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Review

Overview of hyaluronic acid and its development as dermal fillers gel

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ABSTRACT

Since the late 1990s, when the first commercially available hyaluronic acid-based tissue filler appeared, HA fillers have become the "product of choice" of soft tissue augmentation due to their favourable safety profile and minimally invasive nature of the treatment. Recent years have increased the popularity of HA fillers among doctors and patients and the number of available HA fillers and indications for aesthetic treatments. Due to the greater availability, and growing awareness among patients, HA filler treatments have become one of the most frequently performed cosmetic procedures worldwide. For several decades, we have been observing the development and progress of improving fillers to increase the safety and effectiveness of performed treatments.

INTRODUCTION

Hyaluronic acid (HA) is a high molecular weight (1.2 – 10^4 kDa) naturally occurring biodegradable polymer (1). HA is an unbranched non-sulfated glycosaminoglycan (Fig. 1) (2–4). HA can include several thousand-sugar molecules in the backbone. HA is a polyanion that can self-associate and bind to water molecules (when not bound to other molecules), giving it a stiff, viscous quality similar to gelatine (5). In a physiological solution, the backbone of a hyaluronan molecule is stiffened by a combination of the chemical structure of the disaccharide, internal hydrogen bonds, and interactions with the solvent. The axial hydrogen atoms form a non-polar, relatively hydrophobic face, while the equatorial side chains form a more polar, hydrophilic face, thereby creating a twisting ribbon structure. Hyaluronan solutions manifest very unusual rheological properties and are exceedingly lubricious and hydrophilic (5).

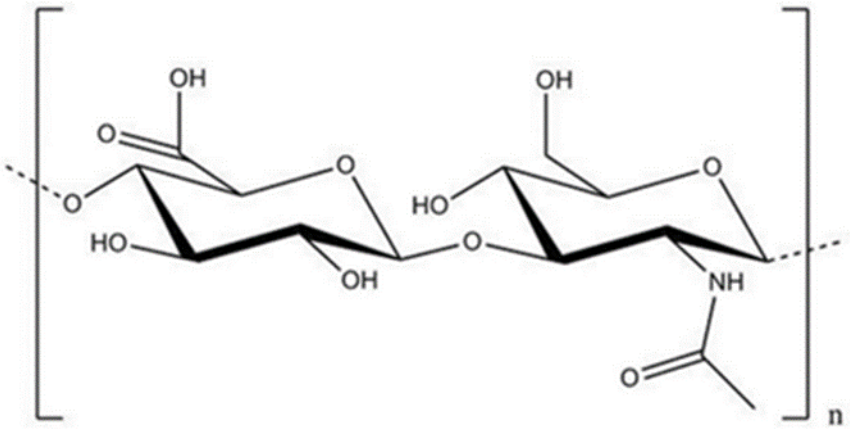

Fig. 1. *Chemical structure of hyaluronic acid (HA), which is made of disaccharide repeats of N-acetylglucosamine and glucuronic acid.*

In an average human body (70 kg), the total HA content is approximately 15 g (3, 5). From this 15 g, the largest amount is in the extracellular matrix (ECM) of skin and musculoskeletal tissue. HA's characteristics, consistency, biocompatibility, and hydrophilicity have made it an excellent moisturiser in cosmetic dermal fillers in dermatology and skin-care products (5). Moreover, its unique viscoelasticity and limited immunogenicity have led to its use in several biomedical applications, such as viscosupplementation in osteoarthritis treatment, as an aid in eye surgery, and wound regeneration (4, 5). In addition, HA has currently been explored as a carrier for different routes such as nasal, oral, pulmonary, ophthalmic, topical, and parenteral (5, 6).

History of hyaluronic acid

In 1934, Karl Meyer and his colleague John Palmer were the first investigators who discovered and isolated HA from the vitreous body of cows' eyes (3, 5). In the 1950s, the chemical structure of HA was solved by this group. They found that HA is composed of two sugar molecules (D-glucuronic acid (known as uronic acid) and synt-acetylglucosamine) and called it hyaluronic acid (hyaluronan). This name is derived from "hyalos" (the Greek word for glass + uronic acid). Initially, they isolated HA as an acid, but it behaved like salt in physiological conditions (sodium hyaluronate) (3, 5, 6). Several years after them, in 1942, Ender Balazs patented the first application of HA as a substitute for egg white in bakery products (5).

Properties of hyaluronic acid

Structural studies showed that the two sugar molecules, D-glucuronic acid and D-N-acetylglucosamine, in the HA disaccharide structure are connected through alternative β-1,4 and β-1,3 glycosidic bonds (Fig. 1) (3, 5). HA's at very low concentrations, chain-entangle each other, leading to a mild viscosity (molecular weight dependent). On the other hand, HA solutions at higher concentrations have a higher-than-expected viscosity due to greater HA chain entanglement that is shear-dependent. For instance, a 1% solution of high molecular weight HA (Mw > ~1000 kDa) can behave like jelly, but when shear stress is applied, it will easily shear thin and can be injected via a thin needle (5). As such, HA is known as a "pseudo-plastic" material. HA solutions' rheological property (concentration and molecular weight dependent) has made HA ideal for lubrication in biomedical applications (5).

Hyaluronic acid performs several structural tasks in the extracellular matrix (ECM), binding with cells and other biological components through specific and non-specific interactions. Several extracellular matrix proteins are stabilised upon binding to HA. Specific molecules and receptors that interact with HA are involved in cellular signal transduction; molecules such as aggrecan, CD44 is a structurally variable and multifunctional cell surface glycoprotein on most cell types and is perhaps the best-characterised transmembrane HA receptor so far. Due to its wide distribution and based on current knowledge, CD44 is currently considered to be the primary HA receptor on most cell types (7).

In vivo synthesis, degradation and elimination of hyaluronic acid

In vivo, Hyaluronic acid is a natural polymer biologically synthesised by cells in the body via an enzymatic process. HA production is a unique, highly controlled, and continuous process; HA is produced and secreted by cells, including fibroblasts, keratinocytes, or chondrocytes.

Degradation of HA is a step-wise process that can occur via enzymatic or non-enzymatic reactions. Three types of enzymes (hyaluronidase, β-D-glucuronidase, and β-N-acetyl-hexosaminidase) are involved in the enzymatic degradation of hyaluronic acid. These enzymes are found in various forms, in the intercellular space and serum. Non-enzymatic mechanisms of HA degradation include thermal or shear stress. Heat is another type of stress leading to HA degradation. Rheological studies on HA solutions showed that increasing temperature resulted in degradation and decreased viscosity exponentially as a function of temperature (8). In addition, chemical reactions such as acidic/alkaline hydrolysis and oxidant degradation are categorised as non-enzymatic degradation pathways for HA (8–10).

Depending on the location in the body, most of the HA is catabolised within days. Studies indicated that the normal half-life of HA varied from 1–3 weeks in inert tissues such as cartilage to 1–2 days in the epidermis of the skin. The half-life in blood was much shorter (2–5 minutes). Besides the enzymatic degradation and non-enzymatic degradation pathways described previously, two more pathways are engaged in HA catabolism:

turnover (internalisation and degradation within tissue) and release from the tissue matrix, drainage into the vasculature, and clearance via lymph nodes, liver, and kidney (Fig. 2) (3).

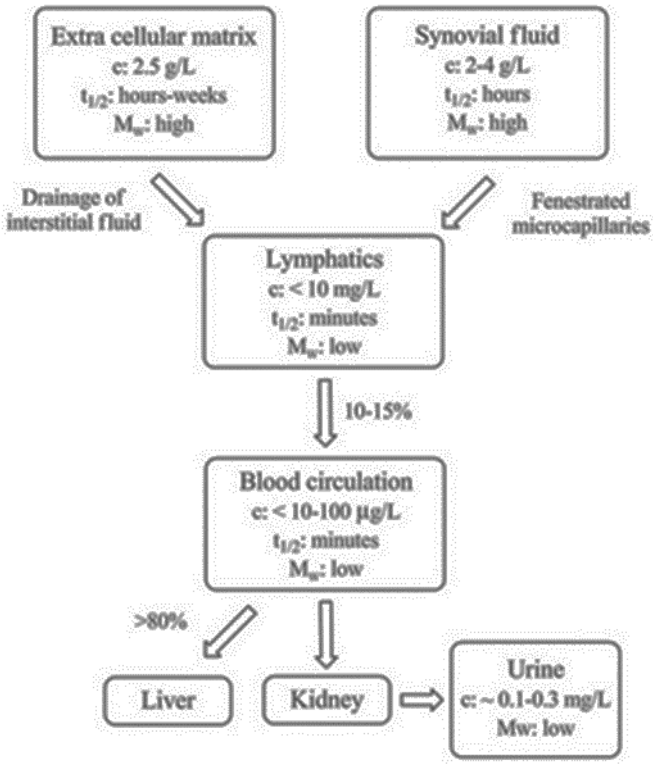

Fig. 2. *HA catabolic pathway in the body. Arrows show the flow of HA. Concentration (c), half-life ($t_{1/2}$), and molecular weight (M_w) of HA within the organ systems are indicated. Figure was regenerated with permission (2). High Mw > ~1000 kDa and low Mw <~450 kDa.*

Application of hyaluronic acid as a dermal filler

The influence of sun exposure, gravity, and years of facial muscle movements appear as wrinkles on the skin. During the ageing process, basic changes in the skin, soft tissue, and skeletal support of the face occur, resulting in a breakdown of the tissues under the skin, leaving lines or other facial defects (11, 12). The development of soft-tissue fillers (dermal fillers) can help lines and wrinkles to be filled temporarily (or `permanently') (11). It seems an ideal dermal filler should be temporary but long-lasting (months to a year or longer), have minimum side effects and no allergenic effect, easy to administer, have minimum pain or no pain upon injection, and have a reasonable cost for both the physician and the patient (11). Depending on the residence time in the tissue, dermal fillers are categorised as temporary, semi-permanent (at least 18 months), and permanent. They are also classified based on their composition with primary ingredients such as collagen (bovine, porcine, or human), animal or bacterial hyaluronic acid, poly-L-lactic acid, calcium hydroxyapatite, polymethyl methacrylate, and polyacrylamide gel (4, 11-15).

HA can be obtained from different sources, including animal tissues and microorganisms. After Meyer and Palmer first isolated HA from bovine vitreous-humor, it was also extracted from other tissues, such as the umbilical cord, rooster combs, and synovial fluid. However, HA from animal sources has sparked concerns due to the high costs of the extraction process, low yields, and side effects. One of the main concerns was the possibility of immune responses and allergies triggered by other substances, such as host proteins and DNA in animal-derived HA (16). Most modern HA fillers are derived from bacterial HA because of their reduced allergenic and immunogenic potential (17).

Hyaluronic acid has been approved by the Food and Drug Administration (FDA) as a dermal filler. In 2006, cosmetic injections of HA were known to be the second most popular non-surgical procedure for women and the third most popular procedure for men (18–21). HA has a very short half-life and is chemically cross-linked to extend duration as a dermal filler (22).

The two main cross-linkers employed in HA dermal fillers currently on the US market are 1,4-butanediol diglycidyl ether (BDDE) and divinyl sulfone (DVS). Both react to hydroxyl groups on HA chains and give similar outcomes, slowing down the drainage and degradation of dermal fillers injected into the skin (12). Also worth mentioning is the natural cross-linker genipin is also worth mentioning, which is a cross-linkers with low cytotoxicity and swelling ratio (23).

New emerging technology is cross-linking HA with Polyethylene glycol (PEG). PEG is a well-known polymer on the pharmaceutical market, but it has been used for the first time in filler. PEG's outstanding advantages are low toxicity and completely degradable in the tissues and reducing the action of proteolytic enzymes, like hyaluronidase (24, 25). The protective action of PEG can mask the implant from the host organism reducing the risk of immunological reaction and formation of the foreign body giant cells (FBGC) (Fig. 3) (25). Since its launch in Europe in 1996, HA has become the "gold standard" in fillers for treating wrinkles, hydrating skin, and increasing volume (4). It is believed to be currently more than 200 brands of dermal fillers in production to date.

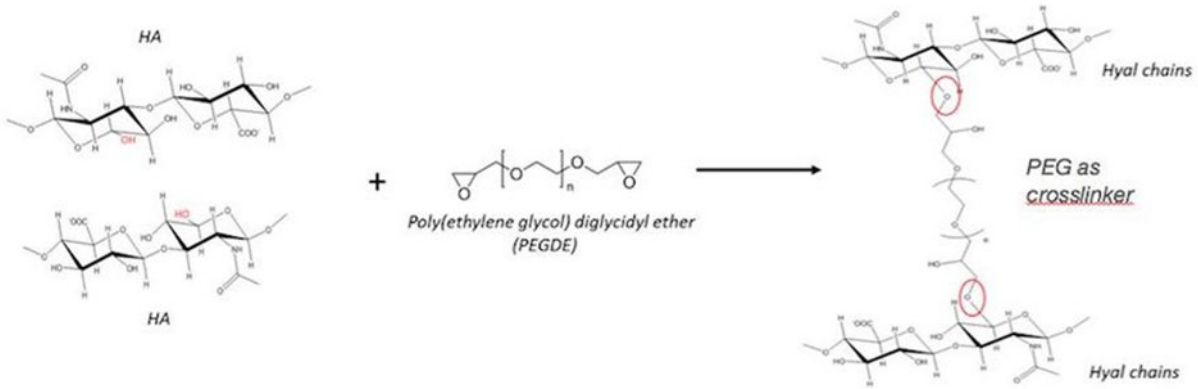

Fig. 3. *Hyaluronic acid (HA) cross-linking with poly (ethylene glycol) diglycidyl ether (PEGDE).*

In the US, Hylaform ® was the first available HA-based filler. Hylaform® is a sterile, colourless gel material in which HA is cross-linked with divinyl sulfone. It is FDA-approved (April 2004) and derived from an avian source (rooster comb). Captique™ is the second FDA-approved (December 2009) manufactured based on Genzyme's patented non-animal stabilised HA technology; this prevented the possibility of immunological issues related to the previous avian source HA. Again, the HA is 20% cross-linked by divinyl sulfone Captique™ was also shown to have a treatment duration anywhere between 3 to 6 months (19).

The next HA-based dermal filler approved by FDA in the US was Restylane® (December 2003). It is a non-animal HA derivative made from the fermentation of equine streptococci and cross-linked with 1,4-butanediol diglycidyl ether (BDDE). The HA (20 mg/ml) is only lightly cross-linked (1%) with a particle size of ~400 μm. Restylane® has a lower degree of cross-linking with different cross-linking chemistry, higher HA concentration, and smaller particle size compared to previous products. The FDA approved this product for mid-dermal applications, including deep wrinkle correction, lip augmentation, nasolabial fold correction, and glabellar creases. The application of Restylane® in clinical trials dates back to the 1990s. In two European clinical trials by Duranti et al. and Olenius (both in 1998), the effect duration was ~8 months. Numerous

doctors have employed Restylane® for several years with acceptable results (~6 to 12 months). It was manufactured by Lea Derm, a subdivision of Corneal Group (Paris, France). It is a non-animal stabilised HA containing high cross-linked HA made by Streptococcus equi bacteria. Six different formulations of Juvéderm™ have been developed with concentrations ranging from 18 mg/g to 24 mg/g. These are known to be highly viscous dermal fillers. The HA is cross-linked (6%) with a patented single-phase BDDE chemistry, phosphate buffered to 6.5–7.3 pH (26). The higher HA concentration and greater cross-linking support the notion that treatment via Juvéderm™ can last between 6 to 12 months (19). The main differences between these dermal fillers are the source of HA, concentration, the particle size of beads (if included in the formulation), degree and type of cross-linking, and inclusion or lack of anaesthetic. For the available dermal fillers, the source of HA is either avian (found primarily in rooster combs) or bacteria (mainly from the synthetic fermentation of the *Staphylococcus equine bacterium*). HA is derived by crushing reaction and extraction, which could result in residual endotoxin and chemical solvent contaminants in the HA. Until recently, Neauvia Filler derives differently from the bacteria *Bacillus subtilis*. This bacterium, which is non-pathogenic to humans, HA is purified in a process based on water usage; this would probably give an advantage to *Bacillus subtilis* hyaluronic acid; however, the HA from *Bacillus subtilis* does not exist any longer because the only company that was manufacturing such a HA shut down the production in 2017. Neauvia is manufactured today with a pure HA from *Streptococcus Equi* (25, 27).

The concentration of HA has a vital role in dermal fillers. Dermal fillers with high HA concentration tend to have better high projection or volumization and longer duration of effect; and, with high viscoelastic properties, less amount of product need to be injected to achieve the same aesthetical correction; however, higher extrusion force will be needed when injecting the gel using fine needles. In all HA fillers, a single ether cross-link was typically utilised. Recently, HA fillers have utilised greater degrees of cross-linking. Cross-linking can improve resistance to degradation, making for a longer-lasting treatment, but increasing the difficulty of injection (28). With PEG cross-linking, the hydrogel has distinct properties; it has less water intake (swelling), more stability and keeping more of the starting rheological properties also with a longer duration in the tissues with a higher viscosity and high cohesivity, which will have less chance to displace or migrate while bio-integrating more in the tissues.

Current HA-based dermal fillers have a maximum treatment duration of up to 12 months. To extend this treatment duration, HA with higher molecular weight or more cross-linking can be used; however, the injectability of the HA solution drops by increasing HA molecular weight and cross-linking, which makes the dermal filler challenging to administer, possibly producing pain for the patient. Therefore, the development of dermal filler products with enhanced injectability and more prolonged duration is desired.

DISCUSSION

Future perspectives

Hyaluronic acid is a naturally occurring biomolecule available in body tissues and fluids. Due to the prevalence of hyaluronic acid in the body and its desirable properties, HA has been utilised in several biomedical products. This article reviewed HA's physical and chemical characteristics applied to dermal filling. In the application, difficulties such as the potential toxicity of cross-linking techniques, the high viscosity of HA solutions, and rapid elimination have been raised as limitations to improving biomedical products derived from HA. In order to overcome these limitations, current and emerging strategies to modify HA were reviewed as potential approaches. In particular, new strategies for safely cross-linking HA with PEG as it reduces inflammation and granuloma formation.

Moreover, it delays the degradation and the bio-absorption of the implant. PEG makes Neauvia fillers the first and only BIOMIMETIC fillers worldwide. Formulations that improve product performance will also utilise physical interactions between components (e.g., interactions between HA nanoparticles). Utilising particle/polymer or particle/particle interactions may provide new approaches to tune the viscoelastic properties of HA formulations as applied to tissue engineering and dermal filling (29, 30).

Given their worldwide success and popularity, HA fillers will likely remain a "golden standard" in the aesthetic and cosmetic proceduralist's toolbox. Innovations in the field – ranging from methods of biochemical engineering to novel techniques for injection and treatment – all lend great promise to the future of HA fillers in aesthetic practice. Continuing improvements in injection technique also offer a promising future for HA filler use. Blunt-tipped cannulas are rising in popularity as an alternative to hypodermic needles, as their use is associated with a statistically significant decrease in bruising and lower pain associated with the injection. Another area that can be subject to continuous development is exactness in volumetric dosing is highly dependent on injector experience, as elements such as HA filler viscosity, needle gauge, and plane/site of injection all influence the force needed to inject a bolus of filler agent. In order to elevate this aspect of treatment beyond volume marking on syringes and "injector feel" while introducing a bolus, companies are working on new technical solutions to ensure the precision of product dosing (31).

CONCLUSIONS

In conclusion, the continuous development and improvement of the physicochemical properties of hyaluronic acid-based fillers will benefit both patients and doctors, significantly improving the safety of treatments, reducing the amount of product used and improving the procedure results.

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