



Oligo-HA

*Hyaluronic acid for better penetration, epidermis
regeneration & keratynocyte stemness maintenance*



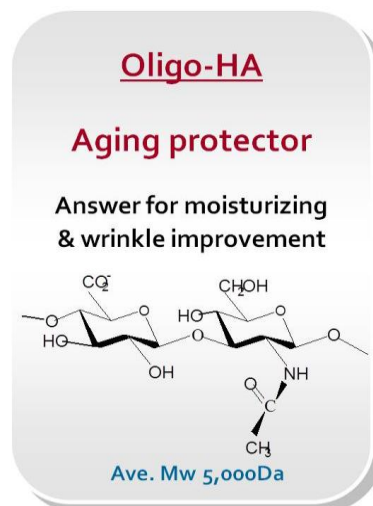
Oligo-HA

Hyaluronic acid for better penetration, epidermis regeneration & keratinocyte stemness maintenance

GENERAL DESCRIPTION

Hyaluronic acid exists naturally in all living organisms and is a universal component of the spaces between the cells of body tissues (extracellular space). It is a polysaccharide that has an identical chemical structure whether it is found in simple bacteria or in human beings. HA can be found in many places in the human body, including:

- skin, where it creates volume (without HA, the skin would appear dry, withered and wrinkled)
- the vitreous body, where it gives volume and shape to the eyes
- cartilage, bone and synovial fluid, where it acts as a lubricant, shock absorber and filter, amongst other things
- blood vessels
- the umbilical cord, where its function is to ensure that the contact between the mother and the fetus is never broken



Hyaluronic acid plays a vital role in connective tissues such as the skin. The dermis of the skin comprises a network of collagen fibers within an interstitial substance composed largely of hyaluronic acid. The elastic properties of hyaluronic acid offer resistance to compression, so that the skin protects underlying structures against damage. At the same time, the non-

Newtonian properties of hyaluronic acid allow the collagen fibers to move easily through the interstitial substance. This lubrication by hyaluronic acid allows the skin to accommodate the changes in shape and volume that occur when the underlying bones and joints move.

The hyaluronic acid gel in the skin also inhibits movement of foreign particles, such as bacteria. This makes an important contribution to the skin's defensive function as a barrier to infection. The barrier also affects the free passage of other exogenous material, such as some drugs. This is the reason why some subcutaneous injections and drugs in creams or ointments include a small amount of the enzyme hyaluronidase. The enzyme degrades the hyaluronic acid gel around it, so that the drug is able to pass more freely through the tissues of the skin.

When we get older, the amount of HA in the skin diminishes as the skin cells lose their ability to produce hyaluronic acid. In addition, the molecular weight of the HA is decreased with age, so that it does not hold water as well as before. This can be demonstrated by squeezing the skin between the fingers. In young people, the skin rapidly restores itself to its original volume. However, as we get older, the skin's ability to restore itself is reduced.

PRODUCT INFORMATION

Oligo-HA is a hyaluronic acid with **molecular weight** around 5,000da developed by Bioland's high bio-fermentation technology for cosmeceutical application with its excellent anti-aging effect.

- INCI Nam: *Hyaluronic acid*
- Efficacies: Anti-aging

COSMECEUTICAL PROPERTIES AND APPLICATIONS

- It has excellent skin permeability, cell proliferation, epidermis regeneration → **Anti-aging**

With its properties, **Oligo-HA** can be used in all kinds of formulation for skin care like eye control cream, serum, skin toner and lotion in general.

TECHNICAL DATA

● Product specification

Description	White ~ Light yellow powder
Appearance of HA 1% Solution	Colorless clear liquid
Heavy Metals	Not more than 20ppm
Arsenic	Not more than 2ppm
Water Content (KF)	Not more than 10.0%
- Uronic Acid Content	45 ~ 51.3%
- Hyaluronic Acid Content	88 ~ 100%
Other acidic	Not Detected
Mucopolysaccharides	
Molecular Weight (Calculated by GPC chromatography)	3.0 ~ 10.1Kda
Total Plate Count	Not more than 1×10^2 cfu/g
Hemolytic Streptococci	Not Detected

● Processing & Dosage

The recommended dosage of **Oligo-HA** is 0.01 ~ 0.5% to obtain a significant anti-aging effect. Oligo-HA is a water soluble ingredient.

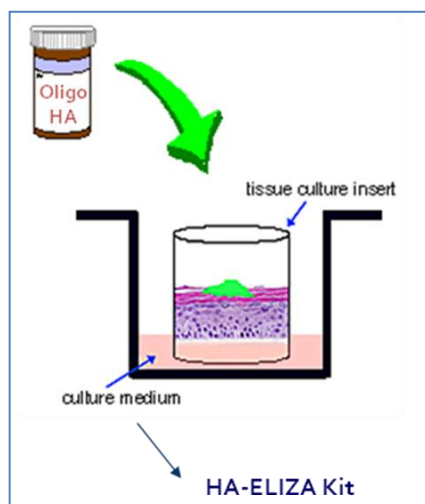
● Storage & Shelf life

Oligo-HA should be stored at room temperature in a tightly closed and lightproof packing. If stored in these conditions, the shelf life is at least 24 months. Once the original packing is open, it is highly recommended to use at once.

EFFICACY DATA

● Skin permeability - In-vivo

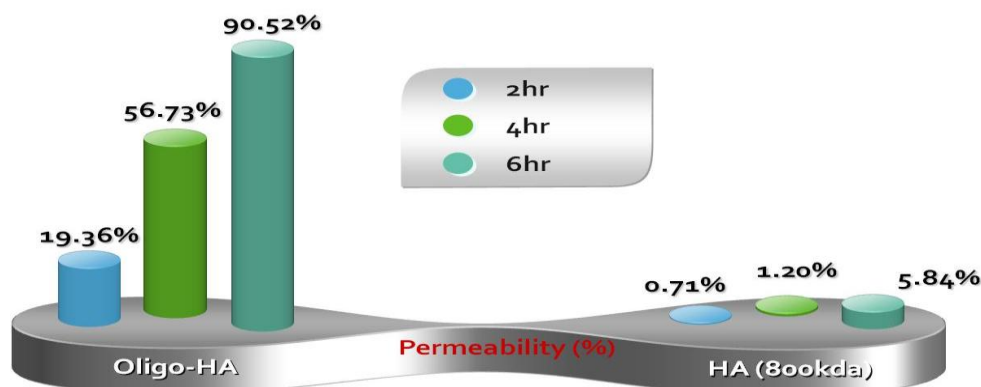
Test Method (I)



Dermal equivalents were made using Type I collagen, 5 X IMDM, raft buffer (2.2% Na₂HCO₃, 200 mM HEPES, 0.05 N NaOH) and 1 X 10⁴ human fibroblasts/ml. The ratio of Type I collagen, 5 X DMEM and raft buffer was 7 : 2 : 1. The cells and collagen mixtures were poured into insert and gelled at 37°C for 1 h. Then 1 ml of ascorbic acid-containing IMDM with 10% FBS was added and changed medium on every 2 days. On 7th day, the normal keratinocytes were seeded on dermis at a density of 2 X 10⁵ cells/ml and incubated for 7 days with supplemented ascorbic acid-containing IMDM with 10% FBS and K-SFM (1 : 1). Then, the medium of insert was discarded for induction of keratinization and cultured for 5 days. Samples were treated on epidermis and medium collected at the end of the 2-h period. The concentration of test compound passed through skin equivalent were analyzed by HA-ELIZA Kit (Tecan).

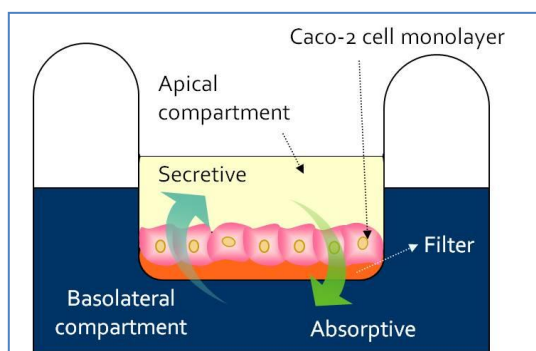
Permeability of Oligo-HA (8,000 Da) was measured in comparison with Sodium Hyaluronate (800,000 Da)

Results



Oligo-HA showed much higher skin permeability compared with sodium hyaluronate (800,000 Da).

Test Method (II)



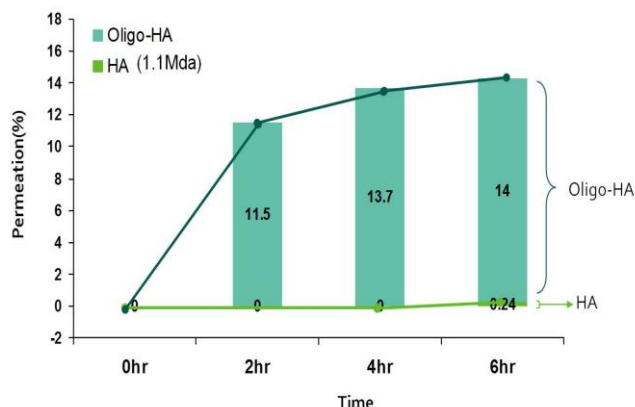
Caco-2 cells (passages 26) were seeded onto Transwell™ cell culture inserts (12 mm diameter, 0.4 mm pore size, 24 well) at a density of 1x10⁵ cells/insert and cultured in MEM supplemented with 10% FBS, 1% NEAA (non-essential amino acid), 100 µg/ml penicillin/streptomycin and 7.5% NaHCO₃. Cells were incubated at 37°C in 5% CO₂/95% air in a CO₂ incubator, with medium exchange on every day. On days 9, the integrity of the cell mono layers was confirmed by TEER measurements (Trans Epithelial Electric Resistance, Millicell-ERS, Millipore). Confluent cell mono layers in culture medium with TEER values greater than

300 Ωcm², after correction for resistance in control blank wells, were used in the transport experiments.

The medium was replaced with HBSS-HEPES transport medium (HBSS with 10mM HEPES and adjusted to pH 7.4 with 5N NaOH) and 2.5 mg/ml of Oligo-HA (M.W. 5,000) and HA (M.W. 1,100,000) were treated, respectively. After the incubation of 2, 4, and 6 hr, upper and down media of inserts was taken and transferred into the receiver chamber and stored at 4°C until analysis.

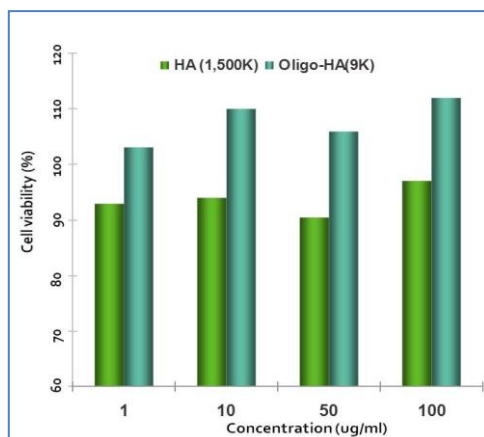
The amount of Oligo-HA and HA of upper and lower part of the inserts was measured by GPC(Gel Permeation Chromatography). % Penetration was calculated by the comparison of the HA amount of upper and lower part of inserts.

Results (II)

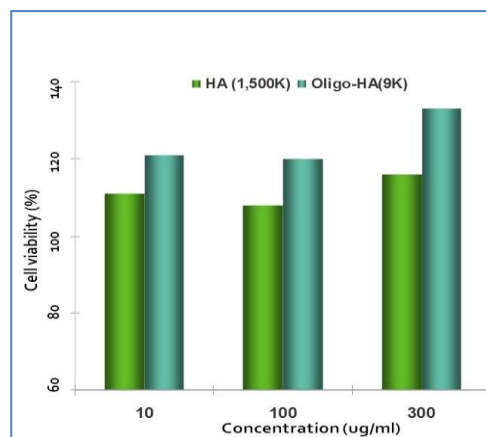


Oligo-HA shows much higher skin permeability compared with sodium hyaluronate (1.1 Mda).

● Cell proliferation



<Fibroblast proliferation>

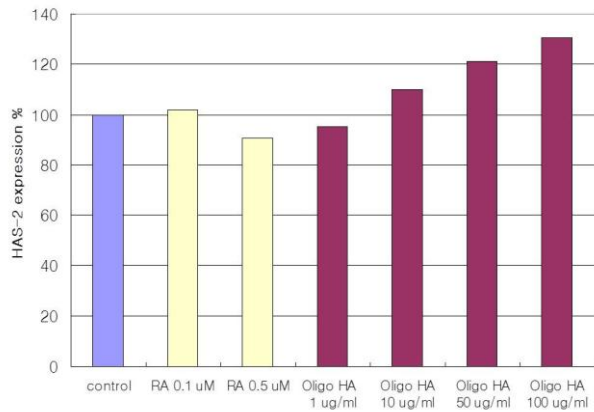


<Keratinocyte proliferation>

Oligo-HA (9,000Da) shows much higher cell proliferation activity in fibroblast and keratinocyte dose dependently compared with sodium hyaluronate (1.5Mda).

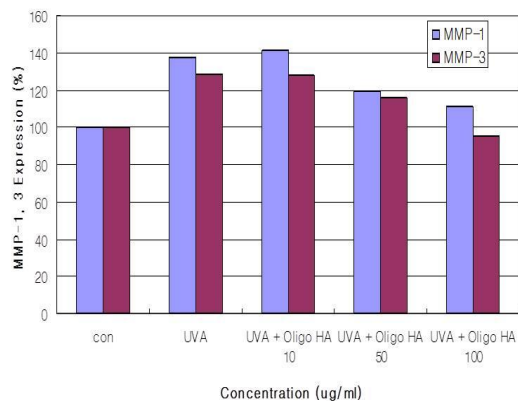
● Anti-aging effect - In-vitro

<HAS-2 Stimulation in fibroblasts>



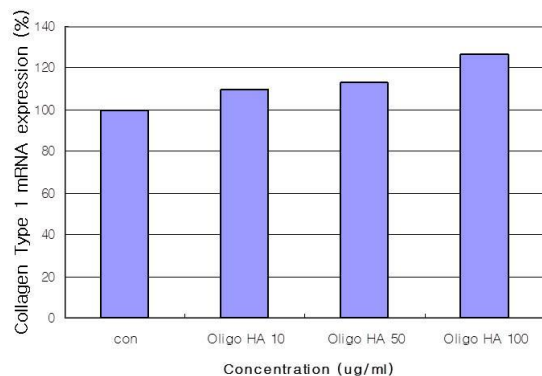
After Oligo-HA penetrated into dermis, it promotes production of hyaluronic acid biosynthesis in the dermis.

<Collagenase expression inhibition>



After Oligo-HA penetrated into dermis, it inhibits collagenase expression .

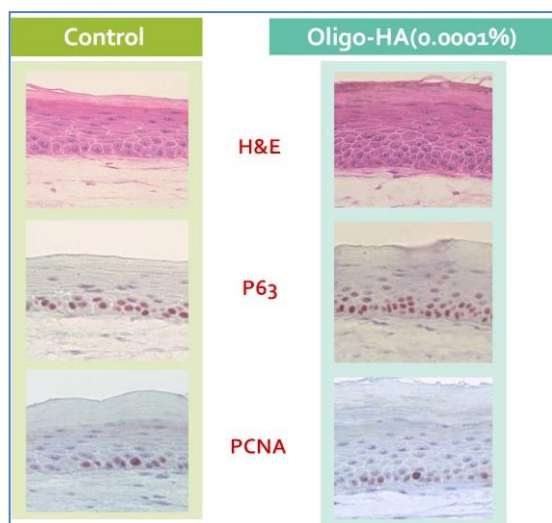
<Collagen Type I expression promotion>



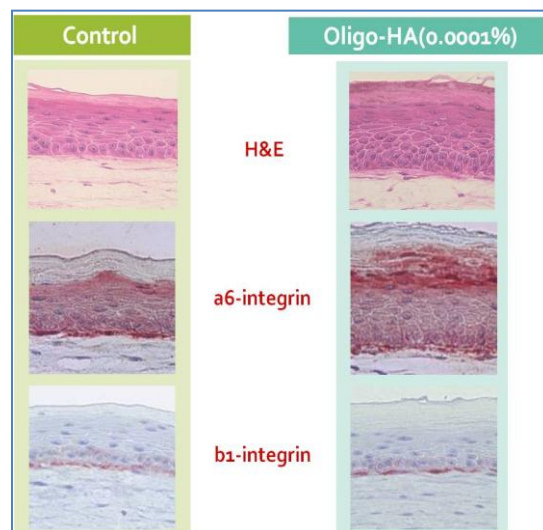
After Oligo-HA penetrated into dermis, it promotes collagen type I expression.

● Epidermis regeneration & Stemness maintenance

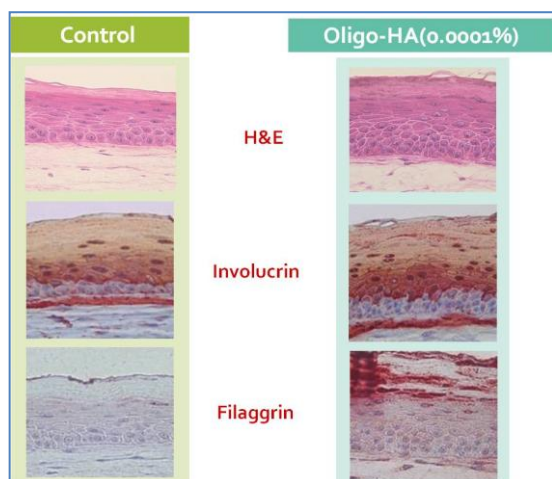
Result-1



Result-2



Result-3



- Oligo-HA increases the number of p63 positive cells and increased regulation of integrin a6 and b2.

- Oligo-HA increases the epidermal thickness and promotes the differentiation of epidermis.

● Anti-wrinkle activity – Clinical trial

Test I Method

Tested by	Ellead Skin Research Center, Korea
Test materials	Oligo-HA (5,000Da) 0.2%
Volunteer	22 females (aged between 30 and 55, average 45.4)
Treatment	All subjects were divided into 2 groups I, II randomly and instructed to apply the Oligo-HA contained lotion and placebo twice a day in the designated crow's feet area, respectively.
Evaluation	To assess the anti-wrinkle effect, the dermatologists visually assessed crow's feet wrinkles, took photos using the face fixing device, lighting sets used for wrinkle photography, and high-definition digital camera (Olympus E-1, JAPAN) and photographic system, image capturing and image analysis via PRIMOS(Phaseshift Rapid In vivo Measurement of Skin, GFMesstechnik GmbH, Germany).

Result-1



Oligo-HA 0.2% shows significant wrinkle improvement.

Result-2-1



Result-2-2

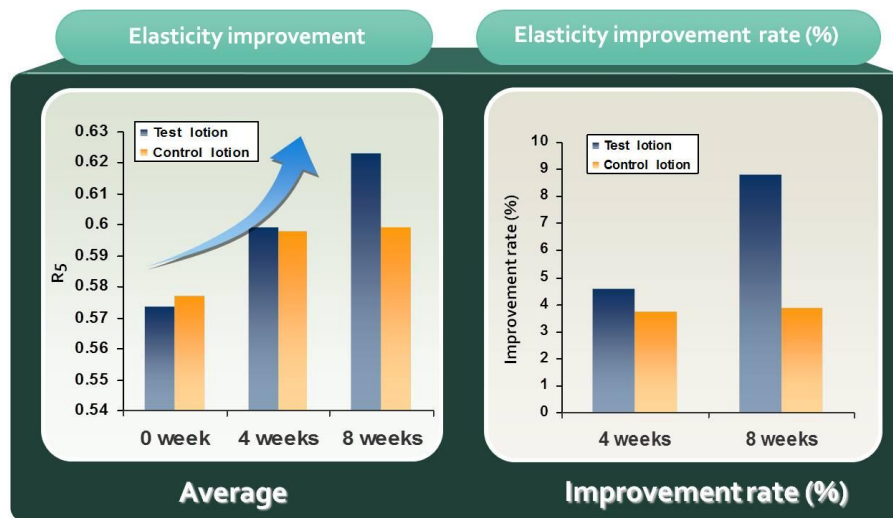


Average depth of wrinkle was significantly improved (22%) after treating lotion containing Oligo-HA 0.2% for 8 weeks.

● Skin elasticity improvement activity – Clinical trial

Tested by	Ellead Skin Research Center, Korea
Test materials	Oligo-HA (5,000Da) 0.2%
Volunteer	22 females (aged between 30 and 55, average 45.4)
Treatment	All subjects were divided into 2 groups I, II randomly and instructed to apply the Oligo-HA contained lotion and placebo twice a day in the designated crow's feet area, respectively.
Evaluation	The degree of elasticity of the eyelid was measured using the cutometer SEM 575(Courage and Khazaka, Koln, Germany). A probe with a diameter of 2mm was mounted on this equipment for the test. Mode 1 or time-strain mode was used as the measurement condition. On-time 2.0s and Off-time 2.0s were then applied to the specific negative pressure of the Mode 1 condition(450mbar), and measurements were taken three consecutive time.

Result-1



Oligo-HA 0.2% shows significant skin elasticity improvement.