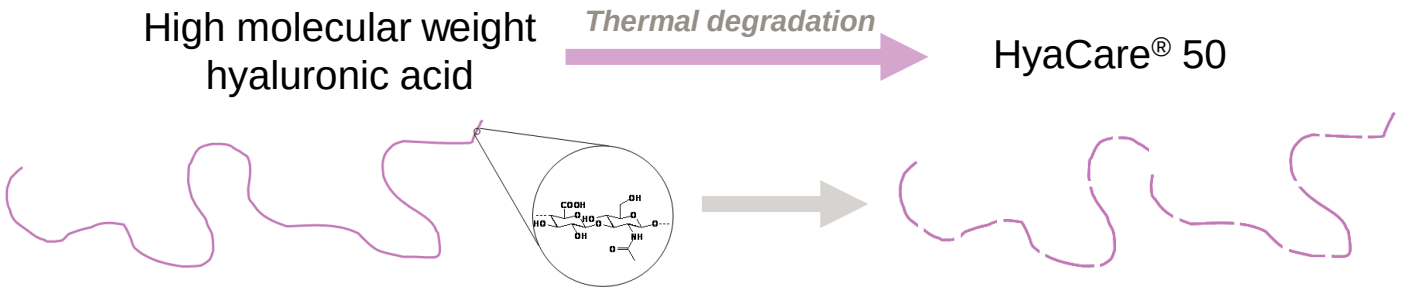
HyaCare® 50

The Tight Junction strengthening Hyaluronic Acid

EVONIK INDUSTRIES

Properties & Features

- Hyaluronic acid is produced by fermentation of *B. subtilis* (non-pathogenic) using an environmentally friendly, solvent free recovery process
- Mild, thermal degradation leads to low molecular weight hyaluronic acid
- HyaCare® 50 has an average molecular weight of 50 kDa
- INCI: Hydrolyzed Hyaluronic Acid

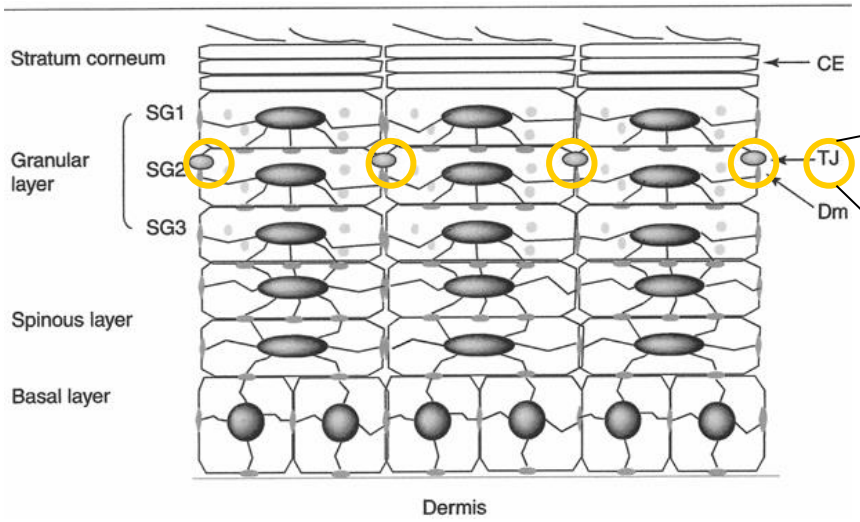


Benefits & Claims

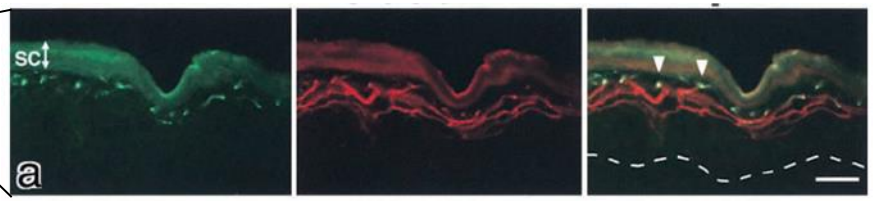
HyaCare® 50

- supplies hyaluronic acid into the skin
- increases the content of hyaluronic acid in the skin
- fills wrinkles from inside
- rejuvenates the skin
- reduces deep wrinkles significantly
- reduces crow feet

Background information: Epidermis & tight junction proteins



Double-immunofluoresence microscopy of frozen section of the skin



Occludin *Claudin 4* *Occludin & Claudin 4*

Epidermis

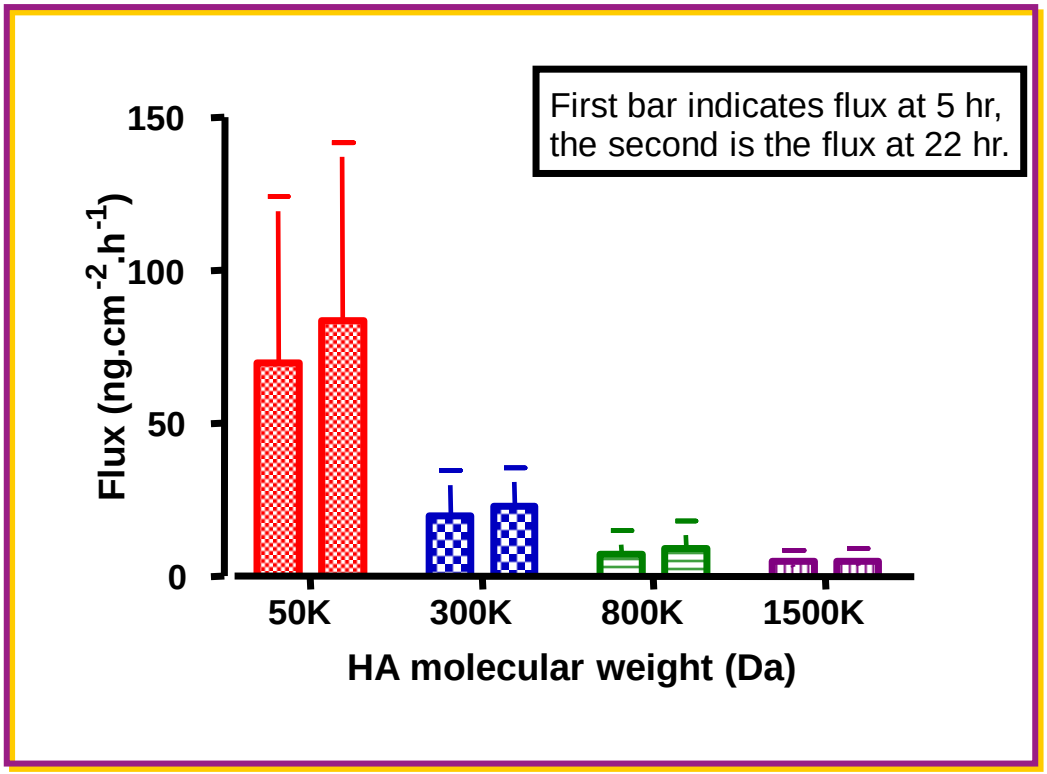
Tight junction proteins

⇨ Tight junctions are important for the barrier function of the skin & the cellular elasticity

Ex vivo permeation study – Test design

Model system:	Dermatomed skin from porcine ear
Selected application:	Tritiated HA of different MW (50; 300; 800; 1500 kDa)
Selected time point:	5 & 22 hours
Analysis:	Franz diffusion cells
	Radioactivity in receptor phase determined using liquid scintillation counter

Ex vivo permeation study – Test result



HyaCare® 50 (= 50 kDa) shows an improved permeation flux into porcine skin

HyaCare® 50 supplies hyaluronic acid into the skin

In vitro gene expression analysis – Test design & result

Model system:	Reconstituted human epidermis (SkinEthic)
Selected application:	thin, liquid formulation
Concentration:	vehicle ± 0.5 % HyaCare® 50 or HyaCare®
Selected time point:	48 hours
Analysis:	DNA gene chip analysis

Result:

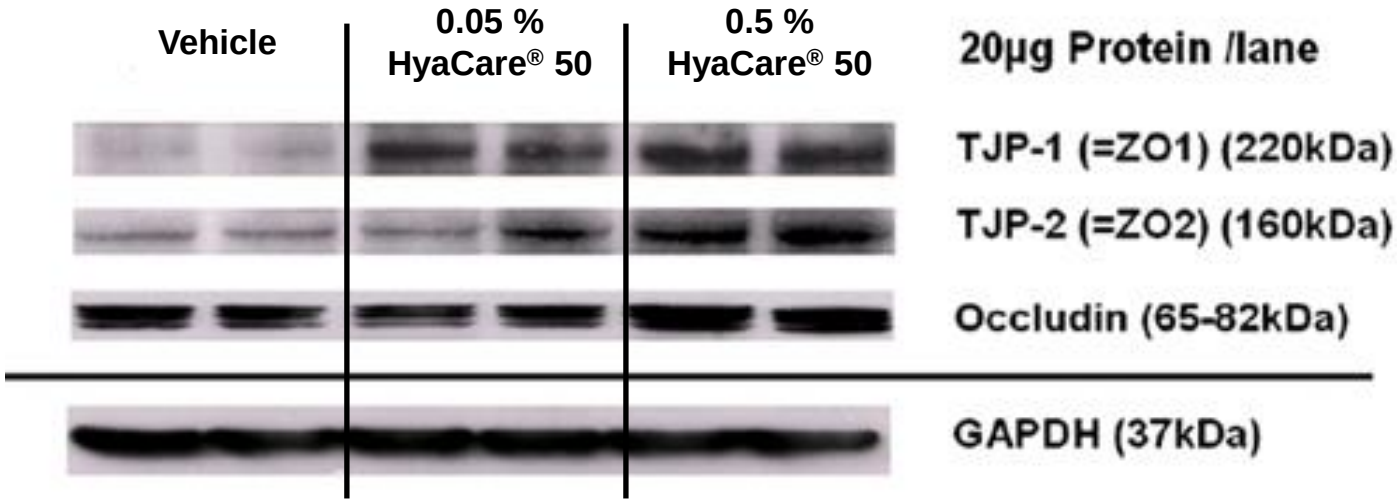
- HyaCare® 50 induces genes involved in
  - junctional control
  - differentiation
  - and other genes related to the aging process

HyaCare® 50 is able to induce these genes stronger than high molecular weight HA

In vitro protein expression analysis – Test design

Model system:	Reconstituted human epidermis (SkinEthic)
Selected application:	aqueous solution
Concentration:	vehicle ± 0.05 or 0.5 % HyaCare® 50
Selected time point:	72 hours
Analysis:	Western Blot analysis normalized to GAPDH - Tight Junction Proteins (TJP) - Adherens Junction Proteins (AJP)

In vitro protein expression analysis – Test result

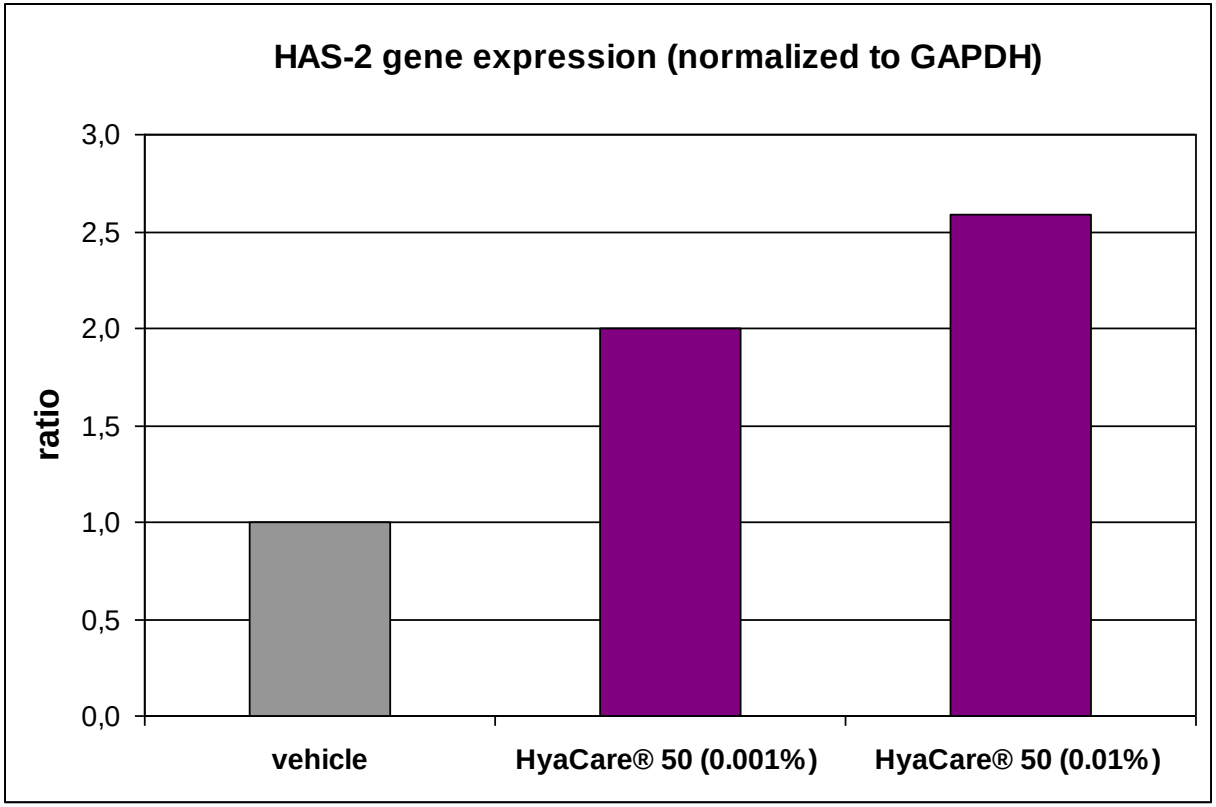


- HyaCare® 50 improves the protein expression of tight junction proteins & adherens junction proteins in a dose dependant manner
- HyaCare® 50
  - improves the balance of ion homeostasis
  - protects the viable layers of the skin
  - contributes to a healthier, younger, tender looking skin

In vitro gene expression analysis – Test design

Model system:	Human dermal fibroblasts
Selected application:	aqueous solution
Concentration:	vehicle ± 0.001 or 0.01 % HyaCare® 50
Selected time point:	24 hours
Analysis:	qRT-PCR on genes involved in the Hyaluronic Acid synthesis (HAS-2) pathway - normalized to GAPDH - compared to vehicle

In vitro gene expression analysis – Test result



HyaCare® 50

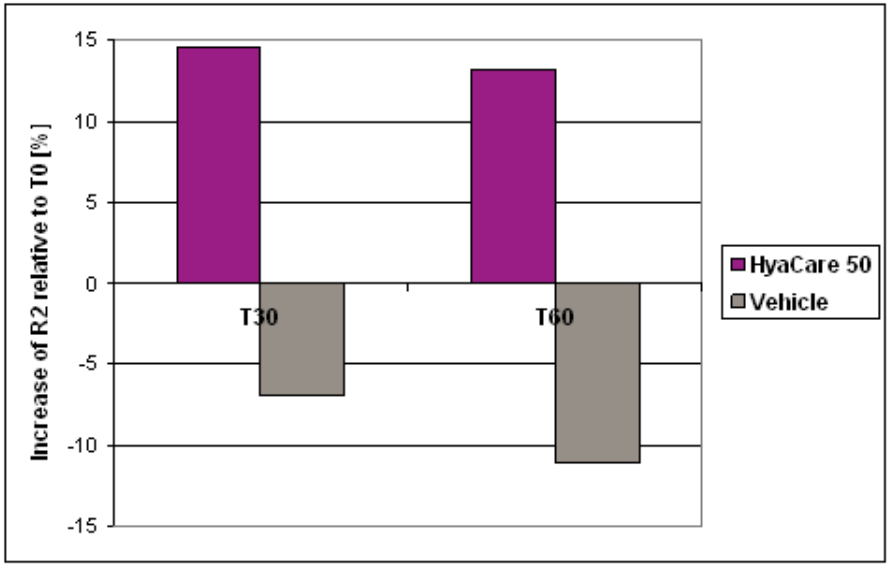
- increases the content of hyaluronic acid in the skin
- fills wrinkles from inside

In vivo evaluation – Test design

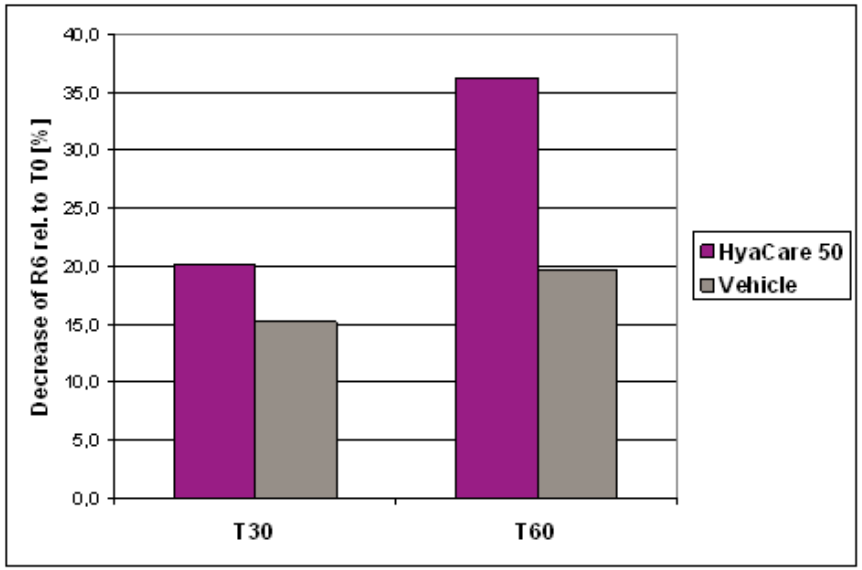
Number of volunteers:	12
Selected application:	O/W formulation
Concentration:	vehicle ± 0.1 % HyaCare® 50
Selected time point:	before and after 4 and 8 weeks of application
Analysis:	Cutometry (skin elasticity)
	Image analysis of skin replica (skin roughness)

In vivo evaluation – Test result

R2 (Overall elasticity)

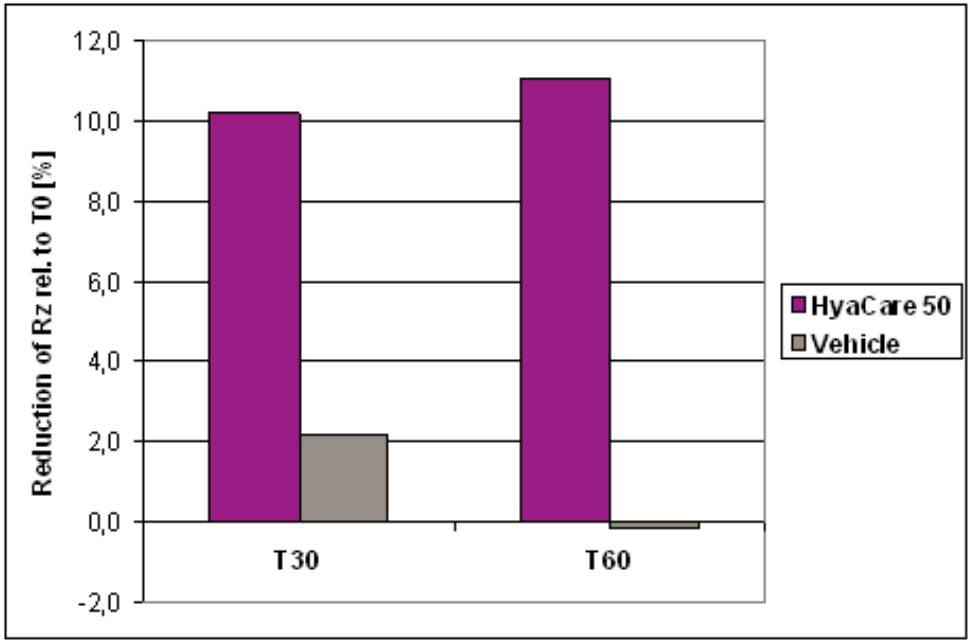


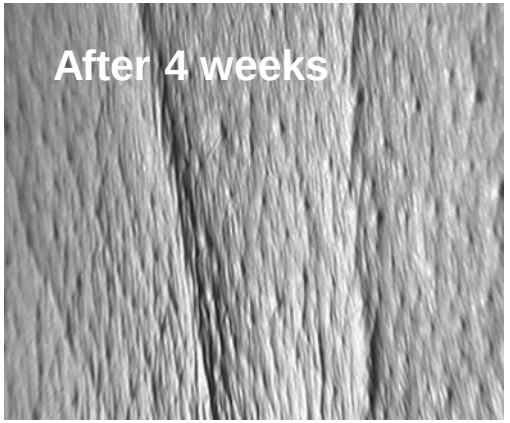
R6 (Viscoelastic ratio)



HyaCare® 50 rejuvenates the skin

In vivo evaluation – Test result





HyaCare® 50 reduces deep wrinkles significantly

HyaCare® 50 – Photos

Before | After 4 weeks | After 8 weeks

HyaCare® 50 reduces crow feet

HyaCare® 50 – Formulation hints

Recommended usage concentration: 0.01 – 0.2 %, clinically tested at 0.1 %

Preparation of an O/W-Emulsion (Cream or Lotion):

HyaCare® 50 can be added to the water phase of the emulsion. Then the emulsion is prepared as usual.

In some cases HyaCare® 50 might disturb the built-up of the viscosity/creamy consistency. Here it is recommended to prepare the emulsion as usual & to add an aqueous solution of HyaCare® 50 during the cooling process at temperatures <40°C.

HyaCare® 50 can decrease the viscosity of an O/W-emulsion. The viscosity can be adjusted by increasing the concentration of consistency enhancers like TEGO® Alkanol, TEGIN® M Pellets or Stearic Acid or hydrocolloids like TEGO® Carbomer or Xanthan Gum.

Preparation of a W/O-Emulsion (Cream or Lotion):

HyaCare® 50 is added to the water phase. The emulsion is prepared as usual.

HyaCare® 50 – Guideline formulation

	O/W Anti-Wrinkle Cream - Filler effect Mac 534/3/3	
A	TEGOSOFT® CI (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	TEGO® Care CG 90 (Cetearyl Glucoside)	1.0 %
	SK-influx® (Ceramide NP; Ceramide AP; Ceramide EOP; Phytosphingosine; Cholesterol; Sodium Lauroyl Lactylate; Carbomer; Xanthan Gum)	5.0 %
	HyaCare® 50 (Hydrolyzed Hyaluronic Acid)	0.2 %
	Glycerin	3.0 %
	Allantoin	0.1 %
	Water	69.2 %
C	TEGO® Carbomer 134 (Carbomer)	0.1 %
	Mineral Oil (30 mPa s)	0.4 %
D	Sodium Hydroxide (10% in water)	q.s.
Z	Preservative, perfume	q.s.

Processing

1. Heat phase A & B separately to approx. 80 °C.
2. Add phase A to phase B while stirring.[1)]
3. Homogenize.
4. Cool with gentle stirring to approx. 60 °C & add phase C.
5. Homogenize for a short time.
6. Cool with gentle stirring & add phase D below 40 °C.

[1)] Important: If phase A has to be charged into the vessel first, phase B must be added without stirring.

HyaCare® 50 – Guideline formulation

	W/O Night Cream – Intensive Repair Mac 534/1/2	
A	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 W80, Paramelt, B.V.)	1.2 %
	Hydrogenated Castor Oil	0.8 %
B	**HyaCare® 50 (Hydrolyzed Hyaluronic Acid)**	0.2 %
	TEGO® Cosmo C 100 (Creatine)	0.5 %
	Sodium Chloride	0.5 %
	Water	76.8 %
Z	Preservative, perfume	q.s.

Processing

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.

HyaCare® 50 – Guideline formulation

	Hyaluron Complex Anti-Wrinkle Cream Mac 689/3/1	
A	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/Sebacate; Sodium Isostearate)	2.0 %
B	HyaCare® (Sodium Hyaluronate)	0.1 %
	HyaCare® 50 (Hydrolyzed Hyaluronic Acid)	0.1 %
	Glycerin	3.0 %
	Water	71.8 %
Z	Preservative, perfume	q.s.

Processing

1. Heat phase A and B separately to approx. 70 - 75 °C.
2. Add phase A to phase B with stirring. [1)]
3. Homogenize.
4. Cool with gentle stirring.

[1)] Important: If phase A has to be charged into the vessel first, phase B must be added without stirring.

HyaCare® 50 – Guideline formulation

	Reviving Anti-Wrinkle Serum MK 3/10-10	
A	Water	81.7 %
	Butylene Glycol	4.0 %
	TEGO® Carbomer 140 (Carbomer)	0.3 %
	TEGO® Carbomer 141 (Carbomer)	0.1 %
B	Sodium Hydroxide (10 % in water)	1.4 %
C	Water	9.9 %
	HyaCare® 50 (Hydrolyzed Hyaluronic Acid)	0.1 %
	TEGO® Pep 4-17 (Tetrapeptide-21; Glycerin; Butylene Glycol; Aqua)	2.5 %
Z	Preservative, perfume	q.s.

Processing

1. Disperse TEGO® Carbomer types in phase A..
2. Add phase B and stir well.
3. Mix ingredients of phase C and add to phase A/B.
4. Stir until homogeneous.

HyaCare® 50 – Your benefits at a glance

HyaCare® 50

- supplies hyaluronic acid into the skin
- increases the content of hyaluronic acid
- fills wrinkles from inside
- rejuvenates the skin
- reduces deep wrinkles significantly
- reduces crow feet

<u>Possible applications:</u>

- Anti-wrinkle eye creams
- Anti-wrinkle face creams
- Anti-aging foundations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Especially concerning Active Ingredients

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In vitro gene expression analysis – Test result

	HA 50 kDa [Fold change vs. vehicle]	HA 800 kDa [Fold change vs. vehicle]
Junctional Control		
Occludin	3.4	2.0
Claudin 4	3.8	2.4
Claudin 7	7.5	2.8
Claudin 17	5.1	3.2
Aquaporin 3	2.3	2.0
Desmocollin 2	1.8	1.4
Tight junction protein 2	1.9	1.3
Striatin, calmodulin binding protein	2.2	1.5
Kinesin family member C3	2.6	1.8
Differentiation/Epidermis development		
Kallikrein 6	3.8	3.3
Kallikrein 14	2.0	2.1
Repetin	4.6	2.3
Keratin 34	3.0	1.4
Forkhead box Q1	4.0	3.2
ATPase, Ca++ transporting, type 2C, member 1	-2.2	-1.6
Ceramide kinase	- 2.5	-2.0
Small proline rich protein 4	2.7	2.2
Biliverdin reductase A	-1.9	-1.3
Mitogen-activated protein kinase kinase 1	2.1	1.5
Calbindin 1	11.3	4.4
S100 calcium binding protein A12 (calgranulin C)	2.0	1.6
Others		
Thrombospondin 1	3.0	2.6
Sirtuin	2.9	1.6
TIMP metallopeptidase inhibitor 3	2.2	1.8
Glutathione peroxidase 2	3.3	2.7
Heparin-binding EGF-like growth factor	3.4	2.2
Interleukin 10 receptor, beta	-2.7	-2.0
Interleukin 18	-1.8	-1.9

Glossary

Tight Junction Proteins/Adherens Junction Proteins:

The cohesion of keratinocytes in the outer layers of the skin (stratum corneum & stratum granulosum) is an important factor that represents a second barrier function of the skin and therefore protection against mechanical stress.

Cohesion of keratinocytes is mainly arranged by special intercellular protein complexes such as desmosomes, Adherens Junctions (AJs), and Tight Junctions (TJs).

The second barrier of the skin underneath the cornified stratum corneum is consisting of so called Tight Junctions.

Tight Junctions are composed of a complex network of proteins that connect the cells with each other by interactions between integral membrane proteins which are in turn anchored to the cytoskeleton.

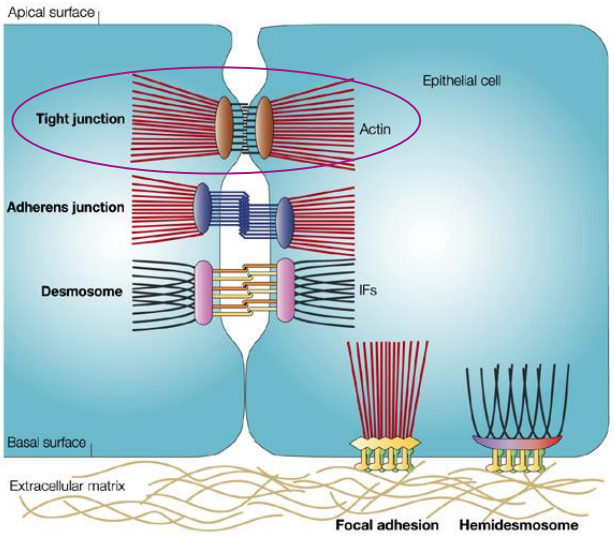
Tight Junctions prevent the passage of molecules and ions through the space between cells and thus protect the viable layers of the epidermis.

Glossary

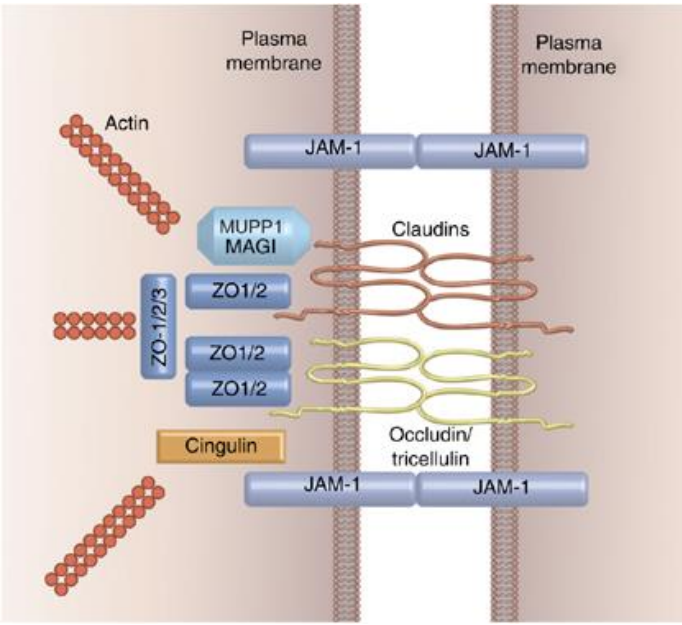
TJP-1 (= ZO-1): tight junction protein 1 (zona occludens 1)

TJP-2 (=ZO-2): tight junction protein 2 (zona occludens 2)

The zonula occludens proteins (ZO-1, ZO-2) interact with Occludin and Claudins and are associated with Actin to form a direct link to the cytoskeleton.



Occludin: integral plasma-membrane protein located at the tight junctions; Together with the **Claudin group** of proteins, it is the main component of the tight junctions.



GAPDH: Glyceraldehyde 3-phosphate dehydrogenase

Enzyme of the energy metabolism; is used as „reference“ molecule because it is known to be expressed at constant levels in all cell types

Glossary

Dermatome: medical device for skin transplantation purposes that cuts of a 1 mm thick layer of the skin

Tritiated HA: radioactively labeled HA; analysis with scintillation (= radioactivity) counter

Glossary

Low molecular weight fragments of HA:

Hyaluronic acid (HA) is a polysaccharide and a major component of the extra cellular matrix. Since it is able to bind large amounts of water it controls tissue hydration and maintains the extra cellular space.

Hyaluronic acid is widely used for cosmetic applications where it is mainly used as natural moisturizer and as anti-aging active. Especially the biological anti-aging activity is limited by the enormous molecular size of HA that can reach up to 2,000 kDa and interferes with its penetration into the skin. Fragmentation of large HA polymers can markedly improve its penetration abilities. Nevertheless pro-inflammatory responses have been reported for very small HA fragments (5-15 kDa) which are recognized by special receptors of the immune-system, therefore size matters and has be above a specific threshold.

Glossary

HAS (Hyaluronan Synthase):

The Extracellular Matrix (ECM) is a three dimensional network of macromolecules which are produced and secreted by different cell-types in human skin.

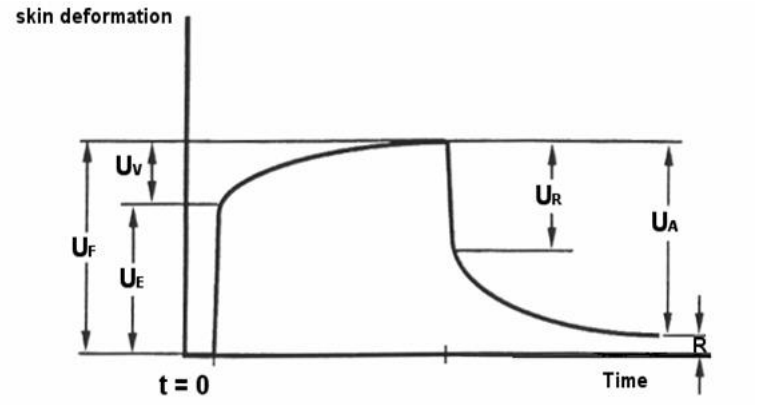
The ECM is very important for intra- and inter-cellular transport pathways and to strengthen the skin structure. Hyaluronic Acid (HA) is the predominant macromoelcule of the ECM.

Hyaluronic Acid is synthesized in epidermal cells (keratinocytes) as well as dermal cells (fibroblasts). In fibroblasts the major enzyme of Hyaluronan synthesis is Hyaluronan Synthase-2 (HAS-2). HAS-1 and -3 play minor roles. The produced HA has a high molecular weight and plays a crucial role in Extracellular Matrix formation as well as maintenance of water balance and nutrient-transport in the dermis. Therefore, it contributes to an individual appearance of skin structure.

Glossary

- ✓ **Overall elasticity of the skin** = UA / UF
  - UA = Total recovery after deformation stress
  - UF = Total extensibility of the skin

- ✓ **Viscoelastic ratio** = UV / UE
  - UV = Viscoelastic creep after the elastic deformation
  - UE = Elastic deformation due stress application



Rz parameter: maximum roughness of the skin. Describes the disctance between highest and the deepest point in the roughness profile of the skin surface.

In vivo evaluation – Test formulation

		% (w/w)
A	Hydrogenated Polydecene	20.0
	Steareth-2	3.0
	Steareth-21	1.0
	Cetearyl Alcohol	1.5
B	Water	ad 100
	Na_2-EDTA	0.1
C	Water	5.0
	Diazolidinyl Urea	0.25
	HyaCare® 50	0.1
D	Phenoxyethanol, Methylparaben, Butylparaben, Ethylparaben, Propylparaben, Isobutylparaben	0.8
		pH 6.4

Publications

„Low molecular weight hyaluronic acid: ist effects on epidermal gene Expression and skin ageing“; SOEFW Nov 2008

„Fifty-kDa Hyaluronic Acid Upregulates Some Epidermal Genes without Changing TNF-Expression in Reconstituted Epidermis“; Skin Pharmacology and Physiology; 2011

„Anti aging – a multifunctional concept“; Cossma Sept 2009