A New Sodium Hyaluronate for Skin Moisturization and Antiaging

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KEY WORDS: *hyaluronic acid, sodium hyaluronate, antiaging, skin moisturization, skin elasticity*

ABSTRACT: *A new cosmetic ingredient, produced by fermentation of* Bacillus subtilis *as a bioidentical polymer for natural sodium hyaluronate, shows moisturizing, antiwrinkle and skin firmness properties in clinical tests.*

In response to growing concerns about animal-derived sources for hyaluronic acid, some researchers have turned to biotech methods to produce this skin moisturizing agent. One example is described here with test results showing its additional anti-wrinkle and skin firmness properties.

Hyaluronic Acid

Hyaluronic acid (HA) is a natural and linear carbohydrate polymer belonging to the class of the nonsulfated glycosaminoglycans. Sodium hyaluronate (SHA) is the sodium salt of HA. Meyer and co-workers extracted HA from the vitreous humor of cattle eyes in 1934[1] and pursued their pioneering efforts 20 years later with the elucidation of HA structure.[2]

HA is a unique biopolymer in that its structure is highly conserved and identical in all species. HA is composed of β-1,3-*N*-acetyl glucosamine and β-1,4-glucuronic acid repeating disaccharide units, which have a molecular mass of approximately 400 Da (**Figure 1**). The number of repeating disaccharides can reach 10,000 or more, resulting in molecular weights of 4 MDa or more.[3]

From the 1980s onward, the understanding of HA properties and functions has blossomed into a truly scientific and industrial enterprise, such that now this biopolymer is recognized as a high-value product and has numerous proven and marketed applications within biomedical areas, and technical areas such as cosmetics.

HA is found in the vitreous body. It also is abundant in the extracellular matrix, especially of soft connective tissue, and in the synovial fluids of articular joints. Skin tissue contains the largest amount of HA, about 7–8 g per average adult human.[3]

The most striking features of HA are its unique hygroscopic, rheological and visco-elastic properties. HA is one of the most hygroscopic molecules in nature and hydrated HA can contain up to 1,000-fold more water than its own weight.[4] Moreover, the high viscosity of HA solutions grants this macromolecule with exceptional lubricating and cushioning effects in the eye and in the synovial fluid.

All these unmatched functions are particularly relevant in skin, where HA holds moisture, controls tissue hydration and contributes to the elasticity of the skin. Finally, HA acts as a radical scavenger[5] and exhibits antioxidative effect,[6] which results in increased protection of the skin against UV irradiation[7] and in positive attributes during wound healing.[8]

A New Sodium Hyaluronate

The concentration of HA in rooster combs and human umbilical cords is very high, reaching 7500 mg/L and 4100 mg/L, respectively.[4] In the early 1980s, Balazs and coworkers developed a procedure to isolate, purify and identify hyaluronic acid from rooster combs and human umbilical cords.[9] Since then, HA has been produced from rooster combs at industrial scale. However, the rooster comb-based extraction process is faced by a growing concern over the use of animal-derived products for technical and especially biomedical applications. Hence, microbial fermentation has emerged as a new technique for the production of HA.[10]

The bacterial production of HA involving a *Streptococcus zooepidemicus* strain was first described in 1989,[11] giving rise to the first commercializa-

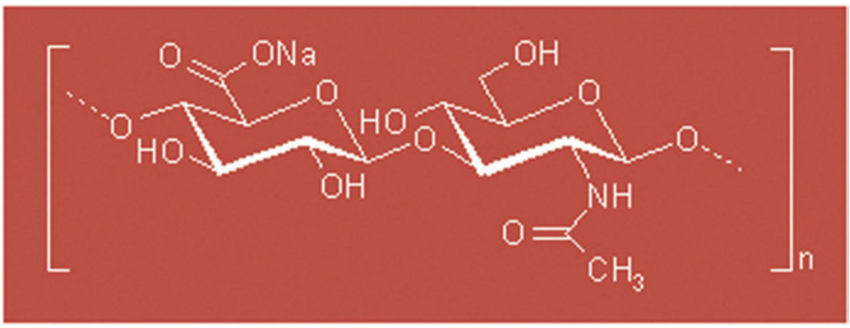

Figure 1. Chemical structure of HA

Formula 1. Skin cream for testing moisturization and antiwrinkle effects

Water *(aqua)*	74.2% w/w
Sodium hyaluronate (HyaCare, Novozymes)	0.1
Hydrogenated polydecene (Nexbase 2006 FG, Fortum)	20.0
Steareth-2 (Brij 72, Uniqema)	3.0
Steareth-21 (Brij 721, Uniqema)	1.0
Cetearyl alcohol (Lanette O, Cognis)	1.5
Propylene glycol (and) diazolidinyl urea (and) methylparaben (and) propylparaben (Germaben II, Nipa)	0.2
	100.0

tion of biosynthetic HA. Nevertheless, streptococci are pathogenic in nature. In addition, they are fastidious lactic acid bacteria with demanding requirements such as media enriched in proteins during the fermentation. The presence of bacterial endotoxins, chondroitin sulfates, proteins, nucleic acids and heavy metals in HA from streptococcal fermentation or rooster combs has also been reported.[10] Finally, both extracted HA and microbial HA are purified using harsh organic solvents.

Using its core competencies in enzymes and microorganisms, Novozymes has developed a method for producing SHA by fermentation of *Bacillus subtilis*, a novel and nonpathogenic strain. Products from this strain generally are regarded as safe. In this article, the term *SHAB* will be used to refer to the sodium hyaluronate[a] produced by fermentation of *Bacillus subtilis*. The SHAB process relies on defined recovery steps, during which no organic solvents are used.

These two latter attributes make the SHAB production process the most environmentally friendly SHA production process developed to date. In addition, the SHAB process produces a fast-dissolving HA powder composed of microspheres and nanospheres. Solubility tests show that SHAB in a 0.2% weight-to-weight (w/w) aqueous solution dissolves efficiently and quickly by mixing for less than 25 min owing to the large surface area of the particles. As a consequence, HA-based cosmetic formulations can be prepared faster and use less energy.

Moisturization Properties of SHAB

The fact that dry skin is one of the most common human disorders has led to the extensive use of moisturizers on a daily basis.[12] Moisturizers are cosmetic products designed to restore and maintain the stratum corneum (SC) perfectly hydrated.[13] Moisturization also evokes a young, soft and elastic skin. SH has been used widely in cosmetics as the premium skin moisturizer due to its remarkable water-binding and film-forming properties. Herein, the unique

[a] *HyaCare (INCI: Sodium hyaluronate) is a product of Novozymes Biopolymer A/S. HyaCare is a registered trademark.*

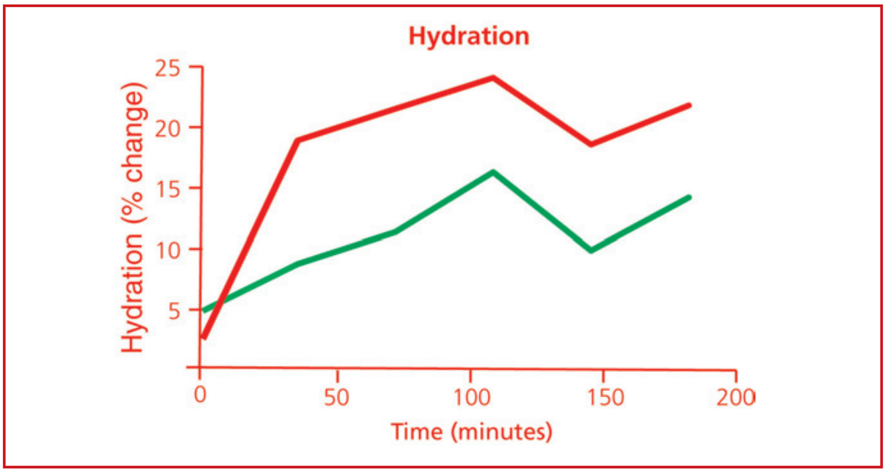

Figure 2. Mean (n=12) percentage change from untreated skin for short-term hydration at SHAB-treated (red) and placebo-treated (green) skin sites

properties of SHAB as the HA of choice were evidenced through the evaluation of its functional properties in cosmetic formulations.

A cosmetic cream formulation containing 0.1% SHAB was prepared as described in **Formula 1** on page 52. Recognized external and independent institutes measured the efficacy of the SHAB cream and compared it to that of a placebo cream and an untreated skin area. The placebo cream was similar in composition to the active cream except that it did not contain SHAB. It also did not show any significant moisturizing or antiwrinkle effects. All tests were performed in a bioclimatic room at 24°C and at 50% relative humidity.

In these studies, mean values and standard deviations were calculated for initial and final instrumental values (hydration, elasticity and image analysis). The differences between the data values were considered significant when the probability P was less than 0.05.

Short-term and long-term hydration: The moisturizing efficacy of a cosmetic product can be assessed by measuring skin hydration. Typically, the skin surface hydration is measured by corneometry, which determines the capacitance of the SC and thereby reflects the relative SC moisture.

The hydration study was carried out by applying the SHAB cream and a placebo cream on the forearms of 12 volunteers (female, mean age 38). The hydration was evaluated at the treated sites and at an untreated site by a corneometer[b] in two separate studies, after a short-term application up to 180 min and after a prolonged application twice a day for eight weeks, respectively.

The results indicate that SHAB efficiently moisturizes the skin, both in short-term (**Figure 2**) and long-term (**Figure 3** on page 55) applications. The SHAB cream induced a significant increase in the mean basal value of the skin hydration upon short-term application. Moreover, an increase in the hydration values of 7% was recorded after an eight-week treatment.

Transepidermal water loss: The measurement of transepidermal water loss (TEWL) of skin is a noninvasive method for assessing the efficiency of the skin as a protective barrier. TEWL measures the water vapor released by the skin surface based on Fick's diffusion formula.

The ability of SHAB to protect the skin by limiting water evaporation was evaluated instrumentally[c] by measuring the TEWL at sites treated by the SHAB cream, or by the placebo cream, or left untreated on 12 volunteers. The study was conducted as a short-term study up to 180 min.

Figure 4 on page 56 illustrates the percentage TEWL decrease compared to an untreated area. The results show that SHAB forms an effective barrier against moisture loss. The SHAB cream induces a significant reduction in the mean basal value of TEWL.

[b] *Combi CM 825, Courage & Khazaka*
[c] *Tewameter TM 210, Courage & Khazaka*

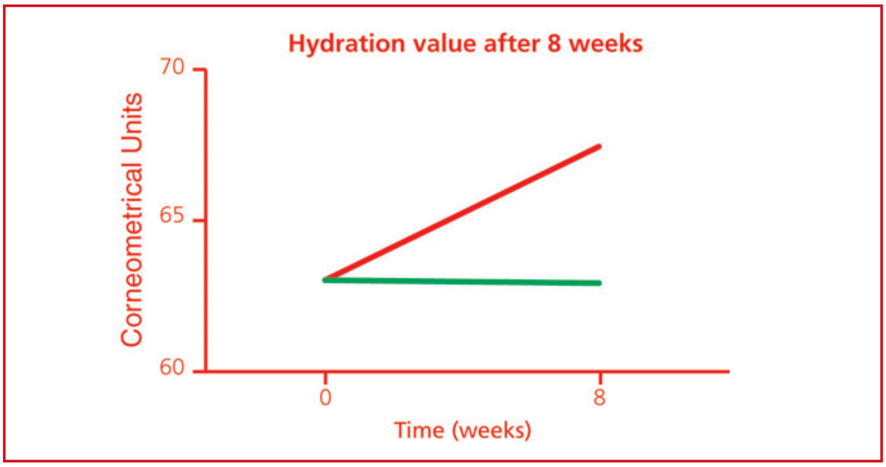

Figure 3. Mean (n=12) change in skin hydration value (corneometrical units) for SHAB-treated (red) and placebo-treated (green) skin sites at start and end of the eight-week treatment period.

Antiaging Properties of SHAB

Skin aging is a natural and universal process that results from physiological alterations in skin functions.[14] Exposure to UV light also is expected to affect the biological processes in the skin and thus to accelerate aging.[15] Aged skin typically looks dry, rough, less flexible and with fine wrinkle lines.[14] A moisturizer such as SHAB can be used to treat dry skin and prevent skin aging due to its water retention capacity and hydration properties.

Antiwrinkle effects: The ability of cosmetic ingredients to reduce fine line wrinkles in the skin can be tested by evaluation of the skin roughness parameters. The topography of the skin surface typically is evaluated by skin surface replicas and image analysis. The SC microrelief is assessed by image analysis of a plastic replica of the skin surface. From this image the standard roughness parameters Ra (mean roughness) and Rz (maximum roughness for deep wrinkles) are calculated. The reduction of the maximum roughness value reflects the decrease of deep wrinkles. Herein, the antiwrinkle properties of an SHAB-containing cream were investigated and compared to those of a placebo cream. The test was performed at treated and untreated sites on the faces of 12 female volunteers (mean age 46) at a frequency of twice a day and during eight weeks.

The skin surface replicas were made using a fast-hardening synthetic polymer[d] and adhesive discs. The skin replicas were analyzed by image processing software[e], which allows a global data analysis of some relief parameters, according to the method described by Carcuff.

The silicon replica of the cutaneous surface was illuminated by a light source with a defined incident angle of 35° with the purpose of generating shadows that are wider when ridges are higher. An image covering an area 12 x 9 mm on each skin replica surface was obtained through a video camera[f].

In the SHAB-treated area, the mean maximum roughness value significantly decreased by 10% after eight weeks of treatment (**Figure 5**) on page 56. The placebo-treated area showed no change in the maximum roughness values. This study clearly demonstrates that SHAB has an antiwrinkle effect. A statistically significant decrease of 10% in the maximum roughness value was detected in the area treated with the SHAB cream.

Skin elasticity: Healthy and young skin appears firm, soft and elastic. It is well-known that with aging, skin tissues lose elasticity. This study demonstrated that topically applied HA can restore the elasticity of the skin.

The elasticity of SHAB-treated skin was measured instrumentally[g]. By applying a vacuum, the skin layers are sucked into the opening of a measuring probe. The vertical deformation of the

[d] *Silflo, Flexico Ltd, UK*

[e] *Quantilines, Monaderm*

[f] *High Performance CCD camera, COHU*

[g] *Cutometer SEM 575, Courage & Khazaka*

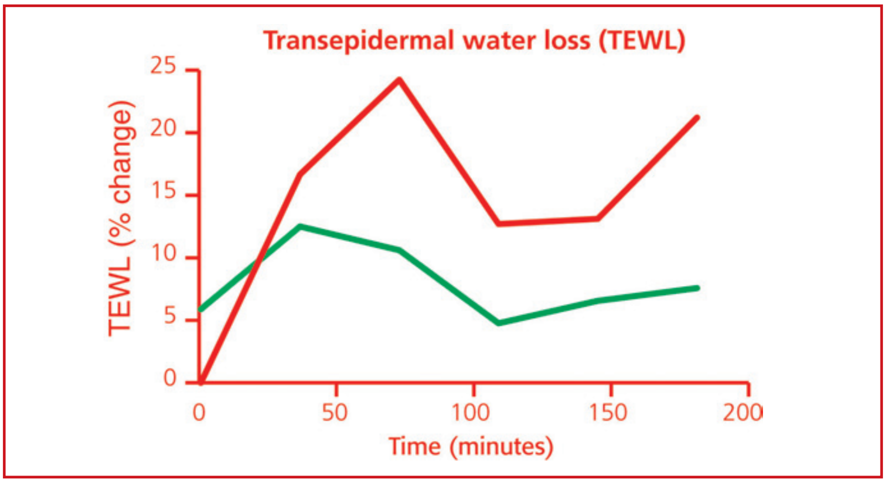

Figure 4. Mean (n=12) percentage change from untreated skin for short-term TEWL following a single application of SHAB-treated (red) or placebo-treated (green) cream

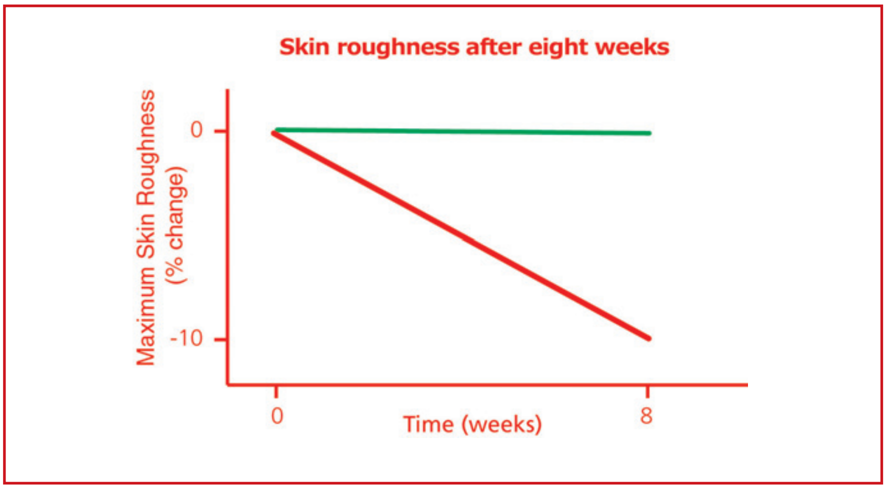

Figure 5. Mean (n=12) percentage reduction in skin roughness of facial skin treated with a SHAB-containing cream (red) or a placebo cream (green) and measured at start and end of the eight-week treatment period

skin is recorded optically, applying multiple cycles of stretching and relaxing. This method provides the deformation parameters related to the skin elasticity: R0 (maximum deformation), R2 (overall elasticity) and R6 (viscoelastic ratio). The study was performed on 12 volunteers (female, mean age 46) by applying the SHAB cream on the face twice a day for eight weeks. The results were compared to the placebo cream (**Figure 6**) on page 58.

This study illustrates that SHAB increases the elasticity of the skin. The area treated with the active cream showed a statistically significant increase of 27% in the overall skin elasticity.

Discussion

The volunteers were asked about irritation and allergy during the treatment. In all cases, application of SHAB did not cause any irritant or allergic reactions. In addition, SHAB does not appear to dehydrate the skin; in contrast, the data regarding the decrease in TEWL shows that it forms a barrier and stimulates hydration of the skin.

The mechanisms behind the observed effects are not well understood. Skin penetration tests have been conducted and the effect of molecular weight on permeation of hyaluronic acid has been studied. The results obtained showed that low-molecular-weight species (50 and 300 kDa) penetrated the skin only to a small extent and accumulated on, in

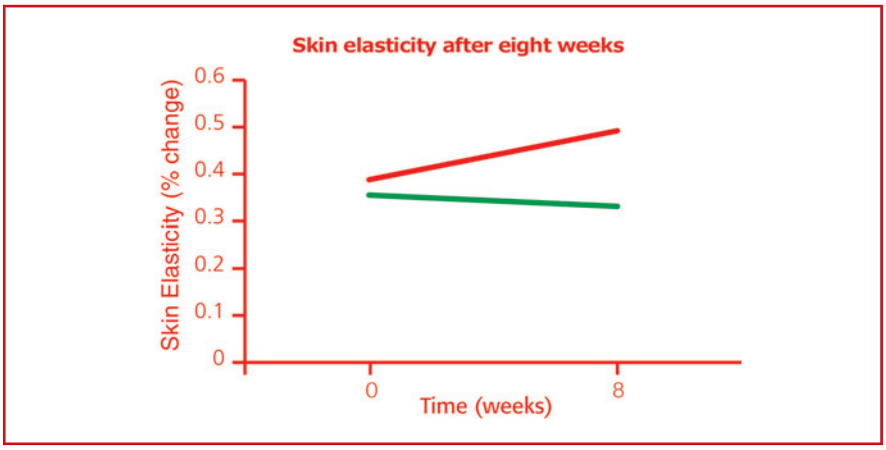

Figure 6. Mean (n=12) change in overall skin elasticity parameter in facial skin treated with a SHAB-containing cream (red) or a placebo cream (green) and measured at start and end of the eight-week treatment period

and around the follicular structures in the skin. The observed effects (enhanced elasticity and antiwrinkle) could result from degradation of SHAB into smaller fractions that are then able to penetrate the skin through the hair follicles.

Conclusion

The SHAB originating from a new and safe fermentation method was shown to have enhanced dissolving properties compared to existing hyaluronic acid products. This is a clear advantage when preparing cosmetic formulations.

Moreover it has been proved that SHAB is an excellent moisturizer. It increases water-binding capacity (data not shown) and decreases TEWL upon short-term and long-term applications. Finally clinical tests demonstrated that SHAB decreases the skin roughness while improving the skin elasticity, suggesting its use as an antiwrinkle agent.

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