Comparative Physical Properties of Hyaluronic Acid Dermal Fillers

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BACKGROUND Hyaluronic acid (HA) fillers are becoming the material of choice for use in cosmetic soft tissue and dermal correction. HA fillers appear to be similar, but their physical characteristics can be quite different. These differences have the potential to affect the ability of the physician to provide the patient with a natural and enduring result.

OBJECTIVE The objective of this article is to discuss the key physical properties and methods used in characterizing dermal fillers. These methods were then used to analyze several well-known commercially available fillers.

METHODS AND MATERIALS Analytical methods were employed to generate data on the properties of various fillers. The measured physical properties were concentration, gel-to-fluid ratio, HA gel concentration, degree of HA modification, percentage of cross-linking, swelling, modulus, and particle size.

RESULTS The results demonstrated that commercial fillers exhibit a wide variety of properties.

CONCLUSION Combining the objective factors that influence filler performance with clinical experience will provide the patient with the optimal product for achieving the best cosmetic result. A careful review of these gel characteristics is essential in determining filler selection, performance, and patient expectations.

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In recent years, hyaluronic acid (HA)-based fillers have become the material of choice for use in soft tissue and dermal correction, for the most part replacing collagen fillers such as Zyderm, Zyplast, Cosmoderm, and Cosmoplast (Allergan, Irvine, CA).[1–3] Although the HA fillers appear to be similar, their physical characteristics and methods of manufacture are not the same.[2] These differences have clinical ramifications for the physician in that they can affect injection technique, usage, and the quality of the outcome. Often fillers are pragmatically evaluated, with consideration given to the results of the application. Questions such as whether the material is easy to deliver; whether the duration of correction is appropriate; whether the material bruises, swells, and creates inflammation; and whether the results look natural are frequently the only means of characterizing a filler.

There is no universal filler that is appropriate for every application or for every patient. Understanding physical properties of HA fillers and how they interact provides significant information about the expected clinical outcome and the corresponding best cosmetic result for a patient. Therefore, it is important to take an objective approach in assessing factors that may influence HA filler performance, such as total HA concentration, modulus, swelling, particle size, cross-linking, and extrusion force.

Scientists and engineers use a variety of methods to design materials that have the desired final proper-

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ties. Consequently, the filler designers make use of characteristics such as raw material properties, cross-linking schemes, HA concentration, and rheological properties to achieve the end results. Although the results may vary, manufacturers take similar approaches to the design of their fillers. Understanding the means employed by manufacturers to design and characterize their fillers should provide useful insight as to the ability to clinically provide the patient an enduring, natural-looking result.

Recent review articles describe important physical characteristics of HA-based fillers.[2,4] In this review, we discuss the key physical properties and methods used to design and characterize dermal fillers. We then employ these methods to analyze several well-known commercially available fillers.

HA Dermal Filler Properties

Hyaluronic Acid

HA is a glycosaminoglycan disaccharide composed of alternately repeating units of D-glucuronic acid and *N*-acetyl-D-glucosamine (Figure 1). At physiologic pH, HA exists mostly as a sodium salt; this is the most common form of commercially available HA. HA is naturally occurring in the extracellular matrix found in many human tissues, including skin, synovial fluid of joints, vitreous fluid of the eye, and scaffolding within cartilage.[5,6] The average 70-kg man has roughly 15 g of hyaluronan in his body, one-third of which is turned over (degraded and synthesized) every day.[5,7] The largest amount of HA resides in skin tissue (7–8 g per average human adult); thus approximately 50% of the total HA in the body is found in the skin.[5,7] HA is a polyanionic polymer at physiologic pH and is therefore highly charged. The highly charged nature of HA renders it soluble and allows it to bind water extensively.

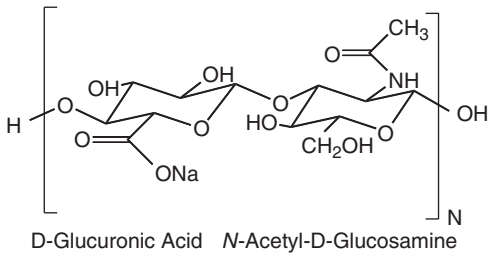

Figure 1. Hyaluronic acid (HA) is a glycosaminoglycan disaccharide composed of repeating units of D-glucuronic acid and *N*-acetyl-D-glucosamine. The molecular weight of HA is proportional to the number of these repeating disaccharides.

Molecular Weight

The molecular weight of HA is proportional to the number of repeating disaccharides in the HA molecule (Figure 1). When discussing the molecular weight (MW) of HA, it is most often the average MW of a sample that is reported. As a result, the polydispersity or range of molecular weights found in a sample is also a consideration. The HA used in manufacturing dermal fillers can range from 500 to 6,000 kDa. Commercial preparations of hyaluronan are usually supplied as the sodium salt and have a disaccharide MW of approximately 401 Da. Therefore, a 1,000,000-MW polymer of HA will have approximately 2,500 repeating disaccharide units, all of which are negatively charged at physiologic pH.

Sometimes the term "MW" is applied generally to properties of dermal fillers. This is technically incorrect, because a typical filler comprises HA molecules cross-linked to form a gel. As a result, the MW of a HA gel is enormous and is essentially immeasurable. Because the MW of the final HA gel is so large, small differences in MW of the starting HA have little effect on the final properties of the gel. Although we cannot effectively speak of the MW of a gel, the number of cross-links and the percentage of modification are important considerations when characterizing HA gels.

Modification and Crosslinking

In its natural state, HA exhibits poor biomechanical properties as a dermal filler. HA has excellent biocompatibility and affinity for water molecules, but it is a soluble polymer that is cleared rapidly when injected into normal skin (Figure 2A).[5,7] Therefore, to provide the ability to lift and fill wrinkles in the

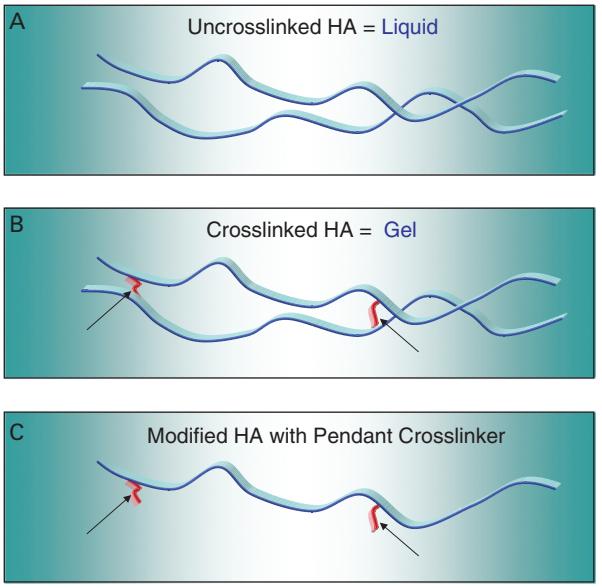

Figure 2. When dissolved in water, hyaluronic acid (HA) behaves as a fluid, with excellent biocompatibility but poor mechanical properties (A). Modification of HA molecules by cross-linking improves mechanical properties by creating gels that have a firmer structure and are able to resist degradation (B). Modification does not necessarily cross-link HA to other HA molecules, resulting in a pendant cross-linker (C). Such structures often result in softer gels.

skin, chemical modification is required to improve its mechanical properties (Figure 2B) and residence time at the implant site. The two most common functional groups that can be modified in HA are the carboxylic acid and the hydroxyl (alcohol). Cross-linking strategies attempt to improve biomechanical properties while maintaining biocompatibility and biological activity. The literature reports many methods for cross-linking HA.[2,8] Biomaterials have been produced through modification to the carboxyl acid group by esterification and through the use of cross-linkers such as dialdehydes and disulfides.[8] The most commonly employed cross-linkers for dermal fillers are divinyl sulfone (Hylaform, Captique, and Prevelle Genzyme Co., Cambridge, MA) and diglycidyl ethers (Restylane, Q-Med, Uppsala, Sweden; Juvederm, Allergan, Irvine, CA; and Belotero, Anteis SA, Geneva, Switzerland) or bis-epoxides (Puragen, Mentor, Santa Barbara, CA).[1,2,4,9]

An assessment of the degree of modification must go beyond determining the amount of cross-links in a material. Bifunctional cross-linkers do not necessarily react at both ends to connect two different strands of HA. Often the cross-linker will bond only at one end, leaving the other end pendant (Figure 2C). Thus the total degree of modification can be defined as;

$$\text{Total \% Degree of Modification} = \text{\% Crosslink} + \text{\% Pendant}$$

Whether chemical modification results in formation of a cross-link (a bond between two strands of HA) or a pendant group is a function of the reaction conditions used by different manufacturers of HA fillers.

The degree of modification can have a significant effect on the properties of a filler material. As the cross-link density of a gel increases, the distance between the cross-linked segments becomes shorter. When a load is applied, these shorter segments require a greater force to deflect. Thus, increasing cross-link density strengthens the overall network, thereby increasing the hardness or stiffness of the gel. However, when the gel comprises all or mostly pendant HA modification, a low cross-link-density network is formed, resulting in softer gels.

In general in vivo degradation of HA occurs through enzymatic degradation and reaction with reactive oxygen species (e.g., superoxide, peroxynitrite). In each case, HA molecular strands are cleaved to smaller oligosaccharides that are more amenable to metabolism and clearance from the body. Thus, a network of cross-linked HA retains its structure until sufficient degradation has occurred at the gel surface to form soluble oligosaccharides that can be metabolized and cleared from the body.[5,7] This simplistic approach provides a general overview of the degradation of HA, although specific cross-linking reagents and conditions used in the cross-linking process can affect the degradation rate of cross-linked HA hydrogels. Also, other physical properties such as gel concentration and degree of swelling can affect the rate of degradation.

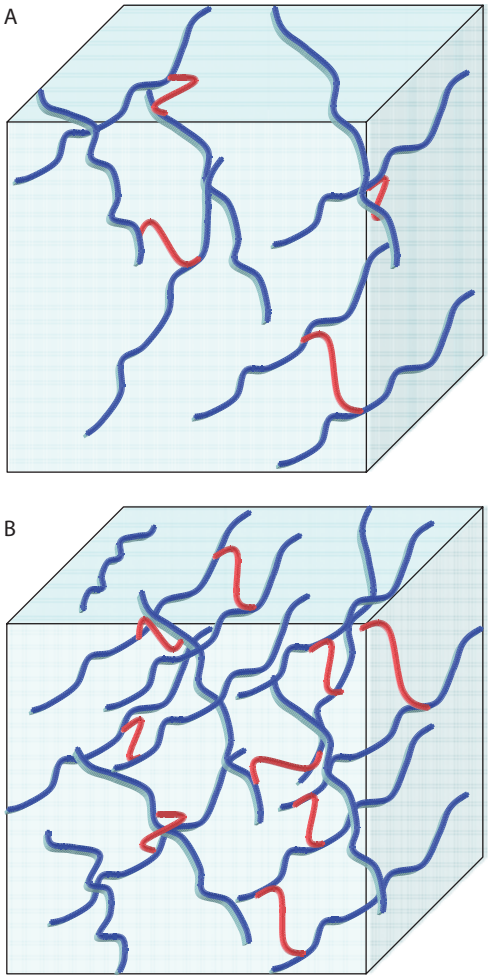

Figure 3. Concentration is a measure of the amount of hyaluronic acid (HA) in a gel. Given the same degree of cross-linking, low concentrations will result in softer gels (A), whereas higher concentration gels result in stiffer gels (B). It also stands to reason that, because there is more cross-linked HA in higher-concentration gels that it should last longer.

Concentration

When manufacturers convey the concentration of a filler, they are articulating the total amount of HA found in the filler, typically expressed in mg/mL (Figure 3). The total HA concentration consists of insoluble HA gel and soluble-free HA. Manufacturers may provide free HA as a soluble fluid component to the gel to facilitate the extrusion of the filler through fine-bore needles. Although not all manufacturers add HA fluid to their fillers, a fluid component is often present. This fluid component contains unmodified and modified soluble HA that is generated during the manufacturing process when HA fragments are formed as a side-product of the chemical modification. These soluble fluids are easily metabolized and do not contribute to the extended duration and effectiveness of the product. Only the cross-linked HA resists enzymatic and radical degradation and therefore extends the filler's presence in the dermis, contributing to its effectiveness. Consequently, it is important to understand how much of the filler's HA concentration is gel or cross-linked HA and how much is soluble fluid or free HA.

Modulus

Most HA-based dermal fillers are viscoelastic, containing elastic (solid) and viscous (liquid) components that can be evaluated using dynamic testing. The rheological characteristic that describes this property is the complex modulus (G^*), which defines the material's total resistance to deformation. G^* can also be defined as sum of the elastic modulus (G') and the viscous modulus (G''). Elastic modulus is also called storage modulus because it describes the storage of energy from the motion in the structure. The magnitude of the G' is dependent upon the elastic interaction and the strength of the interaction in the sample. Viscous modulus is also labelled loss modulus, and it describes the energy that is lost as viscous dissipation. Thus the value of G'' is a measure of the flow properties for a structured sample.

The elastic modulus G' is most often used to characterize the firmness of a gel. Because the elastic modulus or G' of a material describes the interaction between elasticity and strength, it provides a quantitative method for characterizing the hardness or softness of a gel. G' represents the amount of stress required to produce a given amount of deformation.

$$G' = \frac{stress}{strain}$$

Another way of thinking of this is that elastic modulus is a measure of a material's ability to resist deformation. As an example, a stiffer material will have a higher modulus; it will take a greater force to

deform the material a given distance. For most materials, G′ is dependent upon the speed (frequency) at which the force is applied. Intuitively, this makes sense; for instance, a material will resist deformation if the load is applied at a rapid rate, resulting in a higher modulus than if the load were applied at a slower rate. Thus it is important to ensure that there is parity in methods of measurement when comparing modulus values for different materials.

The degree of cross-linking and gel concentration play important roles in defining the modulus of the gel, and many manufacturers use these parameters to influence the hardness or softness of their fillers. Higher gel concentration produces more molecular entanglements and in so doing increases the modulus of the gel. A gel with a lower degree of cross-linking but higher gel concentration could have a similar modulus as a lower concentration gel with a much higher degree of cross-linking. A gel with a lower number of cross-links (covalent bonds) has a greater length of the HA molecule between links, thus requiring less of a force to deform the gel (Figure 4A). As the network is tightened by increasing the number of cross-links, the gel will become stiffer (Figure 4B). HA gels with pendant-type modification have a small effect on modulus because they do not form a cross-linked network (Figure 2).

Gels with higher G′ (higher stiffness) have a better ability to resist dynamic forces occurring during facial muscle movement and thus may provide better support and lift and longer duration of correction in areas such as nasolabial folds and marionette lines. Gels with low G′ are probably better suited to areas with static and superficial wrinkles, where resistance to deformation is not critical, or areas where anatomy does not require stiffness but volume and softness are important, such as in lips. Although all HA gels vary in elastic modulus, even the ones with the highest G′ are much softer than the elastic modulus of human dermis, which has G′ in the 3-MPa range.[10]

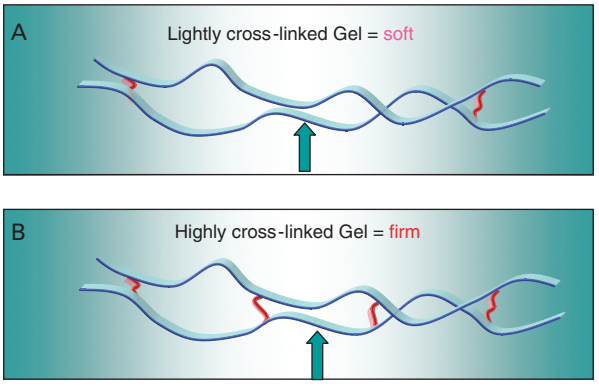

Figure 4. Gels with fewer cross-links have a greater length between links, requiring less of a force to deform the gel (A). Increasing the number of cross-links shortens the distance between cross-links, resulting in a stiffer gel (B).

Swelling

HA at physiological pH is hydrated extensively by water. The three-dimensional structure of HA has a significant influence on the water-binding capabilities of HA. In solution, the coil-like structure of a HA molecule occupies a large domain in comparison with its molecular weight. When in a physiologically neutral solution, water forms hydrogen bonds with the *N*-acetyl and carboxyl groups. The dipole attraction of the hydrogen bond with carboxyl group results in HA's affinity for retaining water. With repeating disaccharide units, the longer the HA molecule, the more water molecules are bound per unit of polymer.

A filler's predisposition for swelling is a function of whether the HA filler has reached its equilibrium for bound water. A HA gel's capacity for swelling will vary from product to product and is dependent upon concentration, cross-link density, and the process used to hydrate the gel. Fully hydrated or equilibrium gels have already reached their hydration capacity; thus they will not swell when injected into the dermis. Nonequilibrium gels tend to swell postinjection, and consideration must be given to underfilling when performing a correction with these gels.

Particle Size and Extrusion Force

The cross-linked gels that constitute dermal fillers must be of sufficient particle size that they can be injected easily through an appropriately sized needle.

In efforts to reduce undesired side effects such as pain, bruising, bleeding, and edema, small-bore needles (27-g and 30-g) are employed. Thus, the gel particles must be appropriately sized to be able to pass through these fine-bore needles with an acceptable extrusion force.

The HA filler manufacturers employ various methods of particulating the gels based on their modulus to obtain an appropriate extrusion force. This results in gels that have various particles sizes and broad or narrow ranges of distribution. The ultimate goal is to size the HA gel particles and define their modulus so that the final gel can be easily administered to the site of application.

When characterizing the particle sizes of a HA gel, consideration must be given to the average particle size, as well as the particle size distribution. Because larger gel particles are more difficult to push through a small-bore needle, a filler with a high average particle size will be more difficult to extrude. The average extrusion force of the filler can be decreased by reducing the average particle size, but if the distribution of particles still includes a number of larger particles, there is the potential that they may cause interrupted or sporadic flow of the product through the needle.

Gel hardness or G′ plays an important role in how the gels must be sized for easy delivery through fine-bore needles. Firm gels, with a high ability to resist deformation, must be sized to small particles and should have a narrow distribution range to be easily injected through a thin-bore needle. On the other hand, soft gels with low G′ can have a broader distribution of particle sizes because the softer particles can be easily deformed to pass through the needle. Regardless of whether a gel is firm or soft, particle size uniformity is preferred to avoid "stop and go" action during injections and for better control of gel placement.

As can be surmised from the previous discussions, it is not particle size alone that affects the extrusion force of a filler. Rheological properties such as modulus of the filler have an effect. The degree of modification, the amount of cross-linked and uncross-linked HA, concentration, and the degree of hydration affect these rheological properties. Thus, extrusion force is the result of a combination of properties that are integral to the design of the filler.

Methods

Percentage Modification Measurement

HA filler samples were degraded using *Streptomyces*-derived hyaluronidase (VWR Scientific, Bridgeport, NJ) for 72 hours at pH 5.0 (acetate buffer) and 37°C. This species of hyaluronidase depolymerizes HA using a unique mechanism that introduces a double bond into the resulting oligosaccharide.[11] Exhaustive digestion of unmodified hyaluronate results in a mixture of tetra- and hexasaccharides.[11] These resulting oligosaccharides were analyzed using high-performance liquid chromatography (HPLC).[12,13] When chemically modified or cross-linked HA is subjected to this enzymatic digestion and analysis, one observes higher-MW oligosaccharides that reflect the chemical modification of the gel.

Conditions of HPLC analysis:

Column: Anionic exchange (4 × 250 mm, CarboPac PA 100, Dionex Corporation, Sunnyvale, CA)

Mobile Phase- A: water
B: 0.4 M sodium phosphate, pH 5.8

Flow rate: 0.8 mL/min
Gradient: step linear

Gradient Table:

Time (min)	*% A*	*% B*
0	90	10
5	90	10
55	20	80
57	90	10

UV detection—232 nm

Injection Volume: 50 μL/each injection

The method separates the digest fragments based on the overall anionic charge of the oligosaccharides. A HPLC analysis of an incomplete hyaluronidase digest was used to generate an elution profile based on oligosaccharide size. Because the hyaluronidase could not digest the cross-linker, after complete digestion of the modified HA, the detection of those peaks that elute at retention times greater than or equal to those of the octasaccharides are the result of covalent cross-linking of these particular oligosaccharides. Therefore, percentage of cross-linking is determined to be the sum total for all of these late-eluting peaks. Because the integrated peak area is proportional to the concentration of each fragment, the relative percentage of cross-linked or pendant modification was determined.

Rheology Measurements

Rheological characterization was performed using an automated Controlled Stress Rheometer (Malvern Instruments LTD, Worcestershire, UK), using a parallel-plate, cone-and-plate, or cylinder-and-cup measuring system at 25°C. The elastic (G′) and viscous (G″) moduli and phase angle (°) were determined using a frequency sweep test. The experiments were performed within the range of the linear viscoelastic region. The phase difference between the stress and strain in an oscillatory deformation is measured as a phase angle that is equal to tan-1 (G″/G′). The G′ measured at frequency 5 Hz for these gels were compared in this study.

Swelling–Dilution Durability Assay

In the dilution study, test samples were diluted with various volume ratios of sample to phosphate buffered saline (PBS) ratio ranging from 1:0.33 (33% dilution) to 1:4 (400% dilution). For each sample lot, three to four different dilutions were made. The diluted gels or solutions were mixed and then tested for rheological properties. The phase angle of the sample at different dilutions was determined on a Bohlin CVO-50 rheometer (Malvern Instruments LTD) using an oscillation test at a frequency of 1 Hz. The percentage change in phase angle for each sample was calculated and plotted against the percentage dilution. The percentage dilution at which the phase angle increased to 50% of its original value was defined as the dilution durability. The dilution durability can be interpreted as the maximum swelling of the gel before phase separation.

Concentration Measurement According to Hexuronic Acid Assay

All samples were diluted with 2N sulfuric acid and then heated in an oven for 1 hour at approximately 95°C. After being cooled to ambient temperature, the samples were diluted with deionized water to a final concentration of approximately 10 to 75 μg/mL hexuronic acid.

A Bran Luebbe Flow Injection Autoanalyzer 3 System (SEAL Analytical Inc., Mequon, WI) was used to measure the total concentration of hexuronic acid as glucuronic acid. The sulfuric acid–treated HA samples and various concentrations (10, 25, 50, and 75 μg/ml) of glucuronic acid standards were injected in sequence through the autoanalyzer. In the autoanalyzer, each sample is first mixed with sulfuric acid–borate and heated at 95°C and then mixed with 0.1% carbazole–ethanol and heated at 95°C again. At the end, a pink color forms that is quantified by measuring the absorbance at 530 nm. The HA concentration of each injected sample was calculated by comparison with authentic standards of glucuronic acid.

Gel-to-Fluid Ratio Using Size-Exclusion Chromatograph with the Multi-Angle Laser Light Scattering Measurement

Size Exclusion Chromatograph (SEC) with the Multi-Angle Laser Light Scattering (MALLS, Wyatt Technology Corporation, Santa Barbara, CA) and refractive index detection can provide direct MW and concentration measurement of soluble polymer in the sample. Dermal filler products were diluted with PBS, thoroughly agitated, and then centrifuged to separate the gel phase from the supernatant. The

supernatant, which corresponds to the fluid portion of each sample, was filtered through a 0.45-μm filter and then injected into the SEC/MALLS system to determine MW and HA concentration. The gel-to-fluid ratio could be calculated using the following equation:

Gel/Fluid Ratio =
{[Total HA Conc] − [Soluble HA Conc]}/
[Soluble HA Conc]

Particle Size

Particle size and distribution measurements were performed on a Malvern Master Sizer Longbed-S particle analyzer (Malvern Instruments LTD). Test samples were placed in a saline suspension in the particle analyzer and scanned for mean particle size and distribution.

Results

A summary of the properties of various fillers is available in Table 1

Discussion

HA Filler Performance

For many years, scientists and physicians have debated which parameter has the most influence on HA filler duration. In the past, HA concentration and gel particle size were thought to be the most important differentiating parameters.[1,3,4,14] As a result, companies provided line extensions that purported to extend product duration by increasing the HA concentration or particle size. Examples of products with greater HA concentration include Juvederm 18 and Juvederm 24 by Allergan (formerly Corneal) and Belotero Soft/Belotero Basic by Merz, whereas Hylaform/Hylaform Plus by Genzyme and Restylane/Perlane by Q-Med are examples of differentiation by particle size.

The clinical evaluation of Hylaform and Hylaform Plus as well as Restylane and Perlane, the HA gels with the same chemical formulation but different particles sizes, demonstrated that larger particle size does not extend duration of those formulations.[15–20] One explanation for these results is that the particle sizes are not sufficiently different (~700 m for Hylaform Plus and Perlane, vs 500 and 300 m for Hylaform and Restylane, respectively) to translate into a discernible clinical effect. Therefore, large-particle-size fillers could be beneficial for filling deeper wrinkles, although one should not expect a longer duration than with a small particle filler of the same composition.

HA concentration is a principal parameter in influencing product duration, although as previously discussed, it is not the total HA concentration that affects duration, but rather the amount of cross-linked HA gel that plays an important role in filler performance. Unmodified HA is completely metabolized a few days after injection.[5] Table 1 lists values for the free HA concentration and cross-linked HA

TABLE 1. Properties of Fillers in the Study

	Hylaform	*Hylaform Plus*	*Prevelle*	*Restylane*	*Perlane*	*Juvederm 30 HV*
Total HA concentration (mg/mL)	5.5	5.5	5.5	20	20	24
Gel-to-fluid ratio	98:2	98:2	98:2	75:25	75:25	60:40
HA gel concentration (mg/mL)	5.4	5.4	5.4	15.0	15.0	14.4
Degree of HA modification (%)	23	23	23	3	3	10
Percentage cross-linked HA	12	12	12	1.2	1.4	2
Dilution durability/percentage swelling	<25	<25	<25	50	50	300
G′ modulus (Pa)	140–220	140–220	230–260	660	588	105
Average particle size (μm)	500	700	350	300	650	300

HA = hyaluronic acid.

gel concentration for commercially available dermal fillers. Hylaform/Prevelle has 98% or 5.4 mg/mL of cross-linked HA gel, whereas Restylane/Perlane has 75% or 15.0 mg/mL, and Juvederm 30 HV has only 60% or 14.4 mg/mL of cross-linked HA gel component contributing to their duration.

Another important characteristic that affects clinical performance is the degree of cross-linking that was introduced earlier. Quite frequently, the degree of cross-linking is used interchangeably with the degree of total modification when describing HA dermal fillers. We need to remember that total modification includes the percentage of cross-link plus the percentage of pendant. The cross-link ratio can be defined as the ratio of percentage of cross-linking to the percentage of total modification and can be used as a way of characterizing a particular gel. For example, the ratio of cross-linked HA to modified HA is approximately 50% for Hylaform/Prevelle, 40% for Restylane, and as low as 20% for Juvederm 30 HV, as described in Table 1. This ratio is dependent on reaction conditions used to produce these products. The HA modified with predominantly pendant groups forms gels that are held together by physical entanglement due to interchain hydrogen bonding. These gels are not as strong as the ones produced by creating a covalently cross-linked network. Therefore, when comparing HA gels with the same concentration and total degree of modification, gels with a high cross-link-to-pendant ratio should provide better resistance to degradation and deformation and thus should maintain longer duration of effect than those with predominantly pendant groups.

To further understand performance of the HA fillers in the clinical setting, it may be useful to combine the HA gel concentration and degree of cross-linking together. Table 1 shows that Restylane/Perlane and Juvederm 30 HV have similar HA gel concentration (15.0 and 14.4 mg/mL, respectively) and percentage cross-linking (1.3% and 2%, respectively). Evaluation of these three products in the controlled clinical studies showed duration of effect of 6 months in the majority of patients.[17,18,21] Although these products were not tested side by side in the clinic, the clinical trial designs were similar, allowing us to postulate that comparable results are due to similar concentration and percentage cross-linking exhibited in the two products.

Pendant modification is a result of the reaction conditions and is not specific to a bifunctional cross-linker. Pendant modification can change the conformation of the HA molecule, rendering it less soluble than unmodified HA, although this type of modification does not produce strong covalent bonds to retard the degradation and deformation of HA network and therefore is more likely to contribute to gel swelling than to its longevity. This could partly explain high swelling of Juvederm 30 HV, as shown by our in vitro testing, because this product mostly contains the pendant type of HA modification (8%) and less cross-linked type (2%) (Table 1) and is supported by the clinical experience.[9,21]

Another reason for swelling of Juvederm 30 HV is its nonequilibrium state that forces the formulation to hydrate by attracting fluids after injection.[9] The same is true for Restylane/Perlane, although in this case, the nonequilibrium hydration state is most likely the reason for continued HA gel swelling when implanted.

Hylaform/Hylaform Plus and Prevelle also have a substantial percentage of pendant-type modification (11%), which constitutes 50% of the total modification of the HA in these formulations. However, these products do not swell as much (Table 1), in part because of the high degree of cross-linked HA network holding the structure together and because of the fully hydrated state of the product.

The analysis of HA fillers is not complete without understanding a role of gel hardness or G′ in their performance. This parameter depends on HA concentration, starting MW, type of modification, and presence or absence of unmodified HA in the finished product. The elastic modulus can be used to help define the particular application of the HA

filler. Firm gels with high G′ provide better resistance to deformation but may feel stiffer or lumpier when injected than a softer gel. Firm gels may induce more trauma to the tissue than the soft gels, thus potentially leading to more pain, inflammation, edema, and erythema postinjection. Soft gels with low G′ do not resist deformation as well as the firm gels but could provide a more natural feel when injected. Softer gels may be better suited for use in less-dynamic wrinkles such as tear troughs or the soft tissue found in lips and the periorbital region, where a soft feel is important to a patient.

The data on the modulus G′, presented in Table 1, shows that Juvederm 30 HV is the softest of the HA fillers reviewed (G′ 105 Pa), closely followed by Hylaform/Hylaform Plus (G′ 140–220 Pa) and Prevelle (G′ 220–260 Pa). The modulus for Restylane/Perlane (G′ 600–700 Pa) is six times as great as that of Juvederm 30 HV and three times as great as Hylaform, Hylaform Plus, and Prevelle. The FDA has approved all of these products for the same indication, but these differences in the modulus may be used as guidance to physicians to refine the use of each product to better suit the needs of their patients. Arguably, the difference in G′ values may be too small to drive a need to change the current practice of using these HA fillers.

It is important to remember that no single parameter defines a use of a HA filler; therefore a careful review of these gel characteristics is essential to proper understanding of each filler's performance, selecting the correct filler for an application, and setting correct expectations for the patients to meet their needs.

Conclusions

Commercially available HA-based fillers have a wide variety of properties that have an extensive effect on their use and clinical outcomes. Combining the objective factors that influence filler performance with clinical experience will result in providing the patient with the optimal product for achieving the best cosmetic result. In this article, we have provided the reader with objective in vitro methods and parameters for characterizing the HA fillers they may employ in their practice. Understanding these characteristics can be important when selecting a filler for a given application and patient. It should be remembered that actual clinical results are dependent not only on HA filler characteristics, but also on the response of the biological host. Degradation of the implant, whether due to enzymes or free radicals, will vary from patient to patient. Injection depth and technique can have a profound effect on the degree of hydration and inflammatory response to the implant. Consequently, patient satisfaction is not solely dependent upon physical properties of the filler, implantation technique and biological host response contribute to final outcome. Finally, it is important to remember that no single parameter defines a use of a HA filler; therefore, a careful review of these gel characteristics is essential for proper understanding of each filler performance and in setting correct expectations for patients to meet their needs.

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