

# Rheological Evaluation of the Physical Properties of Hyaluronic Acid Dermal Fillers

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## ABSTRACT

**Background:** Hyaluronic acid (HA) gels are commonly injected into the skin to lift rhytides and to improve facial appearance. The different processes used in their manufacture and formulation yield products with unique physical characteristics that play an important role in predicting their clinical performance.

**Objective:** The following rheologic evaluation was performed to objectively measure the physical characteristics of HA dermal filler products derived from similar bacterial sources and containing the same butanediol diglycidyl ether cross-linker, but formulated using different manufacturing techniques. The objective of this study was to evaluate the physical characteristics of two distinct families of HA products, thereby providing clinicians with a greater understanding of these products' attributes and the ability to optimize their use in the treatment of patients seeking facial rejuvenation.

**Materials and Methods:** The physical properties of commercially-available dermal fillers containing HA were evaluated using rheologic testing methods under clinically-relevant conditions. Additionally, light microscopy was used to assess the particulate nature of each product.

**Results:** The gels tested demonstrated a broad range of elasticity, firmness and viscosity. Light microscopy confirmed the particulate nature of each product and revealed HA particles of varying size and distribution.

**Conclusion:** This rheologic evaluation demonstrates that differences exist among the HA products tested including gel elasticity, viscosity, and the range and distribution of gel particle sizes. Understanding the distinct physical characteristics of different HA dermal fillers and how these characteristics may predict their clinical behavior can assist clinicians in achieving the desired results in patients seeking facial rejuvenation.

*J Drugs Dermatol.* 2011;10(9):974-980.

## INTRODUCTION

**H**yaluronic acid (HA) is a glycosaminoglycan that consists of repeating units of glucuronic acid and N-acetyl-glucosamine (hyaluronan) and which readily dissolves in water to form a viscous gel. It is found in the extracellular matrix of many tissues, and approximately one-half of the HA in the human body is found in the skin where it plays an important role in providing structure and maintaining normal moisture content.<sup>1</sup> During the aging process, the HA content of the dermis decreases and this contributes to volume loss, diminished dermal water-binding capacity and the development of rhytides.<sup>2</sup> As HA is a physiologic component of human skin, HA-containing products are well-suited for use as dermal fillers in treating patients seeking facial rejuvenation.

Commercially-available HA dermal fillers are similar in many respects. They all contain HA obtained through the fermentation of *Streptococcus* sp. bacteria and the HA molecules are joined together using cross-linkers such as butanediol diglycidyl ether (BDDE) to prevent rapid in vivo enzymatic and oxidative degradation.<sup>1,3</sup> Despite these similarities, HA dermal fillers differ in their physical characteristics and therefore may behave clinically in different ways, based upon the manufacturing methods used,<sup>2,3</sup> which determine the type and extent of HA cross-linking and the size and concentration of HA particles.<sup>1,3</sup>

For example, five HA dermal fillers contain HA derived from similar bacterial cultures and are stabilized with the same BDDE

cross-linker. One family of products contains HA that is manufactured using a patented NASHA™ process while another utilizes a proprietary Hylacross™ technology.<sup>2</sup> Regardless of the manufacturing methods employed, each produces gels containing particulate HA.<sup>1,5</sup> Differences include particle size distribution,<sup>1,5</sup> total HA concentration<sup>1,3</sup> and the extent of crosslinking.<sup>1,2,6,7</sup> Fully hydrated gels that have reached their maximum water-binding capacity will not swell following injection while there is a tendency for less hydrated gels to swell after injection.<sup>1</sup> Together, these properties may alter the firmness, elasticity and viscosity of the gels and impact the way they behave in the skin, including the amount of structural support or lift they provide which is related to the extent to which they improve the appearance of rhytides.

Rheology is a branch of physics that studies how materials such as HA gels behave in response to applied forces. The physical properties of HA gels are described using a variety of rheologic terms (Table 1). One of the most frequently discussed characteristics of HA gels is the elastic modulus, represented by the symbol  $G'$  (G prime). This is the ability of a gel to resist deformation by an applied force. A gel with a numerically higher value for  $G'$  is better able to resist alterations in shape and is described as being firmer, harder or more elastic than a gel with a lower  $G'$ . As gels with a higher  $G'$  store a greater amount of the energy being applied to them, this property may also be referred to as the storage modulus. More stored energy allows a gel to

TABLE 1.

Rheology Terms Used to Describe Hyaluronic Acid Gels

Elasticity	The ability of a gel to “spring back” to its original shape after being deformed.
Elastic modulus	A measure of stored energy in a viscoelastic material, represented by the symbol $G'$ .
Viscosity	The flow characteristics of a gel; used to define gel thickness.
Viscous modulus	A measure of dissipated energy in a viscoelastic material represented by the symbol $G''$ .
Complex modulus	The total resistance to deformation of a HA gel determined by the vector sum of $G'$ and $G''$ , represented by the symbol $G^*$ .
Complex viscosity	The viscosity calculated from frequency sweep, represented by $\eta^*$ , the Greek letter eta.
Viscoelastic	A term used to describe HA gels which possess both elastic and viscous properties.
Shear force	An external force which is applied parallel to a gel by placing it between two plates that twist in opposite directions
Shear thinning	Decreasing gel viscosity with increasing rate of deformation.

“spring back” to its original shape to a greater extent after being deformed. Clinically, dermal fillers with higher  $G'$  values are expected to provide greater structural support and volumization.<sup>2</sup>

Understanding the distinct physical characteristics of different HA dermal fillers and how these characteristics may predict their clinical behavior can assist clinicians in achieving the desired results in patients seeking facial rejuvenation.

Another important property of HA gels is viscous modulus, represented by the symbol  $G''$  (G double prime). Gels with numerically higher values of  $G''$  are described as being thicker or more viscous than gels with a lower  $G''$ . Since a greater amount of energy applied to gels with a higher  $G''$  is lost as dissipated heat,  $G''$  is also referred to as loss modulus. A related property is complex viscosity, represented by the symbol  $\eta^*$  (Greek letter eta). This is the ability of the gel structure to resist shear forces. A shear force may be applied to a gel by placing it between parallel plates rotating in opposite directions. If the shear rate is steadily increased, the gel will reach a critical point where  $\eta^*$  will gradually decrease. This phenomenon is known as shear thinning. Although the physical properties of HA dermal fillers are often reported in terms of  $G'$  and  $G''$ ,<sup>1,8</sup> a more comprehensive measure of the total resistance to deformation is known as the complex modulus which is the sum of  $G'$  and  $G''$  and is represented by the symbol  $G^*$ .<sup>9</sup>

Previous rheologic studies have evaluated the physical properties of a variety of commercially-available HA-containing fillers, demonstrating that these products possess a range of elastic<sup>1,8,9</sup> and viscous characteristics.<sup>8,9</sup> Understanding these differences is relevant to the practicing clinician as it impacts filler product selection and injection location, based on the needs and desired results of the patient. Numerous clinical studies have established the effective use of HA dermal fillers for soft-tissue augmentation<sup>10-17</sup>; however, understanding of the differences in the physical characteristics of these products may enable clinicians to better predict their clinical performance and thus to optimize patient outcomes.

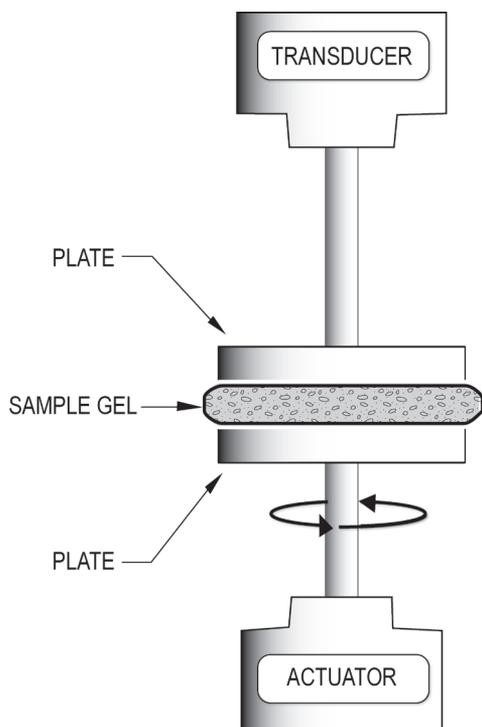
The following rheologic study was performed to better understand the physical properties of two widely used families of HA dermal filler products and to objectively evaluate their distinct characteristics.

## METHODS

### Materials

The HA dermal fillers tested were Restylane® and Perlane® (Medicis Aesthetics, Inc., Scottsdale, AZ); and Juvéderm Ultra,® Juvéderm Ultra Plus® and Juvéderm Voluma® (Allergan, Inc., Irvine, CA).

**FIGURE 1.** Testing apparatus. The behavior of dermal fillers in response to applied shear forces was measured using a rotational or shear rheometer. Each gel was applied between two parallel circular plates. The actuator applied a variable rotational strain to each gel by oscillating at varying frequencies. The resistance of each gel to these applied forces was measured by the fixed transducer.



### Rheologic Methods

The behavior of each gel in response to an applied shear force was measured using a rheometer (Rheometrics ARES; TA Instruments Ltd., West Sussex, England). A sample of each gel was squeezed between two parallel circular plates and a variable rotational strain was applied to the gel by rotating one plate at varying frequencies while the other remained fixed (Figure 1). The rheometer measures the resistance of the gel to these applied forces. To approximate the anticipated effects of facial muscle movement and gravity on implanted fillers, a gradually increasing shear force (measured in strain sweeps) was applied to each gel at a constant rate of 10 rads/sec (~1.6 Hz).

Testing was performed within the linear viscoelastic range of each product, defined as the maximum amount of deformation each gel can withstand without changing its physical properties in an unpredictable and potentially irreversible manner. Increasing rates of deformation (1-100 rads/sec) at a fixed strain (frequency sweeps) were then applied to each gel followed immediately by decreasing rates of deformation (100-1 rads/sec). During both procedures, the range and rates of deformation remained the same. The results of this experiment established the viscosity ( $G''$  and  $\eta^*$ ), elasticity or firmness ( $G'$ ) and total resistance to deformation ( $G^*$ ) of each product.

Using these data, the percent elasticity, or the extent to which each product returned to its original shape after deformation, was determined based on the relationship: percent elasticity =  $100 \times G'/(G'+G'')$ . The recovery coefficient was then calculated for each gel by dividing the viscosity value obtained during the increasing sweep frequency by the viscosity value obtained during the decreasing sweep frequency. Values for the recovery coefficients were interpreted as follows ("structure" refers to short- or medium-range interactions between molecules in a fluid by physical or chemical processes):

- $\sim 1$  = the gel retained its structure despite applied forces.
- $> 1$  = the gel experienced structural breakdown.
- $< 1$  = the gel experienced increased structure.

### Light Microscopy

Light microscopy was performed using a Nikon SMZ-10A Stereoscopic Zoom microscope (Nikon Corporation, Japan) to characterize the particulate nature of the gels with respect to particle size and distribution. To enhance visual contrast, some samples were diluted in water and stained with toluidin blue.

### RESULTS

Restylane and Perlane were firmer than Juvéderm Ultra, Juvéderm Ultra Plus and Juvéderm Voluma as demonstrated by higher elasticity modulus ( $G'$ ) and complex modulus ( $G^*$ ) values (Table 2). The  $G'$  and  $G^*$  for Restylane and Perlane were higher than that of Juvéderm Ultra, Juvéderm Ultra Plus and Juvéderm Voluma. Percent elasticity for Restylane and Perlane was also higher than for Juvéderm Ultra and Juvéderm Ultra Plus at both tested frequencies but was highest for Juvéderm Voluma (Table 3). These five products demonstrate variable abilities to resist deformation and return to their original shape or "spring back" after a force has been applied. During controlled stress measurements, all samples behaved in a similar manner at stresses between 10 and 100 Pa. The recovery coefficients were found to be approximately 1 for all samples, meaning they retain their internal structure.

**TABLE 2.**

Elasticity and Complex Modulus at Test Frequency of 10 rads/sec		
	$G'$ (Pa)	$G^*$ (Pa)
<b>Restylane</b>	<b>293.9</b>	<b>301.6</b>
<b>Perlane</b>	<b>337.9</b>	<b>344.4</b>
<b>Juvéderm Ultra</b>	111.2	119.6
<b>Juvéderm Ultra Plus</b>	159.2	166.9
<b>Juvéderm Voluma</b>	219.9	221.0

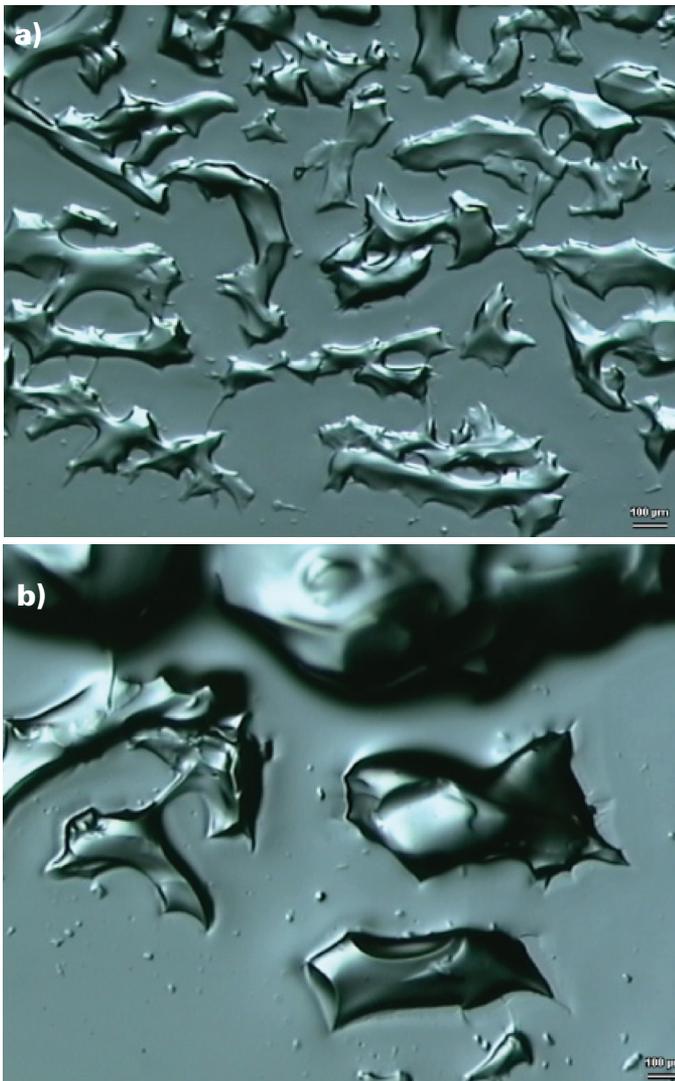
Each experiment was performed at a fixed strain amplitude (1%) and temperature (24° C).

Photomicrographs illustrate that samples of Restylane and Perlane (Figure 2) both contain well-defined particles of very similar size with Perlane having larger-sized particles. Juvéderm Ultra, Juvéderm Ultra Plus and Juvéderm Voluma are also particulate in nature; however, their broader range of sizes make the particles in these products appear less obvious under light microscopy due to the similar refractive indices of the HA particles and surrounding liquid phase (Figure 3). Visualization of HA particles in a sample of Juvéderm Voluma with light microscopy was possible with the use of a staining technique. Toluidine blue revealed the particulate nature of this gel as shown in high- and low-magnification photomicrographs (Figure 4).

## DISCUSSION

Soft tissue augmentation with HA fillers has become one of

**FIGURE 2.** Photomicrographs of Restylane and Perlane. Photomicrographs of (a) Restylane and (b) Perlane. Each scale bar=100  $\mu$ M.



the most commonly performed cosmetic procedures in the United States.<sup>18</sup> Advantages of HA dermal fillers include an excellent safety profile characterized by low immunogenicity following administration,<sup>19</sup> durability,<sup>13,20</sup> and reversibility with hyaluronidase.<sup>21</sup> Additionally, Wang and colleagues demonstrated that the injection of a small gel particle HA filler (Restylane) stimulates natural collagen production that is presumed to be induced by the mechanical stretching of the dermis and activation of dermal fibroblasts.<sup>10</sup> Current FDA-approved indications for the use of HA fillers include soft tissue correction of moderate to severe facial rhytides and folds such as nasolabial folds; however, HA fillers are also commonly used for improvement of lip rhytides, marionette lines and nasojugal folds, lip filling and contouring, and chin and cheek augmentation.<sup>22</sup> For these varied clinical applications, it may be beneficial to utilize HA fillers that display a range of physical characteristics in order to meet individual patient needs.

In this study, the higher  $G'$  and  $G^*$  values for Restylane and Perlane indicate that they are firmer gels and are able to resist deformation to a greater extent when a force is applied. These results are consistent with previously reported data demonstrating that Restylane and Perlane possess higher  $G'$  values than Juvéderm Ultra and Juvéderm Ultra Plus<sup>1,8</sup> (Table 4). Expressed as  $G^*$  (Pa), other investigators also found the firmness of Restylane (306.6) and Perlane (334.5) to be greater than Juvéderm Ultra (36.7) and Juvéderm Ultra Plus (51.46).<sup>9</sup> Although the absolute values for  $G^*$  and  $G'$  vary across published reports, their relative magnitudes remain similar. As with any testing method or experiment, differences in reported values represent variations in the rheologic testing parameters such as the oscillation frequencies employed and the temperature at which testing was performed.

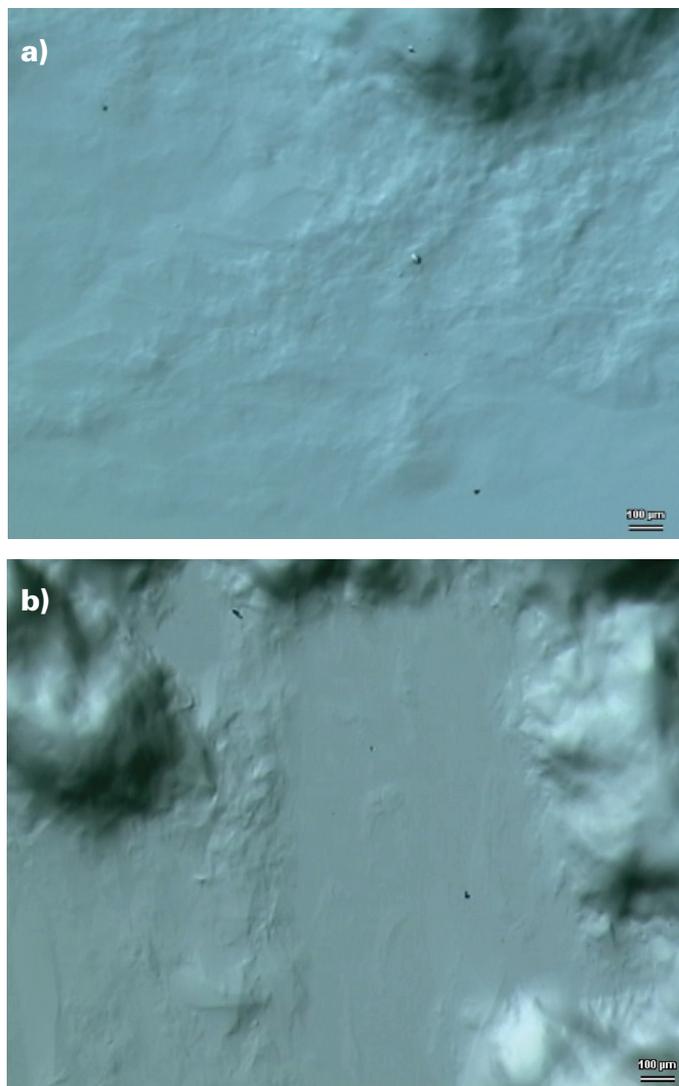
In a recent publication comparing the lifting capacity of HA fillers, Borrell and colleagues concluded that Juvéderm Ultra and Juvéderm Ultra Plus are able to achieve high lifting capacity by combining lower relative  $G'$  with higher "cohesivity," which is described as "the tendency of a gel to stick together and hold its form or shape under stress".<sup>23</sup> The authors use the term "cohesive" to describe greater crosslinking

**TABLE 3.**

	Percent Elasticity Test Frequency (rads/sec)	
	1	10
Restylane	81.6	81.3
Perlane	83.9	83.5
Juvéderm Ultra	72.9	71.7
Juvéderm Ultra Plus	78.6	76.1
Juvéderm Voluma	88.6	90.8

Each experiment was performed at a fixed strain amplitude (1%) and temperature (24° C).

**FIGURE 3.** Photomicrographs of Juvéderm Ultra (a) and Juvéderm Ultra Plus (b). Each scale bar=100  $\mu$ M.



which holds the HA particles together and compensates for decreased lifting capacity of a low  $G'$  gel. While a higher degree of crosslinking might confer benefits upon a HA filler in some clinical situations, the concept that this can alter the effect of lower  $G'$  on lifting capacity is not supported by other studies reported in the literature.<sup>1,8,9</sup> Additionally, the force and strain levels used for conducting the parallel plate and linear compression rheometry testing were not reported in this study, making interpretation of the results difficult.

The extent of HA gel crosslinking or modification is expressed as the percentage of HA disaccharide monomer that is bound to crosslinker molecules. For example, 5% crosslinking means that there are an average of five crosslinker molecules per 100 HA disaccharide monomers.<sup>3</sup> An increase in the degree of modification has been reported to result in

**TABLE 4.**

Elasticity Modulus ( $G'$ ) Determinations From Three Studies			
	Study 1 <sup>a</sup>	Study 2 <sup>b</sup>	Study 3 <sup>c</sup>
Restylane	588	541	293.9
Perlane	660	513	337.9
Juvéderm Ultra	Not Tested	28	111.2
Juvéderm Ultra Plus	105	75	159.2
Juvéderm Voluma	Not Tested	274	219.9

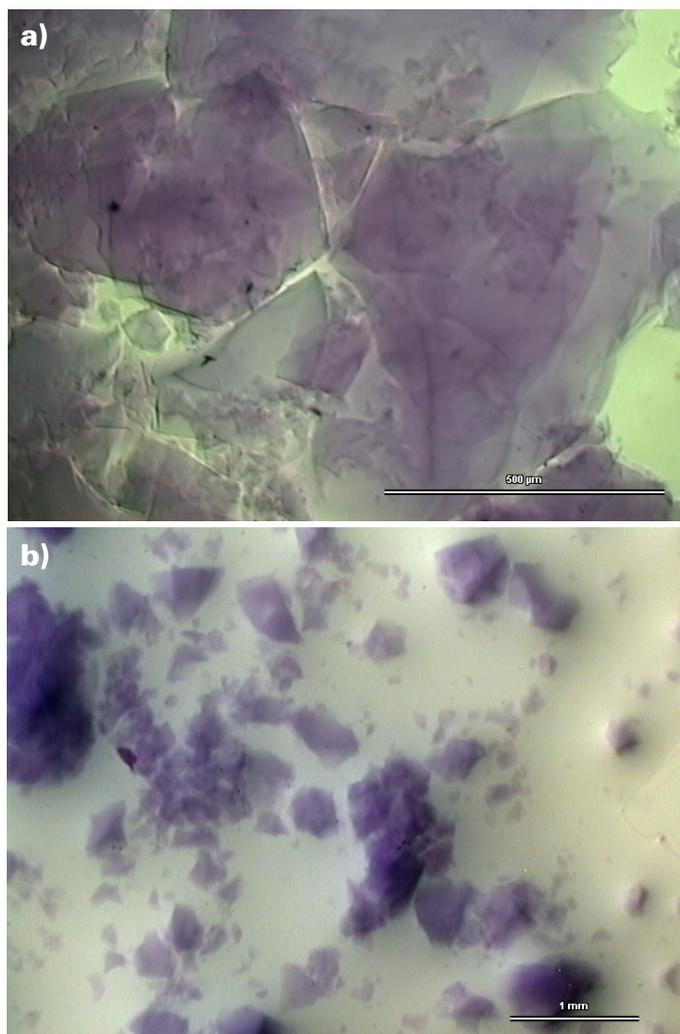
<sup>a</sup>Kablík et al., 2009; <sup>b</sup>Sundaram et al., 2010; <sup>c</sup>Present study. Note that these studies were performed under variable testing conditions.

harder or firmer gels<sup>1,3</sup>; however, we found that measurements of  $G'$  were not exclusively related to cross-linking. Juvéderm Ultra and Juvéderm Ultra Plus contain 24 mg/mL of HA<sup>24,25</sup> which is 6–8% cross-linked. Nuclear magnetic resonance studies have shown that the mean proportion of cross-linking of the 20 mg/mL of HA contained in Perlane<sup>26</sup> is approximately 1%.<sup>4</sup> No free or soluble HA is added during the manufacturing process to this family of dermal fillers, although some breakage of HA chains may occur during the manufacturing process of any gel resulting in the presence of small amounts of soluble HA.<sup>4</sup>

As demonstrated by their higher values for elasticity modulus ( $G'$ ) and complex modulus ( $G^*$ ), Restylane and Perlane are firmer than the Juvéderm family of dermal fillers. Percent elasticity values are calculated based on a ratio of  $G'$  and  $G''$  using the formula  $100 \times G'/(G'+G'')$ . Therefore, a gel with high  $G'$  and  $G''$  may have the same value for percent elasticity as a gel with low  $G'$  and  $G''$ . Together, the fluid and particle components in Juvéderm Voluma as the finished product demonstrated greater percent elasticity than the other fillers in this analysis due to the greater concordance between  $G'$  and  $G''$ ; however, it is the elasticity of the gel particles alone which contributes to the lift effect of a HA dermal filler.

The extent of HA modification also influences water absorption, which is inversely proportional to the degree of cross-linking.<sup>27</sup> The physical properties of HA gel can be modified by the proportion of BDDE molecules that attach two strands of HA together (cross-linked) and the proportion that are attached to only one strand of HA (pendant). Thus, the physical characteristics of each HA filler product and its clinical performance are not dependent on any single factor but result from a combination of numerous factors related to its manufacturing process. Newer HA fillers containing lidocaine to minimize patient discomfort during injection, such as Restylane-L, Perlane-L, Juvéderm Ultra XC and Juvéderm Ultra Plus XC are manufactured using processes that ensure homogenous distribution of the anesthetic without altering the physical properties of the products.<sup>18,28</sup>

**FIGURE 4. a-b)** Photomicrographs of Juvéderm Voluma under high (a) and low (b) magnification. The particulate nature of the gel is demonstrated using a toluidin blue staining technique. Left scale bar=500  $\mu$ m, right scale bar=1 mm.



Photomicroscopy confirmed previous reports describing the particulate nature of the gels tested.<sup>1</sup> Although the average particle size was similar for the HA filler products tested here,<sup>1</sup> there was considerable variation in the distribution and range of particle sizes. The size of the HA particles in Restylane and Perlane is more uniform, while Juvéderm Ultra, Juvéderm Ultra Plus and Juvéderm Voluma contain HA with a wide range of particle sizes. It is probable that these differences are due to the different processes employed in the manufacturing of the products. Using laser diffraction techniques, other investigators have also demonstrated that Juvéderm Ultra and Juvéderm Ultra Plus contain HA particles of more varied sizes.<sup>5</sup>

While the dermal filler products in this study demonstrated different degrees of elasticity or firmness, viscosity, and distribution of particle size, the value of various dermal fillers ultimately depends on how they are used clinically.<sup>29-31</sup> The structural support and lift-

ing capacity of HA fillers that possess a high degree of firmness ( $G'$ ) and viscosity may be more desirable in treating deep folds and creating lift and volume while products that are less firm and viscous may be better suited for treating shallower folds and lines. Also, dermal fillers with greater viscosity and elasticity will tend to resist spreading after implantation.<sup>8</sup> Brandt and Cazzinga suggest using firmer HA dermal fillers for treating the glabella, cheeks, jawline, periocular and perioral areas while softer fillers may be more useful in treating the labiomental crease and lips.<sup>30</sup>

## CONCLUSION

The results of this rheologic study indicate that five commonly-used HA soft tissue fillers possess a range of physical properties, including firmness and viscosity. Microscopy and staining confirmed the particulate nature of each product with varying ranges related to particle size and distribution. Although in vitro studies provide less direct evidence than comparative clinical trials, the objective methods employed in this study are of value to measure, describe and understand the physical attributes of HA soft tissue fillers. Appreciation of these properties and their potential impact on clinical performance may better equip the clinician to select appropriate HA filler products based on patient needs and desired outcomes.

## ACKNOWLEDGEMENTS

The authors acknowledge the assistance of Dr. Carl S. Hornfeldt during the preparation of this manuscript.

## DISCLOSURES

This study was sponsored by Medicis Aesthetics, Inc., Scottsdale, AZ, USA. The rheological and microscopic analyses of samples in this study were performed by the independent laboratory Intertek Measurement Science Group, Redcar, UK (David Stocks, BSc [Hons]) with funding from Medicis Aesthetics Incorporated, Scottsdale, AZ.

Hema Sundaram, MD, has served as a consultant for, and has received grants and/or research support from: Medicis Aesthetics Incorporated, Scottsdale, AZ; Mentor Worldwide, LLC, Santa Barbara, CA; Merz Aesthetics, Inc., San Mateo, CA; Suneva Medical, Inc., San Diego, CA.

Jason Michaels, MD, has served as speaker and consultant for Medicis Aesthetics Incorporated, Scottsdale, AZ and as consultant for Allergan, Inc., Irvine, CA.

Mitchell S. Wortzman, PhD and Diane B. Nelson, BSN, MPH are employees of Medicis Aesthetics Incorporated, Scottsdale, AZ. Manzer J. Durrani, PhD, was an employee of Medicis Aesthetics Incorporated at the time this study was conducted.

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