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Review

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The interaction between hyaluronidase and hyaluronic acid gel fillers - a review of the literature and comparative analysis

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Abstract

Hyaluronic acid (HA) is the most common component of aesthetic fillers. Many formulations exist, each exhibiting properties that are manifestations of individual molecular modifications. The enzyme hyaluronidase degrades hyaluronic acid and can therefore be injected into soft tissue to reduce suboptimally placed HA fillers or to reverse local ischemic complications. The clinically available varieties of hyaluronidase may be derived from crude animal extracts or genetically engineered from recombinant human DNA. Different HA fillers are not uniformly dissolved by a single source hyaluronidase, and hyaluronidase from different sources may have varying efficacy in the degradation of HA. Previous studies of subsets of HA fillers and hyaluronidases have provided limited and often conflicting data regarding these differences, and a more comprehensive scientific study is needed. In this review, the authors describe commonly available formulations of HA and hyaluronidase and review all studies of HA-hyaluronidase interaction available via a PubMed and Google Scholar search from 2005 to present, exploring trends in the data. Factors determined to confer increased resistance to degradation included higher concentration of HA, higher crosslinking density, and status as monophasic versus biphasic. Fillers of the Juvéderm family were generally found to be more resistant to degradation than members of the Restylane family. Results are less consistent for Belotero Balance. No variety of hyaluronidase was consistently superior at dissolving any variety of HA filler. More research is needed to clarify these clinically relevant relationships.

Keywords: Hyaluronic acid, hyaluronic acid gel, hyaluronidase, dermal fillers, enzymatic degradation, filler complications

INTRODUCTION

Hyaluronic acid (HA) in various configurations of density and crosslinking is commonly used as a substrate for dermal fillers. These widely available and popular fillers augment volume and smooth contour irregularities in cosmetic and age-related changes of the face, hands and other anatomic areas[1]. Though many filler materials, including collagen, autologous fat, calcium hydroxyapatite, and poly-L-lactic acid are used in facial rejuvenation, HA accounts for more injections than all other filler varieties combined[2].

One of the main advantages of HA fillers is their easy reversibility. This quality is useful as a means of addressing patient dissatisfaction from superficial or inappropriate placement following filler injection[3]. Additionally and most importantly, these injections have been associated with rare but serious ischemic complications including local soft tissue necrosis as well as blindness from possible embolic occlusion of the central retinal artery[3]. Hyaluronidase is a naturally occurring enzyme that catalyzes the degradation of HA by hydrolyzing the bond between *N*-acetylglucosamine and glucuronic acid[4,5]. In addition to its off-label use as a subcutaneous adjuvant that increases the dispersion of drugs, it has been shown to be effective in both reversing the volumetric effects of HA filler injections and treating local ischemic complications[6-9], although the reversibility of HA filler associated blindness with hyaluronidase has not been established[10,11]. Understanding the interactions between the various fillers and the enzymes available for dissolution might optimize outcomes in the event of a filler misplacement or occlusive event.

Since the first FDA approval in 2003 of the HA-containing filler, Restylane, additional HA formulations have become commercially available. Aiming to increase stability and longevity, manufacturers of HA fillers chemically modify the molecules by crosslinking them into larger conjugated derivatives[12]. The different HA fillers vary according to the method and extent of crosslinking, concentration of HA, particulate size, HA source, and status as monophasic or biphasic[13]. This influences the properties of each gel filler, including hydrophilia, cohesivity, hardness, and viscosity[2]. Clinically, knowledge of these differences can be used to select the optimal HA filler for a given application[6]. Additionally, the structural differences between HA fillers are known to alter their response to hyaluronidase[2,6,12,14-18].

Hyaluronidase, similarly, is available in several formulations, broadly categorized as purified crude extracts from ovine or bovine testicular tissue, and products of recombinant technology from human DNA[19]. Though all major formulations are dosed equivalently, each type has its own optimal pH and is considered therapeutically distinct by the FDA[19].

In the clinical setting, it has been reported that some fillers are more or less responsive to enzymatic degradation as compared to others[16]. Thus, the interaction between different HA fillers and the varieties of hyaluronidase is an area of research with important implications, especially for the choice of reversal agent in treating suboptimal outcomes or complications from filler injections. Although multiple studies have explored the effect of hyaluronidase on different types of HA fillers, few have examined more than a single type of hyaluronidase[6,16]. A comprehensive analysis of the full range of commercially available HA fillers and hyaluronidases is needed. Unfortunately, the literature to date concerning this topic has been noted to exhibit significant and unresolved heterogeneity in both experimentation and results[20]. To address the incompletely answered question of how different HA fillers respond to different types of hyaluronidase, the authors first summarize the known literature available on PubMed and Google Scholar of HA-hyaluronidase interactions. The authors then attempt to clarify, across studies, the relationships between commercially available HA-based fillers and ovine-, bovine-, and recombinant-sourced hyaluronidase. Results from experiments using different fillers and hyaluronidases are cross-referenced and information describing how different HA fillers respond to hyaluronidase will be presented. Additionally, possible explanations for discrepancies found across studies are explored. Through this review, we attempt to provide a summary of foundational knowledge and highlight the need for future clarifying studies.

Table 1. Properties of the Three Major Families of HA Filler

Family name	Crosslinking agent	Monophasic/ Biphasic	Crosslinking technology	Sub varieties	Concentration (mg/mL)	Percent crosslinking
Restylane	BDDE	Biphasic	NASHA	Restylane	20	1%
				Restylane-L	20	1%
				Restylane Lyft	20	1%
				Restylane Silk	20	1%
			XpresHAn	Restylane Refyne	20	6%
				Restylane Defyne	20	8%
Juvéderm	BDDE	Monophasic	Hylacross	Juvéderm Ultra	24	6%-9%
				Juvéderm Ultra Plus	24	8%-11%
			Vycross	Juvéderm Voluma	20	Unreported
				Juvéderm Vollure	17.5	Unreported
				Juvéderm Volbella	15	Unreported
				Juvéderm Volite	12	Unreported
Belotero	N/A	Monophasic	CPM	Belotero Balance	22.5	Polydensified gel
				Belotero Soft	20	Polydensified gel
				Belotero Intense	25.5	Polydensified gel
				Belotero Volume	26	Polydensified gel
			Uncrosslinked	Belotero Hydro	18	N/A

BDDE: 1,4-butanediol diglycidyl ether

REVIEWING THE LITERATURE

A literature search of published reports from 2005 to April 2020 was performed on the interactions between different types of hyaluronic acid fillers and hyaluronidases. The databases of PubMed, Ovid MEDLINE, and Google Scholar were searched using keywords including: "hyaluronic acid fillers and hyaluronidase," "degradation of hyaluronic acid," "hyaluronic acid and hyaluronidase interactions," "hyaluronic acid filler comparison," and "hyaluronic acid filler sensitivity." The search was limited to the literature in English. In addition, references in the identified articles were reviewed to identify additional reports, if any.

LITERATURE FINDINGS

More than one hundred experiments, reviews, and reports regarding hyaluronic acid-hyaluronidase relationships and relevant topics were identified and reviewed. Eight relevant studies were identified and analyzed in detail. The authors attempted to include all relevant data in this report.

Classification of hyaluronic acid dermal fillers and hyaluronidases

Varieties of hyaluronic acid filler

At the time of this writing, 18 individual formulations of HA have been approved for use as dermal fillers by the FDA[21]. The subtypes and properties of three major filler families, Restylane, Juvéderm, and Belotero Balance, are described below and summarized in Table 1.

Restylane is manufactured by Q-Med AB (Uppsala, Sweden) and produced from HA generated via fermentation[22]. Produced entirely from non-animal sources, it is classified as a non-animal stabilized hyaluronic acid (NASHA) and is stabilized though a crosslinking process to the compound 1,4-butanediol diglycidyl ether (BDDE)[22,23]. It has a HA concentration of 20 mg/mL and a biphasic formulation with a gel particulate size of 330-430 micrometers[22,24]. The degree of crosslinking is relatively low at 1%[24]. Subsequently introduced members of the Restylane family include Restylane Lyft (formerly known as Perlane), which contains fewer, larger gel particles per milliliter (8,000 per mL *vs.* 100,000 per mL); Restylane-L, which contains lidocaine; and Restylane Silk, which contains lidocaine, is less viscous, and was formulated specifically for lip augmentation[22,24]. Restylane Refyne, Restylane Defyne, and Restylane Kysse are manufactured with XpresHAn Technology, which varies the degree of crosslinking and gel particle size to create softer gels[25,26].

Juvéderm, initially produced by Lea Derm (Paris, France) and subsequently manufactured and distributed by Allergan (Irvine, CA), is also a BDDE-crosslinked NASHA produced from equine streptococci[24]. In contrast to Restylane, it is a monophasic gel without distinct particles, originally produced through Hylacross technology that allows for a high concentration of crosslinking. First approved by the FDA in 2006, the Juvéderm family of fillers has expanded to many formulations that vary in concentration of HA and crosslinking density. The two main varieties available for facial wrinkles and folds in the US market are Juvéderm Ultra (Juvéderm 24 HV) and Juvéderm Ultra Plus (Juvéderm 30 HV), which both have a concentration of 24 mg/mL but differ in their crosslinking percentages (9% and 11%, respectively)[27].

The more recent formulations of Juvéderm are manufactured using Vycross technology, which combines low- and high-weight HA molecules to improve crosslinking efficiency[24]. Juvéderm Voluma XC (20 mg/mL) is a formulation with high cohesivity and viscosity used for deep injections and cheek augmentation. Juvéderm Vollure XC (17.5 mg/mL) is slightly less concentrated and designed for volumizing nasolabial folds[28]. Even denser crosslinking is present in Juvéderm Volbella XC (15 mg/mL), which is manufactured with a higher proportion of low molecular weight HA and designed for lip augmentation and perioral rhytids[29]. Juvéderm Volite, the least concentrated variety at 12 mg/mL, is for superficial cutaneous depressions and fine lines[30].

Members of the Belotero family, in contrast to the above monodensified varieties, are polydensfied compounds that contain continuously crosslinked HA in a single phase[31]. Manufactured by Anteis S.A. (Geneva, Switzerland), fillers of the Belotero family are made with a cohesive polydensified matrix (CPM) technology that creates a gel with nonuniform crosslinking[15]. This creates a low-viscosity filler that exhibits homogenous intradermal distribution as compared to other fillers, theoretically allowing for increased injection precision[24,32]. Like Juvéderm, the Belotero family has expanded into many different formulations that vary in concentration[31]. However, the only one currently available in the US is Belotero Balance, which has a concentration of 22.5 mg/mL and is approved for facial wrinkles and folds[31].

Several other filler varieties, including RHA 2, RHA 3, RHA 4 (Teoxane SA), Revanesse Versa, and Revanesse Versa Plus, are also available in the US, but are less commonly studied and used clinically. Hydrelle (previously known as Elevess) is manufactured by Anika Therapeutics (Bedford, MA) and marketed through Coapt Systems (Palo Alto, California). Though also sourced from equine streptococci, it is crosslinked with p-phenylene bisethyl carbodiimide (BCDI)[22]. With a concentration of 28 mg/mL, it has amongst the highest available content of HA[22]. Prevelle Silk, the second generation of now-unavailable Captique, is manufactured by Genzyme Corporation (Cambridge, MA) and marketed by Johnson & Johnson (Skillman, NJ); it has a concentration ranging from 4.5-6 mg/mL and is cross-linked with di-vinyl sulfone[22]. Hylaform (manufactured by Genzyme Biosurgery in Ridgefield, NJ), was an avian-sourced formulation with concentration ranging from 4.5-6 mg/mL of HA. Notably, it is no longer available on the market in the US[21,27].

Varieties of hyaluronidase

There are four varieties of hyaluronidase currently available in the United States: two derived from purified bovine testicular hyaluronidase, one derived from purified ovine testicular hyaluronidase, and one manufactured from recombinant human DNA[19].

Vitrase, manufactured by STA Pharmaceutical (Irvine, CA), is derived from purified ovine testicular hyaluronidase. Amphadase, manufactured by Amphastar Pharmaceuticals, Inc. (Rancho Cucamonga, CA), and Hydase, manufactured by PrimaPharm, Inc. (San Diego, CA), are derived from purified bovine testicular hyaluronidase. Hylenex, manufactured by Halozyme Therapeutics (San Diego, CA), is made from recombinant human DNA[33]. Though one experimental study has suggested these varieties are

Table 2. Summary of reviewed experiments of HA-hyaluronidase interactions

Study	Type	Measurement	Hyaluronidase(s) used	Findings: x > y (x dissolves more than y)
Rao *et al.*[6]	*In vitro*	Visual comparison	Recombinant	Res > Juv Voluma > Juv Ultra > Belo
			Ovine	Res > Juv Voluma > Juv Ultra > Belo
Jones *et al.*[2]	*In vitro*	Chromatography	Ovine	Hylenex > Res > Juv Ultra
Flynn *et al.*[15]	*In vitro*	Chromatography	Ovine	Res > Juv Ultra > Belo
Sall *et al.*[12]	*In vitro*	Absorbance measurement	Bovine	Res > Res Lyft >> Surg 18 > Juv 30 > Juv 24 > Juv 30 HV > Juv 24 HV > Surg 30 XP > Surg 24 XP > Surg 30
Cavallini *et al.*[14]	*In vitro*	Visual comparison	Bovine	Juv Volite > Teosyal RHA > Teosyal Ultra = Juv Voluma > Macrolane = Res
Buhren *et al.*[17]	*In vitro*	Fluorescence Measurement	Bovine	Belo > Res >>> Juv (didn't degrade)
Shumate *et al.*[16]	*In vivo* - Rat model	3D Image Quantification	Recombinant	Res-L > Juv Ultra = Juv Voluma
			Ovine	Res-L = Juv Ultra > Juv Voluma
Juhasz *et al.*[18]	*In vivo* - Human subjects	5-Point Palpation Score	Ovine	Belo >> Juv Ultra > Res Lyft > Juv Ultra Plus > Res-L > Res Silk > Juv Voluma

Res: Restylane; Juv: Juvéderm; Belo: Belotero Balance; Surg: Surgiderm

equally potent and can be used interchangeably[6], subsequent tests and prevailing clinical perceptions have questioned this assumption[16,34]. Clinically, it is noteworthy that animal-derived enzymes, though less expensive and more readily available, generally have a less favorable immunogenic profile than those that are recombinantly produced[34].

Interactions between different types of hyaluronic acid filler and hyaluronidase

Prior studies examining the relationship between different HA fillers and hyaluronidases vary broadly with respect to the products tested and methodology. Experimental designs have included qualitative *in vitro* studies[6,14], quantitative *in vitro* studies[2,12,15,17], an animal model[16], and clinical testing of human subjects[18]. Twenty-one individual formulations of HA-containing fillers have been examined, with over 75% being members of the families Restylane, Juvéderm, or Belotero Balance[2,6,12,14-18]. We focused our analysis on these three product families, excluding exploration of fillers such as Surgiderm and Teosyal that have not been studied widely or comparatively. A summary of the relevant literature, including type(s) of HA filler and hyaluronidase tested, can be found in Table 2. Although there are some inconsistencies in the reported susceptibility of specific HA fillers to dissolution by hyaluronidase, these studies collectively suggest several guiding principles of HA filler dissolution by hyaluronidase.

Greater concentrations of hyaluronic acid are associated with greater resistance to degradation

The concentration of HA in currently available fillers range from 4.5 mg/mL to 30 mg/mL, with the most commonly injected varieties falling between 20-24 mg/mL[13]. A higher concentration of HA generally corresponds to increased stiffness and longevity[27]. In nearly every study of HA-hyaluronidase interaction, filler varieties with a lower concentration of HA tended to dissolve more quickly than fillers with higher HA concentrations. For example, in comparing three filler varieties with different HA concentrations (24 mg/mL, 20 mg/mL, and 5.5 mg/mL), Jones *et al.*[2] measured the generation of soluble HA to demonstrate that a lower concentration of HA correlated with increased susceptibility to dissolution by ovine hyaluronidase. Rao *et al.*[6] observed similar findings in an *in vitro* study with ovine-derived hyaluronidase and validated this finding for recombinant hyaluronidase. Cavallini *et al.*[14] studied the response of six fillers to bovine-derived hyaluronidase and observed the most rapid and homogenous dissolution in the two varieties with the lowest concentrations of HA, Juvederm Volite (12 mg/mL) and Teosyal RHA 1 (Teoxane Laboratories, Geneva, Switzerland; monophasic; 15 mg/mL). *In vivo*, using a rat model, Shumate *et al.*[16] demonstrated faster degradation by ovine hyaluronidase of Restylane-L (20 mg/mL) when compared to two filler varieties from the Juvederm family (20-24 mg/mL). Altogether, the improved dissolution of lower concentration HA by hyaluronidase was validated across all major HA filler varieties and with all three sources of hyaluronidase, in both *in vivo* and *in vitro* studies.

Greater degree of crosslinking is associated with greater resistance to degradation

HA is stabilized by crosslinking individual particles to each other with covalent bonds in all HA-containing fillers[12]. Across all studies of HA-hyaluronidase interactions, a higher degree of crosslinking generally correlated with greater resistance to degradation by hyaluronidase. Sall *et al.*[12] examined the response of 11 individual formulations of HA filler to bovine hyaluronidase and found that the slowest to dissolve, Surgiderm 30 (Allergan, Irvine, California; monophasic; 24 mg/mL) had the greatest degree of crosslinking. The fastest to dissolve, Restylane and Perlane, had the lowest degree of crosslinking. These findings were further validated by comparisons of fillers within the same family, which eliminated possible confounders such as the monophasic/biphasic status that differ between filler classes such as in Surgiderm and Restylane. Within the Juvéderm family, for example, less-crosslinked fillers such as Juvéderm 18 dissolved more quickly than their more-crosslinked counterparts.

These findings were validated in additional *in vitro* experiments of bovine hyaluronidase[14,17], ovine hyaluronidase[2,6,15], and recombinant hyaluronidase[6]. *In vivo*, these findings were validated in human subjects by Juhasz *et al.*[18], who found less dissolution of Juvéderm Ultra Plus than Juvéderm Ultra. Although this difference was small and the sample size was too small to achieve significance, these two filler varieties are ideal for comparing this variable as both have the same concentration of HA (24 mg/mL), but Juvederm Ultra Plus has an increased density of crosslinking[27]. The decreased dissolution observed makes scientific sense, as a higher density of crosslinking decreases the access hyaluronidase has to its enzymatic substrate[12].

Monophasic hyaluronic acid formulations are more resistant to degradation than biphasic formulations

HA fillers are classified as monophasic (cohesive gels without distinct particles) or biphasic (particles suspended in gel)[35]. Because the individual particles of a biphasic filler create a greater surface area for enzymatic attack, these formulations, e.g., Restylane, have been predicted to exhibit increased susceptibility to degradation compared to their monophasic counterparts[12]. Although this and other principles may have origins in manufacturer claims or manufacturer-sponsored studies, this trend is upheld by the reviewed articles on HA-hyaluronidase interactions. Sall *et al.*[12] studied both major biphasic compounds (Restylane and Perlane) and found these fillers significantly more susceptible to degradation by bovine hyaluronidase than monophasic varieties of Juvéderm. In other studies, Restylane was routinely found to be more dissolvable than comparably concentrated monophasic fillers with all three varieties of hyaluronidase[2,6,15,17], in both *in vitro* and *in vivo* experiments[16,18].

Response of hyaluronic acid fillers to different types of hyaluronidase

In the two studies that individually examined the effect of multiple types of hyaluronidase on different varieties of HA filler[6,16], only Restylane-L demonstrated a source-dependent response to hyaluronidase. In their rat model, Shumate *et al.*[16] found that, at lower concentrations of hyaluronidase (10 U/0.1 mL), Restylane-L was degraded more by ovine hyaluronidase than by recombinant hyaluronidase. Notably, no difference in dissolution efficacy between hyaluronidases was observed for Juvéderm Voluma XC (which has the same HA content, 20 mg/mL, as Restylane-L) or Juvéderm Ultra Plus XC (24 mg/mL) at any concentration of hyaluronidase. However, it is also important to note that at higher doses of hyaluronidase (30 U/0.1 mL), all tested fillers exposed to each type of hyaluronidase were reduced to undetectable levels within 6 hours, leading the authors to conclude that any responsive differences due to HA filler structure or hyaluronidase type are clinically insignificant.

Rao *et al.*[6] also studied *in vitro* both ovine and recombinant hyaluronidase and found no hyaluronidase-based difference in the response of Restylane, Juvéderm, Juvéderm Voluma, or Belotero at any concentration. Interestingly, the ratio of ovine hyaluronidase to Restylane used by Rao *et al.*[6] (5 U/mg) was identical to that used by Shumate *et al.*[16] The differences in the experimental design of these two studies may suggest

that the activity of ovine hyaluronidase, when interacting with a biphasic HA filler, is measurably altered by the conditions of an *in vivo* system as compared to an *in vitro* system. More studies are needed for clarification.

Trends observed in individual fillers and filler families

Across all studies considered together, it is difficult to compare the response of individual filler types to different types of hyaluronidase due to the variation in the amount of filler studied, quantity of hyaluronidase used, and the method of measuring degradation. However, when the same types of HA filler were used in different studies, it is possible to compare their response to different types of hyaluronidase relative to each other. Three filler families were studied in half or more of the experiments reviewed: Restylane, Juvéderm, and Belotero Balance.

Restylane

Restylane varieties were studied in all ten[1] (8 studies, 2 of which examined 2 different hyaluronidase types) reported experiments of HA-hyaluronidase interactions and were observed to be the most responsive to hyaluronidase in six studies and the least responsive to hyaluronidase in one. When compared directly to Belotero in the same experiment, Restylane was found to be more susceptible to degradation 60% of the time. When compared directly to any member of the Juvéderm family, it was found to be more susceptible to degradation 90% of the time.

A biphasic formulation with relatively little crosslinking, Restylane and its derivatives were unsurprisingly almost always found to be more susceptible to degradation than the Juvéderm family of fillers. In an outlier study, Cavallini *et al.*[14] qualitatively found Restylane (20 mg/mL) to be the least responsive of six fillers to bovine hyaluronidase. Using a ratio of 30 U hyaluronidase per 0.1 mL filler (15 U hyaluronidase per mg Restylane), which exceeds established recommendations of 5 U hyaluronidase per 0.1 mL Restylane for ischemic complications[34], they noted that Restylane needed both additional time and enzyme to reach complete liquefaction, as compared to other fillers, including Juvéderm Volite (12 mg/mL), which dissolved instantly. The results of this study contrast with other studies of similar design. Rao *et al.*[6], using a qualitative *in vitro* model, also found Restylane to be the most susceptible compound to ovine and recombinant hyaluronidase using lower ratios of 5 U per 1 mg and 3.75 U per 1 mg, respectively. In addition, other studies of *bovine* hyaluronidase also found that Restylane dissolved more rapidly than comparably concentrated varieties of Juvéderm[12,17]. The reason for Cavallini's experimental discrepancy is unclear, but may be attributable to subjective errors in qualitative interpretation of filler gel consistencies. Interestingly, Restylane is the only one of the six tested fillers to not be pictured in this study's photographic figure of its experimental results.

With regard to Restylane's response to different types of hyaluronidase, there is no evidence that any one enzyme is best at dissolution. Again, using Juvéderm as a comparison, Restylane was found to be more degradable in 2/2 studies of recombinant hyaluronidase, 5/5 studies of ovine hyaluronidase, and 2/3 studies of bovine hyaluronidase. While this might superficially suggest a relatively poor response to bovine hyaluronidase, this single anomaly (Cavallini *et al.*[14]) should be considered in the context outlined above. The slight predilection of Restylane for ovine hyaluronidase over recombinant hyaluronidase reported by Shumate *et al.*[16] is not clearly replicated across all studies considered together, though the small number of studies examining recombinant hyaluronidase limit the conclusions that may be drawn.

Juvéderm

Juvéderm varieties were also studied in all ten reported experiments and were observed to be the least responsive to hyaluronidase in five and the most responsive to hyaluronidase in one. When compared directly to Belotero, Juvéderm was found to be more resistant to degradation 40% of the time. When compared directly to Restylane, Juvéderm was found to be more resistant to degradation 90% of the time.

Overall, the Juvéderm family exhibits many of the qualities described to be associated with resistance to degradation by hyaluronidase: it is a monophasic compound and its formulations generally have higher concentrations of HA and more crosslinking than other HA fillers[22]. It is not unexpected then that it resists degradation more effectively than Restylane in a wide variety of experiments, including both *in vivo* and *in vitro* designs and with all three different sources of hyaluronidase.

In the single study in which a Juvéderm filler was observed to exhibit the most degradation of its studied subset[14], the specific variety was Juvéderm Volite (12 mg/mL), a formulation with significantly lower HA concentration than the majority of the Juvéderm family of fillers. Juvéderm Voluma (20 mg/mL) demonstrated significantly more resistance to degradation in this experiment. No other studies examined the Volite member of the Juvéderm family.

When studies looking for differences in Juvéderm's response to different varieties of hyaluronidase are compared, no variety of hyaluronidase is clearly better at dissolving Juvéderm than others. In the recombinant hyaluronidase experiments where direct comparison is possible, Juvéderm fillers are universally more resistant than Restylane (2/2) and less resistant than Belotero (1/1). In ovine experiments, Juvéderm fillers are more resistant than Restylane (5/5) and again, generally less resistant than Belotero (2/3). In bovine experiments, Juvéderm fillers are generally more resistant to dissolution than Restylane (2/3) and, interestingly, more resistant than Belotero (1/1). Though this last result may appear to suggest that bovine hyaluronidase is less effective against Juvéderm (relative to Belotero), it is also notable that the one instance in which Juvéderm dissolved more than Restylane also occurred with bovine hyaluronidase, which would suggest the opposite.

When comparing studies that examined both Juvéderm and Belotero, the single *in vitro* study in which Juvéderm was less resistant utilized bovine hyaluronidase to compare the fillers[17]. However, no effective degradation at all was seen in Juvéderm Ultra 3 in this experiment, despite degradation of this formulation being observed with bovine hyaluronidase in other experiments[12]. This discrepancy makes it difficult to draw any specific conclusions about Juvéderm's response to bovine hyaluronidase, and no additional data from other studies is available as a point of comparison.

Belotero balance

Belotero balance was studied in 5 experiments and was observed to be the least responsive to hyaluronidase in 3 and the most responsive to hyaluronidase in 2. In 3 out of 4 *in vitro* experiments[6,15], including 2 with ovine hyaluronidase and 1 with recombinant hyaluronidase, Belotero was observed to be the most resistant to degradation, likely in part due to its monophasic status, more extensive CPM crosslinking, and higher molecular weight than other common fillers like Juvéderm Ultra and Restylane[15].

In an experiment on human subjects[18], with ovine hyaluronidase, Belotero was observed to be the least resistant to degradation as compared to Restylane and Juvéderm Ultra Plus. This discrepancy might be attributable to factors introduced by the *in vivo* system or the experimental design of the study, in which HA degradation was measured clinically via a standardized palpation scale following hyaluronidase injection. Belotero, with its unique polydensified matrix structure, has a lower viscosity than other HA fillers and thus a greater homogenous intradermal distribution of injection[31]. Per the authors' own acknowledgement, these properties may have confounded measurements of Belotero relative to its more viscous counterparts.

Interestingly, the *in vitro* experiment in which Belotero was found to be the least resistant to degradation (Buhren *et al.*[17]) was the only experiment that studied Belotero (22.5 mg/mL) with bovine hyaluronidase. In this experiment of Belotero, Emervel (Restylane, 20 mg/mL) and Juvéderm Ultra 3 (24 mg/mL),

50 microliters of filler was combined with a fluorescent linker dye and exposed to 10 units (U/ml) of hyaluronidase, with degradation measured by recorded changes in fluorescence intensity. This can be contrasted with the work of Flynn *et al.*[15], who used a different experimental design (chromatography) but the same ratio of hyaluronidase to filler (16 U per 0.08 mg) to show Belotero was more resistant to ovine hyaluronidase than Juvéderm Ultra 3. When compared directly, these studies may suggest that Belotero is more susceptible than Juvéderm to ovine hyaluronidase than bovine hyaluronidase.

However, Buhren *et al.*[17] notably observed no effective degradation of Juvéderm Ultra 3 by bovine hyaluronidase, even after 24 h. Sall *et al.*[12] did observe degradation of Juvéderm Ultra 3 at a lower concentration of bovine hyaluronidase than Burhen *et al.* Though Sall *et al.* did not study Belotero, this difference in results with the same Juvéderm variety using the same enzyme suggests that the Burhen experiment may have had some confounding feature either in measurement methodology or other experimental factors such as pH or temperature. More studies are needed to clarify whether Belotero is less responsive to bovine hyaluronidase than ovine hyaluronidase.

CONCLUSION

Overall, the literature suggests that different varieties of hyaluronidase and HA fillers vary in their interactions. Factors associated with higher resistance to degradation include increased concentration of HA, increased crosslinking, and monophasic formulation. Unsurprisingly, filler varieties that are monophasic, highly concentrated, and exhibit a high density of crosslinking (such as Juvéderm Ultra Plus) are less responsive to hyaluronidase and require a larger quantity of enzyme to achieve complete dissolution.

Clinically, these differences should be considered when following established guidelines for the quantity of hyaluronidase required for filler dissolution. This amount needed depends on the nature of the complication. In cases of suboptimal injection placement or overcorrection, 5-10 U of hyaluronidase has been cited as the appropriate dose[36]. This recommendation is not specific to the volume of filler, which must be considered. Additionally, this recommendation established using Restylane, may not be appropriate for other fillers. In the treatment of ischemic complications as opposed to misplacement, higher doses of hyaluronidase are required, with 5 units suggested per 0.1 mL of Restylane and 10 units per 0.1 mL of Juvéderm[34]. Thus, for any given 1 mL of filler injected, the suggested starting dose of hyaluronidase varies from 5 to 100 U depending on the type and volume of filler injected and the nature of the complication. The general principles of degradation susceptibility outlined above in conjunction with clinical context and volume of filler injected should provide injectors with an estimated starting reversal dose.

In addition to presenting the accepted knowledge to date, this review highlights the information that remains uncertain and in need of further investigation. While members of the Restylane family appear to respond best to all varieties of hyaluronidase and members of the Juvéderm family appear to be the most resistant, experimental results are less consistent for Belotero Balance. There are no clear trends established across multiple studies to support the claim that any one type of hyaluronidase is better at dissolving a specific variety of HA filler.

More research is needed to substantiate these results and explore individual efficacy. Such experiments might include an *in vitro* study that examines all major HA fillers and all varieties of hyaluronidase under uniform volumetric, temperature, and time exposure conditions. Once a preliminary set of individual relationships between filler types and hyaluronidases is established, further studies in animals and human subjects can determine whether these trends remain valid *in vivo*. In definitively establishing these relationships, clarification of this issue will prepare injector clinicians to better address filler complications that arise from the use of a given HA product.

DECLARATIONS

Authors' contributions

Made substantial contributions to the conception and design of the study; performed literature review, background research, and data analysis; drafted initial version of the work and contributed to revisions; drafted initial version of Table 1; edited and augmented Table 2; provided administrative support: Paap MK
Made substantial contributions to the conception and design of the study; reviewed and substantially revised initial and subsequent drafts of manuscript; reviewed and analyzed relevant literature; contributed additional background research; drafted initial version of Table 2; edited and augmented Table 1: Silkiss RZ

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Both authors declared that there are no conflicts of interest.

Ethical approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

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