

## Research

# Comparison of the Degradability of Hyaluronic Acid by Ovine and Recombinant Human Hyaluronidase

Tyler Safran, MD, MSc<sup>®</sup>; Roy Khalaf, MD; Andrei Metelitsa, MD; Julie Woodward, MD; and Andreas Nikolis, MD, PhD<sup>®</sup>

Aesthetic Surgery Journal  
2025, Vol 45(10) 1086–1093  
Editorial Decision date: June 20, 2025; online  
publish-ahead-of-print June 30, 2025.  
© The Author(s) 2025. Published by Oxford  
University Press on behalf of The Aesthetic  
Society.  
This is an Open Access article distributed under  
the terms of the Creative Commons Attribution  
License (<https://creativecommons.org/licenses/by/4.0/>), which permits unrestricted reuse,  
distribution, and reproduction in any medium,  
provided the original work is properly cited.  
<https://doi.org/10.1093/asj/sjaf127>  
[www.aestheticsurgeryjournal.com](http://www.aestheticsurgeryjournal.com)

**OXFORD**  
UNIVERSITY PRESS

### Abstract

**Background:** The mainstay of treatment for adverse events to hyaluronic acid filler is the use of hyaluronidase (HYAL); however, the dose and dilution are not standardized.

**Objectives:** The objective of this study was to examine differential dilutions and concentrations of HYAL, and to compare the effectiveness of ovine and human HYAL.

**Methods:** Fillers were selected for study based on a variety of rheologic factors. A 0.2-mL dose of product was selected for use based on previous studies. Degradation was assessed by comparing both ovine and recombinant HYAL over a range of concentrations and dilutions.

**Results:** In the 2:1 dilution group, Restylane Lidocaine (Galderma, Lausanne, Switzerland) was degraded by 100 U of ovine HYAL after 50 minutes. No other filler was completely degraded; Restylane Lyft (Galderma) was partially degraded after 1 hour of treatment with 100 U of HYAL. In both the 3:1 and 4:1 dilution groups, Restylane Shayepe (Galderma) and Restylane Lyft fillers were most susceptible to degradation, dissolving within 30 minutes with 100 U of recombinant HYAL and within 40 minutes with 100 U of ovine HYAL. Juvéderm and RHA4 fillers were the most resistant, requiring 300 U of HYAL for degradation within 1 hour. Beyond a 3:1 dilution ratio, no further improvement was observed.

**Conclusions:** This study demonstrates that 300 U of hyaluronidase is sufficient to degrade 0.2 mL of the most resistant hyaluronic acid fillers within 1 hour. Importantly, a minimum dilution of 3:1 should be used to provide adequate fluid for dissolving filler. Ovine HYAL appears to be just as effective as recombinant HYAL in terms of dissolving product.

The use of hyaluronic acid (HA)-based fillers for facial shaping and rejuvenation has continued to grow year over year.<sup>1</sup> As their use increases, so does innovation in product type, rheology, and injection technique. Given the widespread use of HA by many varieties of practitioners, it is of paramount importance to understand management of adverse events. Although such events are rare, HA fillers are associated with both vascular and nonvascular adverse events of varying degrees of severity that require treatment.<sup>2</sup> The mainstay of treatment is the use of hyaluronidase (HYAL), an endoglycosidase that breaks down HA into monosaccharides by cleaving its glycosidic bonds.<sup>3</sup> In North America, 3 HYAL formulations are currently available: Vitrase (ovine testicular HYAL; Bausch & Lomb, Laval, Canada), Amphadase (bovine HYAL; Amphastar Pharmaceuticals, Rancho Cucamonga, CA), and Hylenex (recombinant human HYAL; Halozyme Therapeutics, San Diego, CA).<sup>4</sup>

For HYAL to dissolve a HA filler, it must interact with its binding sites within the HA itself.<sup>3</sup> The reaction of a filler to HYAL depends on many factors, including but not limited to the HA concentration, the number of crosslinks, and the form of the filler.<sup>3</sup> HYAL is rapidly degraded and deactivated in the body, with some reports

demonstrating a loss of in vivo effect after 3 to 6 hours.<sup>5</sup> Therefore, to dissolve a HA filler, enough HYAL must be injected close to the filler. Both animal-derived and recombinant human HYAL have been shown to efficiently degrade HA.<sup>6,7</sup> Differences in their enzymatic structure and their immunogenicity may impact their efficacy and safety profiles.<sup>8</sup> While animal HYAL has demonstrated efficacy,

Drs Safran and Nikolis are plastic surgeons, Division of Plastic & Reconstructive Surgery, McGill University Health Centre, Montreal, QC, Canada. Mr Khalaf is a medical student, Faculty of Medicine, McGill University, Montreal, QC, Canada. Dr Metelitsa is a dermatologist, Division of Dermatology, University of Calgary, AB, Canada. Dr Woodward is an oculoplastic surgeon, Department of Ophthalmology, Oculofacial Plastic and Reconstructive Surgery, Duke University Eye Center, Durham, NC, USA.

### Corresponding Author:

Dr Andreas Nikolis, 376 Victoria Avenue, Suite 400A, Westmount, QC H3Z 1C3, Canada.

E-mail: [an@nikolis.net](mailto:an@nikolis.net)

**Table 1.** Characteristics and Indications of Various Hyaluronic Acid Fillers

| HA filler         | Manufacturing technology | Concentration of hyaluronic acid (mg/mL) | G' <sup>1,2</sup> | G'' <sup>1,2</sup> | Tan delta <sup>1,2</sup> | Cohesivity/ Normal force <sup>1,2</sup> | Maximum water uptake <sup>1,2</sup> | Indications for use                                                      |
|-------------------|--------------------------|------------------------------------------|-------------------|--------------------|--------------------------|-----------------------------------------|-------------------------------------|--------------------------------------------------------------------------|
| R <sub>L</sub>    | NASHA                    | 20                                       | 977               | 198                | 0.203                    | 32                                      | <100                                | Moderate-severe facial folds and wrinkles <sup>13</sup>                  |
| R <sub>Lido</sub> | NASHA                    | 20                                       | 544               | NA                 | NA                       | NA                                      | NA                                  |                                                                          |
| R <sub>S</sub>    | NASHA                    | 20                                       | NA                | NA                 | NA                       | NA                                      | NA                                  | Skin smoothness and appearance <sup>13</sup>                             |
| R <sub>SC</sub>   | NA                       | NA                                       | NA                | NA                 | NA                       | NA                                      | NA                                  |                                                                          |
| R <sub>H</sub>    | RHA                      | 23                                       | 346               | 62                 | 0.179                    | 115                                     | 366                                 | Injection in deep dermis to superficial subcutaneous tissue              |
| B <sub>V</sub>    | CPM                      | 26                                       | 438               | 103                | 0.235                    | 97                                      | 370                                 | Restoration of facial volume <sup>13</sup>                               |
| J <sub>VO</sub>   | Vycross                  | 20                                       | 398               | 41                 | 0.103                    | 40                                      | 227                                 | Restores volume of the face<br>Deep dermis, subcutaneously <sup>14</sup> |
| J <sub>VX</sub>   | Vycross                  | 25                                       | 665               | 49                 | 0.074                    | 93                                      | 253                                 | Injection in deep dermis<br>Jawline and chin contouring                  |

B<sub>V</sub>, Belotero Volume; CPM, cohesive polydensified matrix; HA, hyaluronic acid; J<sub>VO</sub>, Juvederm Voluma; J<sub>VX</sub>, Juvederm Volux; NA, not applicable; NASHA, non-animal stabilized hyaluronic acid; R<sub>H</sub>, RHA, resilient hyaluronic acid; RHA4; R<sub>L</sub>, Restylane Lyft; R<sub>Lido</sub>, Restylane Lidocaine; R<sub>S</sub>, Restylane Shayepe; R<sub>SC</sub>, Revanesse Sculpt+.

bovine-derived HYAL has been associated with hypersensitivity reactions and, in rare cases, anaphylaxis.<sup>8,9</sup>

Currently there is a lack of standardization and knowledge of HYAL dosing, dilutions, administration, and treatment algorithms. To answer this question, many authors have performed both in vitro and in vivo studies examining the various HA products available and their response to varying concentrations of HYAL (both animal and human derived).<sup>7,10</sup> These studies mainly examined how linearly increasing units of HYAL dissolved fixed amounts of HA. Unsurprisingly, increasing the HYAL dose yielded more robust degradation of product, but this increase in HYAL was also accompanied with an increase in the overall volume of the reconstituted HYAL.<sup>7,10</sup> Additionally, these studies all either manually agitated the filler/HYAL mixture by stirring or by direct injection. In vivo, this becomes more of a challenge without advanced ultrasound capabilities or exact knowledge of product location.

No study to date, however, has examined how HA fillers react and degrade when treated with varying ratios of HYAL/saline to filler (ie, liquid-to-solid ratios). Additionally, no study has compared the efficacy of ovine to human recombinant HYAL in this context. The goal of this study was to examine how changing the ratio of HYAL/saline to filler by varying the HYAL concentration influences the degradation of a range of HA fillers, and to compare the efficacy of ovine and human recombinant HYAL at different dilutions.

## METHODS

Multiple experiments were designed to test the efficacy of ovine HYAL, to compare ovine and human HYAL, and to determine optimal dilutions of HYAL to achieve dissolution of HA. These studies were performed between September and November 2024.

### Experiment 1 (1:1 and 2:1 dilutions)

The first experimental study examined the response of 5 HA gels to lower dilutions of ovine HYAL. Five fillers—Restylane Shayepe (R<sub>S</sub>; NASHA-HD, Galderma, Lausanne, Switzerland), Restylane Lyft (R<sub>L</sub>; NASHA, Galderma), Restylane Lidocaine (R<sub>Lido</sub>; NASHA, Galderma),

Revanesse Sculpt+ (R<sub>SC</sub>; Prollenium, Richmond Hill, Canada) and Juvederm Volux (J<sub>VX</sub>; VYCROS, Abbvie, North Chicago, IL)—were selected for study to provide a variety of rheologies. These gels were selected based on previous studies, selecting products that were easily degraded vs those that were not.<sup>11</sup> Table 1 includes all product information.

The dosage of HA was chosen based on previous in vitro studies to provide a comparison.<sup>11</sup> As a result, 0.2 mL of filler was selected. Degradation was measured every 10 minutes until complete degradation to a limit of 60 minutes. This process was repeated with increased dosages of 20, 60 and 100 U of HYAL (3 groups). With a fixed HA volume of 0.2 mL, the volumes of HYAL and normal saline (NS) required to achieve effective degradation were determined while maintaining the ratio of HYAL/NS to filler in the group. To do so, liquid-to-solid ratios of 1:1 and 2:1 were selected for this group. Normal saline alone was used as a control group, respecting the same dilutions (fourth group). Ovine HYAL (1500 U/mL) was utilized to be able to accurately dilute the solution to the appropriate ratios.

All products were injected with low extrusion force into Petri dishes via a 25G needle by the same individual. HYAL solutions were created and placed in perfluid without injecting it intrasubstance and without stirring.

## Experiment 2

The second experimental study examined the response of 7 HA gels to 2 kinds of HYAL available in North America, recombinant human HYAL and ovine HYAL, at higher dilutions. Based on previous findings in the literature,<sup>11</sup> two non-animal stabilized hyaluronic acid fillers, R<sub>S</sub> and R<sub>L</sub>, were selected, as well as the 5 most resistant fillers identified: R<sub>SC</sub>, Belotero Volume (B<sub>V</sub>; Merz Aesthetics, Raleigh, NC), RHA4 (R<sub>H</sub>; Teoxane, Geneva, Switzerland), Juvederm Voluma (J<sub>VO</sub>, Abbvie) and J<sub>VX</sub>. Table 1 includes all filler information.

Two experiments were designed to assess degradation of each respective HA filler by each substrate of HYAL, as well as different doses and dilutions. In this study, NS was used as a control, as well as HYAL Hylenex 150 U/mL and ovine HYAL 150 U/mL. The same injection protocol as Experiment 1 was utilized.

**Table 2.** Dilutions of HYAL and Study Group Make-up

| Group number   | 1:1 Volume ovine HYAL (1500 U/mL)         | 2:1 Volume ovine HYAL (1500 U/mL)        | 3:1 Volume human and ovine (150 U/mL) | 4:1 Volume human and ovine (150 U/mL) |
|----------------|-------------------------------------------|------------------------------------------|---------------------------------------|---------------------------------------|
| 1 (Saline)     | 0.2 mL HA + 0.2 mL NS                     | 0.2 mL HA + 0.4 mL NS                    | 0.2 mL HA + 0.66 mL NS                | 0.2 mL HA + 0.83 mL NS                |
| 2 (20 U HYAL)  | 0.2 mL HA + 0.013 mL HYAL + 0.187 mL NS   | 0.2 mL HA + 0.013 mL HYAL + 0.374 mL NS  | 0.2 mL HA + 0.13 mL HYAL + 0.53 mL NS | 0.2 mL HA + 0.13 mL HYAL + 0.70 mL NS |
| 3 (60 U HYAL)  | 0.2 mL HA + 0.04 mL HYAL + 0.16 mL NS     | 0.2 mL HA + 0.04 mL HYAL + 0.32 mL NS    | 0.2 mL HA + 0.39 mL HYAL + 0.27 mL NS | 0.2 mL HA + 0.39 mL HYAL + 0.44 mL NS |
| 4 (100 U HYAL) | 0.2 mL HA + 0.0667 mL HYAL + 0.1333 mL NS | 0.2 mL HA + 0.0667 mL HYAL + 0.267 mL NS | 0.2 mL HA + 0.67 mL HYAL              | 0.2 mL HA + 0.67 mL HYAL + 0.17 mL NS |

HA, hyaluronic acid; HYAL, hyaluronidase; NS, normal saline.

Like the first experiment, each group was designed to maintain precise liquid-to-solid ratios of 3:1 and 4:1. Four groups were utilized to ensure consistency in volume control.

Again, both experiments were completed using both human and ovine HYAL. Table 2 lists all dilutions and filler quantities.

### Experiment 3: Higher Dose Experiment

In the event of nondegradability of a filler, a subsequent trial was conducted with higher doses of HYAL. To maintain the same ratios (3:1 and 4:1), only ovine HYAL (1500 U/mL) was used. The dose of HYAL was incrementally increased by 50 units/per experiment. The same injection and HYAL placement procedures as used in the other experiments were followed.

### Macroscopic Photography

Photography of the HA fillers were taken immediately postinjection (1, 3, and 5 minutes), and at intervals of 10 minutes for 1 hour, using a camera with a macroscopic lens. Each photograph was taken with the same lighting and at the same distance from the sample.

### Microscopic Assessment

In addition to the macroscopic photography, high-powered microscopy was utilized by the evaluators to determine if any residual filler was present (see next section).

### Degradation Assessment

To evaluate the degradation of HA fillers, a visual assessment was conducted using both macroscopic photography and high-powered microscopy at predetermined time intervals (30 minutes, 40 minutes, 50 minutes, and 1 hour) after the injection of HYAL by 3 independent reviewers (2 board-certified plastic surgeons and 1 oculoplastic surgeon). The primary outcome measured was the visible dissolution (complete degradation) or reduction (partial degradation) in the volume of the HA filler at each time point. Complete degradation was defined as no evidence of physical filler remaining. No stirring was performed, as seen in previous studies, because it is physically impossible to manipulate filler *in vivo*. A Fleiss's kappa analysis was performed to assess the level of agreement among the 3 evaluators in determining whether the product was degraded at each time point. This statistical test was selected given the binary (yes/no) input of each reviewer at the selected time intervals.

## RESULTS

### Low Dilutions (1:1 and 2:1)

For the 1:1 ratio trial, at time points of 30 minutes, 40 minutes, 50 minutes, and 1 hour, no degradation (complete or partial) was seen in any of the 5 fillers with up to 100 U of ovine HYAL. Additionally, no degradation was seen in any of the control samples treated with pure NS (Figure 1).

For the 2:1 ratio, no degradation was seen at 30 and 40 minutes in any dilution. By 50 minutes,  $R_L$  was totally degraded with 100 U of ovine HYAL. No other complete degradation of fillers was noted. In terms of partial degradation,  $R_L$  was noted to undergo partial degradation by 1 hour with 100 U of HYAL (Figure 2).

### Higher Dilutions (3:1 and 4:1) and Comparison of Substrates

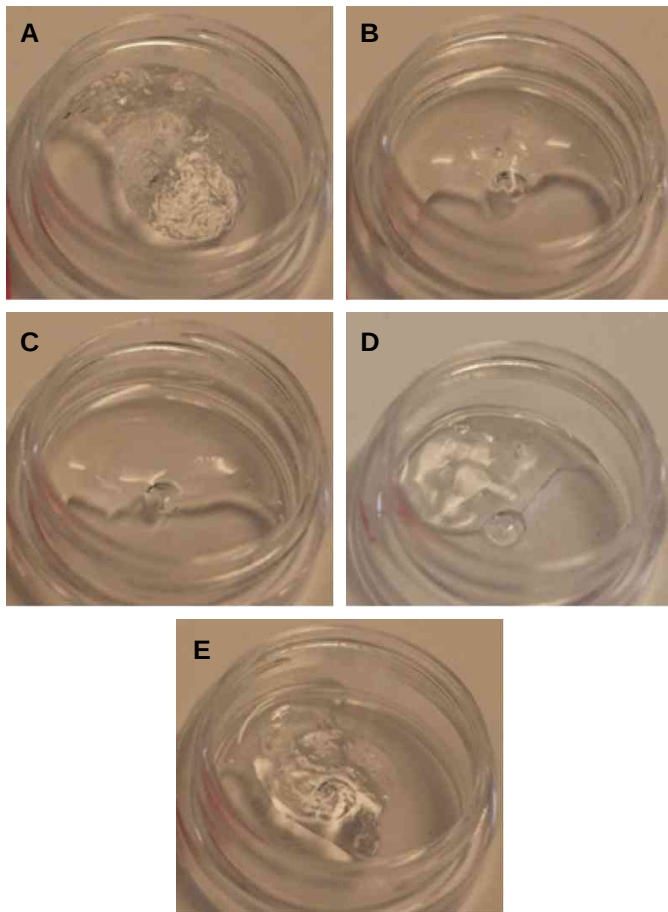
In both the 3:1 and 4:1 experiments using ovine HYAL,  $R_L$  and  $R_S$  were the first to dissolve, achieving complete degradation at 40 minutes following the injection of 100 U of ovine HYAL. No degradation was observed for the remaining 5 HA fillers after 1 hour of observation with 100 U. Figure 3 illustrates the dissolution rates of all 7 HA fillers at 40 minutes following injection of ovine HYAL.

For recombinant HYAL, in both the 3:1 and 4:1 groups,  $R_L$  was degraded first by 60 U at 30 minutes, while  $R_S$  required 100 U of recombinant HYAL and was degraded after 1 hour. The remaining 5 fillers did not show signs of degradation after 1 hour of observation with up to 100 U. Figure 4 illustrates the dissolution rates of all 7 HA fillers with human HYAL.

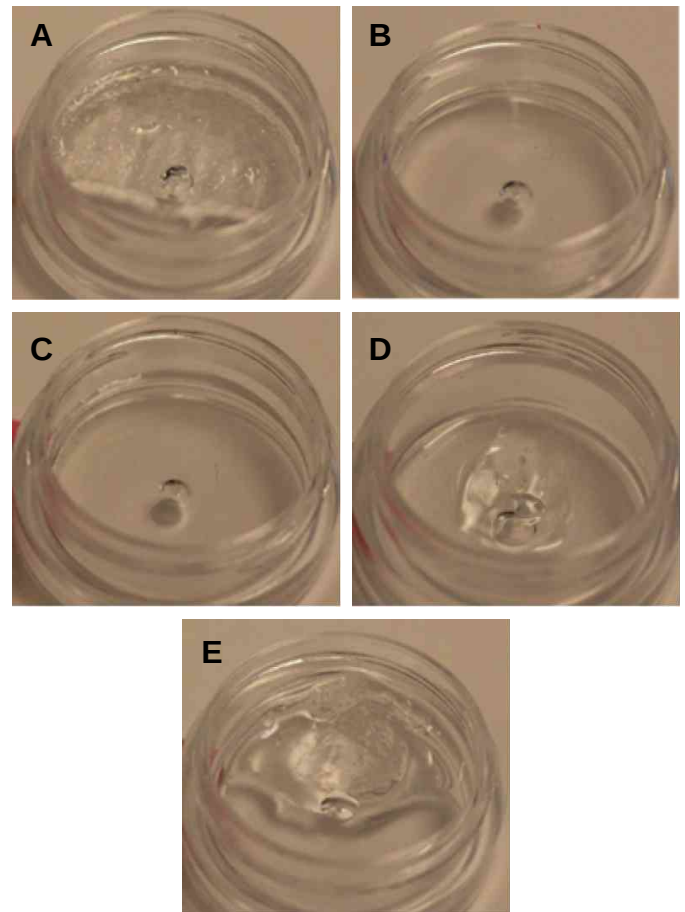
### Higher-Concentration Experiment

For this phase, the 5 fillers that had not been degraded by 100 U of either ovine or recombinant HYAL were injected with 250 and 300 U of ovine HYAL. This resulted in Group 5 (250 U: 0.167 mL HYAL + 0.493 mL NS) and Group 6 (300 U: 0.2 mL HYAL + 0.46 mL NS) in Experiment 1 (3:1), and Group 5 (250 U: 0.167 mL HYAL + 0.663 mL NS) and Group 6 (300 U: 0.2 mL HYAL + 0.63 mL NS) in Experiment 2 (4:1).

The results are summarized in Table 3. Following the injection of 250 and 300 U of ovine HYAL in both the 3:1 and 4:1 groups,  $R_{SC}$  and  $B_V$  were the most susceptible to degradation, with both dissolving by 40 minutes.  $J_{VO}$  was degraded by 50 minutes by 300 U of HYAL, while  $J_{VX}$  was degraded after 1 hour with 300 U of HYAL.



**Figure 1.** Comparison of the degradability of 0.2 mL of 5 different hyaluronic acid fillers by 100 U of ovine hyaluronidase at a liquid-to-solid ratio of 1:1: (A) Restylane Shayepe, (B) Restylane Lyft, (C) Restylane Lidocaine, (D) Revanese Sculpt+, and (E) Juvederm Volux.



**Figure 2.** Comparison of the degradability of 0.2 mL of 5 different hyaluronic acid fillers by 100 U of ovine hyaluronidase at a liquid-to-solid ratio of 2:1: (A) Restylane Shayepe, (B) Restylane Lyft, (C) Restylane Lidocaine, (D) Revanese Sculpt+, and (E) Juvederm Volux.

Lastly,  $R_H$  was also degraded after 1 hour by 300 U of HYAL. All results were seen in both the 3:1 and 4:1 groups (Figures 5, 6).

## Fleiss Kappa Score

All 3 reviewers provided identical ratings at all time points, indicating complete agreement across all assessments. As a result, the Fleiss kappa score was calculated to be 1, reflecting perfect interrater agreement.

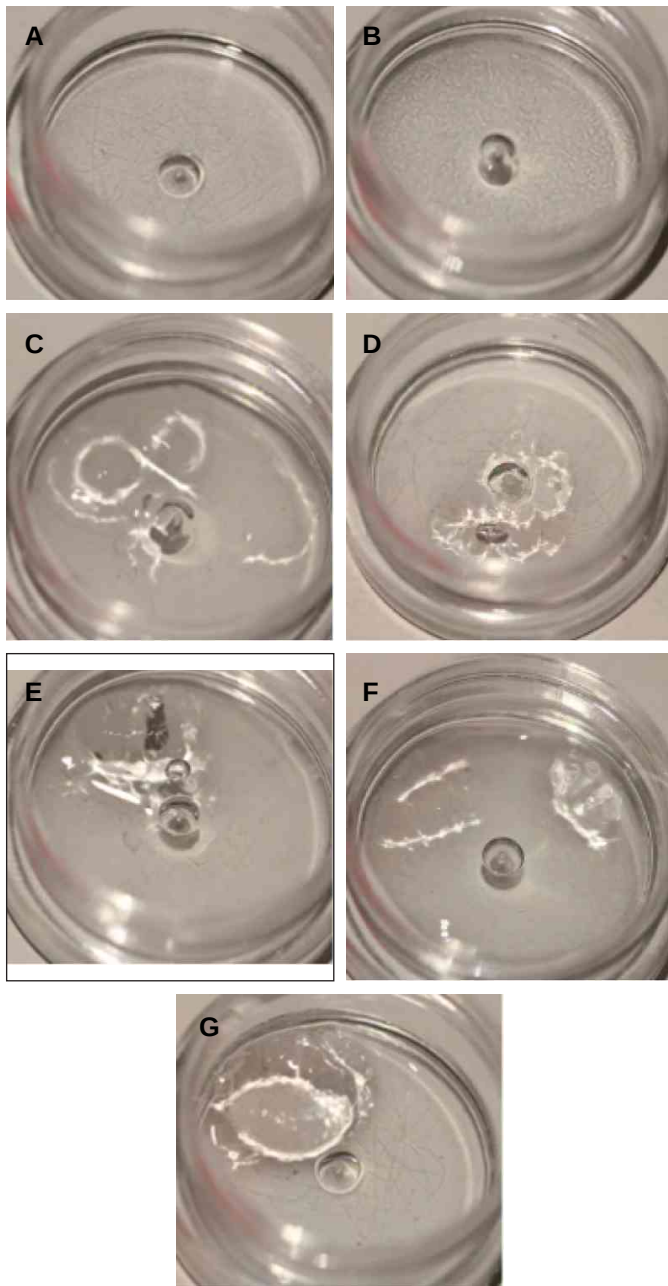
## DISCUSSION

This study was successful at demonstrating a difference in degradation of HA products based on differences in dilution and dose of the HYAL (liquid-to solid ratio), with no further subsequent improvement seen after a 3:1 dilution. Interestingly, all reviewers provided identical ratings (complete concordance) for every time point, indicating complete agreement across all assessments. No filler was dissolved in the 1:1 group even at concentrations of 100 U of ovine HYAL. This differed from the 2:1 group with 100 U of HYAL successfully dissolving  $R_{Lido}$  and partially degrading  $R_L$ . Interestingly, previous studies have demonstrated degradation of both  $R_L$  and  $R_{Lido}$  products with less than 100 U of product.<sup>11</sup> Park et al, however, used volumes to a maximum of 0.9 mL (120 U of human recombinant HYAL) which represents a liquid-to-solid ratio of 4.5:1.<sup>11</sup> Interestingly, in another study,

when lower ratios (0.1 mL with 0.15 mL of HYAL reconstituted) were considered, only partial degradation of the products was visualized, and more degradation of product was visualized with an increased concentration 40 U of HYAL, but that also included a larger liquid-to-filler ratio of 3:1.<sup>15</sup> Lastly, in terms of manipulation of product, one study also used smaller volumes of reconstituted HYAL (100 U in 0.05 mL) to degrade 0.1 mL of product (ratio, 0.5), however only partial degradation of  $R_L$  was seen after stirring of the HYAL/filler mixture ( $P < .001$ ).<sup>16</sup>

The study was also successful at demonstrating similar effectiveness when using either human recombinant or ovine HYAL. Both were able to dissolve the same products in similar timeframes. In terms of individual products,  $R_L$  and  $R_S$  were the most susceptible to degradation by both recombinant and ovine HYAL. Specifically, 100 U of ovine HYAL degraded  $R_S$  within 30 minutes, whereas 100 U of recombinant HYAL took 1 hour to achieve the same result. One study demonstrated that ovine HYAL significantly dissolved  $R_{Lido}$  more effectively within 4 days postinjection, compared with recombinant human HYAL.<sup>12</sup> In contrast, Rao et al observed no difference in the degradation of Restylane, Juvéderm, or Belotero by either recombinant or ovine HYAL, despite using similar concentrations of Restylane.<sup>13</sup>

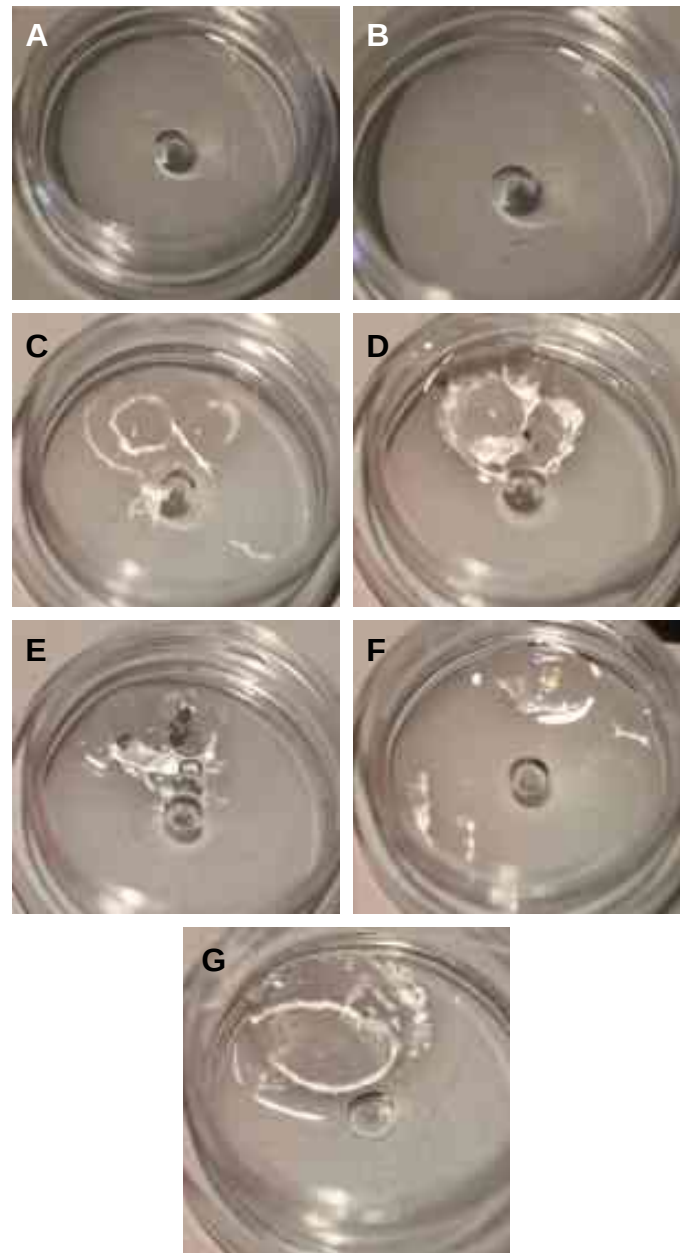
The fillers that were the most resistant to degradation in this study were  $R_H$ ,  $J_{VX}$ , and  $J_{VO}$ . This observation aligns with the findings of Park et al, who ranked  $J_{VX}$  as the most resistant.<sup>11</sup> Despite the common belief that a higher  $G'$  correlates with greater resistance to



**Figure 3.** Comparison of the degradability of 0.2 mL of 7 hyaluronic acid fillers by 100 U of ovine hyaluronidase at 40 minutes: (A) Restylane Shaype ( $R_S$ ), (B) Restylane Lyft ( $R_L$ ), (C) Revanesse Sculpt+ ( $R_{SC}$ ), (D) RHA4 ( $R_H$ ), (E) Belotero Volume ( $B_V$ ), (F) Juvederm Voluma ( $J_{VO}$ ), and (G) Juvederm Volux ( $J_{VX}$ ).

degradation, the fillers that had higher  $G'$  values, such as  $R_L$  and  $R_S$ , were the easiest to degrade in the present study. In this study, both  $R_H$  and  $J_{VX}$  demonstrated similar levels of resistance, being degraded at 300 U of ovine HYAL 1 hour postinjection. Although their  $G'$  values were lower than that of  $R_L$ , other factors likely contributed to their resistance. This can be partially attributed to their higher concentrations of HA, 26 mg/mL for  $J_{VX}$  and 23 mg/mL for  $R_H$ . In this study,  $B_V$  was more easily degraded by ovine HYAL than  $J_{VX}$  and  $R_H$ . This differs from a study by Bühren et al who reported that  $B_V$  maintained its form better than Juvederm and Restylane fillers after degradation by ovine HYAL, suggesting that  $B_V$  may exhibit greater resistance.<sup>14</sup>

Overall, this study's findings suggest that 300 U of HYAL is sufficient to degrade 0.2 mL of the most resistant HA fillers within



**Figure 4.** Comparison of the degradability of 0.2 mL of 7 hyaluronic acid by 100 units of recombinant human hyaluronidase at 60 minutes: (A) Restylane Shaype ( $R_S$ ), (B) Restylane Lyft ( $R_L$ ), (C) Revanesse Sculpt+ ( $R_{SC}$ ), (D) RHA4 ( $R_H$ ), (E) Belotero Volume ( $B_V$ ), (F) Juvederm Voluma ( $J_{VO}$ ), and (G) Juvederm Volux ( $J_{VX}$ ).

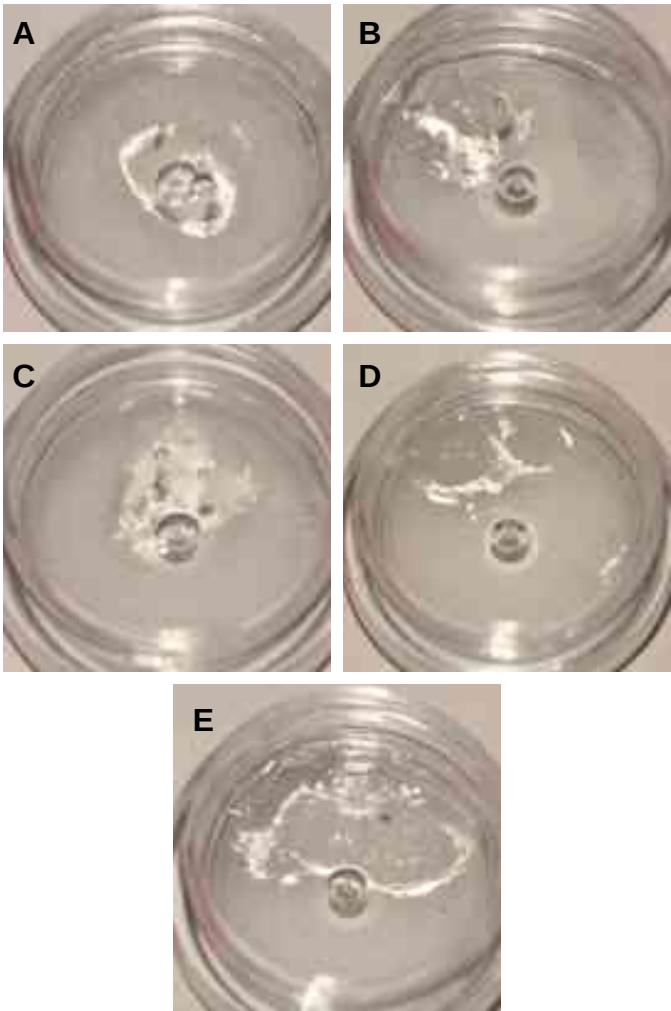
1 hour. It is recommended that clinicians consider this dose to achieve efficient degradation of HA fillers in under 1 hour. Of note, the 300 U of HYAL was administered in a 3:1 ratio. If a standard 150 U/mL reconstitution is used, for 0.2 mL of filler, this would surpass the 3:1 ratio, and thus provide ample dilution.

Previous consensus and review articles have suggested that to treat vascular events, injecting the area with around 400 to 600 IU of HYAL is required to adequately bathe the HA and provide ample dissolution to restore blood flow.<sup>17</sup> One consensus panel on the management of adverse events in HA injections found that 93% of participants view the Delorenzi protocol to be the gold standard for vascular events.<sup>17</sup> The Delorenzi protocol involves HYAL dosing based on the volume of ischemic tissue, with hourly repeated dosing to maintain high concentrations of HYAL throughout the ischemic

**Table 3.** Time to Degradation of Various HA Fillers by Different Concentrations of Ovine and Recombinant HYAL

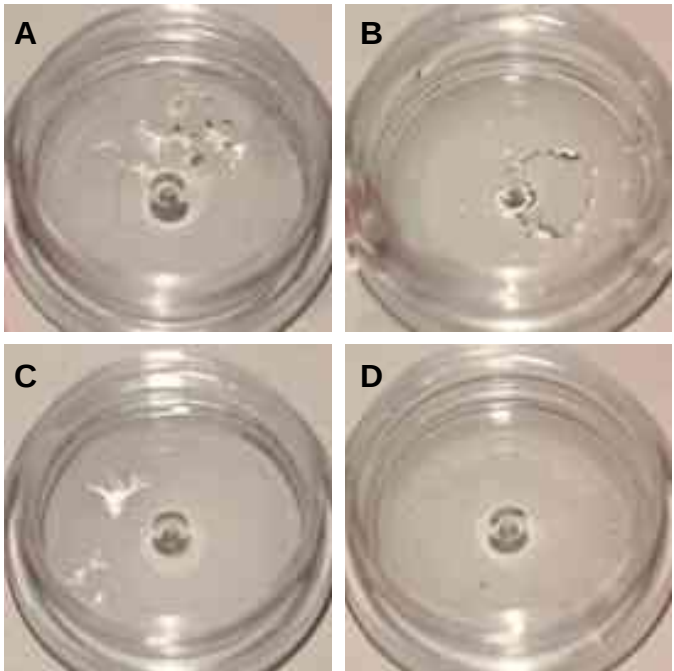
| HA filler       | Ovine HYAL (100 U) | Recombinant HYAL (100 U) | Ovine HYAL (250 U) | Ovine HYAL (300 U) | Fleiss's kappa |
|-----------------|--------------------|--------------------------|--------------------|--------------------|----------------|
| R <sub>L</sub>  | 40 minutes         | 30 minutes (60 U)        | Not tested         | Not tested         | 1              |
| R <sub>S</sub>  | 40 minutes         | 1 hour                   | Not tested         | Not tested         | 1              |
| R <sub>SC</sub> | No degradation     | No degradation           | 40 minutes         | 40 minutes         | 1              |
| R <sub>H</sub>  | No degradation     | No degradation           | No degradation     | 1 hour             | 1              |
| B <sub>V</sub>  | No degradation     | No degradation           | No degradation     | 40 minutes         | 1              |
| J <sub>VO</sub> | No degradation     | No degradation           | No degradation     | 50 minutes         | 1              |
| J <sub>VX</sub> | No degradation     | No degradation           | No degradation     | 1 hour             | 1              |

All values represent the time to visible degradation as assessed independently by 3 raters. Fleiss's kappa = 1.0, indicating complete interrater agreement. B<sub>V</sub>, Belotero Volume; HA, hyaluronic acid; HYAL, hyaluronidase; J<sub>VO</sub>, Juvederm Voluma; J<sub>VX</sub>, Juvederm Volux; R<sub>H</sub>, RHA4; R<sub>L</sub>, Restylane Lyft; R<sub>S</sub>, Restylane Shaype; R<sub>SC</sub>, Revanesse Sculpt+.



**Figure 5.** Comparison of the degradability of 0.2 mL of 5 hyaluronic acid fillers by 250 U of recombinant human hyaluronidase at 60 minutes: (A) Revanesse Sculpt+ (R<sub>SC</sub>), (B) RHA4 (R<sub>H</sub>), (C) Belotero Volume (B<sub>V</sub>), (D) Juvederm Voluma (J<sub>VO</sub>), and (E) Juvederm Volux (J<sub>VX</sub>).

zone.<sup>18</sup> This new theory was based on the idea that an affected vessel must be sufficiently bathed with the enzyme solution to be able to work. An example given is for a low-volume vascular embolic event (defined as <0.1 mL): a usual dose would be 3 mL of HYAL (about



**Figure 6.** Comparison of degradability of the 0.2 mL of four hyaluronic acid fillers by 300 U of recombinant human hyaluronidase at 60 minutes: (A) RHA4 (R<sub>H</sub>), (B) Belotero Volume (B<sub>V</sub>), (C) Juvederm Voluma (J<sub>VO</sub>), and (D) Juvederm Volux (J<sub>VX</sub>).

450 U in a reconstituted dose) and this dose increases with the size of the affected area.<sup>18</sup> This represents a 30:1 ratio of liquid (solution) to solid (filler), much greater than seen in the present study.

With the novel use of ultrasound-based devices, delivery of HYAL to the affected area may be more reliable, especially for nonvascular correction, but nevertheless no clear consensus exists on dosage or dilution. Additionally, as demonstrated in previous studies, intrafiller injection of HYAL may increase the ability to dissolve filler using less HYAL when compared with perfiller injection.<sup>11</sup> Unfortunately, ultrasound technology is not carried in a vast majority of clinics where HA filler is administered and its use involves a learning curve.

Understanding appropriate dosing and dilution is critical, especially in nonvascular cases, given the possible side effects from large doses of HYAL. One adverse event that has been described is referred to as post-HYAL syndrome, which may include hollowing of the facial tissues, loss of skin elasticity, or discoloration of the skin.<sup>19</sup> Post-HYAL

syndrome was described in a 90-patient case series treated with HYAL over a 4-year period for swelling (52%), lumpiness (20%), and filler dissolution prior to blepharoplasty (17%).<sup>19</sup> The study did conclude, however, that post-HYAL syndrome may be related to previous filler volume and duration, rather than the dose of HYAL used. Variable doses were used: 150 IU/mL in 66% patients, 75 IU/mL in 31%, 37.5 IU/mL in 3%, and 100 IU/mL in 1%. Additionally, HYAL can be associated with an allergic reaction, although this is rare.<sup>20</sup> Previous studies have described allergic reactions, hypersensitivity, and angioedema related to the use of ovine HYAL. The incidence of allergic reactions has been reported to be 0.05% to 0.69%, and urticaria and angioedema have also been reported to occur at an even lower frequency.<sup>3</sup> One review found that these allergic reactions can present up to 120 hours after injection.<sup>21</sup> If warranted, a skin test can be performed with 3 IU of HYAL.<sup>3</sup> Consensus panels have not demonstrated much utility in previous skin testing with the incidence of allergic reaction being so low.<sup>17,20</sup> Of note, injection of ovine HYAL should be avoided in patients who are allergic to bovine collagen and bee stings due to possible cross-reactivity.

It is important to note that HYAL has previously been added in varying quantities to local anesthesia to help with tissue dispersion.<sup>22</sup> It is therefore hypothesized that, in vivo, even lower ratios may be successful at partially degrading product if the HYAL can be adequately spread around the filler, although this has not been demonstrated. Additionally, given the pain associated with filler dissolving, some authors have advocated for the use of lidocaine to be added to the HYAL mixture.<sup>23</sup> Although some authors argue this may change the pH of the surrounding tissue, rendering HYAL less effective, similar to what is seen in the context of infection and biofilm, it may contrarily also render the mixture more dilute and increase the ratio of solid to liquid.<sup>24</sup>

In terms of aesthetic correction or for partial degradation, previous studies have demonstrated that lower concentrations of HYAL (20 U) may be as effective as higher concentrations (40 U), with a general rule of 20 U for 4 to 6 mg of HA.<sup>25</sup> Regardless, when correcting for nodules or the Tyndall effect, less can be administered and the patient may return for further treatment. The same, unfortunately, cannot be said about vascular events. One panel survey, which surveyed 264 healthcare professionals, found that the majority used a concentration of 500 to 750 IU/mL for vascular events and 150 to 250 IU/mL for nonvascular adverse events, but there was wide variability in the group.<sup>26</sup> Additionally, 5 percent of respondents admitted to observing events related to the HYAL itself, including swelling, anaphylaxis, skin laxity, and soft tissue atrophy.<sup>26</sup>

This study demonstrated that the amount of fluid injected (ratio of HYAL mixture to filler) may play a larger role than previously expected, especially in patients who are referred for aesthetic correction. This trial demonstrates that the liquid-to-solid ratio is likely as important as the concentration of HYAL, especially when injected into the surrounding tissues. This effect is further compounded when vascular injection is seen, as the HYAL needs to not only penetrate the filler, but also the vessel wall. One ex vivo study examined portions of the anterior jugular vein and facial artery from neck dissections. The vein and artery specimens were filled with 25 mg/mL of crosslinked HA filler. Each specimen was soaked in 0.5 mL of HYAL (300 IU/mL), in its own test tube, for 4 hours, after which the remaining HA volume was found to be 0.02 mL in the vein segment and 0.002 mL in the artery segment.<sup>27</sup> While the efficacy of intravascular injection of HYAL is still up for debate, with some injectors feeling that this may in fact propagate the embolic filler forward, it is still of upmost importance to ensure an adequate amount of HYAL is injected perivascularly to help with clot breakdown and restoration of flow.

Clinically, these findings provide a foundation for future trials to explore various concentrations, ratios, and filler types to better understand the impact of ratio on filler dissolution. This is particularly relevant when examining different formulations of HYAL. For example, Hylenex recombinant is supplied as a 150 U/mL vial, which may require multiple vials for certain fillers. In contrast, ovine-derived formulations are often available in 1500 U/vials, allowing for easier storage and dilution. In aesthetic adverse events, this could result in more effective dissolving with fewer side effects. In ischemic events, it may facilitate better resolution of embolism and faster restoration of blood flow.

## Limitations

This study is not without limitations. First, considering the reliance on visual assessments for degradation, there was potential for subjectivity, despite attempts at using multiple reviewers. Second, only ovine HYAL was used for higher-concentration experiments due to limited availability of higher concentrations of recombinant HYAL; this may introduce variability that limits direct comparison at these elevated HYAL doses. Moreover, the controlled experimental study may not fully replicate real-world conditions. Factors such as individual patient variability, tissue type, and injection technique could affect HA degradation in clinical practice and should be taken into consideration. Lastly, as mentioned, HYAL itself can help the spread of fluid in human tissues and exhibits its effect for longer than 1 hour.

## CONCLUSIONS

This study demonstrates that 300 U of HYAL is sufficient to degrade 0.2 mL of the most resistant HA fillers within 1 hour, offering a clearer guideline for clinicians treating HA-related complications. Additionally, a minimum dilution of 3:1 (liquid-to-filler ratio) should be used to provide adequate fluid for dissolving filler. Lastly, ovine HYAL appears to be just as effective as recombinant HYAL in terms of dissolving product.

## Acknowledgments

Dr Safran and Mr Khalaf contributed to this work equally as co-first authors.

## Disclosures

Dr Nikolis is or has been a consultant and/or speaker for Galderma (Lausanne, Switzerland), Merz (Frankfurt, Germany), Allergan (Irvine, CA), and Prolenium (Montreal, QC). Dr Metelitsa is a consultant and/or speaker for Galderma, Allergan, and Crescita Therapeutics (Laval, Canada). Dr Woodward has been a consultant and/or speaker for Galderma, Merz, Allergan, Prolenium, and SkinCeuticals (New York, NY). The remaining authors have nothing to disclose.

## Funding

The authors received no financial support for the research, authorship, and publication of this article.

## REFERENCES

1. Aesthetic Plastic Surgery National Databank Statistics 2023. *Aesthet Surg J.* 2024;44:1-25. doi: [10.1093/asj/sjae188](https://doi.org/10.1093/asj/sjae188)
2. Safran T, Swift A, Cotofana S, Nikolis A. Evaluating safety in hyaluronic acid lip injections. *Expert Opin Drug Saf.* 2021;20:1473-1486. doi: [10.1080/14740338.2021.1962283](https://doi.org/10.1080/14740338.2021.1962283)

3. Jung H. Hyaluronidase: an overview of its properties, applications, and side effects. *Arch Plast Surg*. 2020;47:297-300. doi: [10.5999/aps.2020.00752](https://doi.org/10.5999/aps.2020.00752)
4. Reuther T, Bayrhammer J, Kersch M. Effects of a three-session skin rejuvenation treatment using stabilized hyaluronic acid-based gel of non-animal origin on skin elasticity: a pilot study. *Arch Dermatol Res*. 2010;302:37-45. doi: [10.1007/s00403-009-0988-9](https://doi.org/10.1007/s00403-009-0988-9)
5. Kim HJ, Kwon SB, Whang KU, Lee JS, Park YL, Lee SY. The duration of hyaluronidase and optimal timing of hyaluronic acid (HA) filler reinjection after hyaluronidase injection. *J Cosmet Laser Ther*. 2018;20:52-57. doi: [10.1080/14764172.2017.1293825](https://doi.org/10.1080/14764172.2017.1293825)
6. Faivre J, Wu K, Gallet M, Sparrow J, Bourdon F, Gallagher CJ. Comparison of hyaluronidase-mediated degradation kinetics of commercially available hyaluronic acid fillers in vitro. *Aesthet Surg J*. 2024;44:NP402-NP410. doi: [10.1093/asj/sjae032](https://doi.org/10.1093/asj/sjae032)
7. Gerber PA, Buhren BA, Bölke E, Philipp-Dormston WG, Homey B, Schrupf H. Time- and dose-dependent effects of hyaluronidase on the degradation of different hyaluronan-based fillers in vitro. *Plast Reconstr Surg*. 2023;151:560-567. doi: [10.1097/PRS.00000000000009916](https://doi.org/10.1097/PRS.00000000000009916)
8. Beatrous BP, Liddell PH, Turner LM, Raucher D, Jordan JR. In vitro comparison between ovine and human recombinant hyaluronidase on hyaluronic acid fillers. *Facial Plast Surg Aesthet Med*. 2023;25:391-395. doi: [10.1089/fpsam.2022.0082](https://doi.org/10.1089/fpsam.2022.0082)
9. Ebo DG, Goossens S, Opsomer F, Bridts CH, Stevens WJ. Flow-assisted diagnosis of anaphylaxis to hyaluronidase. *Allergy*. 2005;60:1333-1334. doi: [10.1111/j.1398-9995.2005.00891.x](https://doi.org/10.1111/j.1398-9995.2005.00891.x)
10. Roohani I, Zaki D, Firriolo J, et al. Hyaluronidase availability in emergency rooms: a state-wide census. *Plastic Reconstr Surg Glob Open*. 2021;9:7-8. doi: [10.1097/01.GOX.0000799116.81668.2e](https://doi.org/10.1097/01.GOX.0000799116.81668.2e)
11. Park KE, Mehta P, Kherani F, et al. Response of 21 hyaluronic acid fillers to recombinant human hyaluronidase. *Plast Reconstr Surg Glob Open*. 2023;11:e5457. doi: [10.1097/GOX.00000000000005457](https://doi.org/10.1097/GOX.00000000000005457)
12. Humate GT, Chopra R, Jones D, Messina DJ, Hee CK. In vivo degradation of crosslinked hyaluronic acid fillers by exogenous hyaluronidases. *Dermatol Surg*. 2018;44:1075-1083. doi: [10.1097/DSS.0000000000001525](https://doi.org/10.1097/DSS.0000000000001525)
13. Rao V, Chi S, Woodward J. Reversing facial fillers: interactions between hyaluronidase and commercially available hyaluronic-acid based fillers. *J Drugs Dermatol*. 2014;13:1053.
14. Buhren BA, Schrupf H, Bölke E, Kammers K, Gerber PA. Standardized in vitro analysis of the degradability of hyaluronic acid fillers by hyaluronidase. *Eur J Med Res*. 2018;23:37. doi: [10.1186/s40001-018-0334-9](https://doi.org/10.1186/s40001-018-0334-9)
15. Mehta P, Ryu C, Park K, Kherani F, Zhang-Nunes S. Response of five different hyaluronic acid gels to varying doses of recombinant human hyaluronidase. *J Plast Reconstr Aesthet Surg*. 2023;76:298-300. doi: [10.1016/j.bjps.2022.10.043](https://doi.org/10.1016/j.bjps.2022.10.043)
16. Germani M, de Queiroz MVGB, Yuri de França Shimizu M, et al. Comparative in-vitro degradation of hyaluronic acids exposed to different hyaluronidase enzymes. *J Oral Biol Craniofac Res*. 2025;15:178-182. doi: [10.1016/j.jobcr.2025.01.001](https://doi.org/10.1016/j.jobcr.2025.01.001)
17. Nikolis A, Cohen JL, Enright KM, et al. Deliberations of the Safety Task Force: risk factors and treatment of adverse events associated with aesthetic injectables. *J Cosmet Dermatol*. 2024;23:3551-3564. doi: [10.1111/jocd.16476](https://doi.org/10.1111/jocd.16476)
18. DeLorenzi C. New high dose pulsed hyaluronidase protocol for hyaluronic acid filler vascular adverse events. *Aesthet Surg J*. 2017;37:814-825. doi: [10.1093/asj/sjw251](https://doi.org/10.1093/asj/sjw251)
19. Wilde CL, Jiang K, Lee S, Ezra DG. The posthyaluronidase syndrome: dosing strategies for hyaluronidase in the dissolving of facial filler and independent predictors of poor outcomes. *Plast Reconstr Surg Glob Open*. 2024;12:e5765. doi: [10.1097/GOX.00000000000005765](https://doi.org/10.1097/GOX.00000000000005765)
20. Wu L, Liu X, Jian X, et al. Delayed allergic hypersensitivity to hyaluronidase during the treatment of granulomatous hyaluronic acid reactions. *J Cosmet Dermatol*. 2018;17:991-995. doi: [10.1111/jocd.12461](https://doi.org/10.1111/jocd.12461)
21. Guliyeva G, Huayllani MT, Kraft C, Lehrman C, Kraft MT. Allergic complications of hyaluronidase injection: risk factors, treatment strategies, and recommendations for management. *Aesthetic Plast Surg*. 2024;48:413-439. doi: [10.1007/s00266-023-03348-5](https://doi.org/10.1007/s00266-023-03348-5)
22. Mohamed AA, Radwan TA, Mohamed MM, et al. Safety and efficacy of addition of hyaluronidase to a mixture of lidocaine and bupivacaine in scalp nerves block in elective craniotomy operations; comparative study. *BMC Anesthesiol*. 2018;18:129. doi: [10.1186/s12871-018-0590-9](https://doi.org/10.1186/s12871-018-0590-9)
23. Murray G, Convery C, Walker L, Davies E. Guideline for the safe use of hyaluronidase in aesthetic medicine, including modified high-dose protocol. *J Clin Aesthet Dermatol*. 2021;14:E69-E75.
24. Kroumpouzos G, Treacy P. Hyaluronidase for dermal filler complications: review of applications and dosage recommendations. *JMIR Dermatol*. 2024;7:e50403. doi: [10.2196/50403](https://doi.org/10.2196/50403)
25. Juhász MLW, Levin MK, Marmur ES. The kinetics of reversible hyaluronic acid filler injection treated with hyaluronidase. *Dermatol Surg*. 2017;43:841-847. doi: [10.1097/DSS.0000000000001084](https://doi.org/10.1097/DSS.0000000000001084)
26. Currie E, Granata B, Goodman G, et al. The use of hyaluronidase in aesthetic practice: a comparative study of practitioner usage in elective and emergency situations. *Aesthet Surg J*. 2024;44:647-657. doi: [10.1093/asj/sjae009](https://doi.org/10.1093/asj/sjae009)
27. Rauso R, Zerbinati N, Fragola R, Nicoletti GF, Tartaro G. Transvascular hydrolysis of hyaluronic acid filler with hyaluronidase: an ex vivo study. *Dermatol Surg*. 2021;47:370-372. doi: [10.1097/DSS.00000000000002773](https://doi.org/10.1097/DSS.00000000000002773)