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ORIGINAL RESEARCH

Evaluation of the Hydrophilic, Cohesive, and Physical Properties of Eight Hyaluronic Acid Fillers: Clinical Implications of Gel Differentiation

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Background: Hyaluronic acid (HA) fillers are used to treat an array of aesthetic indications. Proper filler selection is paramount for successful patient outcomes. However, many important physiochemical and physical properties that impact HA gel behavior remain undefined.

Purpose: To evaluate the hydrophilicity, cohesivity and particle size of eight commercial HA fillers manufactured by either Non-Animal Stabilized Hyaluronic Acid (NASHA) or Optimal Balance Technology (OBT) techniques.

Methods and Materials: Three individual in vitro experiments were performed to assess HA swelling capacity, cohesion, and particle size. Image analyses, blinded evaluation using the Gavard-Sundaram Cohesivity Scale, and laser diffraction technology were utilized, respectively.

Results: Compared to fillers manufactured with NASHA technology, OBT products demonstrated greater swelling capacity, cohesion, and wider particle size distributions. Strong positive correlations between swelling factor, degree of cohesivity, and increasing widths of the particle size distributions were observed.

Conclusions: The hydrophilicity, cohesivity and particle size distributions vary among HA fillers manufactured with different techniques. The creation of new labels identifying products based on their unique combination of physiochemical and physical characteristics may help guide appropriate selection of HA fillers to optimize patient outcomes.

Keywords: Optimal balance technology, OBT, Non-animal stabilized hyaluronic acid, NASHA, aesthetics

Introduction

For the treatment of age-related volume loss and wrinkles, the aesthetic practitioner has a myriad of hyaluronic acid (HA) fillers to choose from in their armamentarium. Perspectives on the proper selection of filler have been debated previously,[1] with consensus statements and treatment guidelines continuously being developed and advanced.[2–4] Various properties that help qualify the use of certain HA fillers for specific indications have been defined and measured.[5] Gel strength/firmness [G prime (G')] has been shown previously to differentiate between gels,[6,7] but other important features of HA include its hydrophilicity (ability to retain water), cohesivity (the force of attraction that holds molecules of a given substance together), and physical characteristics [eg, particle size (mean and distribution), shape]. The aim of the present study was to evaluate these properties in eight commercially available HA fillers, manufactured by two different methods [Non-Animal Stabilized Hyaluronic Acid (NASHA) and Optimal Balance Technology (OBT)]. In addition, the findings of this study aim to provide injectors with a scientific rationale for differentiating fillers based on their physiochemical and physical characteristics.

Methods

Three related experiments were performed using HA fillers from several batches. Each experiment was performed twice, with tests conducted in a private practice and university laboratories.

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Simulating in vivo HA Hydration

In the first experiment, eight fillers (Table 1) commercially available in the Canadian market were loaded from their prepackaged syringes into 10 mL test tubes, and diluted with normal saline in a 1:7 ratio. To ensure sufficient hydration, 3.5 mL of saline was first delivered to the test tube, followed by 1mL of HA, and topped with another 3.5 mL of saline. The low HA to saline ratio was carefully selected to ensure there would be sufficient space in the tube to visualize the interface between the saline and hydrated HA. Additionally, this dilution factor was supported by previously published work that demonstrated certain fillers absorbed up to 400% of their weight in water.[8,9] The volumes used were also limited by the size of the 10 mL vials. The tubes were sealed with a screw top, inverted ten times, then left to rest at room temperature (21.5–23.5° C) for seven days. The room temperature was checked once daily. After the seven-day incubation period, the test tubes were centrifuged for five minutes at 10,000 revolutions per minute (rpm). This allowed the denser hydrated HA to pass to the bottom of the test tube, while the unabsorbed saline rose to the top. The centrifugation configuration was carefully selected using rationale from two previous studies. The first study was an experiment conducted by Goodman et al,[8] which allowed for approximately 3500rpm for ten minutes (total of 35,000 revolutions). In a later study, investigators rotated the centrifuge at 1200rpm for twelve minutes (total of 14,400 revolutions) and raised concerns that longer durations could potentially compress the saline out of the hydrated HA.[10] To ensure our results could be compared to these earlier works and account for the aforementioned concerns regarding the potential compression of the sample, we arbitrarily reduced the centrifuging time to five minutes since our centrifuge rotated at the same speed as that used in the first paper (total of 17,500 revolutions). After the centrifugation was completed, 10 µL of blue dye (DipQuick counter stain # 3) was gently pipetted onto the top surface of the sample. Its diffusion to the interface between the saline and the hydrated HA created a demarcation line. After approximately 90 minutes, two-dimensional photographs were captured to illustrate the saline-hydrated HA interface and used for analyses (Figure 1). The swelling capabilities of each HA were quantified based on the percent increase in volume from the original 1 mL deposited into the vial, as well as the swelling factor [defined as the maximum capacity to take up additional fluid at equilibrium],[11,12] determined as final mL/g and calculated by V_7/V_1, where V_1 is the initial volume of the gel at Day 1 and V_7 is the volume of the fully swollen gel at Day 7. For statistical analysis, correlations between swelling capability and other physiochemical and physical properties were investigated.

Evaluation of Cohesivity

A cohesivity assay was performed under standardized testing conditions, including a room temperature between 22.5–23.5° C. First, 0.1 mL of blue dye (DipQuick counter stain # 3) was added as a coloring agent to 1 mL of HA gel, using a Luer Lock. After mixing the HA and dye for 1 minute, samples were loaded into an 800-mL glass beaker containing 370 mL of distilled water and a 2 cm magnetic bar stirrer, from a fixed height of 2 cm above the water surface. Each sample was loaded using the needle provided in the manufacturer's packaging. The gel and water mixture was stirred at a constant rotational frequency of 160 rpm. Standardized digital images were collected 90 seconds after complete extrusion of each sample into the beaker and commencement of magnetic stirring. The cohesivity of each specimen was assessed visually from these images, defined as the ratio of intact to dispersed gel. Two independent raters blinded to product selection graded all imagery, based on the five-point visual Gavard-Sundaram Cohesivity Scale (GSCS). Both raters were physician-injectors with over ten years of experience using the products under evaluation. The GSCS rates cohesivity using criteria ranging from fully dispersed (1) to fully cohesive (5).[22] A third independent and blinded rater resolved any discrepancies.

Evaluation of Particle Size and Shape

The FLOWSYNC (Microtrac MRB, Pennsylvania, USA) hybrid device was used to perform the particle size and shape analyses, using tri-laser diffraction/light scattering and image analysis measurements. For characterising particle size, the FLOWSYNC reports three values: i) "MA" is the mean diameter of the area distribution, ii) "MV" is the mean diameter of the volume distribution, and iii) "MN" is the mean diameter of the number distribution. In case of conflicting data, it was decided a priori that our discussion would focus primarily on the values of MA, given this was the most relevant

Table 1 Summary Descriptions of the Eight Hyaluronic Acid Fillers Evaluated in the Present Experiments

N°	Code	Manufacturing Technology	HA Concentration	Particle Area [MA (μm)]	Particle Volume [MV (μm)]	Particle Number [MN (μm)]	Sphericity	Swelling Factor	Swelling capacity (%)	Cohesivity Grade*	G' (Pa)[13]	G" (Pa)[13]	Tan δ[13]	Indication(s) for Use
1	HA-L	NASHA	20mg/mL	368.9	430.9	224.1	0.937	2.8	80	2	977	198	0.203	Deep dermis to superficial subcutis for the correction of moderate to severe facial folds and wrinkles, such as nasolabial folds.[14] Subcutaneous to supraperiosteal implantation for cheek augmentation and correction of age-related midface contour deficiencies in patients over the age of 21.[14] Subcutaneous plane in the dorsal hand to correct volume deficit in patients over the age of 21.[14]
2	HA-D	OBT	20mg/mL	182.3	191.9	161	0.937	3.4	140	2	342	47	0.137	Mid-to-deep dermis injection for correction of moderate to severe, deep facial wrinkles and folds (nasolabial folds) in patients over the age of 21.[15]
3	HA-K	OBT	20mg/mL	182.1	199.2	132.7	0.930	3.7	170	2	236	50	0.212	Injection into the lips for lip augmentation and the correction of upper perioral rhytids in patients over the age of 21.[16]
4	HA-R	OBT	20mg/mL	138.9	149.9	116.3	0.931	4.1	210	2	116	50	0.431	Mid to deep dermis for correction of moderate facial wrinkles and folds such as nasolabial folds.[17]

(*Continued*)

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Table 1 (Continued).

N°	Code	Manufacturing Technology	HA Concentration	Particle Area [MA (μm)]	Particle Volume [MV (μm)]	Particle Number [MN (μm)]	Sphericity	Swelling Factor	Swelling capacity (%)	Cohesivity Grade*	G' (Pa)[13]	G" (Pa)[13]	Tan δ[13]	Indication(s) for Use
5	HA-SBV	NASHA	20mg/mL	149.5	159.5	128.2	0.933	1.85	Nil	1	667	172	0.258	Deep dermis injection to improve skin smoothness and appearance and the elasticity of the skin in the lower cheek/jawline in the face and dorsal hands.[18]
6	HA-SBVL	NASHA	12 mg/mL	146.3	154.2	128.8	0.945	1.7	Nil	1	84	49	0583	Dermal injection to improve the elasticity of the skin in the lower cheek/jawline in the face and upper neck.[19]
7	HA-V	OBT	20mg/mL	229.3	244.5	162.8	0.934	3.35	135	2	239	50	0.209	Supraperiostic zone or subcutis injection for correction of facial volume loss such as in the cheeks and chin.[20]
8	HA-REST	NASHA	20mg/mL	189.6	207.6	145.3	0.955	1.55	Nil	1	864	185	0.214	Mid-to-deep dermal injection for correction of moderate to severe facial wrinkles and folds, such as nasolabial folds. Submucosal injection for lip augmentation in patients over the age of 21.[21]

Note: Products are displayed in alphabetical order. All products contain lidocaine. *Based on the Gavard-Sundaram Cohesivity Scale. MA is the mean diameter of the area distribution, MV is the mean diameter of the volume distribution, and MN is the mean diameter of the number distribution. All products were tested under the same conditions.[13]

Abbreviations: NASHA, Non-Animal Stabilized Hyaluronic Acid; OBT, Optimal Balance Technology; HA-REST, Restylane-L; HA-SBVL, Restylane Skinboosters Vital Light; HA-SBV, Restylane Skinboosters Vital; HA-L, Restylane Lyft; HA-V, Restylane Volume; HA-D, Restylane Defyne; HA-K, Restylane Kysse; HA-R, Restylane Refyne; G′, elastic modulus; G″, viscous modulus; Tan delta, a measure of the balance of elasticity versus fluidity.

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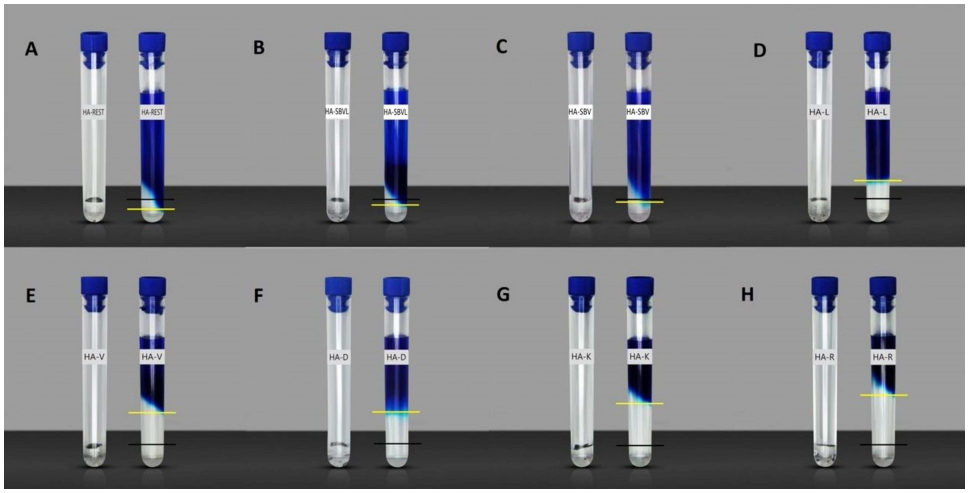

Figure 1 Results of Experiment 1 (hydration test). The swelling capacity of each of the eight hyaluronic acid gels (**A**–**H**) was evaluated based on the difference between the original volume (1mL; black demarcation line on the experimental vials; for reference, 1mL of each product is also depicted in the vial to the right of each sample) and the volume of expanded gel (indicated by the yellow line). The swelling capacity of each filler is thereby reflected by the space between the yellow and black lines and was calculated as the percent increase in volume from baseline (Table 2).

Notes: Samples are displayed from least absorbency (left, top row) to most absorbency (right, bottom row). Samples wherein the black line lays above or near the yellow line have low (samples **A**–**D**) swelling capabilities and those wherein the yellow line lays above the black line have greater swelling capabilities (samples **E** to (**H**).

Abbreviations: HA-SBV, Restylane Skinboosters Vital; HA-L, Restylane Lyft; HA-V, Restylane Volume; HA-D, Restylane Defyne; HA-K, Restylane Kysse; HA-R, Restylane Refyne; HA-REST, Restylane-L; HA-SBVL, Restylane Skinboosters Vital Light.

parameter to our analysis. Nonetheless, all collected values are reported (Table 1). For sample preparation, 0.2 mL of each filler was loaded into a 10 mL vial using the needle provided in the manufacturer's packaging. Two millilitres of distilled water was added to each vial before samples were pipetted into FLOWSYNC's water bath, prefilled with carrier fluid (200 mL distilled water). The FLOWSYNC's automated filling, de-aerating, pre-circulating, and circulating operations were used to ensure consistency and repeatability of samples. Wet operation settings included: Number of rinses: 1; Flow rate: 55%; De-aeration cycles: 3; Ultrasonic power (W): 40%; Ultrasonic time: 60 seconds. During the analysis, particles flowing in the system's stream were backlit by a high-speed strobe light and photographed by a high-resolution digital camera. The subsequent particle size and shape analyses were conducted using the system's internal hardware (Microtrac FLOWSYNC; Leeds & Northrup, St. Petersburg, FL).

Correlations Between Physiochemical and Physical Properties

The potential correlations between swelling factor, cohesivity, and particle size were investigated.

Results

Hydrating HA

Based on the findings of Experiment 1, the following HA fillers did not expand as they bound water: Restylane-L (HA_{REST}), Restylane Skinboosters Vital Light (HA_{SBVL}), Restylane Skinboosters Vital (HA_{SBV}), Restylane Lyft (HA_L) (Figure 1, parts A-D). Each of these fillers were manufactured using NASHA technology. The fillers that did expand (swell) as they bound water included HA_L, Restylane Defyne (HA_D), Restylane Volume (HA_V), Restylane Kysse (HA_K), and Restylane Refyne (HA_R) (Figure 1, parts E-H). Except for HA_L, all of these fillers were manufactured using OBT technology. HA_L was the only NASHA product that displayed a positive swelling capacity, and of note, it is a large-particle HA. The swelling factor (mL/g) and capacity (%) of each filler is displayed in Table 2. Products with a swelling factor between 1 and 2 mL/g resulted in negligible swelling capacities (ie, HA_{REST}, HA_{SBVL}, HA_{SBV}). Swelling factors ranged from 1.55 to 4.1 mL/g and swelling capacities ranged from nil to 210%. Of all products evaluated, HA_{REST} demonstrated the least potential for swelling upon hydration with saline and HA_R displayed the most.

Table 2 Results from All Three Experiments

Experiment	Measurements			
Experiment 1	**Sample**	**Product**	**Swelling factor (mL/g)**	**Swelling capacity (%)**
	A	HA-REST	1.55	Nil
	B	HA-SBVL	1.7	Nil
	C	HA-SBV	1.85	Nil
	D	HA-L	2.8	80
	E	HA-V	3.35	135
	F	HA-D	3.4	140
	G	HA-K	3.7	170
	H	HA-R	4.1	210
Experiment 2	**Product**		**Cohesivity score (1 to 5)**	
	HA-REST		1	
	HA-SBV		1	
	HA-SBVL		1	
	HA-D		2	
	HA-K		2	
	HA-L		2	
	HA-R		2	
	HA-V		2	
Experiment 3	**Product**		**Mean sphericity**	**SD**
	HA-K		0.930	0.060
	HA-R		0.931	0.058
	HA-SBV		0.933	0.071
	HA-V		0.934	0.055
	HA-L		0.937	0.060
	HA-D		0.937	0.058
	HA-SBVL		0.945	0.050
	HA-REST		0.955	0.054
	Product		**MA (μm)**	
	HA-R		138.9	
	HA-SBVL		146.3	
	HA-SBV		149.5	

(*Continued*)

Table 2 (Continued).

Experiment	Measurements	
	HA-K	182.1
	HA-D	182.3
	HA-REST	189.6
	HA-V	229.3
	HA-L	368.9

Notes: Experiment 1: The swelling factor (mL/g) and capacity (%) of each filler is reflected by the space between the yellow and black lines (Figure 1) and was calculated by V_7/V_1, where V_1 is the initial volume of the gel at Day 1 and V_7 is the volume of the fully swollen gel at Day 7. Samples are displayed by increasing swelling factor/capacity. Experiment 2: The cohesivity of each of the eight hyaluronic acid fillers was evaluated by three blinded reviewers, based on the Gavard-Sundaram Cohesivity Scale.[11] Samples are displayed by increasing cohesivity, followed by alphabetical order. Experiment 3: Mean sphericity of the particles of each sample was evaluated. Samples are displayed by increasing sphericity. The mean particle size (MA) of each sample was also assessed. Samples are displayed by increasing size. If the swelling factor is ~1, the gel is at equilibrium in the syringe and will result in negligible swelling capacities. The larger the swelling factor, the further away the gel is from its equilibrium and the greater propensity it will have for swelling. Sphericity is rated from 0 to 1, where 1 = a perfect sphere. MA is the mean diameter of the area distribution.

Cohesivity

The results of Experiment 2 are displayed in Figure 2. Based on Figure 2, the rank order from least to most cohesive was: HA_{SBVL}, HA_{SBV}, HA_{REST}, HA_L, HA_K, HA_R, HA_D, HA_V. Of note, HA_{SBV} and HA_{SBVL} appeared nearly identical on the cohesivity test. Based on blinded review (Table 2), samples consisted of GSCS scores of 1 (fully dispersed) and 2 (mostly dispersed). None of the samples received a cohesivity score of 3 (partially dispersed-partially cohesive), 4 (mostly cohesive), or 5 (fully cohesive). It was observed that in general, NASHA products had a lower degree of cohesivity compared to products manufactured by OBT.

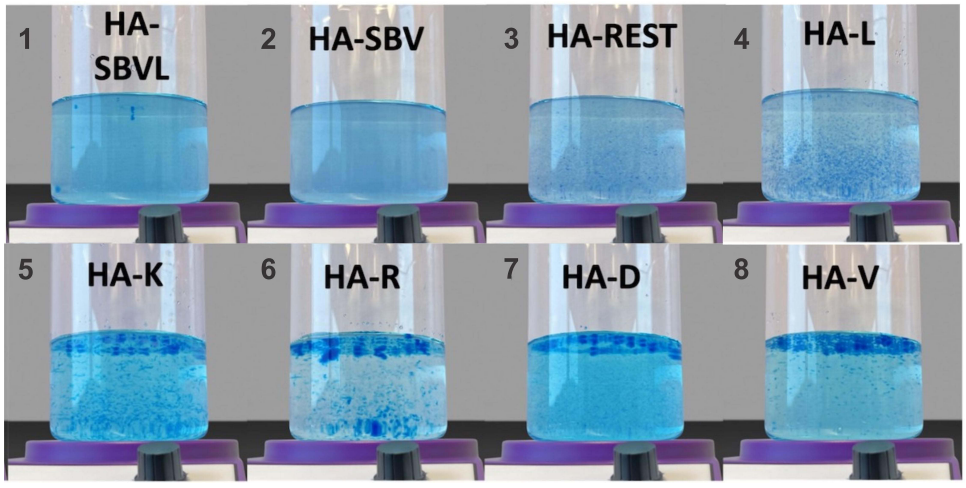

Figure 2 Results of Experiment 2 (cohesivity). After being dyed blue, the cohesivity of each of the eight hyaluronic acid gels was evaluated by adding 1mL of each sample into a beaker containing distilled water and mixing for 1 minute at a constant frequency of 160 rotations per minute. Standardized digital images were collected after 90 seconds and the resulting images were evaluated by three blinded independent raters according to the Gavard-Sundaram Cohesivity Scale.[22] Samples 1 to 8 are displayed by increasing degree of cohesivity.

Abbreviations: HA-SBVL, Restylane Skinboosters Vital Light; HA-SBV, Restylane Skinboosters Vital; HA-REST, Restylane-L; HA-L, Restylane Lyft; HA-K, Restylane Kysse; HA-R, Restylane Refyne; HA-D, Restylane Defyne; HA-V, Restylane Volume.

Particle Size and Shape

Each sample consisted of material containing particle sizes well within the FLOWSYNC's range of sensitivity (ie, 0.01 μm to 4000 μm). Sphericity, which is a measure of how closely a particle resembles a perfect sphere (perfect sphericity = 1.0), was evaluated for each sample. For all samples, the sphericity values were consistently close to 1 (Table 2).

Mean Particle Size

Based on the calculation of MA, the mean particle size of each sample ranged from 138.9 to 368.9 μm (Table 2). From smallest to largest mean particle size, the following rank order was observed: HA_R, HA_{SBVL}, HA_{SBV}, HA_K, HA_D, HA_{REST}, HA_V, HA_L. Based on the mean particle size, three distinct subgroups were evident, including small (M: 144.9 μm; SD: 5.43), medium (M: 184.66 μm; SD: 4.27), and large-particle (M: 275.25 μm; SD: 81.09) HA gels (Supplemental Material 1). There was no statistically significant association between manufacturing technology and mean particle size ($p > 0.05$), and the 95% confidence interval for both products significantly overlapped (NASHA = 115.71 to 295.88; OBT = 124.39 to 241.90). Based on the findings displayed in Table 2 and Supplemental Material 1, the following observations were noted:

- HA_{SBV} and HA_{SBVL} appeared similar in size
- HA_R (small) and HA_L (large) were on opposite ends of the particle size spectrum
- HA_K, HA_D, and HA_{REST} were of moderate particle size
- In addition to HA_L, HA_V consisted of relatively large particles

Particle Size Range

HA_{SBVL}, HA_{SBV}, HA_D, HA_{REST}, and HA_L displayed a narrow range of particle sizes and HA_R, HA_K, and HA_V displayed comparatively wider ranges. In general, products manufactured with OBT resulted in samples with a wider range of particle sizes, compared to those manufactured with NASHA. Of all samples, HA_K displayed the widest range of particle sizes (Supplemental Material 2).

Particle Size Distributions

The particle size distributions are displayed in Figure 3. A simple linear regression was calculated to predict percent (%) pass, based on product and sieve size, b(product) = 0.007, b(sieve) = 0.813; t(2) = 2.925, p = 0.004. A significant regression equation was found [$F(2, 657) = 640.856$, $p < 0.001$, with an R^2 of 0.661, indicating that the regression model

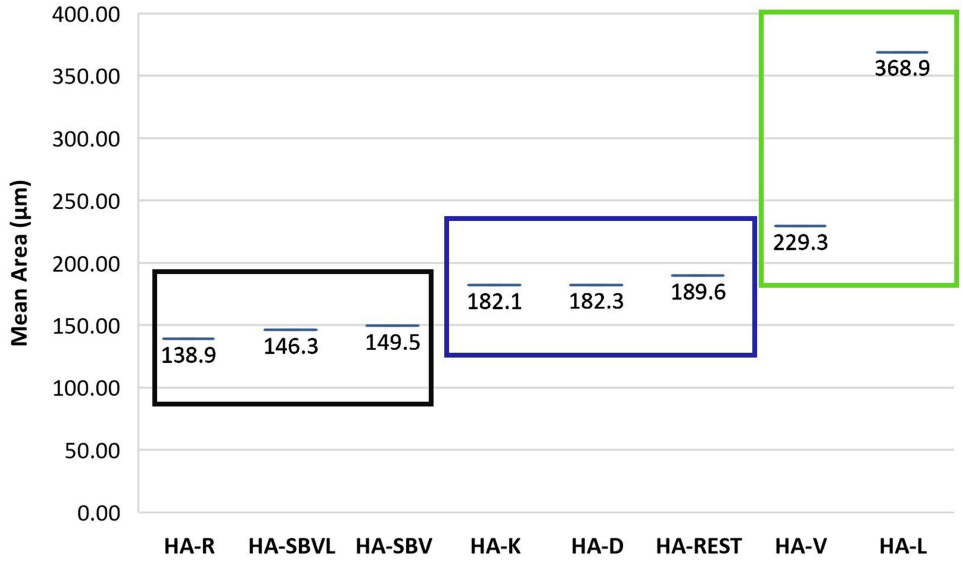

Figure 3 Based on the mean particle size (MA), three distinct subgroups were evident among the nine hyaluronic acid fillers evaluated. This included small (black square), medium (blue square), and large-particle hyaluronic acid gels (green square).
Note: MA is the mean diameter of the area distribution.

Table 3 New Alpha-Numeric Labels Created for Identifying Each Sample, Based on Their Unique Combination of Swelling Factor and Degree of Cohesion

Products	Alpha-Numeric Label (Swelling Factor-Cohesion)	
HA-REST	A-4	Top rows of Figures 1 and 2
HA-SBVL	B-2	
HA-SBV	C-3	
HA-L	D-5	Bottom rows of Figures 1 and 2
HA-V	E-9	
HA-D	F-8	
HA-K	G-6	
HA-R	H-7	

Note: Letters A to I represent increasing values for swelling factor. Numbers 1 to 9 represent values for increasing cohesion.
Abbreviations: HA-REST, Restylane-L; HA-SBVL, Restylane Skinboosters Vital Light; HA-SBV, Restylane Skinboosters Vital; HA-L, Restylane Lyft; HA-V, Restylane Volume; HA-D, Restylane Defyne; HA-K, Restylane Kysse; HA-R, Restylane Refyne.

was better at predicting the % pass than the mean alone (ANOVA $p < 0.001$). Although all samples contained variations of particle sizes, the distributions were far from normally distributed, and all samples displayed one or more clear peaks.

A summary of findings comparing products manufactured with NASHA versus OBT is depicted in Supplemental Material 3. Based on Experiments 1 through 3, it was concluded that NASHA has a low propensity for swelling, as well as a low degree of cohesivity. Conversely, OBT produces products with a higher propensity for swelling and a moderate level of cohesivity. Both methods of manufacturing resulted in HA products consisting of a range of particle sizes (ie, 138.9 to 368.9 μm).

Correlations Between Physiochemical and Physical Properties

There was a strong positive correlation between swelling factor and degree of cohesivity ($R^2 = 0.87$; Supplemental Material 4). This is visually evident by comparing Figures 1 and 2. In each Figure, the four samples presented on the top row are the same, as well as the five samples on the bottom row of each image. While there are a few deviations between Figures in the exact rank order of the samples, samples A to D in Figure 1 correspond to samples 1 to 4 in Figure 2, and samples E to H in Figure 1 correspond to samples 5 to 8 in Figure 2. Given these findings, new labels were created for each sample, based on their unique combination of swelling capacity and degree of cohesion (Table 3).

Particle size did not correlate with swelling factor nor cohesivity ($p > 0.05$). However, the width of the particle size distribution (narrow versus wide) significantly correlated with both cohesivity and swelling factor ($p < 0.05$), with wider distributions (ie, OBT products) associated with greater cohesivity and swelling capacity.

Discussion

The findings of Experiment 1 indicate that in general, NASHA products do not have significant swelling capacities while OBT products do, which is in line with previous findings.[11,12] This indicates that NASHA products have already reached the equilibrium of their swelling potential following the manufacturing process, and therefore have the lowest capacity to take up additional fluid. The only exception to this rule was the large-particle HA (HA_L), although it still had less swelling capacity than all the OBT products. The maximum swelling capacity observed in this experiment was HA_R, which expanded to 210% its original volume. While this increase in volume may seem large, this represents only a moderate level of swelling relative to the swelling capabilities of fillers manufactured with different technologies (eg, Vycross).[8,9]

In Experiment 2, the cohesivity of each of the eight HA fillers under evaluation was demonstrated. The results of this experiment support that the fillers ranged only slightly in degree of cohesivity, from being fully dispersed to partially dispersed (ie, scores of 1 and 2 on the GSCS). Compared to fillers created using different manufacturing technologies (eg, Vycross), the HA fillers evaluated did not possess a high degree of cohesion.[22] Nonetheless, in general NASHA products had a lower degree of cohesivity compared to products manufactured by OBT.

When reviewing the results of the particle size analysis (Experiment 3), shape must be considered along with mean size and width of the distribution. Important parameters related to particle morphology (eg, sphericity) can provide detailed information regarding the behaviour of the HA gels,[23] and these key properties can change drastically with no significant differences reported in the results of the laser diffraction (ie, mean size and size distribution). In theory, production conditions can fuse particles together and cause them to deviate from their desired spherical shape. If defective particles are produced, it may cause negative effects on the flow behavior of an HA gel during injections. Therefore, the finding that all samples contained particles with a high mean sphericity value was promising.

In the literature, typically only mean particle sizes are reported and used to classify products. However, the results of a linear regression supported that particle size distributions offer better predictive value for product identification. Furthermore, the finding that samples did not contain particles of the same size, but rather consisted of a range of particle sizes, should not be interpreted as a limitation of the product's predictability of use or consistency in manufacturing. Instead, this should be understood as an intentional manufacturing practice. Generally speaking, harder gels with a high G' are more difficult to inject and require a greater extrusion force during injections.[24] Therefore, a variety of physicochemical modifications are necessary to facilitate injection while maintaining implant persistence, such as adding uncrosslinked HA to offset injection difficulty.[25]

The positive correlation between swelling factor and cohesion, as previously reported by Edsman and Öhrlund[11] was confirmed by the present experiments. Using drop weight as a measure of cohesion, these investigators found an R^2 value of 0.96, which was slightly higher than the present findings of $R^2 = 0.87$. However, this is likely due to the fact that their calculation of cohesivity was based on a continuous variable (ie, weight in mg) while ours used an ordinal scale (ie, cohesivity grades). Interestingly, despite employing different methods to investigate the products' physiochemical and physical characteristics, our findings are in agreement with each other: Cohesive products are further away from their equilibrium of swelling and therefore, possess a greater propensity for swelling. It is important to note that the swelling factor of a gel is not synonymous with tissue swelling, which is a result of trauma caused by the injection procedure. Further studies are needed to establish the correlation between a gel's swelling factor and subsequent tissue swelling at the injection site.

The findings of these three related experiments can be used by injectors as a guide to selecting the appropriate filler, based on a marriage between the products attributes [eg, hygroscopy, particle size (mean, distribution) and shape, manufacturing technology, HA content, level of crosslinking, viscosity, cohesivity, resistance to deformation (G*), elastic modulus/gel hardness (G′), viscous modulus (G″), phase angle (δ), tissue integration, lift capacity], treatment indications (including a consideration of anatomical location and depth of injection), and patient characteristics (eg, skin thickness, skin elasticity, degree of correction required).[26–29] For example, fillers shown to absorb the least amount of water could be used in areas prone to swelling, such as the periocular area and upper lip rhytids, or they could be used for more superficial injection into the dermis (eg, HA_{SBV}, HA_{SBVL}). Moreover, as water uptake can affect an injector's ability to sculpt and integrate HA into the tissues, the findings of this study may provide additional information regarding the necessary injection technique to employ. Strengths of the present investigation were that all fillers within the NASHA and OBT families were evaluated, methods of differentiating NASHA versus OBT were established based on swelling capacity and cohesivity, and novel correlations were observed.

Cautions and Limitations

1. Considering all three experiments were conducted in vitro, the study design has inherent limitations. For example, this study did not take into consideration the effects of anatomy, shear stress, compression, nor tissue integration on the fillers. Moreover, although we observed some level of HA degradation following the 7-day hydration period, further changes due to breakdown of the products would likely be observed with extended periods of observation.

HA degradation in vivo is largely determined by the enzymatic activity of fibroblasts, which shorten the HA chains and are subsequently ingested by fibroblasts, macrophages and keratinocytes, in addition to the actions of the free radicals, hyaluronidases, thermal hydrolysis, and mechanical stress.[30,31] Therefore, the study findings may not be entirely reflective of in vivo conditions.

2. As 9/10 of the investigated products contained 20 mg/mL of HA, this study could not evaluate the effects of varying HA concentrations with changes in water absorbency.
3. The selected products consisted of two family of fillers (OBT and NASHA), and findings may vary with different manufacturing technologies (eg, Vycross).
4. As particle size varied within each sample, the degree of diffusion of the dye in Experiment 1 was not consistent within the vial (eg, Figure 1, parts A-H). Therefore, the calculation of swelling capability should be interpreted by rank order rather than definite quantitative values.
   (a) Moreover, although the present rank orders were consistent with the findings of Fagien et al,[12] and Edsman and Öhrlund,[11] these previous investigators reported greater values for the relevant swelling factors. Differences in our methodology (ie, hydration time 16 hours versus 7 days) may account for these differences.
   (b) Given that greater values of swelling capacity were observed after 16 hours of hydration compared to 7 days,[11,12] this may indicate that significant decreases in post-injection swelling could be expected in vivo within days of treatment, since decreases were observed by this timepoint.
5. While the authors discuss theories pertaining to potential clinical effects of the observed morphological changes that the HA fillers underwent after a period of hydration, evidencing such processes are beyond the scope of this paper.
6. The proper selection of an HA filler should be dependent on more than just a consideration of the physiochemical and physical properties investigated herein. Therefore, the findings of this study should be used to aid in product selection, rather than to make a definite determination.
7. It is important to recognize that the swelling capability of a filler may not necessarily translate to tissue swelling in vivo. Therefore, choosing to inject fillers known to absorb less water does not guarantee that patients will not experience edema, as this process is affected by additional factors, such as injection technique, rate of injection, depth of injection, health/quality of the tissue, and individual propensity for swelling.[12]

Conclusions

Findings from the current study support the notion that HA fillers manufactured with different technologies exhibit distinct physiochemical and physical properties. For example, products manufactured with OBT were found to exhibit greater swelling potential, levels of cohesivity, and a wider range of particle sizes, compared to NASHA fillers. A strong association between swelling factor and degree of cohesion was evidenced, which enabled the development of new labels to identify HA products based on their unique physiochemical and physical characteristics. In addition to previously reported parameters (eg, G prime), these novel identifiers can be used by injectors to inform product selection and optimize patient outcomes.

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Disclosure

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