

Hyaluronic acid  
HySilk  
HyActive  
OligoHyaferre



# Hyaluronic acid (HA) of different molecular weight (MW)



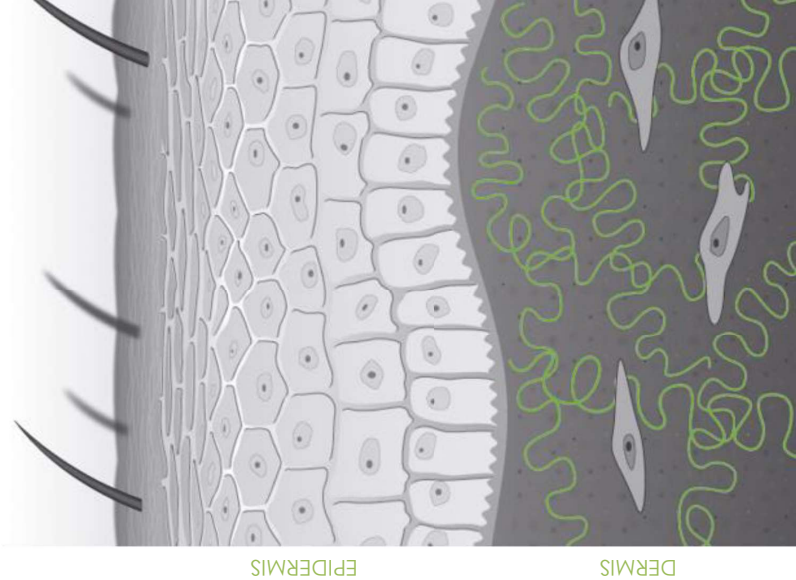
Hyaluronic acid, HySilk, HyActive, OligoHyaferre

## Common properties

- **Biotechnological** production by microorganisms
- **Sustainable**, ecological production
- Nonpathogenic ***Streptococcus equi* subsp. zooepidemicus**
- High MW HA produced first  
=> cleavage by acid hydrolysis=> lower MWs
- **Water-soluble powders**
- **Natural** polysaccharide – COSMOS APPROVED



# HA of different MW



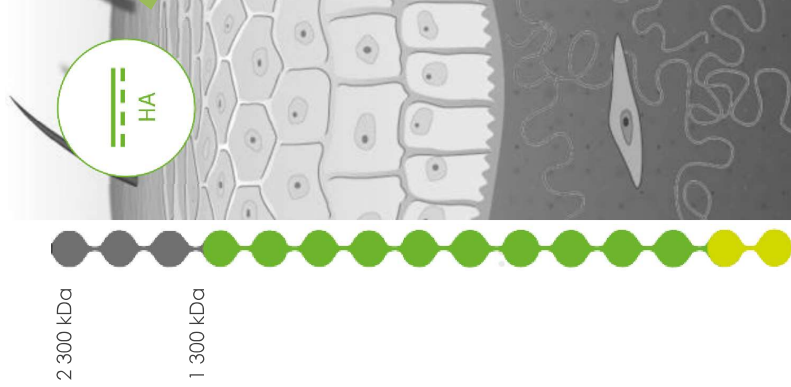
## HYDRATION, FILM-FORMING EFFECT

- Immediate hydration
- Protective superficial film
- Skin microbiome support
- Viscosity

- Active stimulation of skin cells
- Active improvement of skin barrier
- Improvement of skin atrophy
- Wrinkle reduction, elasticity improvement
- Skin nourishment

## SKIN PENETRATION, BIOLOGICAL ACTIVITY

# Hyaluronic acid



## Key properties

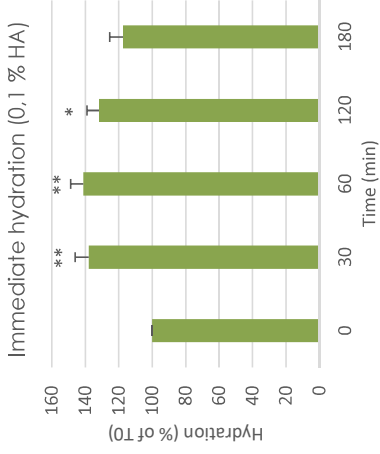
- > 1300 kDa
- Immediate hydration
- Superficial film

=> Protection against pollutants, allergens

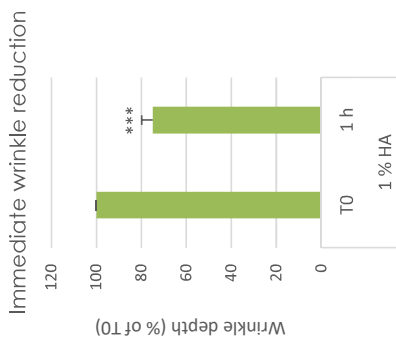
=> Immediate tightening, wrinkle reduction

=> Skin microbiome support

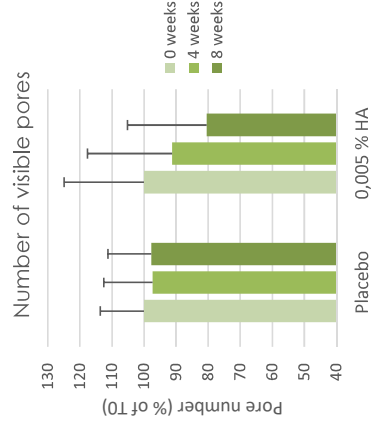
- Reduction of skin pores and sebum



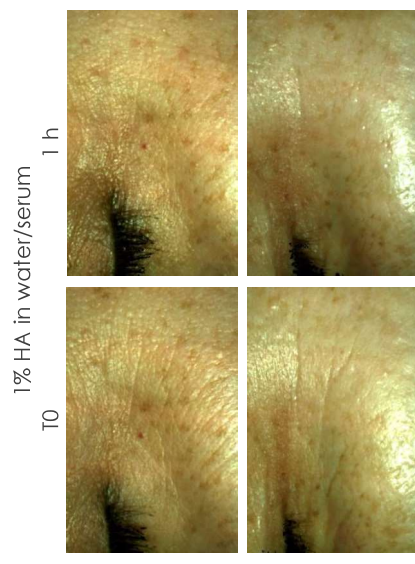
- 6 subjects
- 0,1% HA in emulsion
- Volar forearm
- Corneometer



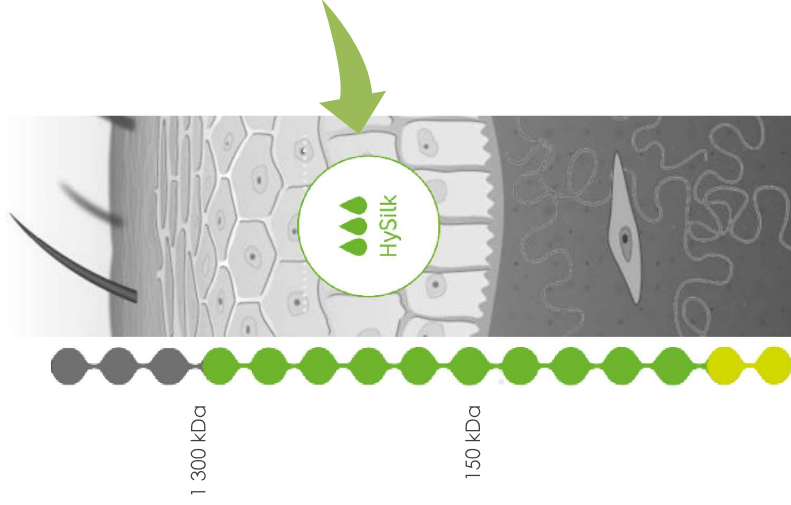
- 11 subjects
- 1% HA in water/serum
- Crow's feet wrinkles
- Primos 3D camera



- 8 subjects
- 0,005 % HA in emulsion
- Visioface (pores)

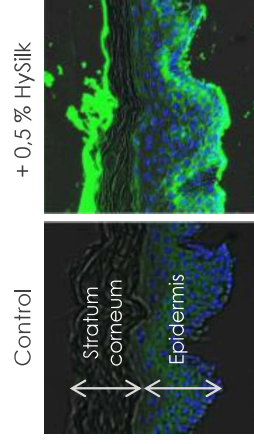


# HySilk

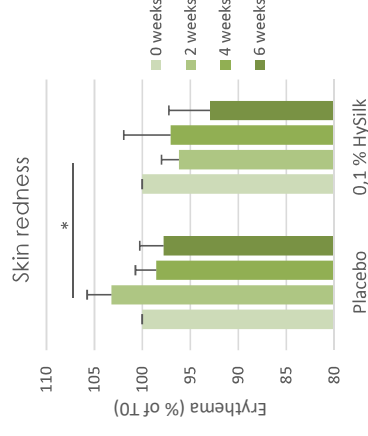
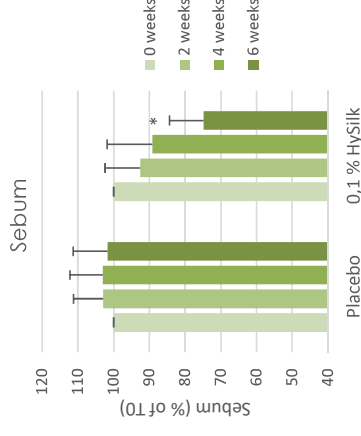


## Key properties

- 150-1300 kDa
- Penetration to epidermis
- Active stimulation of keratinocytes:
  - ↑ Skin barrier components
  - ↑ Natural antioxidant enzymes
- Active improvement of hydration and skin barrier
- Reduction of sebum
- Skin smoothing (decreased roughness)
- Skin calming (decreased redness)

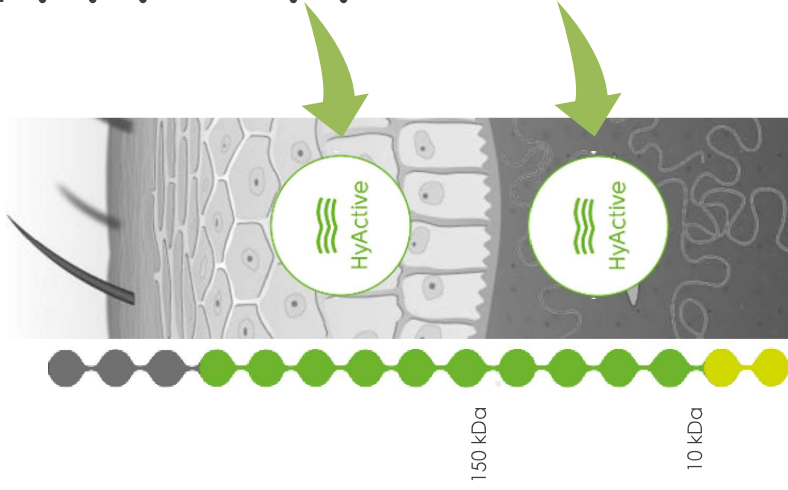


- Skin explants (epidermis only)
- 0,5 % HySilk in buffer
- 24 h, 37 °C
- Immunofluorescence staining (detection of HA-binding protein)



- 6 subjects
- 0,1 % HySilk in emulsion
- Sebumeter, Mexameter (erythema index)

# HyActive



## Key properties

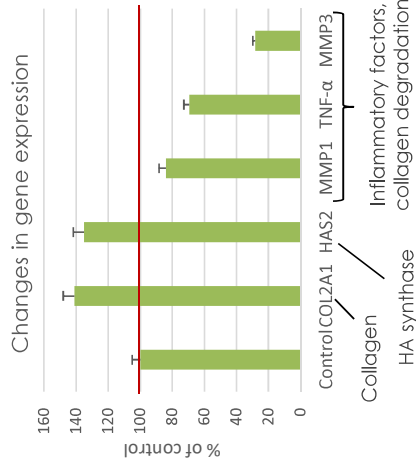
- 10-150 kDa
- Penetration to dermis
- Wrinkle reduction, elasticity improvement
  - ↑ Collagen
  - ↑ Production of body's own HA
- Epidermal atrophy improvement
- Anti-inflammatory effect



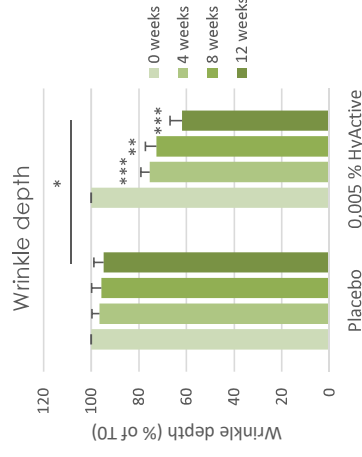
- Skin explants
- 1 % HyActive fluorescently labelled (red)
- 20 h, 37 °C
- Fluorescence microscope



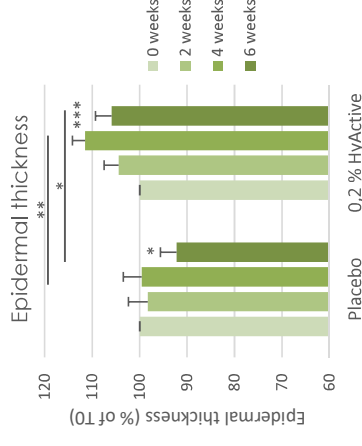
- HaCaT cells
- 0.05 % HyActive, 48 h
- Microarray analysis



0,2 % HyActive  
6 weeks

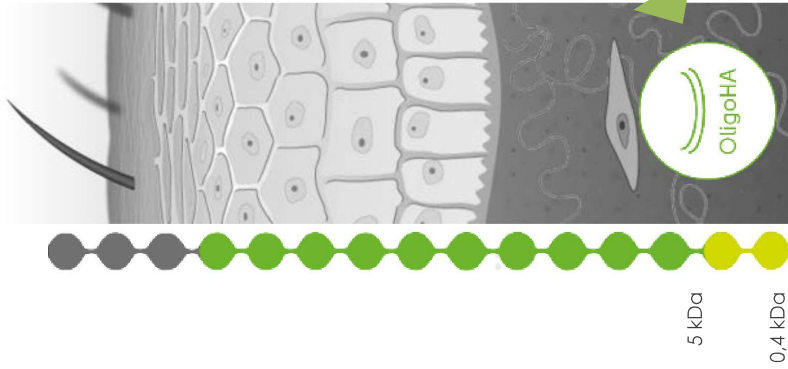


- 8 subjects
- 0.005 % HyActive in emulsion
- 3D LifeViz (crow's feet wrinkles)



- 28 subjects
- 0.2 % HyActive in emulsion
- Vivascope

# OligoHyaferre



## Key properties

- <5 kDa
- Penetration to dermis
- Wrinkle reduction, elasticity improvement
  - ↑ Collagen
  - ↑ Production of body's own HA
- Support of skin regeneration, wound healing
- Angiogenesis (formation of new blood vessels) for skin nourishment
- Skin calming (decreased redness)

The Journal of Molecular Biology, © 2002 by The American Society for Biochemistry and Molecular Biology, Inc. Vol. 321, No. 43, Issue of October 18, pp. 4136-4149, 2002. Printed in U.S.A.

### Angiogenic Oligosaccharides of Hyaluronan Induce Multiple Signaling Pathways Affecting Vascular Endothelial Cell Mitogenic and Wound Healing Responses\*

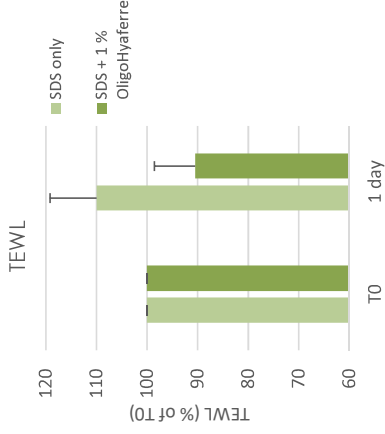
Published: JBC Papers in Press, August 22, 2002; DOI: 10.1074/jbc.M109442002

Mark SleVIN<sup>1</sup>, Shant Kumar<sup>2</sup>, and John Gaffney<sup>2</sup>

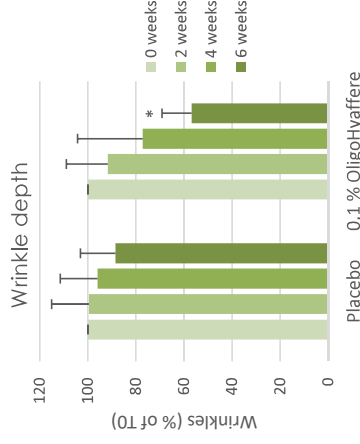
From the <sup>1</sup>Department of Biological Sciences, Manchester Metropolitan University, Manchester M1 5GD, United Kingdom and the <sup>2</sup>Department of Biological Sciences, Stouffville Building, Manchester Victoria University, Manchester M1 5GD, United Kingdom



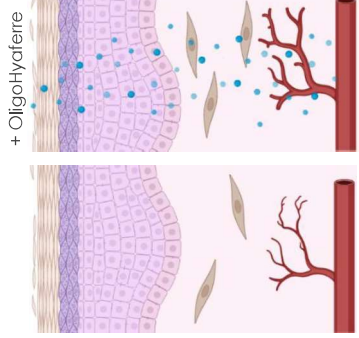
## CONTIPRO



- 3 subjects
- Volar forearm
- Skin damage: 0,75 % SDS in occlusion (patch) 16 h
- Wash
- + 1% OligoHyaferre in water
- 3x daily
- Tewameter



- 6 subjects
- 0,1 % OligoHyaferre in emulsion
- 3D LifeViz (crow's feet wrinkles)



# „Anti-pollution,, effect of Contipro ingredients



## Protection against air pollution-induced skin damage

- Current cosmetic trend
  - Origin in Asia => worldwide
- Reasons:
- Air pollution induces skin damage and premature skin ageing
  - Number of people living in polluted areas is increasing
  - Increased awareness of negative effect of air pollution in the skin



 <p><b>REN Evercalm™ Global Protection Day Cream-</b> An antiaging moisturizer for skin that is delicate or affected by everyday stressors like smoke and pollution</p>	<p><b>AnoRePacific Future Response Age Defense Crème-</b> A soothing cream that protects the skin against environmental aggressors and guards against the appearance of aging.</p>	 <p><b>Tata Harper Purifying Cleanser-</b> A hydrating cleanser that combats the daily wear and tear of environmental exposure and pollution by effectively clearing pores of excess oil, dead skin, and buildup.</p>
 <p><b>Shiseido White Lucent Brightening Protective Cream Broad Spectrum SPF 18-</b> Achieve comprehensive skin protection against UV rays, air pollution, and dryness.</p>	 <p><b>Clarins Extra-Comfort Anti-Pollution Cleansing Cream-</b> The anti-pollution cream cleanser for skin challenged by a polluted urban environment.</p>	 <p><b>Etude House Dust Cut Facial Mist-</b> A facial mist that protects skin from environmental pollutants by skin shield formed with fitting polymer</p>

Non-smoker      Smoker

**Google trends analysis:**  
 Number of searches for keyword „Anti-pollution“  
 Category Face&Body Care (world)





# „Anti-pollution,, effect of Contipro ingredients



## Formation of superficial protective film

- \*\*\*\* HA
- \*\*\* HySilk
- \*\*\* CrossLinked<sup>HA</sup>
- \*\* HyActive
- \*\* OligoHyaferre

## Active improvement of the skin barrier

- \*\*\* HySilk
- \*\* HyActive

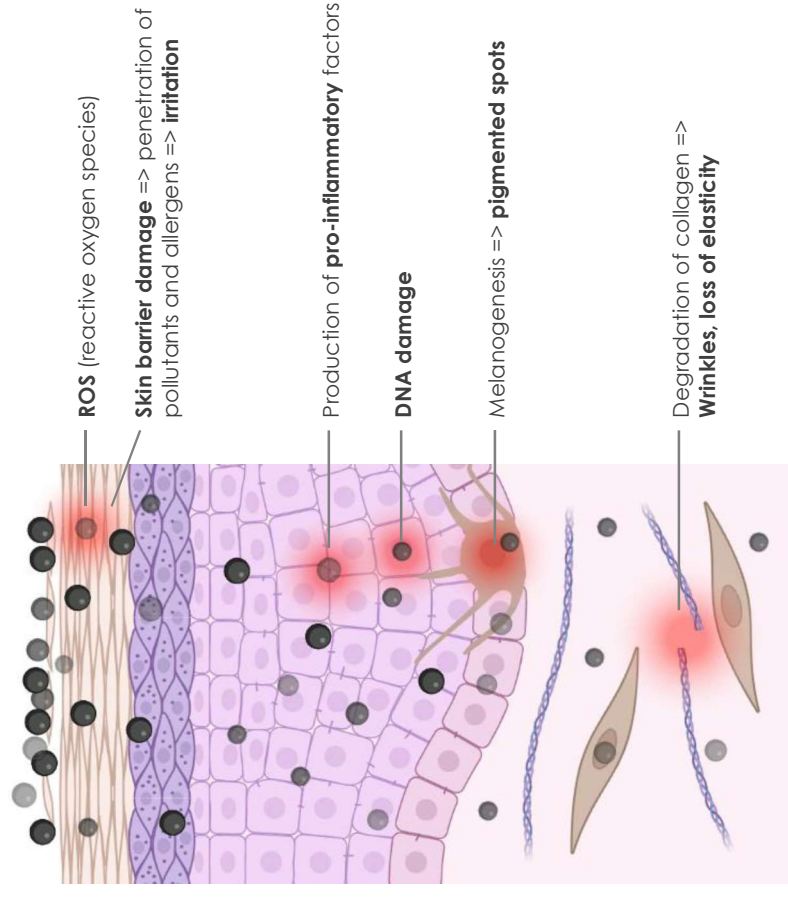
## Anti-inflammatory effect

- \*\*\* Schizophyllan
- \*\* Carboxymethylglucan
- \*\* TanActine

## Other mechanisms

- \*\* Recelline (proteasome activator)

## Air pollution-induced skin damage



# Anti-pollution, effect of Contipro ingredients



**Anti-pollution effect confirmed by all tested ingredients:**

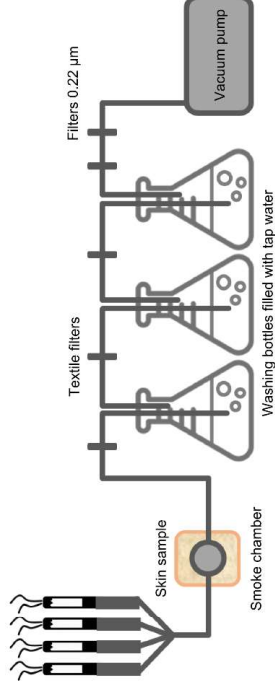
HA (standard)

>

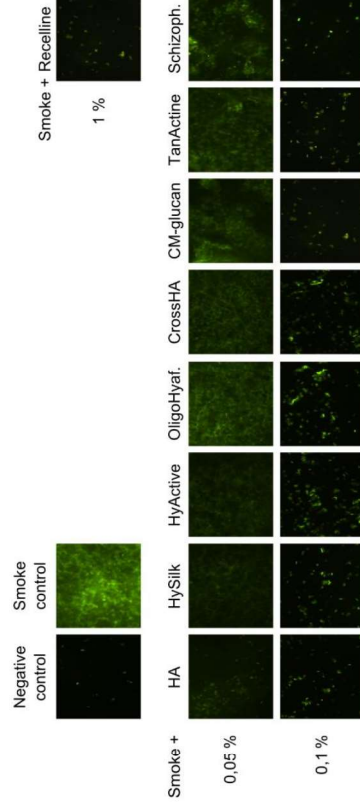
HySilk

>

HyActive, CrossLinked<sup>HA</sup>,  
OligoHyaferré, Schizophyllan,  
Carboxymethylglucan,  
TanActine, Recelline

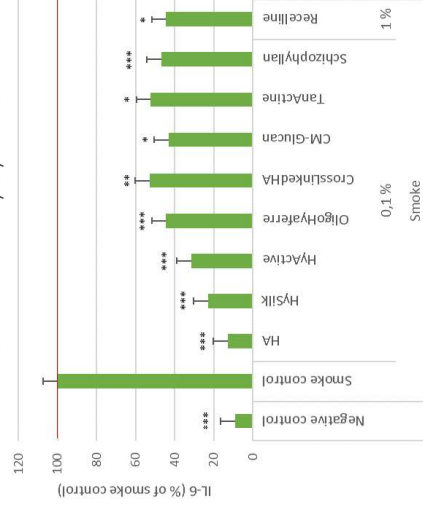


Production of reactive oxygen species (ROS)  
(View on the skin from above (in microscope), ROS green)



- Skin explants
- + Ingredients in a buffer
- 24 h, 37 °C => washing, drying
- Smoke application
- Analyses:
  - ROS (fluorescence microscopy, DCFH-DA)
  - Lipid peroxidation (TBARS from strips)
  - Gene expression of pro-inflammatory cytokine IL6 (qRT-PCR)

Pro-inflammatory cytokine IL6



# Skin microbiome support by Contipro ingredients

## Skin microbiome

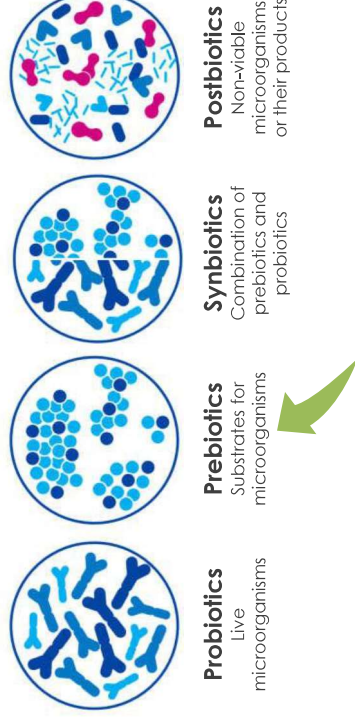
- All microorganisms living on/in the skin
- Current cosmetic trend
- Important for the skin properties, function, health, appearance

## Google trends analysis:

Number of searches for keyword "Microbiome"  
Category Face&Body Care (world)



Ingredients used in cosmetics for the skin microbiome support



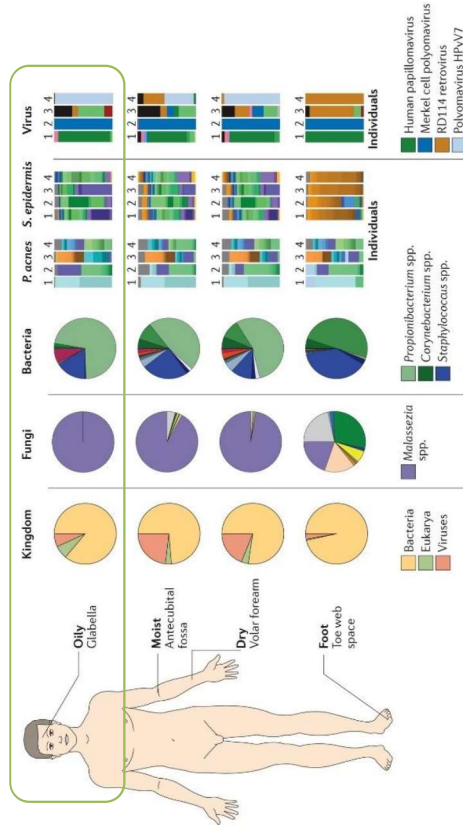
## Contipro cosmetic polysaccharides?



# Skin microbiome support by Contipro ingredients

## Skin microbiome composition

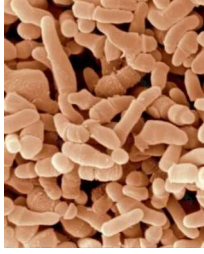
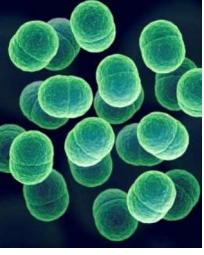
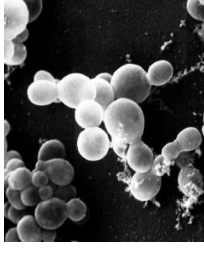

**Bacteria** >>>> viruses > fungi, parasites



### Main function of the skin microbiome:

- Protects against pathogens
- Educates immune system, interacts with it
- Products of microorganisms:
  - => Optimal, slightly acidic pH
  - => Hydration
  - => Skin barrier

Nature Reviews | Microbiology

<b>Bacteria</b> Cutibacterium acnes		<b>Bacteria</b> Staphylococcus epidermidis		<b>Fungi</b> Malassezia		<b>Parasites</b> Demodex folliculorum	
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# Skin microbiome support by Contipro ingredients



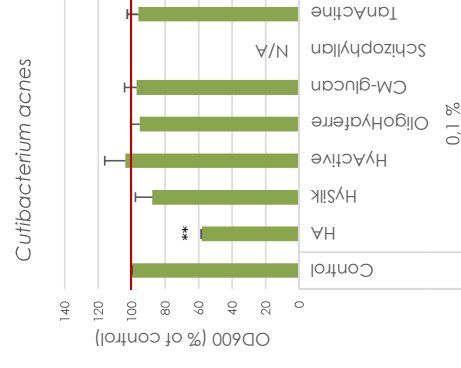
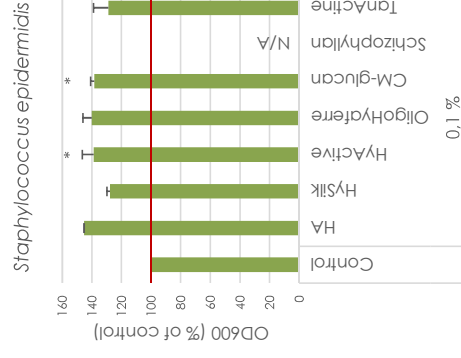
## Contipro polysaccharides tested as the skin prebiotics:

- HA:
  - Hyaluronic acid
  - HySilk
  - HyActive
  - OligoHyaferre
  - Carboxymethylglucan
  - Schizophyllan
  - TanActine
- } Known gut prebiotics

## In vitro cell cultures



- *S. epidermidis*, *C. acnes*
- Cultivation with 0,1 % polysaccharides
- Incubation 4 h (*S. epidermidis*), 40 h (*C. acnes*)
- Optical density measurement



## *S. epidermidis*:

- Growth support

## *C. acnes*:

- No effect
- HA (standard): inhibition

# Skin microbiome support by Contipro ingredients



## Skin cleansing

- All bacteria => significant reduction
- Remaining bacteria:
  - *S. epidermidis* => relative decrease
  - *C. acnes* => relative increase

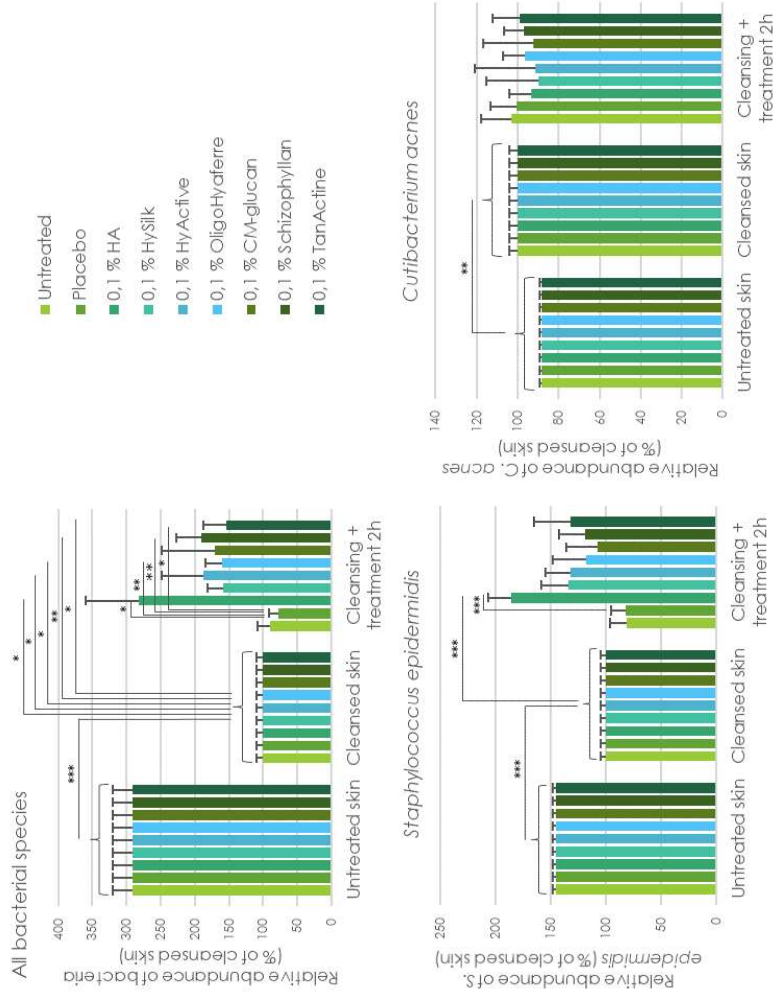
=> Skin is free for colonization by pathogens = risk  
=> Microbial dysbiosis, risk for acne-prone skin

## Effects of polysaccharides

- All bacteria => Faster recovery by all polysaccharides
- Faster normalization of bacterial ratio:
  - *S. epidermidis* => increase
  - *C. acnes* => slight decrease

**Hyaluronic acid** (standard) the best

(Due to its high MW? Stays on the skin surface => more available for microbial consumption)



## In vivo study

- 11 subjects
- 1/2 forehead wiped 5x with cellulose soaked with 1 mL 10 % detergent
- After 15 min: application of 0,5 g of emulsions with 0,1 % polysaccharides for 2 h
- Overall number of bacteria, *S. epidermidis*, *C. acnes*: qPCR

# HA of different MW



## SUMMARY

### Hyaluronic acid

**INCI:** Sodium Hyaluronate

**MW:** 1300-2300 kDa

**Viscosity of solutions:** Highest

**Main place of action:** Skin surface, stratum corneum

- Main effects**
- Immediate hydration
  - Protective superficial film
  - Immediate wrinkle reduction
  - Anti-pollution effect
  - Skin microbiome support

### HySilk

Sodium Hyaluronate

150-1300 kDa

High

Epidermis

- Sebum reduction
- Active improvement of hydration
- Active improvement of skin barrier
- Anti-pollution effect

### HyActive

Sodium Hyaluronate

10-150 kDa

Low

Dermis

- Wrinkle reduction
- Elasticity improvement
- Improvement of epidermal atrophy
- Skin calming, anti-inflammatory effect
- Collagen increase
- Production of body's own HA

### OligoHyaferre

Hydrolyzed Sodium Hyaluronate

<5 kDa

Low

Dermis

- Wrinkle reduction
- Elasticity improvement
- Support of skin regeneration, wound healing
- Angiogenesis, skin nourishment
- Skin calming
- Collagen increase
- Production of body's own HA

## Other effects

Skin pore and sebum reduction, long-term hydration and skin barrier improvement

Decreased skin roughness, skin calming, anti-inflammatory effect, stimulation of natural antioxidant enzymes, wrinkle reduction, elasticity improvement, skin microbiome support

Skin pore reduction, active improvement of hydration and skin barrier function, support of desquamation => decreased roughness, anti-pollution effect, skin microbiome support

Pore and sebum reduction, active improvement of hydration and skin barrier function, anti-pollution effect, skin microbiome support

# HA of different MW – technical information



Hyaluronic acid, HySilk, HyActive, OligoHyaferre

## INCI:

- HA, HySilk, HyActive: Sodium Hyaluronate
- OligoHyaferre: Hydrolyzed Sodium Hyaluronate

**Minimal ordering quantity:** 1 kg

**Samples:** 1 g

**Recommended concentration:** 0.01 – 0.1 %

(up to 2 % for which we have safety data)

**Expiration:** 2 years (OligoHyaferre 3 years)

## Compatibility and processing:

- Incompatible with cationic substances (e.g. surfactants or polymers, Polyquaternium-4, Polyquaternium-10, etc.)
- Prolonged heating and low pH leads to decrease in MW and viscosity (mainly in the case of high MW/standard HA). E.g. 45 min at 90°C => 20 % reduction in MW.

## Solubility

- Fully-soluble in water
- **Always add HA to water little by little while stirring!**
- Higher MW: more viscose solutions, slower dissolution (it can take even hours)
  - HA: commonly 1 % (up to 2 % - turax, short heating)
  - HySilk: commonly 2 % (up to 5 % - turax, short heating)
  - HyActive: commonly 5 % (up to 30 %)
  - OligoHyaferre: commonly 10 % (up to 30 %)
- Soluble in water solutions of alcohols (max. 50 % ethanol, isopropanol etc.) and glycols (max. 50 % propylene glycol, butylene glycol)

- Insoluble in solvents non-miscible with water

**Video of HA dissolution + common mistakes (youtube):**

<https://www.youtube.com/watch?v=iWthYx-qvYY>

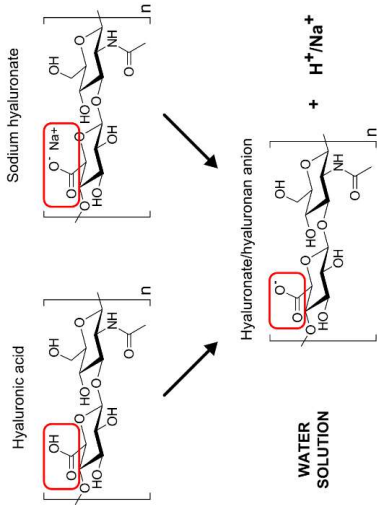


# HA – myths and facts

## 1. HYALURONIC ACID (HA) VS SODIUM HYALURONATE (SH)

INCI „Hyaluronic Acid“ and „Sodium Hyaluronate“ are synonyms in cosmetics

(INCI is not a chemical nomenclature)



Claims found on the internet	True/false	Comment
SH is a salt derived from HA	True	
Only HA can be found in nature including human body whereas SH is not present in nature.	False	Oppositely. You will never find hyaluronic acid in the acid form in nature. It is always present in a form of salt. A salt consists of anion hyaluronan and a cation (most commonly sodium). Hyaluronic acid itself also exists, but only in chemical laboratories because it undergoes fast degradation, and its solutions are very acidic. Our organic chemists sometimes use hyaluronic acid form, but they prepare it by themselves from its salts using ion exchangers which exchange sodium for hydrogen cations. Both HA and SH are soluble in water where they dissociate to the same anion hyaluronan.
SH has a smaller molecular weight (MW) than HA	False	Both molecules can have different MWs.
HA cannot be absorbed when applied topically, it can only be injected, whereas SH penetrates skin.	False	Absorption depends more on the MW of HA. (btw injected HA is usually crosslinked HA)
SH is chemically synthesized from HA.	False	SH is not synthesized chemically, it is always extracted from organisms, usually from microorganisms, or sometimes (rather historically) from rooster combs or eye vitreous. In these organisms, there is only the SH form, therefore, the extracted HA is always in the form of SH (with both possible INCI: „SH“ or „HA“ based on the company decision).

# HA – myths and facts

## Low MW HA/HA oligosaccharides/HA fragments cause inflammation or skin irritation

**NO!!! HA of all MW is completely safe!**

- When human body is in homeostasis, it contains mostly high MW HA. If the skin is injured, HA is cleaved to low MW HA/HA fragments, even oligosaccharides which serve as signaling molecules promoting skin regeneration and wound healing. Because some of these processes are also involved in skin inflammation and irritation, some people misinterpret it and conclude that these HA fragments induce inflammation or irritation when applied on the skin. NO!
- Oppositely, the stimulation of these processes is beneficial for the skin regeneration and healing after skin disruption.
- Also, there is a long history of using low MW HA with no observed negative side effects. HA regardless its MW is considered as safe (Becker et al. 2009)
- We even observed skin calming effect after OligoHyaferre or HyActive long-term treatment.
- Contamination of HA by endotoxins has been shown to be responsible for the pro-inflammatory effects observed in some cases.



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http://ijl.sagepub.com  
hosted at  
http://online.sagepub.com

## Final Report of the Safety Assessment of Hyaluronic Acid, Potassium Hyaluronate, and Sodium Hyaluronate

Lillian C. Becker, MS, Wilma F. Bergfeld, MD, Donald V. Beliso, MD,  
Curtis D. Klaassen, PhD, James G. Marks Jr, MD, Ronald C. Shank, PhD,  
Thomas J. Slaga, PhD, Paul W. Snyder, DVM, PhD, Cosmetic Ingredient  
Review Expert Panel, and F. Alan Andersen, PhD

Hyaluronic acid, sodium hyaluronate, and potassium hyaluronate function in cosmetics as skin conditioning agents at concentrations up to 2%. Hyaluronic acid, primarily obtained from bacterial fermentation and rooster combs, does penetrate to the dermis. Hyaluronic acid was not toxic in a wide range of acute animal toxicity studies, over several species and with different exposure routes. Hyaluronic acid was not immunogenic, nor was it a sensitizer in animal studies. Hyaluronic acid was not a reproductive or developmental toxicant. Hyaluronic

acid was not genotoxic. Hyaluronic acid likely does not play a causal role in cancer metastasis; rather, increased expression of hyaluronic acid genes may be a consequence of metastatic growth. Widespread clinical use of hyaluronic acid, primarily by injection, has been free of significant adverse reactions. Hyaluronic acid and its sodium and potassium salts are considered safe for use in cosmetics as described in the safety assessment.

Keywords: cosmetics; hyaluronic acid; safety

# SCIENTIFIC REPORTS

OPEN

Endotoxin free hyaluronan and hyaluronan fragments do not stimulate TNF- $\alpha$ , interleukin-12 or upregulate co-stimulatory molecules in dendritic cells or macrophages

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Accepted: 24 October 2016  
Published: 21 November 2016

Yifei Dong<sup>1</sup>, Anif Arafat<sup>1</sup>, Mia Olsson<sup>1,2</sup>, Valbona Cali<sup>1</sup>, Blair Hardman<sup>1</sup>, Manisha Dosanjh<sup>1,3</sup>, Mark Lauer<sup>4,5,6</sup>, Ronald J. Midura<sup>1</sup>, Vincent C. Hascall<sup>1</sup>, Kelly L. Brown<sup>1</sup> & Pauline Johnston<sup>1</sup>

# HA – myths and facts

## Plant-based, botanical HA?

**Unfortunately not**

- HA is not produced by plants in nature
- Rather marketing term
- Usually completely different compounds (extracts, other polysaccharides etc.) with ability to stimulate production of HA in our body
- Or they call it botanical because HA is produced by microbial „fermentation“ (probably sounds botanical, but it is incorrect)

