li> <li>Homogenize.</li> <li>Cool with gentle stirring.</li> </ol> <p><sup>1)</sup> Important : If phase A has to be charged into the vessel first, phase B must be added <b>without stirring</b>.</p>	

<b>Reviving Anti-Wrinkle Serum</b> MK 3/10-10	
<b>Phase A</b>	
Water	81.7%
Butylene Glycol	4.0%
TEGO® Carbomer 140 (Carbomer)	0.3%
TEGO® Carbomer 141 (Carbomer)	0.1%
<b>Phase B</b>	
Sodium Hydroxide (10% in water)	1.4%
<b>Phase C</b>	
Water	9.9%
HyaCare® 50	0.1%
TEGO® Pep 4-17 (Tetrapeptide-21; Glycerin; Butylene Glycol; Aqua)	2.5%
<b>Phase Z</b>	
Preservative, perfume	q.s.
<b>Preparation:</b>	
<ol style="list-style-type: none"> <li>Disperse TEGO® Carbomer types in phase A.</li> <li>Add phase B and stir well.</li> <li>Mix ingredients of phase C and add to phase A/B.</li> <li>Stir until homogeneous.</li> </ol>	

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**Especially concerning Active Ingredients**

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