Hyaluronan and the HylaSponge®System for Skin Care

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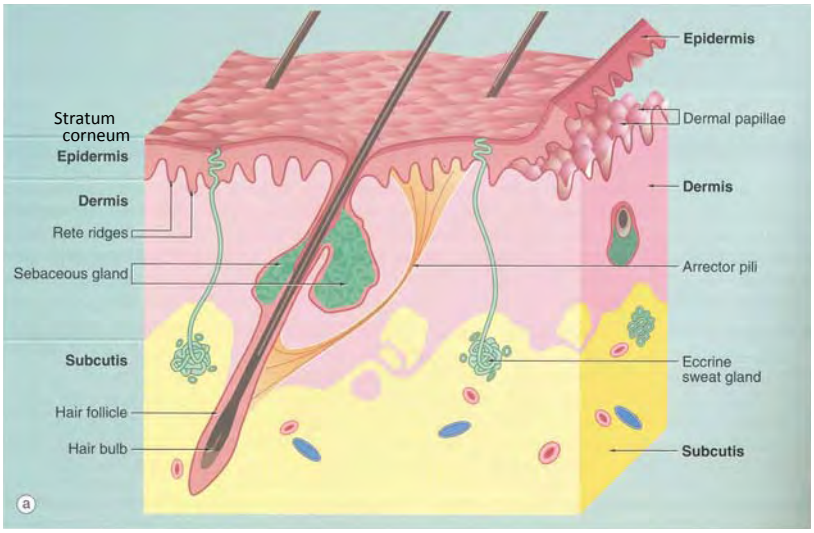
Introduction. Hyaluronan [1] (hyaluronic acid) can be modified in terms of molecular weight, spatial configuration and structural composition. These modifications provide for specific medical or cosmetic (skin care) applications. The most recent, innovative spatial configuration and structural composition of hyaluronan for skin care is the **HylaSponge® System**.

Only the HylaSponge®System acts like an elastic "second skin": it hydrates the skin's surface layer (stratum corneum) and the deeper layer, dermis. The water content maintains a "waterway" to the deeper layers of the skin, guaranteeing the delivery of water, biologically active small water-soluble molecules. The HylaSponge®System in skin care formulations is designed to deliver these benefits:

- Moisturization is provided by the long lasting waterway and equilibrium hydration from the surface to the deeper layers of the skin.
- The intense hydration reduces the appearance of grooves, fine lines and wrinkles while smoothing the skin.
- Continuous delivery of biologically active molecules to the skin.

The following background information will clearly demonstrate how and why the HylaSponge®System works effectively on the skin, and why it can be an essential, exciting and new addition to your skin care formulations. The diagram below illustrates the layers of the skin[2]:



[1] Hyaluronan is also called hyaluronic acid. However, in vivo and in skin care products, this molecule is usually in the sodium (or zinc) salt form, rather than the acid form.

[2] Wheater's Functional Histology. A Text and Colour Atlas, 5th edition, Barbara Young, James S. Lowe, Alan Stevens, John W. Heath, 2006, Elsevier Limited, pp 168.

Where is hyaluronan in the body? Hyaluronan is a very important molecule in our skin. It is present in the "connective tissues" below the epidermis (the topmost cellular layer in the skin). In the skin, there are two connective tissues: the dermis, found below the epidermis and below that, the subcutis. The name connective tissue describes the function of this tissue – namely, connecting the epidermis of the skin to the tissues below it, such as bones, tendons and muscles. In the connective tissues of the skin, high concentrations of hyaluronan fill the space between the collagen and elastin fibers. Grooves, wrinkles, and folds are formed in the dermis and subcutis layers.

One of the most important functions of hyaluronan in the dermis and subcutis of skin is to keep it **hydrated**, controlling its water content. The second important function of hyaluronan is to provide **elasticity** to the skin, thereby augmenting the function of elastic fibers. Both of these functions are based on the structure of hyaluronan and its special relationship to water.

The molecular structure of hyaluronan. Hyaluronan is a very long, string-like polysaccharide molecule, without branches. It is called polysaccharide or more popularly, a "poly-sugar", because it is made up of many small sugar molecules, which are structural modifications of glucose. Two of these sugar molecules form a disaccharide molecule, and many of these disaccharides are bound together in repeating order to form a long molecular chain of hyaluronan. The number of repetitions of this disaccharide unit is very large and there may be as many as 20,000 repeating units, forming a molecule with a weight or mass of 8 million. In the technical literature, the molecular weight (or mass) is used to characterize the hyaluronan molecules. Naturally in the body, or in a solution of a purified hyaluronan, the long, string-like molecule coils up into a structure which is called a *random coil* because there is no specific pattern to its coiled-up structure. When the long chain is coiled up, the size and the volume of this coil are also very important because the space between coils is filled up with water and, the larger the coil, the more water it contains. One can compare this coiled up molecular structure with a sponge that is filled with water and retains water.

History of the use of hyaluronan and its derivatives for skin care. The water-retaining property of hyaluronan is the basis of its biological role in the skin and the reason we suggested its use as a hydrating or moisturizing agent in cosmetic products. Between 1975 and 1981 Dr. Endre Balazs and Dr. Janet Denlinger developed hyaluronan as a moisturizer to be used in skin care formulations. They started a biotechnology company (Biomatrix, Inc.) to manufacture and sell this product worldwide to the skin care industry. In 1981, Endre Balazs received a patent[3] covering this application, the first hyaluronan used as a moisturizing agent. The subject of this patent was the use of viscous hyaluronan solutions mixed with equal amounts of protein in the solution, a combination that was shown to retain water even after it "dried" on the surface of the skin. Ella Bache Cosmetics, Inc. (Paris and New York) was the first skin care company that marketed cosmetic formulations[4] containing this hyaluronan product, first in France and then, through their own distribution system and with Aida Grey (Hollywood) in the US. Biomatrix, Inc. itself developed various formulations with this hyaluronan: Lurogel®, Elastogel® and Dermabien®. Estée Lauder, Inc. followed in 1982, using this viscous, high molecular weight hyaluronan/protein mixture manufactured by Biomatrix in their highly successful product, Night Repair®.

[3] Balazs, E.A. (1981). Hyaluronate Based Compositions and Cosmetic Formulations Containing Same. United States Patent #4,303,676. December 1, 1981.

[4] Developed by Janet L. Denlinger

During the 1980s Biomatix, Inc. developed, patented and marketed other new hyaluronan derivatives under the names Hyladerm/Polyx®, Hyladerm® and in cooperation with Union Carbide, a combination of hyaluronan with a polyanion, which was called Biocare®. The same team of inventors at Biomatrix, Inc. developed, manufactured, and marketed a water-insoluble hyaluronan derivative (hylan B gel) under the name Hylasome®. This was the first gel derivative of hyaluronan available for skin care and therapeutic purposes. Hylan B was later the first hyaluronan gel used worldwide as an injectable skin augmentation product (Hylaform®) for the correction of depressed scars and wrinkles.

By the end of the 1990s, many of the major cosmetic companies utilized hyaluronan compounds in their formulations for a variety of applications ranging from moisturizers to hair care products and to beauty products such as foundations, blushing gels, eyeliner and lip color.

How hyaluronan works in skin care products. The hyaluronan on the surface of the skin contains water, even though it feels dry after application. This water is available for hydration of the skin.

The benefit of using hyaluronan on the surface of the skin is based on three effects: **moisturizing, smoothing** and **masking**, and these three effects depend on three properties of hyaluronan in the final formulation: **average molecular weight, concentration**, and **hydration** of the hyaluronan molecules[5].

1. The **moisturizing** effect. The first hyaluronan product (Biomatrix®) used in skin care was designed in such a way that when applied to the human skin, the skin absorbed ten times more water in the first 15 minutes than the control (measured with a Servo-med evaporimeter). In other words, the hyaluronan content, depending on molecular weight range and its concentration, directly determines a product's moisturizing ability.
2. The **smoothing** effect. One of the most important benefits of high molecular weight, highly hydrated hyaluronan in skin care products is that when it dries on the skin at ambient temperature and humidity, it retains water for a very long time. How long it remains, on the skin in this semi-hydrated state depends on its molecular weight, concentration and the ambient conditions. Since the hydrated hyaluronan molecule occupies a large volume, it fills the static grooves in the skin and, to some degree, the dynamic wrinkles. This hyaluronan, even its "semi-hydrated" form, reflects light. Thus, the grooves and wrinkles that are filled with such hyaluronan appear less deep.
3. The **masking** effect. The "greasiness" of a lipid-containing formulation can be masked with the appropriate concentration and molecular weight of the hyaluronan added to it. Measured with a sebumeter, the masking effect of the first hyaluronan product (Biomatrix®) was measured. It was found that Biomatrix®, with an average molecular weight of 5 million in a concentration of 0.01% in the final product that contained also 10% petrolatum, decreased the "greasiness" by 66%.[6]

[5] Biomatrix, Inc. The use of hyaluronan and hylans for skin care: *Hyaluronan Volume II* (2002), Editors John F. Kennedy, Glyn O. Phillips, Peter A. Williams and Vince C. Hascall. Woodhead Publishing Ltd., Cambridge, England Pages 285-288.

[6] Ibid.

How much is enough hyaluronan in a skin care product?

- The amount of hyaluronan required to be an effective moisturizer depends on the average molecular weight or size of hyaluronan. The **average molecular weight** must be high, and the **concentration** must be above a certain value.
- The amount of water filling the hyaluronan molecules depends on the volume of the hyaluronan molecules, and the volume depends on the molecular weight and the length of the molecular chains, resulting in larger molecular volumes and greater water-retaining or water-controlling properties.

The thicker the hyaluronan layer after application and after "drying", the more water is retained on the surface of the skin and therefore the more effectively the product will moisturize. In other words, the amount (concentration) and the molecular weight of the hyaluronan in the product will define its efficacy – its **moisturizing**, **smoothing** and **masking** effects.

The development of HylaSponge®. In the early 2000s, with Dr. Balazs' initiative and technical support, a new derivative of hyaluronan was developed by a group of chemists[7]. This hyaluronan derivative was produced from highly purified hyaluronan molecules __ the same highly-purified hyaluronan available today worldwide for various therapeutic purposes: in eye surgery, to produce long-lasting analgesia in arthritis, in skin augmentation for filling wrinkles, and in the prevention of post surgical adhesions, among others.

A hyaluronan sponge is an infinitely large hyaluronan molecule created by a patented, free-radical polymerization process that results in binding together many long hyaluronan chains into a very large coil system. In other words, a large, spheroidal particle was created which contained a network of molecular chains with virtually infinite molecular weight. This large volume molecular sphere contains large amounts of water that can be pressed out of the particles as from a sponge. Because of these properties, it was called a molecular *sponge of hyaluronan* or HylaSponge®. This registered trademark, HylaSponge®, is owned by the Matrix Biology Institute.

Five dry HylaSponges® are shown in Figure 1. This picture shows sponges of relatively small size (20-50 µm diameter), but smaller as well as larger sponges can be manufactured. When the dry sponges of any size are exposed to water, they hydrate very quickly and swell. Figure 2 shows three (3) individual (not fully in view) sponges that contain 1% hyaluronan, photographed using a phase-contrast microscope. Note the structure of the crowded hyaluronan molecules connected to form a single molecular structure of approximately 400 x 800 µm.

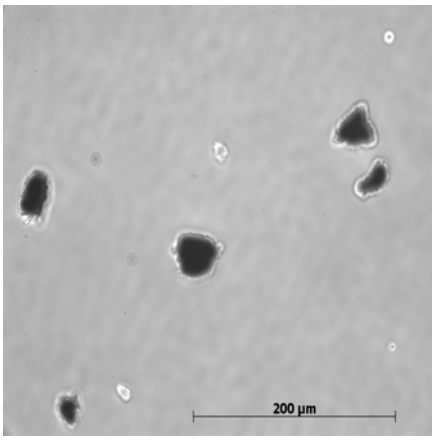


Figure 1. Dry (non-hydrated) sponges

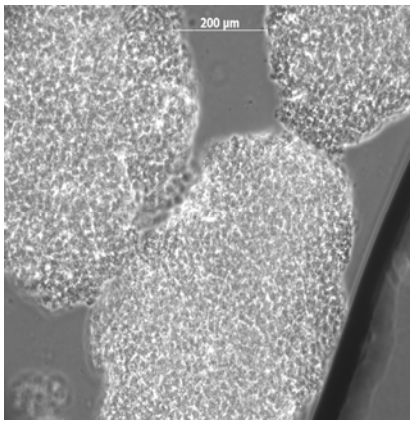


Figure 2. Three hydrated HylaSponges® close to one another. Photograph taken with a phase-contrast microscope.

[7] (Glyn O. Phillips, Tjaart A. du Plessis, Saphwan Al-Assaf, and Peter A. Williams) United States Patent #6610810 (2003 and United States Patent #0841644 (2005).

Figure 3 demonstrates the water uptake of 0.05 g dry sponges. When fully hydrated, this weight of sponge swells to 5 mL volume (approximate weight, 5 g), that is, the HylaSponge® takes up approximately 100 times its weight in water. The figure shows the fully hydrated sponges with excess water at the top, demonstrating this phenomenal hydrating capacity of the HylaSponge®. In essence these fully hydrated sponges represent a hyaluronan gel system that contains 1% pure hyaluronan – insoluble, but fully hydrated in water.

Figure 3. The HylaSponge®System hydrates and swells in water or in the liquid part of a formulation. The tube on the right shows the dry HylaSponge®System (0.05 g in dry form). The tube on the left shows how the dry HylaSponge® System picks up water in the formulation and forms a viscous and elastic liquid. The volume (or weight) of the hydrated HylaSponge®System is 100 times greater than that of the dry particles. This demonstrates the exceptionally high "hydrating properties" of the HylaSponge®System. When it feels dry on the on the surface of the skin, it still contains about 30% water.

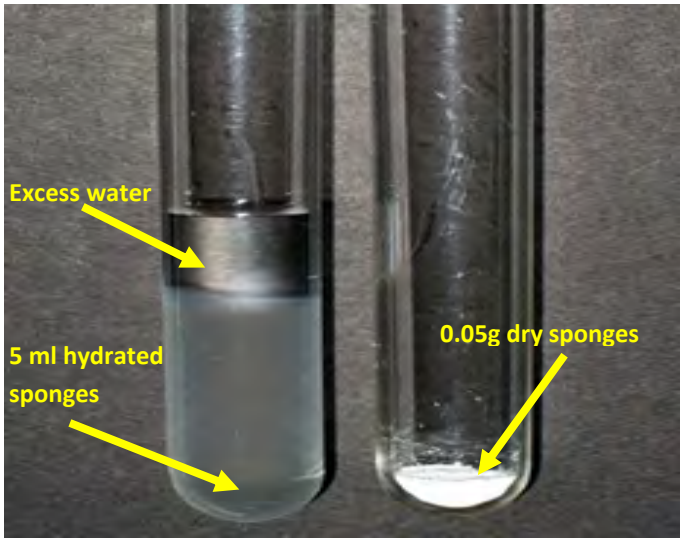


Figure 4 represents a part of the HylaSponge® in Figure 2 under much higher magnification. The molecular structure of the hyaluronan in the hydrated sponge is more visible, with the light area of water between the molecular aggregates. Figure 5 shows one hydrated sponge stained with hyaluronan-specific blue-fluorescing dye. The background is black because the picture was taken using ultraviolet light.

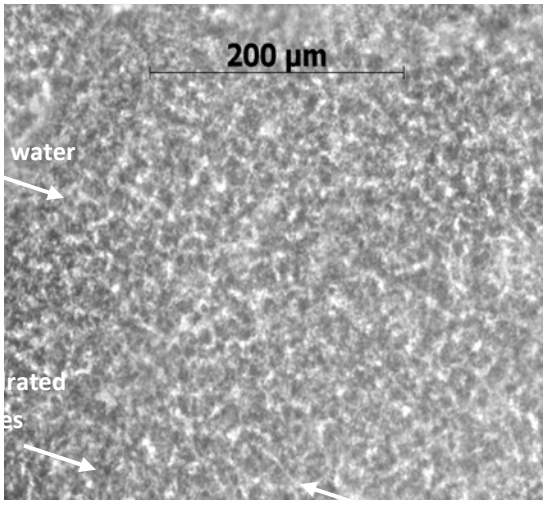


Figure 4. Higher magnification of one of the hydrated sponges from Figure 2.

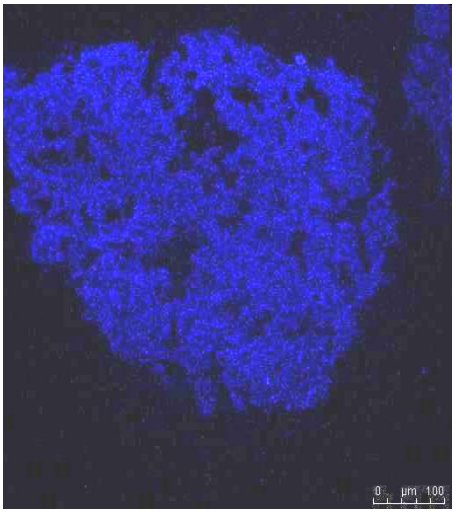


Figure 5. This picture is of a hydrated (water-filled) sponge stained with blue fluorescent dye that specifically stains hyaluronan. Photograph taken with a confocal microscope.

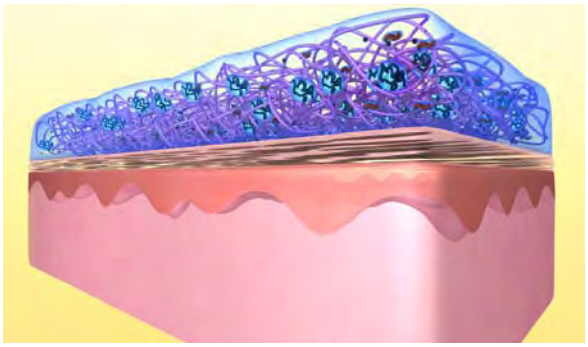
The HylaSponge®System. The HylaSponge®System is a complex hyaluronan molecular system designed and developed by Matrix Biology Institute to be used in skin care and dermatological products. It is the most efficient moisturizer both for dry and normal skin. Most importantly, it is also designed as a "delivery system" for water and water-soluble molecules intended to protect or to stimulate the skin (see Figure 6).

To achieve these functions the HylaSponge®System contains three different pure hyaluronan molecular components, different in their molecular weight. All three hyaluronans are purified to the level required for cosmetic and skin care use.

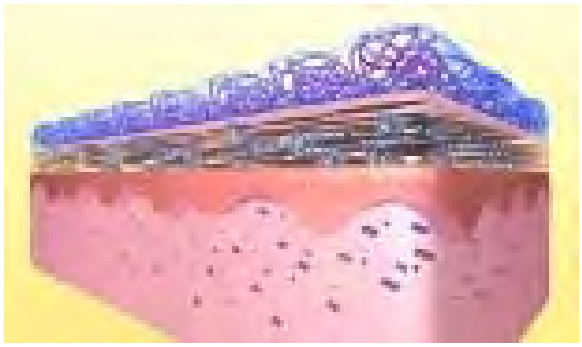
The HylaSponge®System

A second skin on the surface of the healthy and compromised skin

Protects – Hydrates – Fills - Delivers



6A



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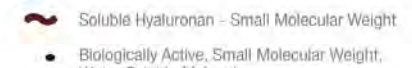
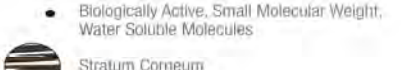
3. 

4. 

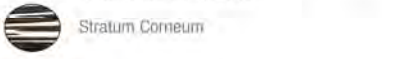
5. 

Figure 6A. This picture demonstrates schematically the **hydrated HylaSponge®System** (approximately 0.1 mm thick). The space in between the sponges is occupied by the large molecular weight, highly hydrated and soluble hyaluronan. The small molecular weight soluble hyaluronan is inside as well around the HylaSponges. The biologically active small molecules are also distributed in the water around and in the sponges.

Figure 6B. This picture demonstrates schematically the **less hydrated HylaSponge®System**, considerably collapsed but still containing approximately half of the water content of the hydrated HylaSponge®System (shown in Figure 6A). Note that the large molecular weight hyaluronan diffuses into the horny layer (stratum corneum) and the small molecular weight hyaluronan diffuses into the dermis. The biologically active small water soluble molecules also diffuse through the horny layer into the dermis.

The three different hyaluronans in the HylaSponge®System.

- **Polymerized hyaluronan (the sponge).** The sponge itself is produced by a patented method of polymerization. Large numbers of hyaluronan molecular chains are polymerized to form a much larger molecular coil—the sponge. In this process the individual molecular coils are permanently hooked together and form a coil system with a very larger volume. We call it a sponge because when the polymerized dry molecular coil is placed in a water or salt solution it will swell to a hydrated, sponge-like molecular entity.
- **Large molecular weight hyaluronan.** The sponges can be hydrated in water that contains large hyaluronan molecules. The average molecular weight of the HylaSponge®System can be changed because the sponges can be hydrated with a solution that contains different sizes of hyaluronan molecules. The concentration of the hyaluronan in the solution can also be varied. The fact is that these large hyaluronan molecules cannot penetrate the sponges; consequently they do not fill the sponges, but connect them by filling the space between them. Thus the rheological properties—that is, the viscous and elastic properties of the sponges suspended in and filled with hyaluronan—are very different from sponges suspended and filled with water only. In other words, the mixture of the sponges with large molecular weight hyaluronan exhibits superior elastic and viscous properties (elastoviscosity). The majority of the large molecules have a molecular weight between 600,000 and 6 million, with an average weight of 2 million. (See Figure 7).
- **Small molecular weight hyaluronan.** The sponges are also filled with a polydisperse hyaluronan molecular population that has an average molecular weight of 50,000. These molecules are not only present in the sponges, but also are dispersed in between the molecular coils of the high molecular weight hyaluronan. The concentration and the average molecular weight of the small hyaluronan molecules can be varied within certain limits. (10,000 - 200,000 molecular weight).

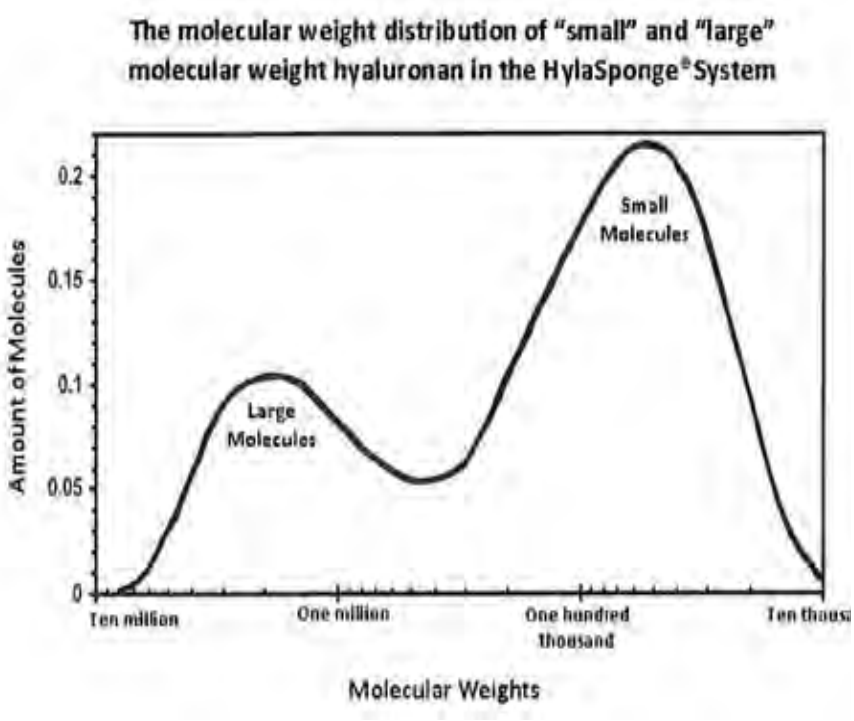


Figure 7. In this picture, the small and large molecular weight water soluble hyaluronans, the two present in the HylaSponge®System are represented as they appear in a gel electrophoresis analysis of the distribution of molecular weights. Hyaluronan is a polymer molecule, which means it consists of a family of molecules with various molecular weights (sizes). That is how they appear in nature as well as in purified forms. The curve above shows the distribution of the small and large molecules. Note that the molecular weights are represented in a logarithmic scale. The peak molecular weight for the small molecules is at 70,000 and the large one is 2,000,000.

The sizes of the two soluble hyaluronan components of the HylaSponge®System are shown in Figure 8 and 9. Figure 8 represents the image of a hyaluronan molecule, taken by the atomic force microscope (AFM), which has an estimated molecular weight of 120,000. The estimate is based on the length of the molecule (0.3 µm) as shown in the picture. From the length one can estimate the molecular weight, that is, approximately 120,000. This size molecule represents a molecule of the small molecular weight population present in the HylaSponge®System. As mentioned above, this small molecular population is spread between 20,000 and 120,000 molecular weight. This means that the smallest molecules in the small molecular weight population are in the range of one sixth of the length shown in the photograph in Figure 8.

Figure 8. The atomic force microscope image of a small molecular weight (120,000) hyaluronan. Note that the size of this picture represents a 1 µm by 1 µm square box. The length of the molecule is approximately 0.3 µm.[8]

The large, about 2,000,000 molecular weight, hyaluronan in a coiled-up form, is shown in a photograph taken with the atomic force microscope (Figure 9). The size of this molecule represents the largest one in the population of the large hyaluronan molecules present in the HylaSponge®System (also see Figure 10).

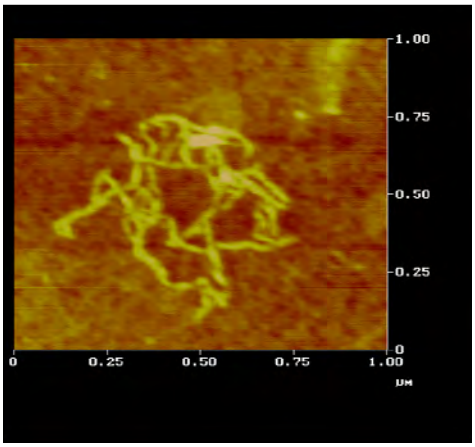


Figure 9. The atomic force microscope image of a coiled-up molecule of approximately 2,000,000 molecular weight. Note that the size of the photograph represents a box of 1 x 1 µm. Consequently the hyaluronan coil occupies a three dimensional domain of approximately 0.5 µm.[9]

[8] This picture is used with permission from the publication by: Spagnoli, C., Korniakou, A., Ulman, A., Balazs, E.A., Lyubchenko, Y.L. and Cowman, M.K. (2005). Carbohydrate Research 340:929-941.

[9] Courtesy of E.A. Balazs and Mary K. Cowman.

Figure 10 shows a representative drawing of the molecule in Figure 9. These hydrated, large molecular coils fill the space between the sponges of the HylaSponge®System.

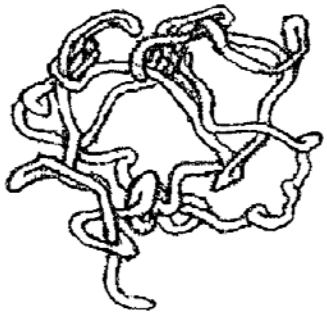


Figure 10. This illustration represents the large molecular coils of hyaluronan pictured in Figure 9.[10]

The large, coiled-up hyaluronan molecular population in an entangled form fills the space between the hyaluronan sponges. This entangled molecular structure is made up of the individual large molecular weight hyaluronan molecules as shown in Figure 11. The space between the hyaluronan molecular chains is filled with water in which small hyaluronan molecules and the biologically active small molecules (like vitamin C) can float.

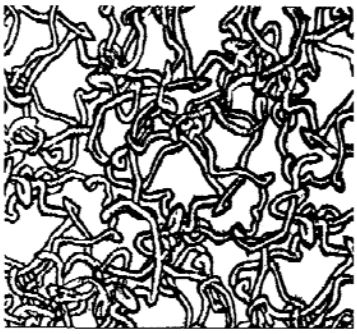


Figure 11. This drawing illustrates the entangled network of the large hyaluronan molecules that fill the space between the sponges.

HylaSponge® with Hyaluronans is a Delivery System. The combination of solid hyaluronan (the sponge) with viscoelastic soluble hyaluronan introduces a new concept for the use of this polysaccharide molecule in skin care. The combination of the high water-retaining capacity of the HylaSponge®System™ filled with two different and highly hydrated hyaluronan molecular populations creates a new dimension of usefulness in skin care technology. This delivery function is based on the design of the System. The specific combination of the three hyaluronans components assures that a large amount of water is retained in the HylaSponge®System for a long time after its application to the skin surface. This retained water provides a "waterway" for the large and small molecular weight hyaluronan as well as the added biologically active molecules (vitamins, among others) to diffuse from the HylaSponge®System into the horny layer and deeper into the skin and exercise their biological functions. This would not be possible without the "water reservoir" created by the HylaSponge®System™. We call it a "waterway", because it creates a path for the hyaluronan molecules and for the biologically active molecules to diffuse from the surface of the skin – from the hydrated sponges – to the horny layer, and from there to the deeper layers of the skin.

Conclusions: The HylaSponge®System combines the benefits of a highly hydrated sponge and the small and large hyaluronan molecules. As a fourth component it can also contain small molecular weight biologically active molecules that penetrate the deeper layers of the skin. (See Figures 6A and 6B)

[10] Courtesy of Matrix Biology Institute

How the HylaSponge®System works on the surface of the skin (see Figures 6 and 12)

- First: the HylaSponge®System does everything that viscoelastic hyaluronan solutions with appropriate molecular weight and concentration can do on the skin. This means that it is a moisturizer, it has a masking effect and a smoothing effect. Consequently, the skin is more hydrated and the wrinkles are filled with partially dried, but still hydrated, hyaluronan which reflects light and gives the skin a smoother appearance. Furthermore it can be used to mask "greasiness".
- Second: the HylaSponge®System does more than viscoelastic hyaluronan solutions. It provides a hydrated "second skin" on the surface of the skin. The large water-insoluble sponges stay on the surface of the skin and hold more water and keep it longer than hyaluronan solutions would. Consequently, the hydration is greater, longer lasting and therefore more effective. The large molecular weight hyaluronan connects the sponges and fills the spaces between them, providing great elasticity and viscosity of the HylaSponge® System. These large hyaluronan molecules are highly hydrated and they penetrate the stratum corneum (horny layer). This extra elasticity becomes important when the HylaSponge® loses some of its water content, because the extra elasticity allows it to remain hydrated for many hours after application.
- Third: the HylaSponge®System is filled with small molecular weight hyaluronan that penetrates into and through the stratum corneum (horny layer) of the skin and provides hydration to this dry surface layer, and eventually reaches the dermis.
- Fourth: One can fill the HylaSponge®System with various small molecular weight, biologically active molecules that are known to penetrate the skin. These molecules, like vitamins and some peptides, can be delivered to the deeper layers of the skin. This is possible because the HylaSponge®System retains its water content for long time. By the structural design of the HylaSponge® one can regulate the extent and duration of its water content. It is important to note that all skin care formulations that do not have hyaluronan can lose water very rapidly on the surface of the skin. When no hydration water is available, the biologically active, water soluble molecules cannot diffuse into the skin. The HylaSponge®System is designed to retain water in order to facilitate the diffusion of water, hydrated small hyaluronan molecules and soluble biologically active molecules into the deeper layers of the skin, as well as the large molecular weight hyaluronan into the horny layer. **The HylaSponge®System forms a highly hydrated elastoviscous "second skin" that protects, fills, hydrates and delivers.**
- Fifth: It is important that a sufficient amount of HylaSponge®System is applied to the skin, thus the amount of HylaSponge®System used in a given formulation is important. **We suggest that the total concentration of hyaluronan in the final product should be between 0.2 to 1.0%.** This range is necessary in order to provide sufficient amounts of all three hyaluronan components (sponges, high and low molecular weight hyaluronan) to provide an appropriate "second skin" and a "waterway" even after the product feels dry on the skin. The HylaSponge®System can be used safely and successfully even at concentrations higher than 1%.

- Figure 12 demonstrates how a drop (0.02mL) of the HylaSponge®System, with a concentration of 0.2% hyaluronan, spreads on the human skin covering the skin and filling the grooves. Use of a higher concentration of the HylaSponge®System will provide more coverage and a longer lasting effect.

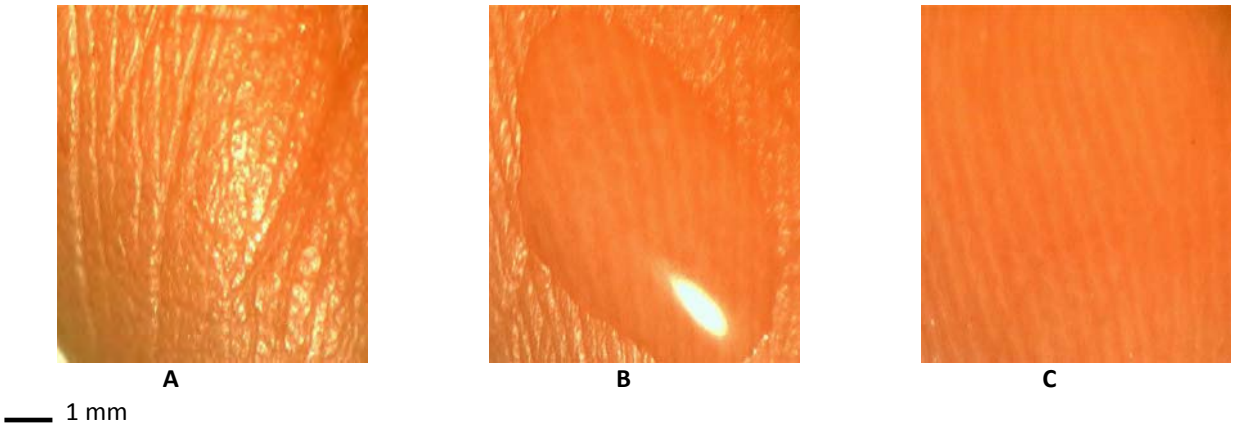


Figure 12. A drop (0.02 mL) of the HylaSponge®System, containing 40 µg of hyaluronan, was spread on the human skin and photographed under 10X magnification. The concentration of hyaluronan in the HylaSponge® System is 0.2%. A. The grooves on the surface of the human skin before application of the HylaSponge® System. B. The drop of the hydrated HylaSponge®System on the skin. C. The drop spread as a thin layer, demonstrating how it fills and masks the grooves.