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Skin Care Effect of Hyaluronic acid as Beauty Foods

R&D Center, Bioland

Abstract



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Hyaluronic acid(HA) is a naturally occurring mucopolysaccharide composed of the repeating disaccharide unit of D-glucuronic acid and N-acetyl-D-glucosamine. HA exists as a high molecular weight polymer in the extracellular matrix of many tissues in the body. HA has been widely used not only for osteoarthritis and ophthalmology but also for cosmetics for skin care. Recently HA has drawn much attention for use in health foods. Bioland Corporation started production of HA by biotechnological process in 1990 for cosmological use. At present Bioland produces HA for cosmetic and food use. We have done many researches to evaluate the helpful effect for skin and safety of HA as food. In order to evaluate the effect of HA on the skin after its oral intake, we measured its bioavailability, skin-care effects on anti-wrinkle, moisturization, elasticity and desquamation by clinical trial. The results showed that HA is very safe and could be helpful to improve anti-wrinkling, moisturization, elasticity, and desquamation on the skin.

Keywords : hyaluronic acid, bioavailability, skin-care effect, safety

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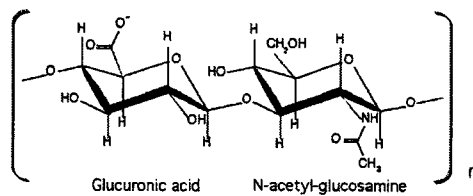


Hyaluronic acid(HA)



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- **K.Meyer and John Palmer(1934)**
 - : Isolate HA from the vitreous of bovine eyes.
 - : Hyaloid(vitreous) + uronic acid → hyaluronic acid
- **Unbranched linear polymer of repeating disaccharide :**
Glucuronic acid and N-acetyl-glucosamine linked by $\beta(1-3)$ glycosidic bond.



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Distribution of HA



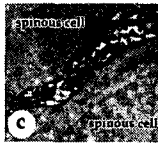
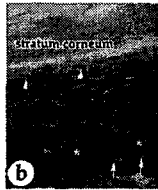
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- **All vertebrates.**
- **Some Microorganisms (eg. Streptococci)**
- **Essential component of extracellular matrices.**
 - Vitreous of Human eye : 0.1~0.4mg/g wet weight
 - Synovial Joint Fluid : 3~4mg/ml
- **The largest amount of HA resides in skin tissue (7~8g per average adult human, ~50% of the total in the body)**
 - Epidermis (~0.1mg/g wet tissue)
 - Dermis (~0.5mg/g wet tissue)

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Function of HA in Epidermis

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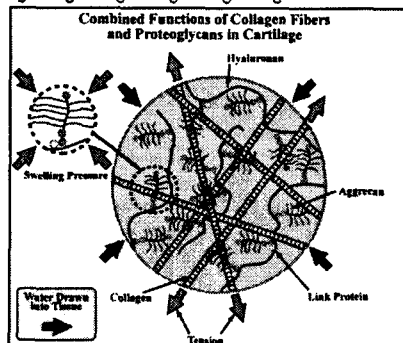


- **Maintenance Extracellular space**
Facilitate Diffusion of Nutritional Supplies to the lower Cells
Waste Products from the Upper Cells.
- **High HA Concentration**
Swelling of Extracellular space
Destabilize Desmosome and Adherens Junctions between Adjacent cells : Enhance Cell Turnover
- **Effective Scavenger of Free Radicals**
Protective role by scavenging reactive oxygen generated by UV

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Function of HA in Cartilage

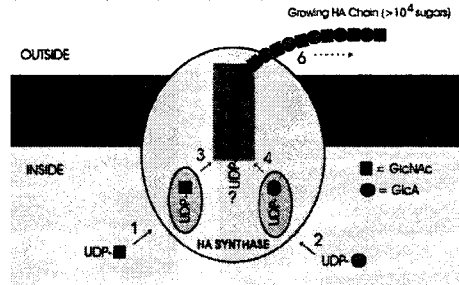
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Aggrecan function in cartilage. The load-bearing properties of cartilage are provided by the tensile properties of the collagen fiber network and the osmotic swelling pressure of the high concentration of aggrecan. The aggrecan is immobilized within the matrix by forming supramolecular aggregates with hyaluronan and link protein. Aggrecan in cartilage occupies less than 15% of its fully expanded volume in solution (see aggrecan in dashed insets).

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Synthesis of HA



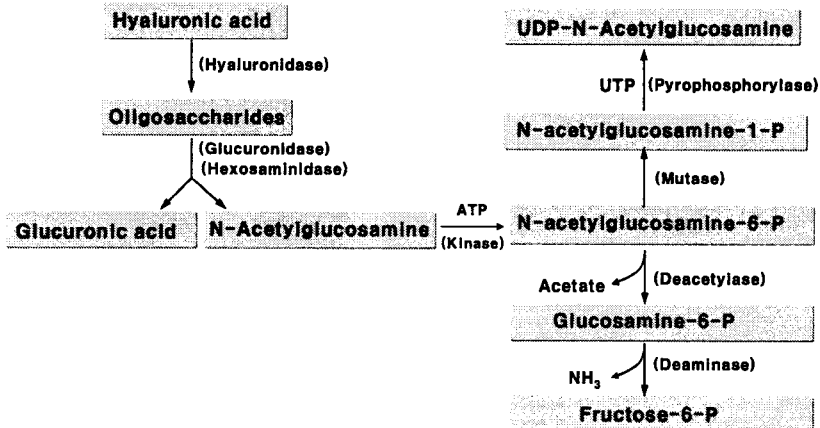
Multiple Functions of Hyaluronan Synthases

- 1) UDP-GlcNAc Binding Site
- 2) UDP-GlcA Binding Site
- 3) beta (1,4) GlcNAc Transferase
- 4) beta (1,3) GlcA Transferase
- 5) HA (acceptor) Binding Site
- 6) HA Transfer (translocation)

Enzyme functions needed for hyaluronan biosynthesis. The diagram shows the membrane-bound hyaluronan synthase and the six independent activities required for the enzyme to make a disaccharide unit and extend the growing hyaluronan chain. Before it is released, the chain can grow to more than 40,000 monosaccharides, corresponding to a mass of more than 8 million Da. The sugar-nucleotide substrates are produced and used by the synthase inside the cell, and the hyaluronan chain is continuously transferred (translocated) so that it is extruded into the exterior of the cell to form the capsule.

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Catabolism of HA in the body

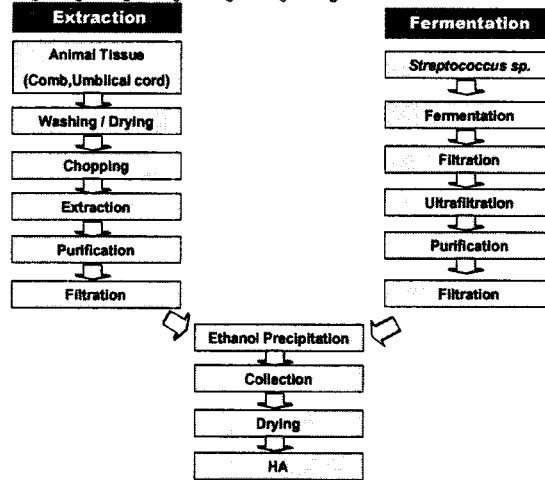


Pathways of hyaluronan catabolism and N-acetylglucosamine utilization in mammalian tissues.

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Production of HA

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Application of HA

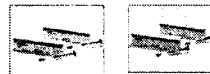
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❖ Pharmaceuticals

**Intra-articular
Injection**



**Eye Surgery
Assistant**



Eye Drop



Wound Dressing

**Injectable
Dermal Filler**



**Drug Delivery
System**

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Application of HA



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❖ Cosmetics

Moisturizer

Emollient



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Application of HA



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❖ Foods



- Soft Capsule
- Tablet
- Drink
- Jelly
- Soup
- Pudding
- Candy

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Clinical Efficacy and Safety of HA

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- Allergen Test
- Bioavailability
- Skin Care Effect
 - Moisturizing, Wrinkle, Elasticity, Desquamation
- Safety

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Allergen test of HA

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: Drug Induced Lymphocyte Stimulation Test
- Biospectrum Life Science Institute

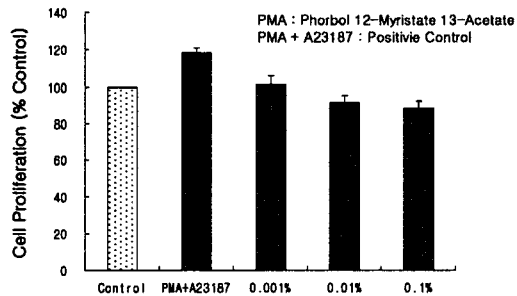


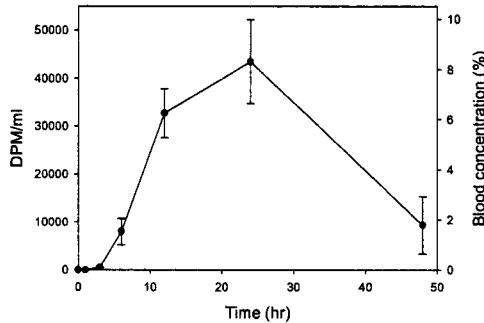
Fig. 1. Effect of Hyaluronic Acid on Lymphocyte Proliferation in T-lymphocyte cells (Jurkat T cells).

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Bioavailability of HA



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Professor Dong-Chul Kim
- Chungnam
National University

- * Test Sample : ^3H -HA, Mw. 80,000
- * Dose : 2.4g/animal
- * Animals : SD Rat (Bw 150g \pm 15) n=3
- * Measurement of Radioactivity : Liquid scintillation counter

Fig. 2. Change in total radioactivity in blood samples as a function of time after oral administration of ^3H -labeled hyaluronic acid

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Skin Care Effect of HA



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- Test Material : Hyaluronic Acid (Bioiland)
- Double Blind Test
- Test group(n=26) : 240mg/day
- Placebo group(n=26) : Cellulose
- Measurement : Moisture, Wrinkle, Elasticity, Desquamation
- Total 12weeks
 - 2weeks before
 - Immediately before
 - 4weeks after intake
 - 8weeks after intake
 - 2weeks after intake terminated

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Skin Moisturizing Effect

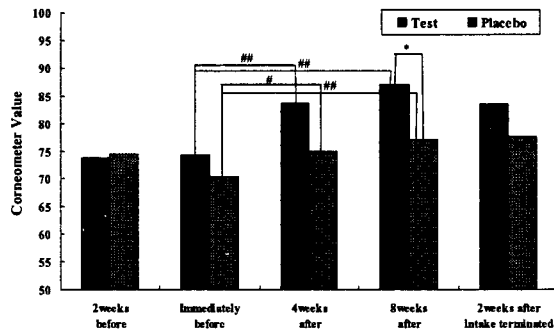


Fig. 3. Skin moisturizing effect of hyaluronic acid as a function of time after its oral intake using a Cerneometer. (paired t-test: # $p < 0.05$, ## $p < 0.01$; independent t-test: * $p < 0.05$).

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Total Wrinkle Amount (TWA)

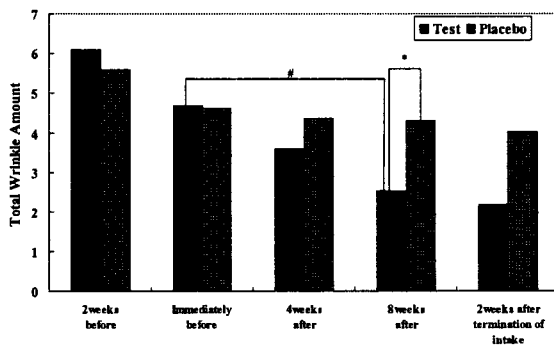


Fig. 4. Comparison of total wrinkle amount (TWA) over time by image analysis performed on the replica using the CCD camera system. TWA was calculated from the area ratio (%) of the area of the shadow to the overall area. (paired t-test: # $p < 0.05$; independent t-test: * $p < 0.05$).

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Photometric Evaluation

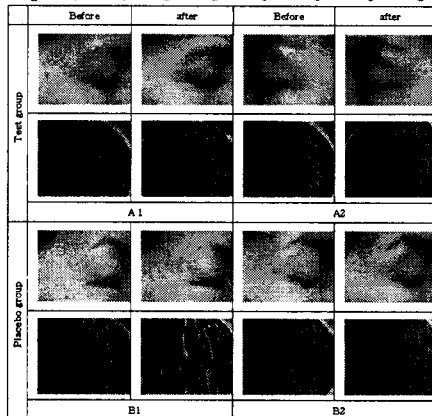


Fig. 5. Photometric evaluation on crow's feet area (up) by a high-definition digital camera (OLYMPUS E-1, JAPAN) after oral intake of hyaluronic acid for 8 weeks. Standard shooting was conducted by the same researcher using fixing direction and position. A replica (down) was manufactured using SILFLO (Flexico, England) and assessed by CCD camera.

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Anti-Wrinkle Effect- Dermatologist' s Assessment

Group	Before intake	4 weeks after intake	8 weeks after intake
Test	3.67 ± 0.82	3.63 ± 0.88	3.42 ± 0.83 ¹⁾
Placebo	3.58 ± 1.02	3.63 ± 0.97	3.63 ± 1.06 ²⁾

¹⁾Paired t-test: * p<0.05 compared with before intake.

²⁾The cutaneous examinations of the crow's feet area were conducted by two dermatologists via a double-blinded method and the values were recorded according to photodamage score (0: none, 1: none/mild, 2: mild, 3: mild/moderate, 4: moderate, 5: moderate/severe, 6: severe, 7: very severe).

Table 1. Results of the visual assessment conducted by the dermatologists on the volunteers.

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Skin Elasticity Effect

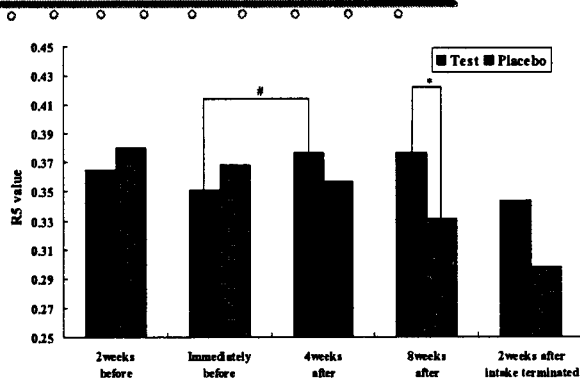
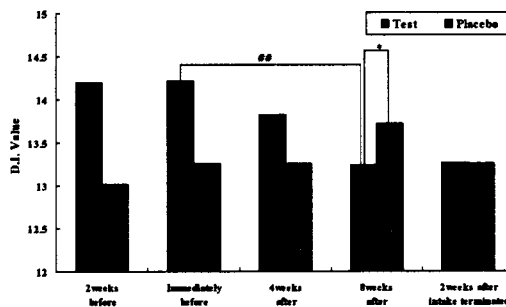


Fig. 6. Skin elasticity effect of hyaluronic acid as a function of time after its oral intake using a Cutometer. (paired t-test: # $p < 0.05$; independent t-test: * $p < 0.05$).

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Skin Desquamation Removal Effect



$$D.I. = \frac{2A + \sum_{n=1}^5 T_n * (n-1)}{6}$$

D.I.: the desquamation index
 A: the percent area covered by corneocytes
 T_n: the percentage of corneocytes in relation to thickness
 n: the thickness level (1-5)

Fig. 7. Skin desquamation removal effect of hyaluronic acid as a function of time after its oral intake using a Visioscan. (paired t-test: ## $p < 0.01$; independent t-test: * $p < 0.05$).

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Safety of HA

Clinical Research Institute

- Seoul National University Hospital

TEST	RESULT
Single Dose Toxicity (SD rat)	LD50 > 2,000mg/kg
Single Dose Toxicity (Beagle Dog)	LC50 > 2,000mg/kg
14 Days Repeated Dose Toxicity (SD rat)	1,000mg/kg
90 Days Repeated Dose Toxicity (SD rat)	1,000mg/kg
Reverse Mutation Test (<i>Salmonella typhimurium</i>)	No Mutagenicity (5mg/plate)
Chromosomal Aberration Test (CHL Cell)	Negative (5mg/ml)
Micronucleus Test (Bone Marrow Cells of ICR Mice)	No Genotoxicity (2,000mg/kg)

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Conclusions

- Hyaluronic acid does not act as an allergen.
- The Hyaluronic acid concentration in blood started to increase rapidly after 3 hr and showed the maximum at around 24 hr after oral intake.
- Hyaluronic acid did not show any particular adverse effects in the safety test.
- Clinical test results showed that hyaluronic acid is very safe and could be helpful to improve anti-wrinkling, moisturization, elasticity, and desquamation on the skin.

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Acknowledgements



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