The first article in this series outlined the methodology of rheologic studies and discussed their value and limitations. This second article presents data from recent rheologic studies of hyaluronic acid and calcium hydroxylapatite fillers and discusses how these data correlate with our current understanding and use of these fillers.

Rheology—the study of flow-related properties—has been recognized for almost a century as being relevant to a number of fields, including materials science, geophysics, and pharmaceutics. Rheologic theory and measurements are considered critical for the manufacture of topical medications, paints and inks, concrete, and even chocolate. The discovery that rheology could shape and guide our clinical use of soft tissue fillers was serendipitous.

Evolution of the Clinical Use of Hyaluronic Acid Fillers
The initial years following FDA approval of the first hyaluronic acid (HA) filler, Restylane (Medicis) in 2003 were marked by an evolution in injection strategy. Although Restylane was initially injected into the dermis, like its collagen predecessors, it soon became apparent that this product was better placed subdermally. One imperative was to avoid what aesthetic specialists call the Tyndall effect (more accurately called Rayleigh scattering), the phenomenon by which a bolus of hyaluronic acid implanted into the superficial dermis scatters the light striking it so that blue light (which has a shorter wavelength) is preferentially reflected back to an observer’s eye. This gives a bluish appearance to the implanted filler, even though it is actually colorless.1,2 Another reason for the move toward deeper injection of Restylane was that it produced more impressive clinical results, allowing the volumization of entire facial zones such as the midface and lower face, rather than the mere filling of individual wrinkles. The effects of panfacial volumization are profound, allowing the restoration of youthful contours and, for some patients, even an improvement on the facial contours of their youth. In retrospect, these effects were a manifestation of the tissue-lifting properties of Restylane, which has relatively high elastic modulus (G prime also written G’).

FDA approval of additional HA fillers brought the realization that, although these products had much in common, they could manifest somewhat different clinical behavior. Hylaform, Hylaform Plus, and Captique were approved in 2004; Juvederm Ultra and Juvederm Ultra Plus (Allergan) in 2006; and Perlane (Medicis) in 2007. The Hylaform products are no longer available, and Captique was reformulated with lidocaine and FDA approved as Prevelle Silk (Mentor) in 2008. The Juvederm and Prevelle Silk products were noted to be softer than Restylane and Perlane—they tended to spread a little more after implantation and were a little less palpable in the tissue. In retrospect, these effects may be attributed largely to the relatively low elastic modulus (G prime) and viscosity of Prevelle Silk and Juvederm Ultra and Ultra Plus.

Even after the introduction of additional HA fillers, many clinicians continued to use a single product to
volumize all facial zones, and many still do currently. However, some began to select different products for distinct clinical applications, based on anecdotal experience and instinct: Perlane and Restylane, for example, might typically be used for zones such as the midface, where lifting was the objective and filler palpability was not problematic, whereas Juvéderm and Juvéderm Ultra Plus might be preferred for areas such as the lips, where palpability after implantation is less desirable and some degree of filler spread into the tissue might be considered aesthetically appealing.

**Rheologic Study of HA Fillers**

The dawning of an evidence-based rationale to define the distinct clinical characteristics of HA filler products came with a seminal paper by Kablik, Monheit, et al. in 2009. This paper presented data pertaining to the physicochemical characteristics of four non animal-derived HA products in current use (Juvéderm Ultra Plus, Perlane, Prevelle Silk, and Restylane) and two animal-derived products that are no longer used (Hylaform and Hylaform Plus). The analysis for each product included total HA concentration (comprising insoluble and soluble HA), elastic modulus (G prime) and gel swelling (a measure of the product’s capacity to absorb water after implantation).

Rheometric measurements at an oscillation frequency of 5 Hz showed Restylane and Perlane to have higher elastic modulus (G prime) than Prevelle Silk and Juvéderm Ultra Plus (Fig. 1).

Total HA concentration was higher for Juvéderm Ultra Plus, Restylane and Perlane (24mg/mL, 20mg/mL and 20mg/mL respectively), which can be classified as high concentration HA products, than for Prevelle Silk (5.5mg/mL).

Insoluble HA gel concentration, a measure of the amount of HA in each product that is cross-linked, was approximately the same for Juvéderm Ultra Plus, Perlane, and Restylane (14.4 mg/ml, 15 mg/ml, and 15 mg/ml respectively), whereas Prevelle Silk had 36 to 37 percent of this concentration (5.4 mg/ml). Evaluation of gel swelling showed that Prevelle Silk had the lowest capacity to absorb water (less than 25 percent dilution durability), followed by Restylane and Perlane (50 percent each), and then Juvéderm Ultra Plus (300 percent). This provides an explanation for the clinical observation that, although any filler can cause tissue swelling...
related to injection technique and other aspects of the injection process. Prevelle Silk, which is a fully hydrated HA, seems to have less inherent tendency to cause swelling than do the other products, which are partially hydrated and tend to produce greater swelling on the second or third day than they do immediately after implantation.

Evolution of the Clinical Use of Calcium Hydroxylapatite Filler

Radiesse (Merz Aesthetics) is a calcium hydroxylapatite (CaHA) filler that was FDA-approved for aesthetic use in 2006. A protocol for mixing Radiesse with lidocaine to produce a final lidocaine concentration of 0.3% was approved in 2009.

Early clinical studies of Radiesse showed it to have significant longevity over HA fillers, with a 12-month corrective effect directly related to the implanted product, and persistent correction after this, presumably from stimulation of neocollagenesis. Radiesse was also observed to have a pronounced lifting effect and to provide efficient volume replacement. Controlled, split-face studies showed that optimal nasolabial fold correction required a significantly smaller volume of Radiesse than of high-concentration HA products.

For these reasons, some clinicians found CaHA filler to be valuable for facial zones where volume-efficient lifting was desired. Deep implantation—ranging from the subdermal or subcutaneous tissue planes in the nasolabial folds and lateral midface to the supraperiosteal plane in the superomedial midface, pre-jowl sulci, chin, and temples—was found to give the best results. The opacity of CaHA and its partial adjustability after implantation, as opposed to the translucence and complete reversibility of HA with hyaluronidase, make it inadvisable for lip augmentation and less suitable for volumization of the anatomically unforgiving periorbital region. Therefore, strategies of differential filler placement have evolved, with CaHA and HA each being placed where they are considered most clinically appropriate and beneficial.

The lidocaine mixing protocol was originally developed to decrease patient discomfort during the injection process. However, a beneficial side effect was that Radiesse with 0.3% lidocaine was found to be easier to extrude from the injection needle than Radiesse alone. As clinicians expanded their use of soft tissue fillers to nonfacial areas, such as the dorsum of the hands, it became common practice to add a slightly larger volume of lidocaine to Radiesse to make it more spreadable. It was discovered that adding lidocaine to the high-concentration HA fillers (Juvéderm Ultra and Ultra Plus, Perlane, and Restylane) also thinned them out and made them more suitable for more superficial implantation into fine rhytides.

Rheologic Study of CaHA Filler

A paper published in 2010 compared the elastic modulus (G prime) and viscosity of CaHA filler (Radiesse) and several HA fillers (Juvéderm Ultra, Juvéderm Ultra Plus, Juvéderm Voluma, Perlane, Restylane, and Restylane Sub Q). Rheometric measurements, taken at 0.7 Hz, were consistent with those obtained by Kablik, et al. for the products common to both studies. The obtained measurements support the concept that these products can be divided into three groups, with Radiesse in the group with the highest G prime and viscosity; Radiesse plus 0.3% lidocaine, Perlane, and Restylane in the medium group; and the Juvéderm product family (Voluma, Ultra, and Ultra Plus) in the low G prime and viscosity group (Figs. 2A, 2B).

It is important to note that this grouping is not intended to rank these products, all of which are safe, efficacious and capable of producing excellent clinical results. It is simply a parsing of their rheologic properties to better understand their behavior during and after injection and optimize their use. Given these data, it can be readily understood that CaHA filler (Radiesse)
provides volume-efficient lifting because it has high G prime and viscosity. The easier extrusion of CaHA plus 0.3% lidocaine from the injection needle can also be explained. Extrusion force is at least partly determined by a filler’s rheologic properties—principally viscosity, which is lower for CaHA plus lidocaine than for CaHA alone although the needle gauge and the size and design of the syringe also play a role. (Fig. 3)

Furthermore, it can be appreciated that the lower viscosity of CaHA with added lidocaine also makes it easier to spread than CaHA alone. Essentially, the adjustment of CaHA and HA spreadability by adding more or less lidocaine is a titration of these fillers’ viscosity.

Study Comparison
The 2009 study of HA fillers employed an oscillation frequency of 5 Hz, whereas the frequency used in the 2010 study of CaHA and HA fillers was only 0.7 Hz. Many researchers now believe that frequencies of less than 2 Hz are more physiologically relevant when evaluating fillers for injection into the skin, given its low-frequency stresses. Some evidence for this concept has been provided by studies of the behavior of Quorn, a mycoprotein-derived meat substitute with a texture and consistency similar to mammalian tissue, when it is exposed to applied shear forces.10

The absolute values of G prime for the products common to both studies vary, and this may be largely caused by the differences in rheometric oscillation frequency. However, the general pattern is consistent, with the same products being found to have relatively high or relatively low G prime. Measurements of G prime and complex viscosity across a range of testing frequencies from 0.1 to 10 Hz have also shown this general pattern9 (Figs. 4, 5).

Interpretation of Rheologic Study Data
In some rheologic studies of gel fillers, flow-related properties are further quantified by secondary measurements derived from the primary measurements of elasticity and viscosity. Two such secondary measurements are the complex modulus and tan delta.

The complex modulus, abbreviated as G*, is calculated as the sum of the elastic modulus (G’) and the viscous modulus (G’’). The complex modulus measures the overall resistance to deformation of a gel, which comprises a recoverable component because of the gel’s elasticity and a non-recoverable component because of the gel’s viscosity.

Tan delta, abbreviated as Tan δ, is calculated as the ratio of viscous modulus to elastic modulus (G’/G’’). It is a measure of the presence and extent of elasticity in a material. Because the tan delta measurement is a ratio, its shortcoming is that materials with high G’ and G’’ and materials with low G’ and G’’ may have similar values. Thus, tan delta may not distinguish adequately between gel fillers with very different flow properties.

In this author’s opinion, the primary rheologic measurements of elasticity and viscosity generally suffice to give a clear picture of how a gel filler will behave when it is exposed to shearing forces, and secondary measure-
ments are of value only when they add to this picture. The HA and CaHA filler products that are currently approved for aesthetic use, and those for which approval is anticipated, all have concordant rheologic parameters—that is, they either have high to medium values for both elasticity and viscosity, or low values for both elasticity and viscosity. Since the complex modulus is the sum of elasticity and viscosity measurements, it will simply parallel these values and, in this author’s opinion, does not significantly enhance understanding or clinical use of these filler products. The potential shortcomings of the tan delta calculation have been discussed in the previous paragraph.

**Clinical Relevance of Rheologic Studies**

Some researchers postulate that the differences in G prime and viscosity between currently available HA products are not sufficient to have clinical impact. Our understanding of the effect of physicochemical properties and other factors on filler behavior in the clinical situation is continually expanding. However, the relevance of G prime and viscosity measurements may become clearer with the advent of two HA products that are currently awaiting FDA approval and lie at opposite ends of the rheologic spectrum.

Belotero Balance (Merz Aesthetics), which is already available outside the United States, has a lower G prime and viscosity than the Juvéderm products. Histopathologic studies have revealed a strikingly homogeneous distribution after Belotero Balance is implanted into the dermis, which may be interpreted as direct microscopic evidence of the effects of viscosity on tissue spread, since the lower a filler’s viscosity, the more readily it spreads after implantation. Belotero Balance has been reported not to cause the Tyndall effect, and this is inferred to be a manifestation of its homogeneous intradermal distribution pattern—and thus of its low viscosity—because the absence of a discrete bolus of HA filler precludes the preferential scattering of blue light. The only other HA filler that does not appear to cause the Tyndall effect is Prevelle Silk, presumably because a bolus of this low-concentration HA product does not contain enough particles to scatter blue light.
The second product awaiting FDA approval in the US and currently approved elsewhere, Prevelle Lift (Mentor), has a higher G prime than Radiesse. A split-face study showed that a smaller volume of Prevelle Lift was required than of a currently available high-concentration HA product with lower G prime to provide equivalent nasolabial fold correction. Over the course of the 36 week study, significantly fewer touch-up treatments were needed to maintain correction with Prevelle Lift than with the lower G prime HA product. The greater volume efficiency of the higher G prime product in this study is consistent with the results from the study comparing Radiesse to a lower G prime HA filler (discussed above), and further support for the concept that high G prime is a predictor of greater lifting capacity and thus better volume efficiency.

Conclusions
Rheologic studies of HA and CaHA fillers have advanced our understanding of these popular products, and provided a rationale for the product selection strategies, based on instinct and anecdotal experience, that have developed since their introduction. Clinical observations initially gave rise to scientific discovery, but it is now science that has the potential to guide us clinically, in optimizing injection strategies—and also in revisiting some previously accepted dogmas. One notable example is that HA filler longevity has been stated to be solely a function of insoluble (crosslinked) HA concentration, with soluble HA playing no role. This may be revealed to be something of an over-simplification as further studies of new and existing filler products proceed. While insoluble HA is undoubtedly important, we may need to better define how the clinical behavior and role of soluble HA are impacted by its molecular weight.

Although rheology is certainly not the sole determinant of how fillers may be best employed, it is a valuable addition to the anatomic and functional considerations that guide their safe and efficacious use. Rheology is helpful in refining our strategies with current products, shortening the learning curve with new products, and providing an evidence-based method of determining a product’s optimal clinical applications. Lifting is a priority for most patients seeking facial rejuvenation, and selection of volume-efficient products for this purpose provides a cost-effective means of meeting or even exceeding patient expectations.

The third and final article in this series will discuss how rheologic data can be used for selection of filler products and injection strategies—a process known as rheologic tailoring.

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