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Rheology of Hyaluronic Acid Dermal Fillers: Understanding the Science to Improve Results in Clinical Practice

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ABSTRACT: Hyaluronic acid fillers are one of the most frequently used in cosmetic procedures. They are popular because of their ability to restore volume to the face, which surgery isn't capable of. Inside the skin, the implant is subjected to various mechanical forces that vary according to the anatomical area. Every filler has specific rheological properties which change the way it performs in the clinical environment. A sound understanding of how the anatomy of the face works, alongside the rheological properties of the filler, is vital for successful outcomes in facial rejuvenation procedures.

1. INTRODUCTION

The effects of aging on the human face include a variety of microscopic and macroscopic intricate volumetric changes. Gravity, unhealthy habits, resorption of the deep three-dimensional structural support, redistribution of subcutaneous fat, as well as environmental factors all worsen these changes over time. (1)

Historically, surgical methods have been the obvious choice in the field of facial rejuvenation. However, since aging of the face can be attributed to repetitive muscle movements that lead to folds and wrinkles, alongside the factor of volume loss, the significance of volume restoration is now the main focus of medical professionals, surpassing the two-dimensional lifting that may be accomplished with surgery. (2) Furthermore, in some instances, surgical interventions may even make the apparent loss of volume more obvious than it was before the procedure. (3)

Dermal fillers add volume, which cannot be achieved through any other cosmetic therapy. First introduced with the invention of the bovine collagen-based dermal fillers in 1981, now dermal fillers made from various innovative compounds can be found. (4) (5) However, the hyaluronic acid (HA)-based ones remain the best option according to professionals and patients alike. Their biodegradability and non-invasive approach are key reasons for the popularity of the procedure ever since the introduction of Restylane®, an HA-based filler in 2003. (6)

Patients often express their concerns about a potential loss of the essential emotional language of the face, which leads to unnatural results and may trigger a loss of self-esteem and a poor quality of life.

As doctors further understand the physiology of facial aging and the rheology of hyaluronic acid, they can make the best choice of fillers according to the desired result. (7). The aim of this article is to help medical professionals choose the right fillers for the needs of their patients.

2. HYALURONIC ACID – INTRODUCTION

Hyaluronic acid (also known as hyaluronan) was first isolated in 1934 from bovine vitreous humor by Karl Meyer and John Palmer. Most of the discoveries relating to hyaluronic acid are, however, attributed to Endre Balazs, who spent half a century studying the substance. (8) For the last 30 years, hyaluronic acid has been essential to procedures such as eye surgery, wound repair and most famously - esthetics. (1)

Chain length and source Hyaluronic acid is the most common glycosaminoglycan in the human dermis and can also be found in the joints, muscles etc. Its purpose in all mammalian connective tissue is to bind collagen and elastin fibers and draw water into the skin, thus maintaining the structure of the skin and also giving it volume. (9)

Hyaluronic acid is a naturally occurring biopolymer. It is an essential part of the extracellular matrix of all adult animal tissues. No matter what the source is, animal or bacterial, the monomeric unit (a non-sulfated disaccharide glucuronate-B-1,3-N-acetylglucosamine-B1,4-) is identical in all hyaluronic acids.

The difference between animal-based and bacterial-based hyaluronic acids comes from the length of the chain, with bacterial-derived HA having a lower degree of polymerization. The length is usually 4000-6000 monomeric units per chain with an average molecular weight of around 1.5 - 2.5 MDa. Animal-derived HA chains consist of about 10 000 - 15 000 monomeric units per chain with an average molecular weight of around 4 - 6 mDA. (10)

3. RHEOLOGICAL PROPERTIES OF HYALURONIC ACID

Rheology is the study of the flow and deformation of materials. It is concerned with the question 'How does a material respond to a force?' (11)

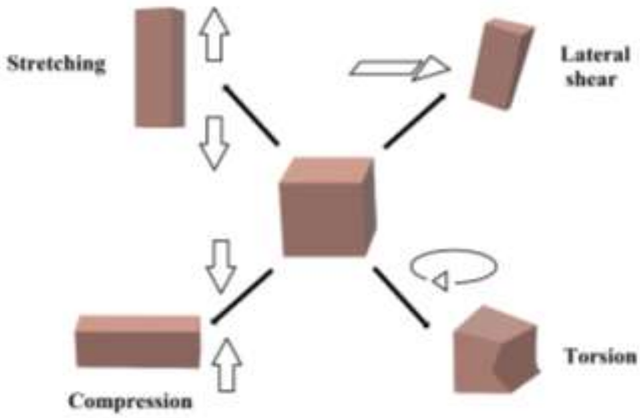


Fig 1. Types of deformation that influence hyaluronic acid

When exposed to shear deformation, HA fillers exhibit both viscous and elastic characteristics, making them viscoelastic materials. After being injected, HA fillers come into contact with a variety of forces, including compression, gravity, and/or relative tissue layer movement. As a result, gathering data with the help of rheology, on how fillers respond to mechanical stress is useful in therapeutic settings. (12)

As a viscoelastic gel, HA has the capacity to deform and flow through a needle or cannula, due to its viscous component, and the capacity to recover its initial shape, due to its elastic component. Thus, during production, hyaluronic acid's elastic and viscous components are mixed to create a gel.

Viscoelasticity

Viscoelasticity is determined by the amount of resistance to shearing forces and/or torsion about the axis. Using a rheometer, four parameters have been defined:

● Shear modulus/Complex Modulus G* - Total energy needed to deform a material, in other words it measures the response of the filler to deformation. (12) G* is a measure of overall viscoelastic properties, referred to as hardness of the gel, that takes into account both the viscous component (G") and the elastic component (G'). The equation is as follows:

$$G^* = \sqrt{(G')^2 + (G'')^2}$$

● Elastic modulus G', also known as storage modulus - The energy fraction of G* being restored after deformation. It measures the elastic properties of the gel particles and their ability to return to their original shape.

Elastic modulus represents a solid-like behavior of the gel once the filler is injected. It is the most common descriptor of HA fillers' rheological properties, typically being used as a measure of a filler's lift capability. However, the relationship between G' and a HA filler's lift capacity isn't always linear, since other variables also influence the performance of the filler.

The softness or firmness of the filler is influenced by the concentration of the gel and degree of crosslinking used by the manufacturer. For higher levels of elasticity (therefore higher levels of G') manufacturers increase the degree of crosslinking. The total matrix becomes stronger as the space between crosslinks is reduced, making the gel stiffer or firmer (higher G′). In contrast, when the number of crosslinks is decreased, the distance between the links of the HA molecules grows, which leads to a softer and less elastic filler (lower G').

A filler with low to medium G' is described as a soft filler. Most HA fillers used in cosmetic procedures are mainly elastic, with G' and G" standing at nearly equal values.

● Viscous modulus G", also known as loss modulus - The energy fraction of G* being lost after deformation. It is a measure that shows the inability of the gel to return to its original shape after deformation and relates to the viscous properties of the gel, therefore its liquid behavior. Viscous modulus is linked to the injectability of the gel. For a filler to be effective, it needs to have low G", but still be viscous enough to be injected and initially molded. G" should not be confused with viscosity, which refers to the flow of the filler during injection and has no bearing on clinical performance.

● Tan δ - The ratio G"/G' that shows if a gel is more elastic or more viscous. Tan < 1 indicates a predominantly elastic filler, whereas tan > 1 indicates a predominantly viscous one. Most HA cross-linked fillers have tan δ <1. It is important to note that while tan helps to determine whether the filler is more elastic or more viscous, it does not reveal the actual magnitudes of G′ and G".

To measure these four parameters, a twisting force via a rheometer, can be applied to a gel between two plates. The test aims to simulate the difference in the degree of dynamic movements across the face, and so they are carried with a range of frequencies, for example from 0.1 to 10 Hz.

Results, similar to any analytical approach, rely on the material (in this case, the filler), the testing equipment, and the experimental circumstances (frequency, amplitude, plate geometry, temperature, etc.) Because comparing data from rheological research that employed various approaches calls for good scientific comprehension and awareness of limits, comparisons between various studies should be done with caution.

According to these variables, for 100% elastic gel, G' tends toward G* and G" tends toward 0, consequently, for a 100% viscous gel (liquid), G" tends toward G* and G' tends toward 0. HA fillers are viscoelastic gels with properties somewhere between the two extremes and are defined by their tan δ.

During cross-linking G' and G* are prone to increase, while G" tends to decrease.

Sliding movements between the various anatomical layers (skin, muscle, fat, and bone) are what generate the shearing forces, and a gel with high G* and G' is characterized by its particles' ability to keep their shape. A bolus of HA, however, is not composed of a sole particle and is instead a complex blend of cross-linked particles with a specific degree of adherence among each other. The degree of adherence is determined by gel cohesivity.

Cohesivity

Cohesivity is the adhesion of the gel to itself. (7) The internal forces, which hold the cross-linked particles of HA together within the gel, define the cohesivity of the filler.

As the degree of cohesivity rises, so does the ability of the injected gel bolus to maintain its macroscopic shape when challenged by the natural movements of the face and the pressure of the surrounding tissue. If we look at the two gels with the same G*/G', the one with higher cohesivity will provide more initial projection per volume than the gel with lower cohesivity. The relevance of this difference in performance depends on the depth of the injection and the facial dynamics of the selected area, and it becomes less relevant over time once tissue integration is complete.

However, the relationship between tissue integration and cohesivity is not that simple, since other factors besides cohesivity have a role in tissue integration, such as other mechanical factors (G'), chemical factors (degradation at variable speed, local stimulation of adjacent tissue, etc.)

The concentration of the HA and certain steps in the cross-linking process define the cohesivity of the HA bolus.

In clinical practice, cohesivity refers to the injected bolus of HA gel's initial vertical projection before it is put under any shearing or compression pressures, and it has a great influence on the modeling capacity of the gel once it has been injected. Therefore, the more cohesive the gel, the less malleable it is.

Other fundamental properties of HA

● Hydrophilia/Water uptake (swelling factor) - Risk of acute edema is determined by water uptake and it is not related to the edema caused by injection trauma. (Edema is swelling caused by excess fluid trapped in a tissue.) (12) It depends both on the concentration of HA and the crosslinking method. The chains are kept securely together as the number of crosslinks grows, which limits their ability to move apart and extend to accommodate the water, lowering the gel's potential to swell.

Different methods for predicting water uptake exist. The values of absolute swelling factor, not unlike other measures, depend on the conditions of the test. However a range of values is observed among fillers. In vitro water uptake assessments will provide results showing the maximum ability of the gel to absorb water. Once the filler is injected in the specific area of the face, other factors such as composition and water content of the adjacent tissues, will lower the filler's ability to reach its full expanding potential.

● HA concentration (mg/mL) - This measure shows the total amount of HA, both insoluble and soluble, found in the filler. Crosslinking technology affects the concentration of HA and the degree of crosslinking.

● Texture (smooth or particular) – This property is important to the Tyndall effect because of the reflection of light on HA, which produces a blue tint if the gel is injected too superficially. (7)

● Tissue integration pattern - The rate of tissue integration pattern and the occurrence itself determine how apparent the filler is and how natural the results are.

● Durability over time

● Tolerance of the filler

There isn't yet a simple association between mechanical properties of the fillers and their potential undesired effects.

4. CROSSLINKING OF HA POLYMERS

In the skin, the half-life of HA that hasn't been cross-linked is around 12 hours. If a non-modified HA filler is injected, it would be rapidly eliminated by enzymes, such as hyaluronidase, as well as free radicals, and removed via the lymphatics and by hepatic metabolism. (10) Therefore, HA is cross-linked to create a viscoelastic substance that has a longer lifespan when applied to the skin. Physicians can currently choose from a variety of dermal fillers based on cross-linked HA technology. (9)

Cross-linkers hold HA polymer chains together in a polymer "network", which results in the typical for fillers, gel texture. The final product now acts as a single unit, and slows the breakdown of the product by months, since enzymes and free radicals can eliminate only a small portion of the gel at a time.

The most frequently used cross-linker is BDDE (1,4-butanediol diglycidyl ether). BDDE reacts with hydroxyl sites on the HA chains to slow down the breakdown of the filler. However, crosslinking can occur with residual cross-linkers in the final product, which can be toxic in high concentrations. To prevent such occurrences, there are certain levels of residual cross-linkers, below which the filler is safe to use.

The degree of crosslinking is essential when comparing HA dermal fillers. It is an indicator of the percentage of HA disaccharide monomer units that are linked to a cross-linker. If a filler has a degree of crosslinking of 4%, for example, it means that on average there are four cross-linker molecules for every 100 HA monomers. If we take two fillers with every other parameter being equal, except for the degree of crosslinking, the one with a greater degree of crosslinking will possess the greater hardness of the gel and longer persistence in the skin. However, the threshold for the highest desirable degree of crosslinking is yet to be identified. A high degree of crosslinking has its setbacks, as it can reduce the hydrophilicity and the lifting capacity of the filler. Moreover, a filler with a crosslinking degree, higher than the desired one, can lead to an immune reaction in the body because of the potentially reduced biocompatibility of the product. Instead of metabolizing the gel, the body might reject it, leading to encapsulation of the gel implant and the formation of a granule or sterile abscess.

For best results, filler manufacturers should produce products with a degree of crosslinking below the threshold.

Gel particle size

Once the HA polymers are merged in a gel mass, the mass must be 'sized' in order for it to be injected in the skin. One method is for the gel mass to pass through a series of sieves or screens. The resulting product is composed of well-defined particles of average size, with different sizes being obtained depending on the sieving method. However, there is a maximum particle size, as well as suspected ideal particle size based on the rate of degradation in the body.

5. THE IDEAL HYALURONIC ACID DERMAL FILLER

The ideal dermal filler should not require allergy testing before injecting, therefore it should be safe, biocompatible, and non-immunogenic. It should be effective, easy to store, distribute and remove if necessary, with low cost and minimal recovery time. Of all the dermal fillers that medical professionals use, HA ones possess most of these properties.

With the simple injection of commercially available hyaluronidase (Vitrase®; ISTA Pharmaceuticals, CA, USA) into the affected area, HA fillers can be simply removed anytime the doctor deems it necessary. (14)

The process of crosslinking is important since it is not only responsible for the prolonged lifespan of the filler in the skin, but it also improves the rheological properties of the filler. (12) The final product is influenced by the molecular weight of the HA at the beginning of the process, the degree of crosslinking conditions (pH, temperature), as well as the post-crosslinking changes (addition of lidocaine, homogenization/sieving, etc). Therefore, variations in the approach that different companies employ lead to differences in the final product.

An initial foundation for predicting treatment outcomes can be created by understanding the variety of HA filler products from the aspect of their rheological and physicochemical properties. With this knowledge, clinicians will be able to choose the HA filler with the appropriate, for the facial area, characteristics.

Performance characteristics (i.e., lift capacity, resistance to deformation, and tissue integration) are influenced by rheological and physicochemical properties of HA fillers, which, along with injection technique (i.e., injection plane, location, volume), and the interaction of the filler with the surrounding tissue, may affect clinical outcomes.

6. FROM RHEOLOGICAL PROPERTIES TO CLINICAL OUTCOMES

Mechanical stresses within the face

The different combinations of compression/stretching and lateral shearing, to which the face is subjected to, vary in every region of the face. (7) There are two types of forces - intrinsic and extrinsic ones.

Intrinsic forces relate to the motions and tensions between the tissues of the face (skin, fat, muscle, bone).

Extrinsic forces are defined by daily life and activities such as sleep, exercise, nutrition, kissing, etc.

Since the mechanical forces have a different degree of effect in every part of the face, it is important to use the right type of HA with the necessary rheological properties for each region of the face.

1. Periocular area and Inferior orbital rim

1.1 Crow's feet

The periorbital region is particularly susceptible to aging, which is frequently accompanied by crow's feet or lateral canthal lines. (1) The underlying collagen and elastin fibers that sustain the skin gradually disappear, causing rhytides. Moreover, wrinkles can develop as a result of repeated facial expressions, UV radiation exposure, and smoking. Their appearance is further emphasized by the movement of the eyelids caused by the contraction of the orbicularis oculi muscles.

In most cases, botulinum toxin therapy is applied. However, for deeper periocular wrinkles the treatment includes HA dermal fillers using retrograde linear threading or serial puncture technique via a 32-gauge needle. Unwanted effects in this area after the procedure may include lumps or bumps, as well as bruising.

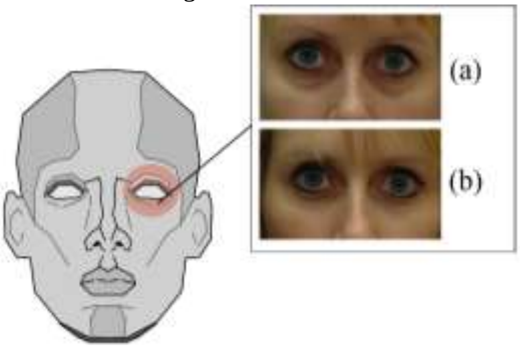


Fig 2. Correction of dark circles using hyaluronic acid 15 mg/mL (VYC-15L). (a) Before correction, (b) After correction (Coll. Th.Michaud)

1.2. Dark circles

The area under the eyes is defined by a thin layer of skin and loose subcutaneous tissue. (7) The lack of major compression forces means that there is little compression or shearing.

The rheological properties of the HA needed for this region are little resistance to compression, high malleability, therefore low cohesivity, and low-to-moderate G'. A low degree of hydrophilia is required to avoid edema after injection. The gel should be smooth and the amount of gel should not be excessive. It is to be injected deep in the correct anatomical layer, close to the bone to avoid the Tyndal effect.

2. The frontal region

The frontal region is defined by rather thick skin, which is tightly stretched on the forehead. The compressive forces are low to moderate and the shearing ones are moderate to strong, the frontalis muscle generates shear stress when contracted.

The HA should have low-to-moderate cohesivity and moderate-to-high G' since the rheological properties should include a significant spreading capacity and a good lift. The filler should not be visible, especially during the frontalis muscle contraction.

For the glabellar lines, the symmetrical lines, which spread between the eyebrows and are caused by the prolonged activity of the corrugator supercilii and procerus muscles, the therapy usually includes botulinum toxin therapy. (1) In cases where the depth of the glabellar furrows is greater, HA fillers can be used alongside botulinum toxins for the best results. The HA filler must be successfully implanted without being overcorrected, and since the blood vessels in this area are extremely susceptible to blockage, fillers must be injected superficially to reduce the danger of cutaneous necrosis.

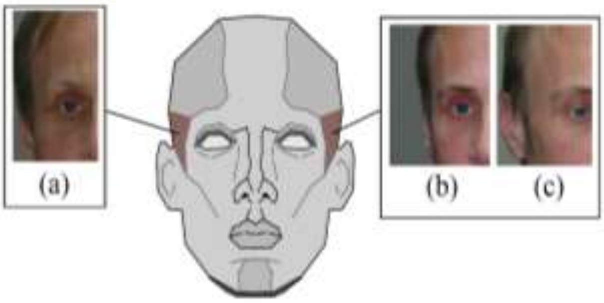


Fig 3. Correction of loss of temporal volume using hyaluronic acid 20 mg/mL (VYC-20L). (a), Before correction, (b,c), After correction(Coll. Th. Michaud)

3. The temporal region

The skin in the area is thick and stretched by the temporalis muscle, which is defined by great strength and large volume. (7) The stress in the temporal region is mainly due to compressive forces, and less due to shearing forces.

The technique applied in the procedure determines the needed rheological properties:

If the filler is injected in the temporal fossa, the HA should possess strong cohesivity and high G’ for a good lift and resistance to compression. If the cohesivity and elasticity are not within the appropriate value, no projection toward the surface will be observed due to the fast flattening of gel particles and gel bolus.

If the filler is injected in the superficial fatty compartment, the HA should have low-to-moderate cohesivity and mild-to-moderate G’. The HA should be malleable with a good spreading capacity to avoid the potential “wave” effect.

4. Rheological specifications of the middle third of the face

4.1 Deep volumization of the middle third

The lateral shearing forces are low, while the compression forces are moderate to high in the deep fatty compartment of the middle third of the face. For a good lift in that area, which is located deeply under the orbicularis muscle, high cohesivity and high elasticity are needed.

For volume restoration, the filler must have high-to-moderate cohesivity, moderate-to-high G’. It should be injected in the correct position close to the bone for it to not be visible or migrate.

For the lifting effect, the HA must be characterized by greater elasticity and cohesivity to lift the adjacent tissue (skin, superficial fatty compartment, and muscle).

4.2 Correction of the superficial fatty compartment

Since there is only skin above the superficial fatty compartment, the degree of compressive forces and shearing forces is lower.

The implant should have a low-to-moderate cohesivity and moderate G’ since the requirements are little projection, good malleability, and good tissue integration for a non-visible effect of the filler. If the filler is too elastic or too cohesive, the final result will be unnatural, such as a “lump” effect.

5. Rheological specification of the lower third of the face

5.1 Lips

The difference between red and white lips should be noted:

● **Red lips:**

High compressive/stretching forces and low shearing forces are typical for this zone.

The rheological specifications are good projection and respect for natural static, with a product that is not visible and has excellent mobility during the movements of the face, as well as good tissue integration. Therefore, HA with good cohesivity and mild G’ should be used.

● **White lips:**

Low compression and low shearing forces are typical for this zone.

The treatment involves superficial correction of helioderm wrinkles and/or expression wrinkles. The HA should have low cohesivity and low G’ since the filler needs to be undetectable and should possess excellent malleability and good spreading.

5.2 Perioral zone

Low-to-moderate compression/stretching forces, low-to-moderate shearing forces, and loose tissue are typical for this zone.

The filler should have low-to-moderate cohesivity and moderate G’ in order for it to have good malleability and not be visible during static state and movements. If the cohesivity and resistance are too high, the filler will produce a “lump” effect. In some cases, such as deep furrows, a HA with very high cohesivity may be needed, but it is difficult to work with and it could be easily detected.

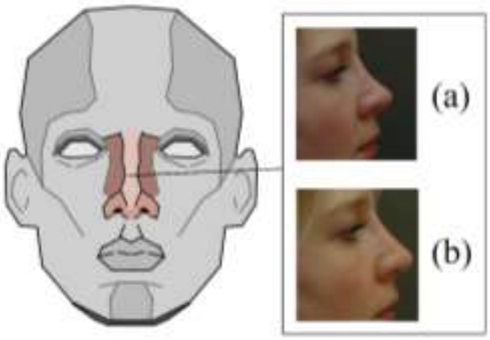
5.3 Chin

The zone is defined by high compressive forces, low shearing forces, and thick skin on the bone.

The HA should have a high cohesivity and high G’ for the filler to have good projection and no migration of the product.

5.4 The Nose

The nose is defined by moderate compressive forces and no shearing, with little tissue with skin lying flat on the bone and cartilage. The HA should have low-to-moderate cohesivity and moderate G’ for fillers with good projection capacity, malleability, and natural effect and no possibility of migration of the product. A filler that has too much elasticity and/or cohesivity would lead to a change in the shape of the nose.



Correction of "trumpet" nose following rhinoplasty using hyaluronic acid 17.5 mg/mL (VYC-17.5L). (a), Before correction, (b), After correction (Coll. Th. Michaud)

5.5 The oval of the face

Compression/stretching forces are moderate, and shearing forces are low to moderate in the area. Depending on the depth of the zone, which is being corrected, the rheological specification differs.

The HA for deep periosteal correction to restore the contour the filler should have high cohesivity and high G'.

The HA for superficial correction should have low cohesivity and moderate G' so the filler is easily modeled and is not visible.

7. Adverse Events

All cosmetic treatments, including the less invasive ones, might have unfavorable side effects. Before any surgery, it is essential to go over all known potential adverse effects with the patient.

Temporary pain, induration, bruising, soreness, itching, edema, and erythema are the most often reported localized adverse effects following treatments with HA dermal fillers. These adverse effects often go away after a few days, and they can be lessened by slow injection techniques, frequent needle changes, choosing the HA filler with the right rheological properties, and warning patients to refrain from aspirin for several days ahead of therapy.

Between 0.0005% and 0.42% of people have experienced hypersensitivity responses, and necrosis or embolization has been reported to happen extremely rarely. The material in the syringe should be pulled back before implantation to prevent this serious adverse event.

7. CONCLUSIONS

Among the various dermal fillers, the HA-based one is the most preferred by professionals and patients, because of the biocompatibility, non-invasive approach, and low cost.

For best outcomes after cosmetic treatment with HA dermal filler, the facial dynamics, as well as the rheological properties of HA must be taken into account. Each area of the face is subjected to specific mechanical forces, which determine the necessary properties of the HA filler. The rheological characteristics of the compound include viscoelasticity and cohesivity, which depend on various parameters, such as the length of the chains, the concentration of the HA, the crosslinking technology, etc.

Every clinical professional, working with HA dermal fillers, should be familiar with these properties as well as the possible adverse events, to provide the best care for their patients.

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