Comparing Water Absorption of Food and Drug Administration–Approved Hyaluronic Acid Fillers

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BACKGROUND To compare the water absorption of 12 FDA-approved hyaluronic acid (HA) facial fillers in vitro in conditions relevant to in vivo injection.

OBJECTIVE The goal of this study was to provide long-term insight into an improved, tailored facial rejuvenation approach and to understand sequelae that could affect preoperative surgical planning.

METHODS In 2 experiments, 12 FDA-approved HA fillers were loaded into test tubes with nonpreserved normal saline and then placed in a 94.5°F–96°F environment for 1 month to allow water absorption by passive diffusion. The test tubes were centrifuged so that the hydrated filler could pass to the bottom of the tube. The tubes were centrifuged for 12 minutes at 1,200 revolutions per minute in the first experiment and for 7 minutes in the second experiment. A blue dye was then instilled to demarcate the filler/saline interface.

RESULTS There was variation in the water absorption of different HAs. Low absorption occurred in non–animal-stabilized hyaluronic acid.

CONCLUSION The pattern of water absorption was similar in the 2 experiments. The results inform us about in vivo conditions and provide guidance for filler selection.

All hyaluronic acid (HA) facial fillers absorb water.[1–3] Delayed tissue swelling after HA injection may take months or even up to 5 to 6 years or longer to become evident in the lower or upper eyelids.[4–11] Such swelling can result in a displeasing aesthetic outcome that may require dissolving the filler with hyaluronidase. This is most important when consulting with patients before blepharoplasty because the long-term presence of filler and associated edema disturb a proper preoperative surgical evaluation. Zoumalan described 23 cases of patients who required dissolution of HA fillers before the true contours of the patient's anatomy could be visualized without confounding edema. Fifteen cases could be dissolved with 1 round of hyaluronidase and 8 required 2 rounds. In 19 of the patients, the edema extended into the malar area.[1] Bernardini published a study on 61 patients with lower eyelid dysmorphia because of a HA filler.[7] Mustak and colleagues[8] reviewed 147 patients who were 5 years out from periorbital injections and found 30.5% had contour irregularities and 11% had malar edema.

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Many authors attribute to the type of filler used and/or the implant technique as well as the occurrence of delayed chronic periocular edema, and despite various articles demonstrating the issue, none have been able to retrospectively track down which fillers were injected, and evidence in support of any specific filler to be safer to prevent long-term complications is lacking. This article is the first to attempt to identify fillers that may carry increased in vitro risk of delayed edema, although the authors stress all HA fillers carry this potential.

Many factors govern the uptake of water by hydrogels such as those based on hyaluronic acid. These relate to the composition and molecular structure of the hydrogel, the degree of ramification, and properties of the solvent in contact with it, for example, the charge distribution and molecular architecture of the gel and the osmolality of the solvent in contact with it.[12–14] In our experiments, the former varied, whereas the latter was constant.

In 2009, Kablik and colleagues published a study that the 2 available non-animal stabilized hyaluronic acid (NASHA) fillers had a percentage swelling of 50%, whereas the filler Hylacross (30HV-24 mg/mL) had a value of 300%.[6] Another publication by Hee and colleagues revealed that the NASHA technology absorbed the least, whereas Hylacross technology absorbed the most water up to 400% of its own weight and that absorption of the Vycross technology was intermediate.[2,11]

Noting that the 12 fillers tested vary in composition and structure, but that many details of their specifics are proprietary, the authors conducted an empirical (rather than deterministic) study of the fillers to provide a direct,

clinically relevant comparison of their propensities with imbibe water.[13–16] The current experimental exposure of fillers to water emulated the body's internal milieu by using preservative free saline (Hospira, Inc., Lake Forest, IL). However, the authors did not attempt to recapitulate all details of fluid exchange with the fillers. Instead, the authors used 2 versions of a simple, controllable protocol for combining fillers and fluid at body temperature for a set time of 1 month to allow passive diffusion of water.

Methods

Two related experiments were performed. In each experiment, the fillers were loaded from their prepackaged syringes into 5 mL test tubes together with nonpreserved normal saline. The tubes were sealed with a screw top and then placed in a temperature-controlled chamber for 30 days at 94.5°F–96°F. Temperature was checked 3 times per week. This allowed the HA to hydrate, simulating, to some extent, in vivo conditions. In the first experiment, 0.8 mL of filler was placed first into the test tube, followed by 4.2 mL of saline. Five mL tubes were chosen because smaller tubes would have been too small to manipulate accurately, and 10 mL tubes have a too large diameter that they would have required a much greater amount of filler to observe the changes. This would have been cost prohibitive for our group. The article by Hee and colleagues,[2] mentioned that Hylacross fillers could absorb up to 400% their weight in water. For this reason, the authors chose .08 mL as our initial volume of filler so that in the event that the filler did absorb 4 times its weight in water, the authors would have space in the tube to visualize the interface line between the liquid and the gel. After incubation for 30 days, the test tubes were centrifuged so that the denser hydrated filler could pass to the bottom of the tube and unabsorbed saline could rise to the top. The tubes were centrifuged for 12 minutes at 1,200 revolutions per minute (rpm). The settings were originally chosen based on the article by Goodman and colleagues[16] Their experiment allowed about 35,000 revolutions by way of a centrifuge of 10 minutes at 3,500 rpm. In this study, the centrifuge only rotated at 1,200 rpm. The authors calculated that to have the same number of revolutions, the authors would have needed to centrifuge for 29 minutes. The authors felt this was too long, so the authors arbitrarily added just 2 minutes to our centrifuge time. A drop of dye was then pipetted onto the top surface of the saline. Its diffusion to the interface between the saline and the hydrated filler demarcated the interface. Photographs were taken so that the filler–water interfaces could be compared.

When the authors saw the results of Experiment 1, the authors did not observe the 400% absorption of Hylacross of water as described in the article by Hee and colleagues.[2] The authors were actually concerned about over centrifugation compressing the water out of the hydrated gel. However, the authors were very encouraged by the incremental "stair-step" absorption of water by the Vycross products increasing their water absorption between 15, 17, and 20 mg/mL, so the authors were very encouraged about being on the right track (Figure 1).

The authors decided to repeat the experiment first to verify our results and second to decrease the centrifuge time in hopes of showing a more dramatic result. Both goals were successfully accomplished. When repeating, the authors believed there would be space in the tubes to introduce the entire 1 mL of filler which left 4 mL for saline. Therefore, 4.0 mL of saline was first placed into the tube followed by 1.0 mL of HA filler carefully placed into the saline.

This also gave the authors a second opportunity to reduce the time of centrifuge to only 7 minutes at 1200 rpm. The results are photographed in Figure 2.

Results

Figures 1 and 2 are composite photographs of results for Experiments 1 and 2. Overall, both experiments showed fairly consistent proportional results. The main difference was that in Experiment 2, contrasts of the results were more striking. This was because the increase in volume due to water absorption was more visible because the fillers were not as compressed by prolonged centrifugation.

In both experiments, the lowest amount of water absorption was observed in the 3 fillers: NASHA

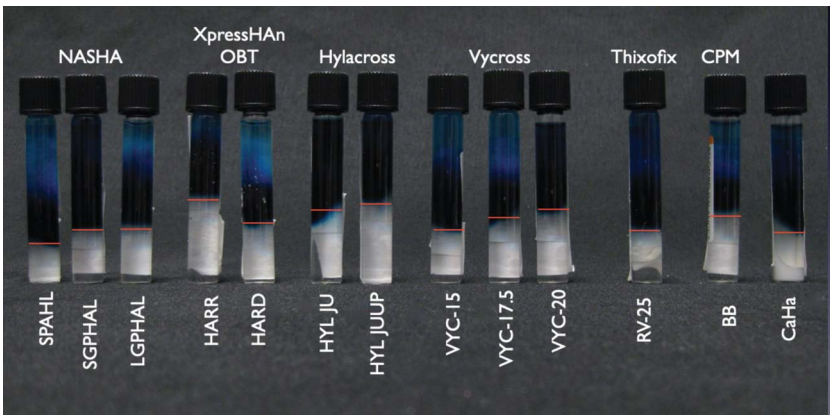

Figure 1. Experiment 1: centrifuge time 12 minutes at 1200 rpm.

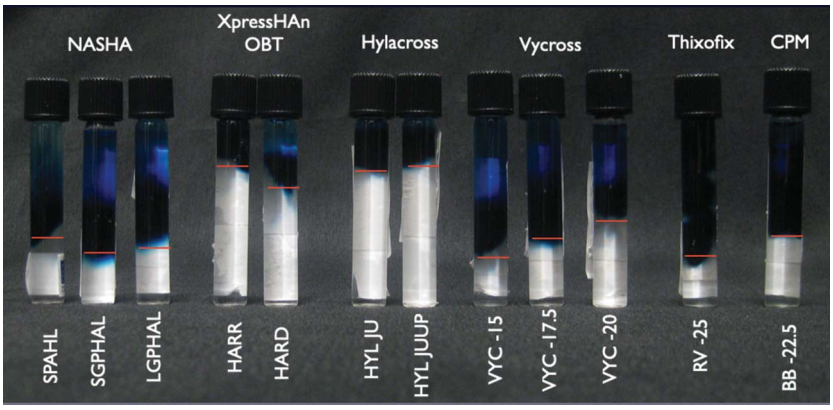

Figure 2. Experiment 2: centrifuge time 7 minutes at 1200 rpm.

technologies, Vycross 15 mg/mL, and Thiofix. Of note, in Experiment 1, the 250-μm small particle hyaluronic acid plus lidocaine (SPHAL) NASHA product had lowest hydrated volume (Figure 1). This was believed to be due to the deposition of 0.05 mL of filler inadvertently along the internal rim of the test tube when placed (Figure 1). This volume may have not reached the saline to absorb water. This was corrected in Experiment 2 (Figure 2). The greatest water absorption was noted in the HARR Optimal Balance/ XpressHAn, as well as in Hylacross technologies.

As previously mentioned, conspicuously encouraging in both experiments was the incremental absorption of water by the 3 concentrations of HA in the Vycross filler. The absorbed volume levels increased in relation to their concentrations of 15, 17.5, and 20 mg/mL, respectively, with the lower concentration absorbing the least water and the highest concentration absorbing the most.

Discussion

Hyaluronic acid fillers have been used in the United States for over 17 years. In this era of aesthetic rejuvenation, many established patients have been receiving HA fillers for several years. Blepharoplasty surgeons should be aware that chronic edema can occur because of long-term filler retention along the periocular area and should be evaluated.[1,4–9] In some cases, costly work-ups with CT, MRI, and biopsy for pathology have been prompted by the sequelae from this long-term retention of filler 9 years after injection.[6] Hyaluronic acid filler in the eyelids and midface has been successfully dissolved with hyaluronidase up to 9 years after the original injection.[1,4–9] Understanding which HA fillers are more prone to volume expansion, and which are more prone to delayed edema over time, is important for injectors to recognize.

In addition to our experiments showing the comparative water absorption of HA fillers, a variety of other theories have been entertained as possible contributors to delayed periocular edema related to fillers. There may be anatomical considerations such as gravity and subsequent pooling of fluid above the malar septum along with greater distensibility of periocular tissue. Slow breakdown of the filler may allow greater filler surface area that is then preserved with microencapsulations, and fibrosis as noted by biopsy in the article by Chang and colleagues[6] may contribute to the delayed presentation in some patients. Compression of the lymphatics by the filler has also been suggested as an etiology of edema.[10]

In Vitro Versus In Vivo Considerations

The technique of measuring water absorption is often performed in professional rheology laboratories by blending the filler and then conducting the centrifuge the same day at room temperature. This experiment was the first to attempt to place an environment more similar to that of in vivo by allowing passive diffusion, rather than blending, at body temperature. Clearly, the in vitro experiments in this study did not fully simulate conditions of filler exposure to fluid, swelling, and integration into tissue in vivo or the effects of the oligosaccharides as a result of breakdown products through dissolution with extended periods of the various hyaluronic acid fillers. The authors were focusing on the primary process of filler swelling because of contact with water and did not attempt to capture filler integration into tissue per se, structural/molecular changes of the filler with longer periods, or associated mechanical factors such as compressive and shear stresses acting against filler in vivo. Limitations of this study can include these differences and changes in water absorption because the longer chain polysaccharides are reduced to smaller oligosaccharides where the effects of these changes are largely unknown. Nonetheless, the contrasts in the in vitro results in this study do inform the authors about the relative contrasts and comparisons in vivo. These results should therefore be interpreted in rank order, with attention to differences in rank values, rather than as quantitative standards. As such, they can serve as a general guide rather than a definitive rule. Delayed edema with Tyndall effect is a specific event limited exclusively to the periorbital area, indicated by that lined by the orbicularis oculi muscle, including the upper lid/ brow region as recently demonstrated.[9] It is all the more

difficult to reproduce the in vitro results with the specific anatomy of this unique zone of risk. However, trying to elucidate the different rheologic features of fillers in rapport to this potential long-term complication should be strongly encouraged, as given the exponential increase of HA injections in the periocular region and the long-time presentation of the dysmorphism to the eyelid, negatively affecting their safety and in turn patients demand.

Differences in results across the test fillers can be explained, at least in part, by differences in their molecular compositions and structures. These correlate, in principle, with macroscale properties and performance measures. Some physicochemical and rheological measurements have been made for some of these fillers, for example, bulk viscoelastic moduli by Hee and colleagues.[2] Empirical in vitro correlates of in vivo performance, for example, lift capacity, have also been measured.[2,3,11–13,15] Fagien and colleagues demonstrated similar amounts of water absorption/swelling factor of the fillers in comparison with this study. The outlier exception was the cohesive polydensified matrix (CPM) technology in this study that demonstrated a midlevel water absorption compared with their study that showed a very high one. Their study involved blending the gels with the saline at 25°C then centrifuging on the same day. This study allowed for a passive diffusion for 1 month at body temperature. The high cohesitivity of the CPM technology may have been broken up by the blending technique in their experiment allowing it to have a high surface area to absorb the water, as opposed to the passive diffusion over time in the experiment in this study. It is unknown if these time and temperature differences make a difference in vitro, but the important point is that the results were fairly similar. Their article also discusses the fact that tissue integration and forces such as shearing, torsion, stretch, and compression cannot be accounted for in vitro experiments.[3]

More complete understanding of the molecular and structural basis both in the short-term and long-term for the results in this study could be undertaken but is not likely necessary for initial clinical interpretation of them. A long-term study is an ideal concept, but unfortunately cost and time constrains would not allow for this at this time. The authors also note that because our data from Experiments 1 and 2, to a good degree seem similar to what has been observed with the articles by Hee and colleagues, [2] Goodman and colleagues, [12] and Fagien and colleagues [17] that this is not necessary to understand the basic concept at hand. One of the values of this study is to have an excellent understandable visual image seen in Figure 3 as a take home message to understand the postinjection possibilities of these fillers that could be considered for preinjection consultation with patients. This would be an excellent goal for a future study. A future study could also contain an update with the new resilient technology of 3 fillers that have been recently introduced in the United States.

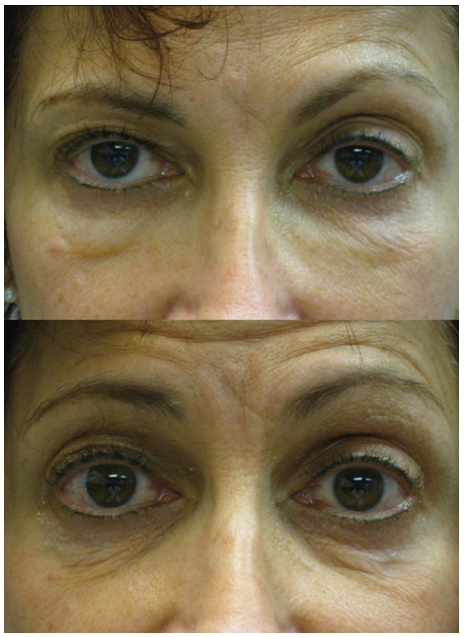

Figure 3. Two years after treatment: prehyaluronidase injection and 2 weeks posthyaluronidase injection.

Clinical Applications

The authors' goals of these experiments were twofold: (1) create a guide for injectors for improved short-term and long-term outcomes with a more tailored approach, including improved understanding of hygroscopic filler properties and (2) better understand long-term sequelae of edema to aid in preoperative surgical planning.

The authors hope that injectors will consider the long-term effects of fillers before treating patients. Fillers that absorb the least amount of water should be used in the periocular areas to avoid eyelid and malar edema. The upper lip rhytids are also an area to consider avoiding high hygroscopic fillers because they can slowly swell and elongate the upper lip. An elongated upper lip that obscures the upper teeth is a sign of aging. Based on the results in this study, lower lid and festoon edema as well as elongation of the upper lip due to edema over time could be minimized by choosing NASHA, Vycross 15 mg/mL, or Thiofix. Fillers that demonstrated midlevel water absorption could be used in a variety of areas but with consideration of their relative abilities to absorb water over time. Other injection procedure characteristics, not specifically studied here, such as integration, lift, resistance to deformation, etc., should also be addressed in the decision of aesthetic placement in the face.

It is important to recognize that choosing fillers that have less absorption of water is not a guarantee that the area will not have edema, which requires dissolution with hyaluronidase in the future.[1,4–9] Even fillers shown here with low water absorption can swell over time in some patients, especially along orbital rims when given a substantial volume and enough time (illustrated in Figures 3 and 4). In

Europe, the short chain (SPHAL) NASHA is used for dermal enhancement over the cheeks with micro boluses termed skin boosters. Although this filler has low water absorption, it is chosen because of its excellent integration into the dermis. It is important to remember that even this NASHA filler still has some capacity for water absorption, in part, because it still lowers the amount of transepidermal water loss.[18]

The clinical literature has included focus on defining and measuring properties that help qualify the use of HA products and their specific indications. A property termed "change in resistance" has been studied in animal models and in vitro. It is associated with filler interaction with tissue including water uptake and integration in vivo, which can account for its ability to sculpt and integrate.[2–6] Anedoctally, short chained, poorly ramified on injection, HA is being used to improve skin quality around the eyes, based on the theoretical "low hydrophilia" and short duration; however, these "skin boosters" to the eyelid are frequent cause of eyelid edema. The authors encourage future studies oriented to discern the impact of complex/more ramified molecules to be riskier or not in respect to this event.

Fagien and Cassuto[19] have described a technique to improve outcomes by prehydrating fillers before injection. Hylacross 22 mg/mL was diluted to 12 to 16 mg/mL with 0.4 mL of lidocaine with epinephrine without complication; this allowed more even distribution of the product without palpable irregularities after procedure. There was no long-term follow-up to determine if there was a decrease in long-term edema. Although this is a reasonable consideration, at present, definitive research on it is lacking, as noted by the authors, and this study justifies why this should be considered with good reason.[16]

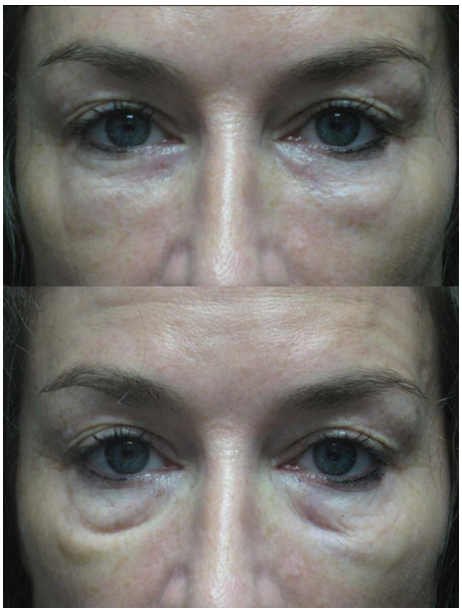

Figure 4. One-year before injection: prehyaluronidase injection and 1-hour posthyaluronidase injection.

Physicians should use their best clinical judgments when evaluating aesthetic patients, especially for surgical procedures such as blepharoplasty. Patients often are unable to recall which fillers they have previously had injected or where such injections were performed, and this can obscure proper patient evaluation. When in doubt, the physician should always have a discussion with the patient about the possible need to dissolve an HA filler in the future.

Conclusion

In summary, this in vitro study was intended to simulate conditions in vivo by observing passive water absorption at body temperature over 30 days. The data are useable as a general guide for improved long-term results to determine how fillers may behave regarding their water absorption over time. The authors suggest that injectors consider avoiding fillers that absorb water in areas where hydration would be displeasing. Fillers with less hygroscopic properties can be considered in the periorbital area and upper lip rhytids to avoid lip swelling and elongation. In contrast, the fillers with greatest hygroscopic properties may be desirable in areas where hydration may be advantageous to create cosmetically appealing results such as the red lip, lower lip rhytids, etched lines on the cheeks, and dorsum of the hands can create cosmetically appealing results. The authors also recognize that all available HA fillers have their own set of unique rheological properties that have value and benefit when used in a variety of locations for rejuvenation of the face. The authors described several other theories that contribute to our knowledge of how HA fillers may absorb water in vivo.

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