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# Hyaluronic Acid Aesthetic Fillers: A Review of Rheological and Physicochemical Properties

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**Synopsis**

Hyaluronic acid's (HA) main functions are absorbing water into the tissues and structuring the skin. It is mostly used in dermal fillers, treatments for certain diseases, and wound healing. This study intends to review current literature of HA's rheology and its physicochemical properties as an injectable filler. Data were acquired from articles concerning HA-based biomaterials published within the last 25 years in PubMed and ScienceDirect. The MeSH terms "hyaluronic acid" and "dermal fillers," were used either alone or combined with "rheology," "physicochemical concepts," "cross-linking reagents," "viscoelastic substances," "cohesivity," and "cosmetic techniques." Some articles not found during the initial search were chosen from the reference lists of previously selected publications. All articles that fit in the theme were considered valid regardless of study type. Available literature describes intrinsic properties of HA as a glycosaminoglycan. As an injectable filler, its rheology (viscoelasticity and cohesivity) and its physicochemical properties (cross-linking, hydrophilia, particle size, and HA concentration) define its clinical behavior by influencing its longevity, lifting capacity, resistance to external forces, and needle extrusion force. HA is promising as a dermal filler and healing agent. Understanding its properties is essential, as each patient benefits from different products. Future research should continue to explore these properties.

## INTRODUCTION

Hyaluronic acid or hyaluronan (HA) is the most abundant glycosaminoglycan present in the human dermis, with around 50% of total body HA being found in the skin, and a component of all mammalians' connective tissue. HA constitutes a compound of cell surfaces and extracellular matrix in the skin, eyes, joints and muscles, and umbilical cord. Its main functions are to draw water into the tissues, volumizing and giving structure to the skin by binding collagen and elastin fibres into a supportive matrix.[1-4] Additionally, HA intervenes in inflammation regulation, drug delivery, angiogenesis, cell migration and proliferation (caused by HA binding itself specifically to proteins that are responsible for these processes, whether they are in the extracellular matrix, on the cell surface, where they're called hyaladherins, or in the cellular cytosol), wound healing (based on its

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antioxidant properties and ability to eliminate free radicals), and cancer progression (based on its hydrodynamics and ability to interact with tumour cell surfaces and influence the porosity and malleability of extracellular and pericellular matrices, and also based on the fact that an increase in HA links to apoptosis, invasiveness, and drug resistance).[5-12]

HA was first discovered in 1934 at Colombia University of New York by two American scientists, Karl Meyer and John Palmer, when they isolated it from bovine vitreous humor.[1,5] HA was then commercialized for the first time by Endre Balazs, who used it as a substitute for egg whites in bakery products. More recently, HA has been used for numerous purposes, such as wound treatment, ophthalmic surgery, drug delivery, arthritis treatment (serving as an intra-articularly injected lubricant), and aesthetic treatment.[1,2,13] The first biocompatible gel (hylan B gel, Hylaform) was created in 1980 by Balaz,[5] and the first HA filler (Restylane) was approved in the United States in December 2003 for the correction of deep wrinkles and folds.[14-17]

In 2006, the American Society of Aesthetic Plastic Surgeons declared HA dermal fillers to be the fastest noninvasive aesthetic procedure in the United States.[1] Currently, there is a wide panoply of different HA-based dermal fillers, each of them manufactured in a distinct way and with different characteristics, with none of them being a universally fitting filler for every situation.[1,5,15,18]

Currently, HA is also used in antiaging treatments, due to its high biocompatibility, volumizing effect, low potential for adverse reactions, reversibility in cases of complications, and possibility of storage without refrigeration for up to two years. Using this polysaccharide as a dermal filler is essential to facial harmonization and can gather many of the desired dermal filler properties. In fact, an ideal filler must be safe, biocompatible, efficient, easy to store, low cost, easily eliminated when necessary, and independent of allergy testing. Thus, clinicians should familiarize themselves with HA's rheological and physicochemical properties as these properties will influence clinical performance. These fillers usually last from 6 to 18 months, depending on several factors such as the HA itself (cross-linking degree, HA concentration, particle size), skin type, medication, age, injection technique, physical activity, and the presence of free radicals in the tissues (particularly in the skin) that quickly degrade uncross-linked HA polymers (depolymerization), among others.[5,14,19-23]

Degradation of HA fillers by free radicals happens due to a transient inflammatory reaction derived from their injection into tissues, and it is caused by the cleavage of glycosidic bonds.[12,23] Oxidative damage and enzymatic degradation represent the two mechanisms through which HA is separated in the body, and these two mechanisms are responsible for the degradation of 30% of the 15g of HA that is locally present. The other 70% of HA is systematically catabolized by the endothelial cells of lymphatic vessels.[12]

## DATA SOURCES AND METHODS

All information used to elaborate the present review was found using the PubMed and ScienceDirect primary databases, where we searched for and selected experimental studies (such as clinical trials and *in vitro* and *in vivo* studies, both in humans and animals) and reviews pertaining to the uses of HA in medicine, particularly those reviews related to aesthetic and healing procedures, and to HA's intrinsic properties. Several research terms were used, such as "hyaluronic acid" and "dermal fillers," both alone and combined with terms such as "rheology," "physicochemical concepts," "water absorption," "cross-linking reagents," "viscoelastic substances," "particle size," "cohesivity," "soft tissue augmentation,"

"aesthetic," and "cosmetic techniques." Any studies using fillers or medicinal products not containing HA were excluded. Research filters were applied for articles published within the last 25 years and written in English or Portuguese. Some of the articles were selected from the reference lists of previously read publications.

## RESULTS

### INTRINSIC PROPERTIES OF HA

HA is a naturally occurring high-molecular-weight polysaccharide belonging to the glycosaminoglycan family and produced in the inner side of the cell membrane, with a natural lifespan in the human body of less than 3 days (24 hours–48 hours), since in its noncross-linked state it is quickly degraded by hyaluronidase in the liver (enzymatic degradation). Whether it is derived from animal or bacterial cultures, its structure is identical, consisting of repeating units of nonsulphated disaccharide, which include molecules of D-glucuronic acid and N-acetylglucosamine, linked by β-(1–4) and β-(1–3) glycosides.[1-3,11,13,18,19] In each monomer, the HA molecule contains a carboxylic acid and a primary alcohol, which are important for recognition by hyaladherins, and an amide, which improves the adhesive properties of the molecule.[12]

Each disaccharide monomer has a molecular weight of around 400 Da, and the complete polymer can reach a total of 10 MDa. There is a proportionate relationship between molecular weight and the number of repeating disaccharides in a HA molecule, and the higher it is, the higher the gel's viscosity. The difference between animal or bacterial HA resides solely in the length of the final polymer chain: bacterial-based HA is usually shorter and has a lower molecular weight than animal-based HA.[14,16,18] Polymer chains of small and medium length usually hold immunostimulant, proangiogenic, and antiapoptotic properties, while larger polymers hold immunosuppressive and antiangiogenic ones.[11,12]

Hyaluronic acid's properties can be divided into rheological and physiochemical. Within rheology, it's possible to identify both viscoelasticity and cohesivity, while physiochemical properties refer to the cross-linking degree, the concentration of HA, the particle size and the water absorption capacity (as is shown in Figure 1).

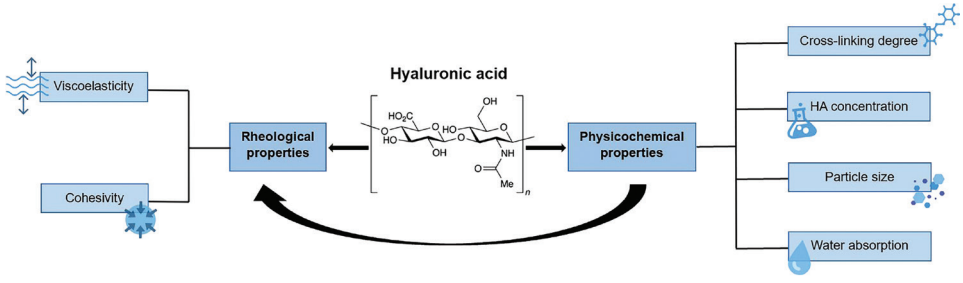


**Figure 1.** The properties of hyaluronic acid.

### RHEOLOGICAL PROPERTIES

*Viscoelasticity.* Rheology is the study of the flow and deformation of materials when subjected to certain forces. This study incorporates different manufacturing processes and HA filler

properties.[24] HA is a viscoelastic gel, which means it has a viscous component and an elastic one. These components, along with its cohesivity, are responsible for defining the gel's capacity to flow through a needle (which decreases with the increase of viscosity) or to return to their original shape after deformation. This means HA will define the filler's properties, such as malleability, extrusion force, lifting capacity, and tissue integration, which in turn will influence its clinical outcomes.[14,25]

The selection of a filler with appropriate characteristics corresponding to each patient also depends on the anatomical area as each area of the face is subjected to different mechanical forces that modify the shape, distribution, duration, and level of correction obtained. There are essentially two types of forces that will act upon the HA: the lateral shear/torsion, which acts in the horizontal plane that is parallel to the skin; and the compressive or stretching force, which acts in a perpendicular plane to that of the skin.[16,19,25,26] The filler's cohesivity, which is responsible for tissue expansion according to a horizontal vector, is determined by the compressive forces. On the other hand, viscoelasticity and the elastic modulus (G'), which are responsible for tissue projection according to a vertical vector, are determined by the lateral shear forces.[24,25]

There are four essential parameters that define a HA gel's viscoelasticity[17-19,22,25027]:

- The shear modulus or complex modulus (G*) is the amount of energy it takes to deform the gel in the horizontal plane. This energy amount determines HA's hardness. It represents the total resistance to deformation.
- The viscous modulus (G") is the fraction of energy that dissipates after deformation, which confirms that the filler is unable to completely restore its initial shape after deformation. G" is clinically related to the filler's injectability (the higher the G", the more difficult the extrusion).
- The elastic/storage modulus (G'), on the other hand, is the fraction of energy that the filler retains after deformation (in other words, its capacity to resist deformation). Fillers with higher G' values are better suited for deeper areas since they are firmer, unlike those with lower G' values, which are softer and better suited for more superficial zones. Higher G' values make a gel harder to inject than lower values.
- Lastly, tan δ corresponds to the G"/ G' ratio and tells us whether a gel is more elastic or more viscous (if G' is higher and the tan $\delta < 1$, then the elastic component is predominant, but if G" is higher and the $\delta > 1$, then the viscous component is predominant).

Most injectable HAs have a lower tan δ, which means they're usually more elastic than viscous. The less viscous gels show higher tissue integration and a more natural appearance, and are thus better suited for more superficial areas.[25,28] Furthermore, these four parameters are influenced by the level of cross-linking shown by the filler. Higher levels of cross-linking usually mean higher levels of G* and G' and lower levels of G".[19,25,26,29] Even though free uncross-linked HA is quickly metabolized (therefore not contributing to the final clinical outcome), it does reduce the filler's viscosity, allowing for an easier injection.[14,18]

It is the different rheological and chemical properties that make it possible to divide the HA fillers into two big groups. The monophasic/cohesive fillers (such as the Juvéderm line) are more homogenous and made up of cross-linked HA chains with varying molecular weights, which make them less elastic and more viscous, while the biphasic/granular fillers (such as the Restylane line) have reticulated HA particles dispersed in either noncrosslinked or very low cross-linked HA, which makes them more fluid and easier to inject. However, there is still debate among the scientific community regarding this division: some authors

argue that all fillers should be considered monophasic as they all have the same composition throughout and believe that there is not a real phase separation.[16,17]

Some fillers also have lidocaine in their composition to reduce the pain that can accompany this treatment, both during the injection and after it. Lidocaine appears to alter some of HA's properties, such as G'.[4,30]

*Cohesivity*. Cohesivity is an essential property for determining the filler's integrity, contributing to it maintaining its microscopic shape after being injected into the patient's tissues.[24] It corresponds to the internal adhesion forces that bind the different cross-linking units together within the gel and translates the resistance to vertical compression/stretching forces. In this way, it defines the initial vertical projection of the dermal filler.[17,19,25] It also has a role in defining the filler's modeling capacity since a less cohesive gel is a more malleable one. However, this property's clinical relevance decreases over time. As the filler is integrated into the tissues, it naturally becomes less malleable and more stuck in place. Cohesivity depends on HA concentration and cross-linking technique, but it is not influenced by the cross-linking degree unlike a lot of other HA properties.[17,25]

It has been suggested that lower cohesivity values contribute to a more uniform distribution of the dermal filler in the tissues, reducing lump formation and allowing for a more superficial placement without inducing the Tyndall effect (when the skin gains a bluish tone due to superficial dermal filler placement),[27] and that the more cohesive a product is, the bigger its tissue integration and lifting capacity are. Still, this property's clinical relevance remains widely debated, due to the lack of standardized measurement techniques and conflicting opinions on cohesivity's true effects on fillers.[16,26]

There are currently four known methods for determining cohesivity, even though none of them tend to provide consistent data: the linear compression test, the average drop-weight, the dye diffusion test, and the Gavard-Sundaram Cohesivity Scale.[16] It has also been noted that measuring a gel's cohesivity before injection is irrelevant, since there is still uncross-linked HA, which will be quickly degraded postinjection making the filler more cohesive.[31]

## PHYSICOCHEMICAL PROPERTIES

*Cross-Linking.* Other deeply important characteristics of HA fillers are their clinical persistence and durability, which are also influenced by the gel's viscous and elastic components in synergy with other important properties. For example, a higher cross-linking degree (which is the percentage of HA disaccharide monomer units bound to a cross-linker molecule), HA concentration, particle size, and molecular weight tend to increase HA's biostability and resistance to degradation over time. Due to these qualities, adding 1,4-butanediol diglycidyl ether (BDDE) or other cross-linking agents to the filler's formula is important as HA's natural duration is one of its main limitations. These cross-linking agents modify the HA by creating "bridges" (ether bonds) between its molecules, which increase the filler's biostability by transforming the filler from a viscous liquid into a gel and making it harder for the filler to be degraded by hyaluronidase and increasing resistance to oxidative stress.[2,3,14,18-21,32] Cross-linking degree is reported by many as the most influential factor for rheological properties, particularly when it comes to gel stiffness.[31]

However, even though they are rare, hypersensitivity reactions tend to happen because of the cross-linking process that is caused by the epoxide groups in the residual BDDE

(a BDDE molecule that hasn't reacted with any other molecules). Because of this, the amount of BDDE used in each filler is maintained at trace amounts (<2 ppm), so that its clinical use can be considered safe. Besides residual BDDE, this agent can be present in dermal fillers in three other states: a fully reacted cross-linker (a BDDE molecule that reacted with HA on both ends), a pendant cross-linker (a molecule that reacted with only one end of HA), and a deactivated cross-linker (hydrolyzed BDDE).[3,5,23] Degrees of cross-linking that are considered too high can lead to problems with biocompatibility because the HA is furthest away from its natural form. As a result, the organism may perceive the HA as more foreign.[4]

In recent years, a different cross-linking agent from BDDE has been studied and more frequently used showing lower levels of cytotoxicity. This agent is part of Neauvia Stimulate, a HA filler of bacterial origin cross-linked with PEG (poly-ethylene-glycol) and 1% of micromolecules of calcium hydroxyapatite. These micromolecules grant the neauvia acid its colagenesis activity, in addition to the filler's volumizing effect. In an *in vitro* study published in 2018, this filler showed no cytotoxicity until at least 24 hours postapplication and caused no alterations to cell viability, morphology, or structure.[33]

*Cross-Linking Technologies.* As has been previously stated, dermal fillers from distinct brands are produced using different cross-linking technologies, which will shape the filler's properties for the target tissue and desired postinjection effect.[31,34] There is a large variety of cross-linking technologies including Vycross, nonanimal stabilized hyaluronic acid (NASHA), and Tri-Hyal.

Vycross, which is used in Juvéderm fillers, is composed of a mixture of high-molecular-weight HA and a significantly higher ratio of low-molecular-weight HA (>1 MDa). These fillers are cross-linked with BDDE at both ends (a fully reacted cross-linker) and show a narrower range of available G' values. They tend to be harder gels and can be noncohesive or partially cohesive.[31,34] Their homogeneous matrix is smoother rather than granular, making them highly malleable with a more even distribution in the tissues. The higher amount of low-molecular-weight HA and a lower overall amount of HA reduces the water absorption of these fillers, thus reducing their swelling. As previously described, the small percentage of noncross-linked HA present helps lower the overall extrusion force.[30] Juvéderm fillers are monophasic and monodensified, which means they are produced by mixing the HAs and cross-linking them in one single moment. In comparison with most other FDA approved fillers, they also present a higher cross-linking degree and lower G' values. Because of these characteristics, they tend to show greater longevity.[35]

Tri-Hyal is a technology applied by Fillmed in their ART FILLER gamma, which is advertised by the manufacturer as consisting of monophasic fillers, and it's characterized by a combination of long chain, very-long chain, and free noncross-linked HA (which facilitates injection extrusion and creates more natural results). The triple cross-linked HAs, all with different molecular weights, provide a more suitable environment for dermal fibroblasts to produce extracellular matrix components, which contributes to skin self-renewal. This makes the filler a good choice as it derives its effect not only from its volumizing action, but also from being a rejuvenating agent. This technology also shows a natural entanglement of HA, which may allow a reduction in the amount of cross-linking agent used (such as BDDE) and a higher sculpting ease, cohesivity, and malleability. This triple cross-linking technique can provide a sustained release of free HA.[36] It also has 0.3% of lidocaine hydrochloride in its composition for anaesthetic properties and a phosphate buffer at pH = 7.2.[37]

NASHA was one of the first technologies used in the dermal filler market, having been used to cross-link Restylane fillers. NASHA fillers contain a HA concentration of 20 mg/mL and consist of low-molecular-weight HA. Restylane fillers are more appropriate for mid-to-deep dermal injections, such as nasolabial folds.[38] In this technology, a small amount of BDDE is added to the filler, meaning the degree of cross-linking ends up being a minute percentage, usually from 1% to 2% in the final product (and 10–15% in the original matrix). After the sizing process, the HA "pearls" (microspheres) obtained are suspended in either a phosphate-buffered solution or noncross-linked HA gel. According to a 2016 study by Micheels *et al*., NASHA fillers are noncohesive and biphasic.[34]

*HA Concentration.* Total HA concentration corresponds to the amount of HA per mL of final product, and it is usually expressed in mg/mL.[14,18] Other than the cross-linking degree and molecular weight, the manufacturing process and total HA concentration (consisting of the insoluble portion of cross-linked HA and the soluble portion of free HA) also influence the gel's viscoelastic and rheological properties (namely its hardness) with lower concentrations creating softer fillers.[14,19,26,29] Fillers with higher HA concentrations also show higher cross-linking degrees and elastic modulus, meaning they are better able to resist deformation and support and volumize the tissues, usually needing to be placed in a deeper plane within them.[24]

It is important to keep in mind that even though there are listed concentrations for each HA filler, sometimes there are variations in the concentration of cross-linking agent and the percentage of cross-linked HA versus uncross-linked HA, which doesn't contribute to the clinical outcome. The same can happen with different batches of the same filler brand. Because of this, the listed concentrations may not always be completely indicative of a product's true performance. Still, there are some brands that share the cross-linking percentage for distinct batches, making it easier for clinicians to correctly choose the most appropriate one for their patients.[32]

*Particle Size.* After a HA filler is cross-linked, it presents itself as a large gel mass. However, it must be able to deform itself to pass through fine-bore needles into the skin. For this to happen, the filler must go through a sizing process, which includes passing it through several sieves. Only after this process is completed, do the HA fillers contain gel particles of a defined average size making it possible for them to flow as easily as possible through a needle. Once the filler is injected, it must regain at least part of its original structure so that it can sustain the tissues (possible thanks to the G').[14,18,22] Fine-bore needles represent an advantage in reducing negative side effects such as edema, pain, tissue trauma or bleeding.[18]

Depending on the sieving method utilized, different dermal fillers can have different particle sizes if they never surpass a stipulated maximum size, beyond which the gel particles could clog the needle. Another way of sizing a gel is through a homogenization process. This results in a smoother and softer gel, thanks to its broader distribution of particle sizes, and lower G' values. Because these gels flow easier, there is no maximum particle size that needs to be respected, as softer particles are easily deformed to pass through the needle. Thus, fillers obtained though the sieving process tend to show higher viscosity and to need a higher extrusion force, that is unless they have uncross-linked HA in their composition.[14,18,22] Still, even when the average particle size is smaller (which would make for lower extrusion forces), there may be sporadic flow of the product through the needle if there is still a considerable number of bigger particles. Because of this, whether a gel is firmer or softer, it is better for the particles' sizes to be uniform.[18] Particle size is also a determinant property for gel hardness as bigger particles also result in a harder gel.[16]

Another important thing to keep in mind is that, as previously mentioned, the particle size will influence the filler's durability. Usually, a filler made up of smaller particles degrades at a faster rate inside the body as smaller particles have a bigger total surface area available for which enzymes to attach themselves. Additionally, they also show lower volumizing abilities. Despite this, most HA fillers currently available in the market show similar particle sizes, so this may not have as big of an impact in clinical differences.[14] Regardless, particle size remains one of the most important elements responsible for defining a filler's characteristics.[17]

*Water Absorption and Hydration Properties.* Another essential property of HA to consider is its hydrophilia, with it being able to retain 1,000 times its volume in water.[1,25] The presence of amine and hydroxyl groups, which form hydrogen bonds with water and negatively charge the HA, is one of the main reasons for HA's high solubility, as it creates a viscous clear liquid when in contact with water.[9,14] HA's hydrophilic properties are what determine the gel's capacity to absorb water and expand, which inherently links these properties to the filler's lifting capacity.[4,25,39] These properties are defined by the insoluble portion of HA.[16]

The lifting capacity itself depends on HA's cohesivity, which was previously described as an important property that maintains a HA filler's integrity and keeps it together. There is also a known connection between the elastic modulus (G') and the filler's water absorption capacity and, consequentially, its lifting capacity.[25] It is then easy to understand that the filler's water absorption also shows an association with HA concentration and the degree of cross-linking, since usually a higher cross-linking density/stronger gel means lower chain flexibility and a lower capacity to absorb water.[2,3,14,15,18,26,29] However, studies also claim that stronger fillers usually show higher lifting capacities,[3] and HA swelling caused by water uptake also depends on surrounding tissues characteristics, such as its pH.[27]

The swelling ratio (or gel fluid uptake) is a measurement that translates the gel's ability to absorb water and expand its volume by binding water while remaining in one single *in vitro* phase (since gels can only absorb a limited amount of water, restricted by the polymer network, before becoming a two-phase system in which there would be HA gel particles suspended in excess water). This ratio is thus used to determine a gel's hydration/saturation level.[15,26,29,40] If the swelling ratio is 1, the gel is at equilibrium, and as it gets higher, the gel gets further away from equilibrium and becomes more cohesive.[15,16,31]

HA fillers achieve equilibrium hydration (full saturation) when a balance is struck between the elastic forces of the swollen HA and its osmotic forces. So, when a filler with a regular cross-linking degree (and around 5.5 mg of HA for every mL of water) is injected, we can consider it to be near equilibrium, which means it will not swell any further. Unlike dermal fillers with higher concentrations of free HA, which are below equilibrium and will swell more postinjection, they have the capacity to absorb water from the surrounding tissues, granting the filler its volumizing effect. This means that the higher the HA concentration, the more a filler will absorb water and swell.[4,14,18,26,40] In the research, the majority of swelling ratio and water absorption capacity studies are related to different biomaterials and not always specifically related to HA fillers.[2-9,13,15,20,26,29,39-43] In fact, there is little evidence comparing water absorption and expansion capacity of different HA fillers.

However, it is still important to keep in mind that there is a possibility that excessive water absorption may lead to tissue trauma, overcorrection, and greater edema. This is especially important when working on dark circles as using an incorrect HA filler may lead to increased eyelid edema. In short, if a filler is more saturated (nearer to equilibrium), it

will most likely cause less edema, but a larger volume of gel needs to be injected to achieve the same results as a less saturated gel.[5,27,28]

## CONCLUSIONS

This review intended to gather the most relevant information on the properties of HA present in available literature. Besides being the most abundant polysaccharide in the human skin, HA as a biomaterial shows a promising role in different areas of medicine (most predominantly from an aesthetic point of view), with HA fillers being used to perform tissue lifting, but also being used as a healing agent, thanks to its effects on fibroblasts and other cells. Therefore, it is crucial to have an intricate understanding of HA's rheology and physicochemical properties, which will influence its clinical outcomes.

There is not one universal dermal filler adequate for all patients and anatomical areas, rather there are multiple products with slight differences in said properties. Viscoelasticity (and especially the elastic modulus—G'), cross-linking, HA concentration, cohesivity, particle size, and hydrophilic expansion are some of the most important and influential factors to consider, especially since they all impact one another. All these factors have a role in defining HA's longevity, expansion and lifting capacity, biocompatibility, and behavior in the patient's tissues. Despite this, there is little research that presents a truly detailed and integral description of all of HA's characteristics. Due to this and the growing concern for aesthetics and effective tissue regeneration methods, it is important for future research to further explore this area and establish better defined protocols.

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