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Review Paper

A Review on Hyaluronic Acid

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Abstract: *Hyaluronic acid is one of the important compound which and has many applications in our body. Hyaluronic acid is found in the connective tissues of humans and animals. It plays a key role in cushioning and lubricating the body and is abundant in the eyes, joints and heart valves. A powerful antioxidant, hyaluronic acid is perhaps best known for its ability to bond water to tissue. The unique viscoelastic nature of HA along with its biocompatibility and non-immunogenicity has led to its use in a number of clinical applications, This paper reviews on properties, production, routes of injection, sources and applications of hyaluronic acid.*

Keywords: Hyaluronic acid, Sources, Properties, Applications, Streptococcal sp.

Introduction

Hyaluronic acid (HA) is a glycosaminoglycan (GAG), which is a substance that attaches to collagen and elastin to form cartilage. HA not only helps keep the cartilage that cushions joints strong and flexible, but also helps increase supplies of joint-lubricating synovial fluid. Hyaluronic acid is present in every tissue of the body, and it performs many important functions. It helps deliver nutrients to and carry toxins from cells that do not have a blood supply, such as those found in cartilage, without adequate amounts of HA, the joints will become brittle and deteriorate. Not only does it keep joints lubricated, but hyaluronic acid also encourages water retention in other bodily tissues. It is found in large concentrations in the extracellular matrix, which is the fluid-filled space between cells. HA locks moisture into the Extra cellular matrix, keeping collagen and elastin moist and promoting a youthful appearance.

Hyaluronic acid can be found in high levels in the eyes, cartilage, heart, inter-vertebral discs of the spine and in the fluids of the middle and inner ear. Hyaluronic acid plays an important role in tissue dehydration, lubrication and cellular function. It is produced in the body naturally, however, over time, as with all vitamins and nutrients in the body, the synthesis of Hyaluronic Acid diminishes. In fact, the half-life of Hyaluronic Acid in the cartilage is 2-3 weeks, and only 1 day in the skin. The importance of HA's role in the human body can also be examined by virtue of its distribution in the human body. A 70 kg human contains around 15 g total of HA, all of which is distributed in varying concentrations around the body: about 4 g/kg in the umbilical cord, 2-4 g/L in the synovial fluid, 0.2 g/kg in the dermis, about 10 mg/L in the thoracic lymph, and approximately 0.1-0.01 mg/L in normal serum.

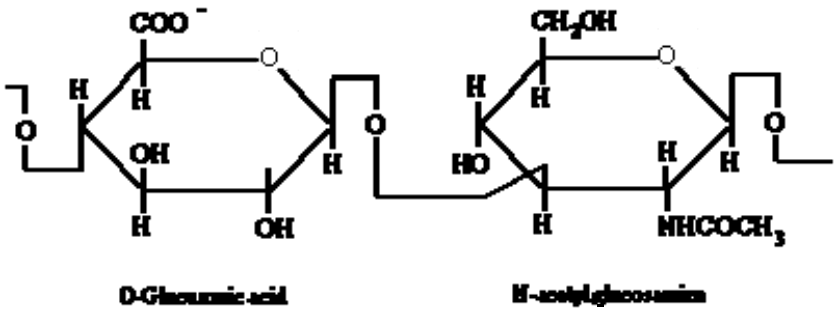

Figure 1: Monomer of hyaluronic acid

When the decline in hyaluronic acid synthesis begins to happen, one may experience joint discomfort, wrinkles and a tendency to get sick frequently, So, if interested people can inject hyaluronic acid in the body to retain back the health up to an extent, but the injection of hyaluronic acid depends on the interest a person[1] .

Hyaluronic acid is a polymer that is made up of many units of disaccharides. A disaccharide is made up of two units called monosaccharides, which are the smallest sugar units. In the case of hyaluronic acid, the disaccharide is glucoronic acid and acetylglucosamine linked together into one unit. Hyaluronic acid can be up to 25,000 disaccharides long.

Hyaluronic acid is fairly stable, in part because of the way that its disaccharide components are positioned. The bulkier parts of the molecule are spaced far apart. By lowering this crowding, which is also known as "steric hindrance," the molecule is able to be flexible, but also resistant to breaking down[2]

Properties of Hyaluronic Acid: Hyaluronic acid (HA) is a biopolymer found naturally in the body. It is a linear polysaccharide (‘many sugars’) with unique physical and biochemical properties such as viscoelasticity and water-binding ability. The body uses many different types of molecules for its supportive tissue, especially cartilage. In addition to being present in the joints, it is also used to hold cells together and as a signal for other cells within the body.

When incorporated into a neutral aqueous solution hydrogen bond formation occurs between water molecules and adjacent carboxyl and N-acetyl groups. This imparts a conformational stiffness to the polymer, which limits its flexibility. The hydrogen bond formation results in the unique water-binding and retention capacity of the polymer. It also follows that the water-binding capacity is directly related to the molecular weight of the molecule. Up to six liters of water may be bound per gram of HA .

Water Absorption: One of the most biologically relevant properties of hyaluronic acid is its ability to absorb water. Hyaluronic acid, because of its many carbohydrate subunits, is able to absorb large quantities of water, which causes it to become somewhat gel-like. As a result, it is used by the body to increase the viscosity of fluid in joints.

Molecular Weight: The molecular weight of HA from different sources is polydisperse and highly variable ranging from 10^4 to 10^7 Da. The extrusion of HA through the cell membrane as it is produced permits unconstrained polymer elongation and hence a very high molecular weight molecule.

Specific Gravity: The Density of hyaluronic acid is: 30 - 200 kg/m3 (Water = 1)

Sources of Hyaluronic Acid: Hyaluronic Acid (HA) is not in the diet in any appreciable amounts. The only way one could receive HA was through a synthetic HA that is injected. It is commonly used for knee problems, wound healing, and cosmetic surgery. A new breakthrough in research has found a natural Hyaluronic acid, at a much more reasonable price than these costly synthetic injections, which is absorbed orally Conquer HA.

Hyaluronic acid is mostly found in animals, though there are a variety of non-meat options that can help increase hyaluronic acid production in the body.

- soy is a vegetable option, Because of its tendency to increase levels of estrogen in the body which in turn increases levels of hyaluronic acid. Tofu, or soy bean curd, is the most versatile soy food. Soy milk, soy ice cream or soy yogurt are a few other options. Soy can also be found as textured vegetable protein.
- Magnesium is essential for hyaluronic acid synthesis, a lack of magnesium in the diet may be part of the cause of low hyaluronic acid levels. Soy is rich in magnesium, as is spinach, green beans, broccoli, cauliflower, asparagus, potatoes, green lettuce and carrots.
- A variety of magnesium-rich fruits are available: apples, bananas, strawberries, tomatoes, avocados, pineapples, oranges, papayas, melons, peaches and pears. Legumes such as kidney beans, pinto beans, black-eyed peas and lentils are rich in magnesium. Peanuts and almonds also contain magnesium.
- Low levels of hyaluronic acid have also been found in individuals with low zinc levels. Zinc is utilized for a variety of bodily processes. Usually associated with foods rich in protein, such as beef, lamb, pork or chicken, there are a few non-meat options.
- The best vegetable source for zinc is pumpkin seeds. Potatoes are also a good source, as is yeast, peanuts, beans, whole grains or brown rice.

Production Media

Medium composition preferably for per liter
glucose----------12 g
magnesium sulfate----------0.2 g
potassium dihydrogenphosphate ------0.4 g
yeast extract ----------1.0 g
yeast peptone ----------3.0 g
uridine ----------0.15g
Water ----------1 L

Optimum Conditions for Growth: Optimum conditions are the ones where the optimum activity is affected by Temperature, pH of culture media. The optimum condition for *Streptococcus zooepidemicus* are
Temperature: 33° C. to 38° C
pH: 7.0 to 7.5
Aeration rate: 0.1 to 1.0 VVM.
Agitation : 600 rpm

Production of Hyaluronic Acid: *Streptococcus zooepidemicus* was inoculated in 25 ml of Todd-Hewitt

liquid medium, and cultured with shaking at 37° C. for 14 hours. Then, 2.5 ml of the resulting culture was inoculated again in 25 ml of Todd-Hewitt medium and cultured with shaking at 37° C. until exponential growth phase was reached. Thereafter, 1 ml of the culture was centrifuged at 4° C.(5,000×g, 5 min.), and the precipitate was collected and washed twice respectively with 1 ml of Tris-maleate buffer(Tris 6 g, pH 6.0, maleic acid 5.8 g, ammonium sulfate 1 g, ferrous sulfate 0.25 mg, magnesium sulfate 0.1 g, calcium nitrate 0.005 g per 1 L). The resulting cell pellets were resuspended in 1 ml of the above buffer solution. 1 ml of the resulting suspension was inoculated in 25 ml of Todd-Hewitt medium and then cultured with shaking at 37° C. for 18 hours to increase the number of viable mutant cells[3] .

The resulting culture was diluted with physical saline, and the diluted solution was plated on a Todd-hewitt agar medium containing 5% blood and incubated at 37° C. for 48 hours. and stored at 4° C. until use.. Since colonies having hemolytic activity form clear zones around them, the colonies without clear zones were selected as mutant colonies lacking hemolytic activity.

Then into a 5 L fermenter 3 L of culture medium containing 60 g of glucose, 1.0 g of magnesium sulfate, 2.0 g of potassium dihydrogenphosphate, 5.0 g of yeast extract, 15.0 g of yeast peptone and 0.75 g of uridine per 1 l of medium, and the medium was steam-sterilized at 121° C. for 20 min. Then, 150 ml of the above seed culture was added thereto, and cultured while maintaining the pH of 7.1 and temperature of 35° C. with aeration rate of 0.5 vvm and agitation of 600 rpm for 24 hours.

Applications: The three main categories of uses for hyaluronic acid are: anti-aging treatments, nutritional supplements, and medical treatments.

Hyaluronic Acid and Cartilage: Hyaluronic acid is one of the primary "lubricating" parts of joints. Hyaluronic acid binds to a protein called aggrecan. This complex is what makes cartilage so resilient to compression. Hyaluronic acid also gives skin its elasticity

A healthy joint contains a constant amount of hyaluronic acid, which has several very important functions:

- Hyaluronic acid gives the synovial fluid its characteristic viscoelastic properties. It enables the fluid to act as a lubricant, shock absorber and a filter controlling the movement of cells and large molecules within the joint
- Hyaluronic acid forms a coating over the entire inner surface of the joint, which acts as a viscoelastic shield over the articular cartilage and protects it from mechanical damage. This coating also protects the cartilage and synovium from free radicals and other inflammatory factors. In addition, hyaluronic acid protects from pain by masking the local nociceptors.
- Hyaluronic acid forms the backbone of the proteoglycan aggregates that are essential for the structural and functional integrity of the articular cartilage[4,5].

Anti-Aging Supplements and Creams: As we age, hyaluronic content in the skin changes due to two separate clinically proven factors, decrease in HA synthesis and shift from concentration from the epidermis to the dermis. These changes can leave the epidermis depleted in hyaluronic acid resulting in thinning, aging, and decreased moisture in the skin. Nutritional supplement and beauty products such as Episilk contain ultra-high concentrations of hyaluronic acid in order to provide intensive moisture for aged skin that suffers from the dryness and wrinkles. When applied to the skin HA can penetrate the skin surface and replenish the natural levels already present. Like hyaluronic acid produced in the body, hyaluronic acid used in this form moisturizes from the dermis to the epidermis from deeper layers of the skin to the outer layer.

Nutritional Supplements: Hyaluronic acid extracted from rooster combs cannot be absorbed by the intestinal tract. But, Japanese scientists have developed a proprietary enzyme-cleaving technique to lower the molecular weight of hyaluronic acid without altering its chemical nature. This allows hyaluronic acid to be taken orally as a nutritional supplement, many varieties of which are readily available for commercial use.

Facial Injections: Hyaluronic acid based gel injections are a new option for the on-spot treatment of facial wrinkles. These shots must be administered by a dermatologist and last up to 4 to 6 weeks[6].

Medical Treatments: Products containing hyaluronic acid are in use or are being researched to aid in the prevention and treatment of symptoms related to connective tissue disorders such as: fractures, hernias, glaucoma, keratoconus, detached retinas, osteoarthritis, TMJ, prevention of scarring, vocal chord repair insufficiency, wrinkled skin, cartilage damage, and wound and ligament healing. Of these, osteoarthritis has recently become a particularly popular area for HA treatment. Physicians have injected hyaluronic acid directly into the synovial fluid in the knee as a treatment for osteoarthritis of the knee for the past 20 years[6].

Benefits and Possible Dangers of Hyaluronic Acid: As hyaluronic acid is a naturally occurring chemical already present in skin, there is little chance of an allergic reaction or the body rejecting the injection. As a result, one of the benefits of hyaluronic acid treatments is that no skin test is required to determine the patient's reaction, as with collagen, the treatment can simply be administered when the patient desires. Hylaform injections immediately remove wrinkles, and the results last for many months. Unlike artificial substances, hyaluronic acid is not only natural but known for its health-promoting qualities, ABC

News documented a village in Japan whose residents lived particularly long lives, which was at least in part attributed to diets rich in hyaluronic acid.

There are potential dangers of certain hyaluronic acid treatments, however. Patients with avian allergies may not be eligible for Hylaform or Hylaform Plus, as the hyaluronic acid is harvested from rooster combs. There are potential reactions to the injection as well, including temporary discomfort, discoloration, and swelling. However, in general these reactions are less severe and pass more quickly than those from other injectable treatments.

Different Microbial Sources: HA has been produced commercially since the early 1980s through fermentation of group C streptococci, in particular *Streptococcus equi* subs. *equi* and subs. *zooepidemicus* (Yamada and Kawasaki, 2005).Table 1 gives information about different streptococcal strains used in the production of HA

Routes of Injection: HA has also been extensively studied in ophthalmic, nasal and parenteral drug delivery. In addition, more novel applications including, pulmonary, implantation and gene deliver have also been suggested which are tabulated in Table 2.

Table 1
Different Microbial Sources

S. No.	Microorganism	Biomass and HA yield HA molecular weight	References
1.	*S. equi* subsp *zooepidemicus* (ATCC 35246)	X: 3.67 g/L, [HA]: 2.46 g/L MW: 2.5MDa	Vazquez et al., 2010[7]
2.	*Streptococcus* sp. ID9102 (KCTC 1139BP)	X: 3 OD600, [HA]: 6.94 g/L MW: 5.9 MDa	Im et al, 2009[8]
3.	*S. zooepidemicus* (ATCC 39920)	X: 2.43 g/L, [HA]: 2.15 g/L MW: n.d.	Wu et al., 2009[9]
4.	*S. zooepidemicus* WSH 24	X: 16.3 g/L, [HA]: 6.6 g/L MW: n.d.	Liu et al., 2008[10]
5.	*Streptococcus*		Blank et al. 2008[11]

Table 2
Routes of injection

S. No.	Route	Justification	Publications
1.	Ophthalmic	Increased ocular residence of drug, which can lead to increased bioavailability	Jarvinen et al., 1995, [12] Sasaki et al., 1996, [13]Gurny et al., 1987, [14] Camber et al., 1987, [15] Camber and Edman, 1989, [16]
2.	Nasal	Bioadhesion resulting in increased bioavailability	Morimoto et al., 1991, [17] Lim et al., 2002. [18]
3.	Pulmonary	Absorption enhancer and dissolution rate modification	Morimoto et al., 2001, [19] Surendrakumar et al., 2003. [20]
4.	Parenteral	Drug carrier and facilitator of liposomal entrapment	Drobnik, 1991, [21] Sakurai et al., 1997, [22] Luo and Prestwich, 1999, [23] Luo et al., 2000, [24] Prisell et al., 1992, [25] Yerushalmi et al., 1994, [26] Yerushalmi and Margalit, 1998, [27] Peer and Margalit, 2000, [28] Eliaz and Szoka, 2001, [29]Peer et al., 2003. [30]
5.	Implant	Dissolution rate modification	Surini et al., 2003, [31] Takayama et al., 1990. [32]
6.	Gene	Dissolution rate modification and protection	Yun et al., 2004, [33] Kim et al., 2003. [34]

Conclusion

Hyaluronan is a strategic biopolymer of primary scientific interest also because of the multiplicity of applications in cosmetic and biomedical fields. For this reason research is continuously growing in many interdisciplinary fields attempting on one side to the improvement of biotechnological production processes on and another side to the development of new hyaluronan formulations/HA-based new materials. Research is promoted by the commercial demand for satisfying improvements in any established application or foreseen novel uses. Future clinical therapies of HA-derived materials critically rely on a more detailed understanding of the effects of HA molecular weight and concentration and how this biomolecule specifically interacts with cells.

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