Cohesion of Hyaluronic Acid Fillers: Correlation Between Cohesion and Other Physicochemical Properties

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BACKGROUND There are several published articles on characterization of fillers, describing methods for both chemical and physicochemical characterization. Recently a lot of focus has been on the development of methods for measuring cohesion of hyaluronic acid (HA) fillers.

OBJECTIVE The aim of this study is to investigate and compare the drop-weight method and the correlation between cohesion and other physicochemical properties using a variety of HA fillers.

MATERIALS AND METHODS HA fillers covering several product families and manufacturing techniques were used. The HA fillers also covered a range of HA concentrations from 12 to 24 mg/mL. Cohesion was determined using sensory evaluation and the drop-weight method. Other physicochemical properties evaluated were rheology and the swelling factor.

RESULTS In this study, it was verified that values obtained by the drop-weight method reflect the perceived cohesion very well. The correlation with rheology is affected by the HA concentration in the products. A remarkably good correlation between swelling factor and cohesion was found.

CONCLUSION Cohesion correlates with other physicochemical methods. It could be discussed whether there is a need for a separate cohesion method because other already established physicochemical methods such as rheology and swelling factor can describe the underlying properties that affect cohesion.

The authors are employed by Galderma.

Hyaluronic acid (HA) fillers have been on the market for many years; the first HA-filler registered in the United States was Restylane in 2003. The number of HA-fillers has since then increased; in November 2016 there were 14 FDA-approved HA products, but not all were available on the US market. According to the American Society for Aesthetic Plastic Surgery, the number of nonsurgical aesthetic procedures in 2014 using HA fillers was 1,696,621, and it is expected to grow further to about 2,068,000 in 2016.¹ In Europe, the device approval regulations are different from the United States leading to a much larger number of approved products. In the United Kingdom alone, there are more than 150 different dermal filler products available of which ~91% are HA fillers. The expected number of procedures with HA fillers

in Europe in 2016 is 1,458,000.² With the increasing number of fillers on the market, there has also been an increase in the interest to differentiate the fillers. A scientific way to describe and differentiate the products is by their physicochemical properties. With the use of well-characterized fillers, it may in the future be possible to better understand the importance of the different properties for the in vivo performance.

There are several articles on the characterization of fillers published, describing methods for both chemical and physicochemical characterization.^{3–10} When it comes to physicochemical characterization, the most common and widely accepted among manufacturers are the rheological properties. The rheological

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properties relate to the polymer network, the cross-linking density, concentration of polymer, etc.¹¹ Other parameters that describe the gel properties are the swelling ability of the polymer network, extractable HA, etc.^{3,4} Recently, a lot of focus has been on the cohesive properties of HA fillers. Several methods have been suggested for measuring cohesion.^{5,6,12–14} In an earlier article,⁶ a dissolution method,¹² a compression force method,^{5,12,13} and the drop-weight method⁶ were compared with the perceived cohesion. It was found that the drop-weight method was the preferred method because there was a good correlation with the perceived cohesion, the results do not depend on subjective assessments, and the method was closely related to the scientific definition of cohesion.¹⁵

There have been suggestions that different physico-chemical properties of the HA-gel relate to the clinical outcome. For example, the gel strength as measured by rheology has been said to relate to the lifting capacity and tissue integration. It has been suggested that firm gels have a better ability to resist deformation,^{3,8,13,16,17} whereas softer gels have been said to better integrate into the tissue because they deform more easily.⁹

There have also been some suggestions on how the cohesion affects the clinical performance. Falcone and Berg¹⁸ suggested that cohesion would not be an advantage for fillers; others have suggested that it is important for the lifting capacity.^{5,13} There have also been some studies on the integration of the product into the tissue that describe the high-cohesive products to have a larger integration.^{19,20} Other investigators have suggested that low-cohesive gels spread or migrate in the tissue dependent on the injection depth.⁵

The aim of this study is to further investigate and compare the correlation between cohesion measured using the drop-weight method and other physico-chemical properties using a variety of HA fillers.

Materials and Methods

Materials

The HA products tested in this study are shown in Table 1.

Rheology

The rheological properties were measured in a frequency sweep from 10 to 0.1 Hz at 0.1% strain (the strain within the linear viscoelastic region). The measurements were made using an Anton Paar MCR 301 (Anton Paar, Graz, Austria) equipped with a PP-25 measuring system with a gap of 1 mm at 25°C. A 30-minute period was used for relaxation of the sample between loading and measuring. Two measurements were performed per sample.

Swelling Factor

The swelling factor of each product was determined by dispersing 0.5 g gel in saline to a total volume of 10 mL. The solution was allowed to swell to equilibrium for 3 to 5 hours before being mixed a second time. The volume of the swollen gel was measured after 16 hours. The swelling factor (SwF) at equilibrium was calculated as follows: V/V_0 , where V_0 is the initial volume of the gel and V is the volume of the fully swollen gel.

Perceived Cohesion

A test panel of 8 scientists performed a blinded sensory analysis of the different HA gel products (maximum number of gels each time was 7 gels). The test comprised manual handling of the gels and rating the gels on a scale of 1 to 5 (where 5 is the highest cohesion). Gels with a rating of 1 (RES_L) and 5 (RES_F) were used as references during the handling.

Cohesion by Drop Weight

The weight of an average fragment/drop of an HA gel that is pushed through a defined vertical orifice at a constant speed was determined. The gel was filled in a 1 mL BD glass syringe (Becton, Dickinson, Franklin Lakes, NJ) and the air was removed by centrifugation. An 18G cannula with a plane orifice (Intramedic Luer Stub Adapter 18 G; Becton, Dickinson) was mounted on the syringe and with the use of a Zwick material tester, Zwick BTC-FR 2.5 TH.D09 (Zwick Roell, Ulm, Germany); the gel was extruded at a constant speed of 7.5 mm per minute, yielding a volume flow of 0.24 mL per minute. When a constant force was achieved, at least 10 fragments/drops

TABLE 1. Products Tested in This Study

Product (Old Name Within Brackets)	Abbreviation Used in Paper	Hyaluronic Acid Concentration (mg/mL)	Technology	Batch
Belotero Balance	BEL _B	22.5	CPM	321205
Juvederm Ultra Plus XC	JUV _{X+}	24	Hylacross	H30LA50007
Juvederm Ultra XC	JUV _X	24	Hylacross	H24LA50161
Juvederm Volbella	JUV _{Vb}	15	Vycross	V15L625642
Juvederm Volift	JUV _{Vi}	17.5	Vycross	V17LA30119
Juvederm Voluma XC	JUV _{VuX}	20	Vycross	VB20A50135
Restylane Defyne (Emervel Deep Lidocaine)	RES _D	20	OBT	Several batches
Restylane Fynesse (Emervel Touch)	RES _F	20	OBT	Several batches
Restylane Kysse (Emervel Lips Lidocaine)	RES _K	20	OBT	Several batches
Restylane Lidocaine	RES	20	NASHA	Several batches
Restylane Lyft Lidocaine (Restylane Perlane Lidocaine)	RES _L	20	NASHA	Several batches
Restylane Refyne (Emervel Classic Lidocaine)	RES _R	20	OBT	Several batches
Restylane Silk	RES _S	20	NASHA	Several batches
Restylane Skinboosters Vital Light Lidocaine	RES _{SVL}	12	NASHA	Several batches
Restylane Volyme (Emervel Volume Lidocaine)	RES _V	20	OBT	Several batches
Teosyal RHA 1	TEO ₁	15		TPRL-142704C
Teosyal RHA 2	TEO ₂	23		TP30L-143601A
Teosyal RHA 3	TEO ₃	23		TP27L-143503C
Teosyal RHA 4	TEO ₄	23		TPUL-143501A

were collected and weighed and the average weight per drop was calculated.

Results

Several physicochemical properties of the investigated HA products were measured; the rheological properties, the swelling ability of the gel, and the cohesive properties.

To keep it simple, the rheological properties are described by the elastic modulus (G') and the viscous modulus (G''), which gives information on the viscoelastic properties of

the gels. The investigated HA products cover a large range with elastic modulus (G') at 0.1 Hz from around 10 Pa (RES_F) to above 500 Pa (RES_L), Figure 1. All products have an elastic modulus that is higher than the viscous modulus.

The swelling ability of the gel depends on the structure of its polymer network. With a more cross-linked, tighter network, there is less ability to expand the network during saline uptake compared with a looser polymer network. A gel that is further away from its equilibrium swelling in the syringe will be able to

Figure 1. Elastic (blue) and viscous modulus (light blue) of the different hyaluronic acid products at 0.1 Hz.

Figure 2. Swelling factor (SwF) for all tested products.

absorb more saline. The swelling factor is determined from the saline uptake by the gel that is required to reach equilibrium swelling in the laboratory. If the swelling factor is 1, the gel is at equilibrium swelling in the syringe. The higher the swelling factor, the further away the gel is from equilibrium. The swelling factor varied from ~ 2 (RES_{SVL}) to more than 17 (RES_F) for the studied products, Figure 2.

Cohesion describes the ability of the gel to stick together. In the current study, both the sensory analysis (perceived cohesion) and the drop-weight method were used to determine the cohesion of the samples; the results are shown in Figure 3.

Discussion

In the earlier investigation,⁶ only 2 different manufacturing techniques, OBT (Emervel) and NASHA (Restylane) were used, both having an HA concentration of 20 mg/mL. When using a small variation in products, one could easily find relationships that are not true when increasing the variation in samples. In the current paper, the scope was extended to include several manufacturing technologies having a range in HA concentration from 12 to 24 mg/mL, Table 1.

Cohesion describes the ability of the gel to stick together. This property has gained a lot of interest

Figure 3. (A) Cohesion measured as drop weight of the samples. (B) Cohesion determined by sensory analysis (perceived cohesion).

lately. Gels have been described as cohesive, often without a measure of the cohesion, merely an estimation of its cohesivity by manually handling the gel. It has also sometimes been discussed as if a gel is either cohesive or not and as if there is not a continuous scale from low to high cohesion. To give a value on the cohesion, there is a need for an analytical method. Recently,⁶ several suggested methods; a dissolution method,¹² a compression force method,^{5,12,13} and the drop-weight method⁶ were evaluated by comparing with sensory analysis. The preferred method for cohesion measurements was the drop-weight method. The drop-weight method was preferred because it had a good correlation with the sensory analysis, it was easily reproducible, it was not dependent on subjective assessments, and it was close to the IUPAC definition of cohesion.¹⁵ In the current study, both the sensory analysis (perceived cohesion) and the drop-weight method were used to determine the cohesion of the samples; the results are shown in Figure 3. When comparing Figure 3A, B, one can see similarities. The correlation between drop weight and perceived cohesion is shown in Figure 4. The correlation shown earlier⁶ is still valid, although the number of samples, manufacturing technologies, and HA concentrations represented have been increased.

A relationship between cohesion and rheology was found earlier.⁶ In that study, only 2 different manufacturing techniques OBT (Emervel) and NASHA (Restylane) were used, both having an HA concentration of 20 mg/mL. In this study, the

Figure 4. Correlation between drop weight and perceived cohesion. Samples with hyaluronic acid concentration 20 mg/mL (blue), higher than 20 mg/mL (green) and lower than 20 mg/mL (red).

relationships are tested with an increased number of product families and manufacturing techniques. The HA products also cover a variety of HA concentrations, Table 1. The correlations shown earlier between G' and perceived cohesion and between G' and drop weight⁶ are still valid, although the number of samples, manufacturing technologies, and HA concentrations represented have been increased, Figure 5. However, with the larger variation in samples, the correlation is not as strong as earlier. It seems that it is mainly the products with lower HA concentrations that do not correlate so well. Looking only at the samples with 20 mg/mL (blue diamonds), the correlation with G' is better for the drop weight than for the perceived cohesion. A larger scatter in perceived cohesion is expected as perceived cohesion relies on assessments.

In the previous investigation,⁶ the gels with higher G' values showed less cohesivity, and the gels with lower G' values demonstrated higher cohesion values. For the current data set, this relationship seems to hold for gels with an HA concentration of 20 mg/mL or more. For the gels with lower HA concentrations, however, low cohesion values were observed regardless of the G' value.

Based on the observations described above, it was interesting to investigate potential correlations between cohesion measured by drop weight and other physicochemical measures of the gel. The swelling ability is a measure of gel properties and should not be confused with the swelling that can occur as an adverse event after injection of fillers. The swelling ability of

Figure 6. Correlation between the swelling factor and the cohesion as measured by drop weight. Samples with hyaluronic acid concentration 20 mg/mL (blue), higher than 20 mg/mL (green) and lower than 20 mg/mL (red).

a gel depends on the nature of its polymer network; a tighter network has less ability to expand during saline uptake compared with a looser network. The correlation between cohesion and the swelling factor as measured in vitro in the laboratory was found to be very good, Figure 6. There seems to be no outliers due to variations in concentration because gels with both higher and lower concentrations fit the correlation very well. It seems that the further away a product is from equilibrium swelling, the more cohesive the product is. This strong correlation implies that it will be difficult to separate the effect of cohesion from the effect of the swelling factor when trying to understand how these parameters affect the behavior in vivo.

Conclusion

In this study, it was verified that values obtained by the drop-weight method reflects the perceived cohesion

Figure 5. Correlation between elastic modulus and the 2 cohesion measures; perceived cohesion (to the left) and drop weight (to the right). Samples with hyaluronic acid concentration 20 mg/mL (blue), higher than 20 mg/mL (green), and lower than 20 mg/mL (red).

very well also when using a larger variation in samples. Cohesion also correlates with other physicochemical methods: the previously observed correlation with G' from rheology⁶ was confirmed for gels with an HA concentration of 20 mg/mL or higher, where higher G' gels showed lower cohesion and lower G' gels showed higher cohesion values. However, gels with HA concentrations lower than 20 mg/mL showed low cohesion values regardless of the G' value. A remarkably good correlation was shown with the measured swelling factor regardless of the HA concentration.

It could be discussed whether there is a need for a separate cohesion method, as other physicochemical methods such as rheology and swelling factor seem to be enough to describe the underlying properties that affect cohesion.

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