Clinical, Cosmetic and Investigational Dermatology

Dovepress
Taylor & Francis Group

Open Access Full Text Article

ORIGINAL RESEARCH

Rheology as a Tool to Investigate the Degradability of Hyaluronic Acid Dermal Fillers

Giulia Grimaldi[1], Giulia Galasso[1], Maria Chiara Capillo[1], Giuseppe Alonci[1], Stefano Bighetti[2], Luca Bettolini[2], Sabrina Sommatis[1], Roberto Mocchi[1], Andrea Carugno[3], Nicola Zerbinati[4]

[1]UB-CARE S.r.l.-Spin-Off, University of Pavia, Pavia, Italy; [2]Dermatology Department, University of Brescia, ASST Spedali Civili di Brescia, Brescia, Italy; [3]Department of Medicine and Surgery, University of Insubria, Varese, Italy; [4]University of Insubria, Department of Medicine and Technology Innovation, Varese, Italy

Correspondence: Stefano Bighetti, Dermatology Department, Piazzale Spedali Civili 1, Brescia, 25123, Italy, Tel +39 030 3995301, Fax +39 030 399505, Email s.bighetti@unibs.it

Background: In the current landscape of aesthetic medicine, the use of hyaluronic acid (HA)-based dermal fillers is increasingly prevalent. Although HA is widely acknowledged for its safety and efficacy the study of its degradability represents a critical aspect in ensuring safety.

Methods: Rotational rheology was chosen to study the HA-based fillers interaction with hyaluronidase enzyme; this allows to establish a correlation between the viscoelastic parameters of the sample and its degradation.

Results: The obtained results indicate that the products exhibit sensitivity to the enzyme and that the rheological parameters vary depending on the contact time and dosage of administration.

Conclusion: Our findings propose a standardized rheological protocol for assessing the degradability of HA-based dermal fillers, offering an alternative to existing methods.

Keywords: hyaluronic acid, adverse drug reaction, aesthetic dermatology, cosmetic dermatology

Introduction

Dermal filler injections are a widely sought-after procedure in aesthetic medicine. Among the various options available, hyaluronic acid (HA) stands out as the most extensively utilized material due to its acknowledged safety and efficacy.[1] HA is considered bio-safe due to its natural high-viscosity, non-sulfated glycosaminoglycan composition, consisting of units of D-glucuronic acid and N-acetyl-D-glucosamine connected by a β (1,4)-glycosidic bond.[2,3] The use of HA-based fillers is generally considered safe and minimally invasive; however, adverse events (AEs) may still occur as a result of these aesthetic procedures. The most frequently observed complications are mild, self-limiting, and reversible reactions at the injection site, such as edema, pain, erythema, itching, and ecchymosis.[4] Other reported AEs include hypersensitivity reactions, infections, surface irregularities, nodules, and, in rare cases, more severe complications like vascular occlusion leading to skin necrosis.[5] Due to these potential complications, along with the limited immunological response, the long-lasting effects, and the ease of reversibility with the administration of a specific antidote (eg, hyaluronidase), become crucial for managing complications during and after filler injections.[6,7]

Hyaluronidase is an endoglycosidase responsible for breaking down HA into monosaccharides by cleaving its glycosidic bond. Human hyaluronidase is found in various organs (testis, spleen, skin, eyes, kidneys, uterus) and body fluids (tears, blood, and semen).[8] Hyaluronidase enzymes are categorized into three main classes based on their final products and mechanism of action: mammalian hyaluronidases, which break down β-1,4 glycosidic linkages to form tetrasaccharides; leech/hookworm hyaluronidases, which break down β-1,3 glycosidic bonds to form pentasaccharides and hexasaccharides; and microbial hyaluronidases, classified as hyaluronate lyases, that produce unsaturated disaccharides through a β-elimination reaction at β-1,4 glycosidic linkages.[9] The enzyme's ability to degrade HA, a key

Received: 24 January 2025
Accepted: 10 May 2025
Published: 31 May 2025

component of the extracellular matrix, makes it a valuable tool in aesthetic medicine for treating nodules, preventing complications related to improper injections, or correcting HA overfilling.[10] Additionally, it is used in medical practice to enhance tissue absorption of various drugs, prevent tissue damage after extravasation by reducing substance concentrations, decrease edema, and improve drug penetration into malignant tissue in chemotherapeutic medicine.[11] Various characteristics, including total HA concentration, gel hardness, viscosity, type of crosslinker used, degree of crosslinking, and lifespan in the skin, influence how HA-based dermal fillers interact with hyaluronidase enzyme.[12] The mechanisms governing the pharmacokinetics of hyaluronidase are not yet fully elucidated; however, existing literature widely describes its interactions with furosemide, benzodiazepines, phenytoin, dopamine, and α-adrenergic agonists. Additionally, anti-inflammatory drugs, antihistamines, mast cell stabilizers, heparin, vitamin C, and various plant-based medications are known to antagonize hyaluronidase. Allergic reactions constitute the primary reported complications following the injection of hyaluronidase, the occurrence of which depends on the administration region.[11] The off-label use of hyaluronidase to dissolve HA in order to manage and resolve post-injection complications has been documented, with a multidose approach proving beneficial in improving the degradation kinetics of the hydrogel. This is attributed to the observed progressive slowdown in the enzymatic activity of hyaluronidases over time, likely due to the enzyme's progressive inactivation. Furthermore, repeated injections may facilitate precise administration of the enzyme. The first documented usage of hyaluronidase to correct misplaced Restylane for periocular rhytids was reported by Saporkar in 2004, involving a 65-year-old woman.[13] The scientific literature discusses various enzymatic degradation tests for HA fillers, encompassing in vitro quantification of released HA fragments, visual analyses, palpation, and imaging in both human patients and animal models.[14] The absence of regulation regarding optimal degradation procedures and the wide range of techniques makes evaluating HA filler degradability and safety particularly challenging. In vitro assessment necessitates the use of different instruments (centrifuge, oven, spectrophotometer, chemical hood) and hazardous reagents like acetic acid. These factors make the experiment costly, time-consuming, and potentially risky.[6] Moreover, in vitro hyaluronidase tests are unable to indicate the physicochemical changes in HA gel properties during degradation. Colorimetric assays and Size Extrusion Chromatography require several steps of sample preparation, while with the proposed method, the sample can be used as it is, directly extruding the filler from the syringe and adding a hyaluronidase solution on top.

A rheological approach could be employed to monitor real-time gel degradation through viscoelastic properties. Utilizing rheology for degradation assessment offers several advantages compared to traditional in vitro methods.[15] Firstly, the entire experiment can be conducted using a single instrument, resulting in savings in terms of materials and analysis time. A comprehensive rheological characterization of the sample can be obtained without requiring additional equipment or solvents, apart from a sodium chloride solution and hyaluronidase. The instrument's software enables the creation of customized sequences suited to the sample type and specific experimental conditions, mimicking stress and frequency conditions resembling in vivo scenarios.[16] The sample is loaded onto the lower geometry and analyzed by a single instrument and sequence operating independently, minimizing sample manipulation and reducing operator-induced variability. The degradation process is monitored in real-time by studying the elastic modulus (G′), providing insight into how sample elastic properties evolve during the analysis.

Additionally, to closely mimic vivo conditions, the rheometer allows for the temperature to be set at 37 °C, maintained constant throughout the analysis.[17]

Given the potential complications of filler injections, it is essential to develop an analytical methodology that can simulate filler behavior under conditions to mimic the in vivo environment.

This is crucial for managing unexpected events effectively and ensuring patient safety. A change in rheological parameters is the direct outcome of the effect of hyaluronidase on dermal fillers in clinical settings, so the use of this method can be beneficial.

Consequently, the aim of our study is to present an alternative to conventional laboratory tests for studying the degradability of HA-based hydrogels using rheological techniques, leading to increased reproducibility and reliability of the collected experimental data.

Table 1 Collection of HA Hydrogel Dermal Fillers Selected for the Degradability Study and Provided by Matex Lab S.p.A. (Brindisi, Italy)

Product	Description	Cross-Linker
Neauvia® Stimulate Man	HA hydrogel 28 mg/mL with CaHA*	PEGDE
Neauvia® Intense	HA hydrogel 28 mg/mL	PEGDE
Neauvia® Intense Rheology	HA hydrogel 22 mg/mL	PEGDE

Note: *Containing 1% relative to HA of hydroxyapatite (CaHA).

Materials and Methods

Sample Collection and Study Objectives

This overview presents an experimental protocol designed to examine the degradability of HA-based dermal fillers in relation to their elastic modulus (G′). Even if G′ is not completely representative of the gel's rheological properties, it was selected due to the direct relationship between the persistence of mechanical properties and the three-dimensional structure of the gel.[18]

During the injection process under physiological conditions, the dermal filler's G′ represents its ability and firmness to resist deformation. Post-injection, G′ indicates how the HA filler withstands skin tightening forces resulting from facial movements.[19] For this investigation, the fillers reported in Table 1, were chosen as considered representative of three different categories, in terms of HA concentration and rheological properties (ie high viscosity with Calcium Hydroxyapatite (CaHA) – Neauvia® Stimulate Man; medium viscosity – Neauvia® Intense; low viscosity - Neauvia® Intense Rheology).

To evaluate the statistical significance, each time-point of each product was analyzed compared to the Control. Values of 0.12 (ns), 0.033 (*), 0.002 (**) and <0.001 (***) were considered statistically significant using GraphPad Prism 10.2.3. (403) One-way ANOVA analysis. Šidák's statistical hypotheses test correction was used for multiple comparisons. The multiplicity was the adjusted P value for each comparison with a 95% confidence interval.

Evaluation of Endured Strain by Preliminary Amplitude Sweep Test

A preliminary Amplitude Sweep test was conducted to establish the Linear Viscoelastic Region (LVER) for the samples, ensuring that they could be manipulated without compromising their internal structure. A rotational rheometer Kinexus Plus (Malvern Panalytical, Worcestershire, UK), with a working gap of 1.0 mm and a 20-mm plate-plate geometry (PU20 SR2467 SS) was employed. The test parameters included a shear strain ranging from 0.1 to 1000% and a frequency of 1 hz. A temperature of 37 °C was maintained to mimic internal body conditions and assess the behavior of HA dermal fillers within the LVER, aligning the experimental conditions with those used to investigate the degradation of hyaluronidase filler. Within the LVER, the applied stress does not compromise the inner structure of the cross-linked HA filler, resulting in constant values for G′, G″, G*and tan δ.[3]

Rheological Filler Degradation by Hyaluronidase

After the LVER determination with Amplitude Sweep test, filler degradation with hyaluronidases was evaluated by setting the shear strain value (%) in the LVER. An internal time sweep sequences were configured for acute and non-acute protocols at 37 °C, utilizing the 20-mm plate-plate geometry (PU20 SR2467 SS). A 1 hz frequency was selected to simulate the physiological movements of the skin and facial muscles.[17] To replicate the medical hyaluronidase injection, literature studies recommended initial injections of 5 to 10 U to reduce the risk of allergic reactions in non-acute reactions, while some experts recommended using 500 U of hyaluronidase per milliliter of HA for removal.[20] Hyaluronidase from bovine testes (Sigma Aldrich, Missouri, USA) and NaCl 0.9% (B. Braun Melsungen AG, Melsungen Germany) were selected to perform all the experimental tests.

The hyaluronidase solution was prepared daily, diluted in NaCl 0.9%. A solvent trap and environmental saturation with NaCl 0.9% were implemented in all experiments to prevent solvent evaporation and sample drying. Control experiments were conducted using NaCl 0.9% solution instead of the enzyme solution. Every 10 minutes 50 µL was

Table 2 Experimental Setting in Acute Protocol Condition

Experimental Condition	Strain (%)	Time (min)	Enzyme (U/mL)
Acute protocol	0	2	–
	1	5	–
	10	1	500
	1	10	–
	10	1	500
	1	10	–
	10	1	500
	1	10	–
	10	1	500
	1	T0 G′≤ 30 Pa	–

added on top of the gel until complete degradation. Even in vivo during injection, it is not assured that the clinicians are able to inject the solution exactly into the gel mass, but the progressive degradation of the filler shows that the surface contact between the enzyme and the mass is good enough. G′ was selected as the key value because it is inversely proportional to the gel's degradation kinetics, with gels having G′ values under 30 Pa considered as liquefied and fully degraded.[21]

Acute Protocol

To investigate the degradability of the HA-based hydrogel in the event of an acute protocol, 0.2 g of dermal filler was extruded onto a plate geometry pre-warmed to 37 °C. The sample was pre-equilibrated for two minutes and then 5 minutes of fixed 1% strain was set in order to establish the G′ (Pa) value of the sample without adding hyaluronidase. After 5 minutes, a pre-set sequence was performed as follows: 1 minute at 10% of strain (in order to simulate the doctor's massage after injection) followed by 10 minutes at 1% strain (to simulate the facial movement). This cycle of measurements was repeated four times, with 50 µL of a 500 U enzymatic solution added to the gel between each cycle (Table 2). Each test was conducted in triplicate.

Not-Acute Protocol

In order to examine the degradability of the HA-based hydrogel in non-acute protocol, 0.2 g of dermal filler was extruded onto a plate geometry that had been pre-warmed to 37 °C. The sample was pre-equilibrated for two minutes, and then a fixed 1% strain was set for 5 minutes to establish the G′ (Pa) value of the sample without the addition of hyaluronidase. Subsequently, to simulate multi-dose administration, a pre-set sequence was implemented as follows: 1 minute at 10% strain (to simulate the doctor's massage) followed by 10 minutes at 1% strain (to simulate facial movement). This cycle of measurements was repeated ten times, and between each cycle, 50 µL of fresh enzymatic solution at increasing concentrations (10, 20, 30, 40, 50, 60, 70, 80, 90, and 100 U, as shown in Table 3) was added to the gel. Each test was conducted in triplicate.

Results

Amplitude Sweep Test and LVER Range Determination

A preliminary Amplitude Sweep test was conducted at 37 °C to illustrate the viscoelastic properties of the dermal filler and to determine the LVER and the crossover point. This was done to ensure that the amount of deformation applied, during the degradation test, falls within the LVER. The data obtained indicates that Neauvia® Stimulate Man has a LVER ranging from 0.1 to 19.30%, while Neauvia® Intense has a LVER between 0.1 and 26.83%, and Neauvia® Intense Rheology ranges from 0.1 to 13.89%. LVER represents the stress or strain range within which all rheological parameters measured are characteristic of the system regardless of the analysis conditions.

Table 3 Experimental Setting in Not-Acute Protocol Condition

Experimental Condition	Strain (%)	Time (min)	Enzyme (U/mL)
Not – acute protocol	0	2	–
	1	5	–
	10	1	10
	1	10	–
	10	1	20
	1	10	–
	10	1	30
	1	10	–
	10	1	40
	1	10	–
	10	1	50
	1	10	–
	10	1	60
	1	10	–
	10	1	70
	1	10	–
	10	1	80
	1	10	–
	10	1	90
	1	10	–
	10	1	100
	1	T0 G′ ≤ 30 Pa	–

Table 4 LVER Strain Range and Crossover Point Obtained for Each Sample Analyzed. Average and Standard Deviation are Calculated

Product	LVER Strain Range (%)	Crossover Point Strain (%)
Neauvia® Stimulate Man	0.1–19.30	925.50 ± 16.40
Neauvia® Intense	0.1–26.83	1000.00 ± 0.00
Neauvia® Intense Rheology	0.1–13.89	887.40 ± 7.70

At the end of LVER, the original structure of the material cannot be recovered. In rheology, this point is called the yield point, while the crossover point is where G′ becomes equal to G″, so the viscoelastic dermal filler begins to behave more like a liquid than a solid.

The LVER regions and the crossover points of each HA dermal filler are summarized in Table 4.

Rheological Filler Degradation by Hyaluronidase: Acute and Not-Acute Protocol

All the tested hydrogels exhibited sensitivity to enzyme degradation, with differing degradation kinetics observed among different concentrations of HA (Figures 1–3). Specifically, we found that lower HA concentrations led to faster degradation of the gel by the enzyme. Additionally, we demonstrated that the speed of gel degradation accelerated with an increase in the number of enzymatic units.

Data collected revealed that the initial G′ mean value of Neauvia® Stimulate Man was approximately 219 Pa. Upon the addition of 50 µL (500 U) of hyaluronidase (acute adverse reaction), it decreased to 25.28 Pa in 42 minutes. The initial decrease was significant and reached a plateau after about 30 minutes, with G′ falling below 30 Pa at 25.28 Pa, with an RSD minor than 11.6%, indicating 88.25% degradation. In the case of non-acute protocol, the enzyme additions were more frequent, but each contained fewer units than in the acute reaction simulation. G′ decreased to under 30 Pa after 103 minutes, with the degradation curve showing a smaller slope than in the previous case, resulting in dermal filler degradation of 85.50% with an 18.94% maximum variability during the test and extrapolated from the triplicate. The G′

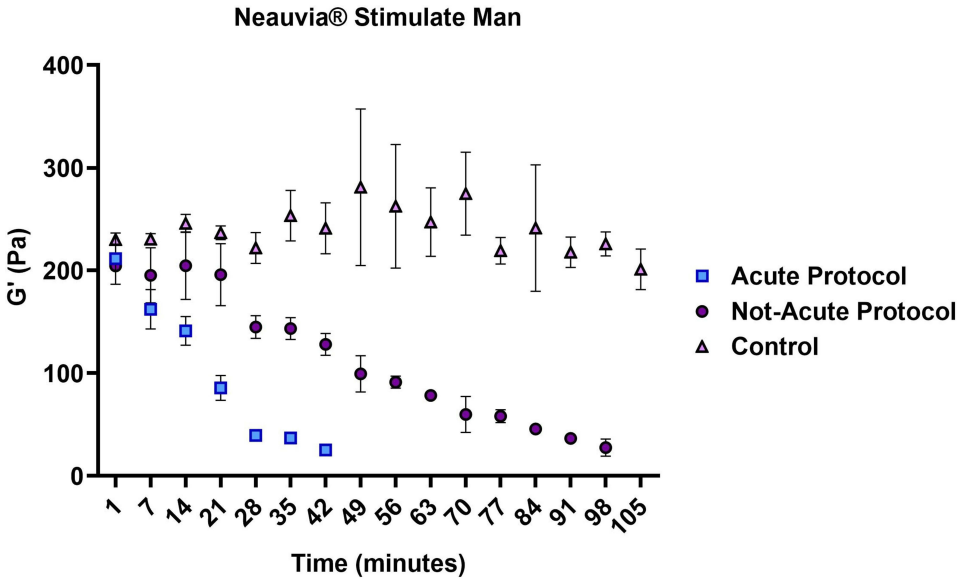

Figure 1 Graphical trend of the Neauvia® Stimulate Man degradation in acute protocol (square), not-acute protocol (circle) and in control condition (triangle, sample added with NaCl 0.9%).

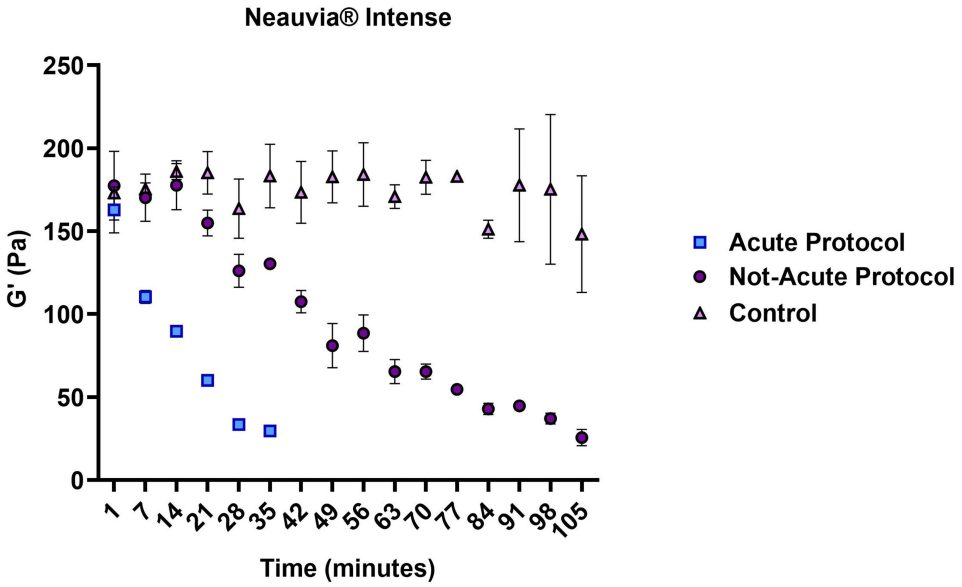

Figure 2 Graphical trend of the Neauvia® Intense degradation in acute protocol (square), not-acute protocol (circle) and in control condition (triangle, sample added with NaCl 0.9%).

value in the control condition showed a minimal 1.41% degradation due to the experimental strain, never reaching 30 Pa even after 103 minutes and 10 additions of the enzymatic solution. The data variability during the test was 20.85% maximum.

The initial G′ for Neauvia® Intense at T0 was lower than Neauvia® Stimulate Man, leading to 81.71% degradation after 34 minutes in the acute adverse reaction simulation with a variability of 6.92% calculated on triplicate. After three additions of 50 µL of hyaluronidase (500 U), G′ decreased from 165.05 Pa to 30.18 Pa. In the non-acute protocol, smaller quantities of enzyme were used in each addition, resulting in the G′ value < 30 Pa being reached after 109 minutes, the same period analyzed in the control condition, with a final G′ value of 155.63 Pa and 11.61% degradation caused by experimental strain and a variability of 15.86% in the triplicate results elaboration. Neauvia® Intense Rheology, with the lowest HA concentration of 22 mg/mL, initially had a G′ value of 145.71 Pa, which decreased after hyaluronidase

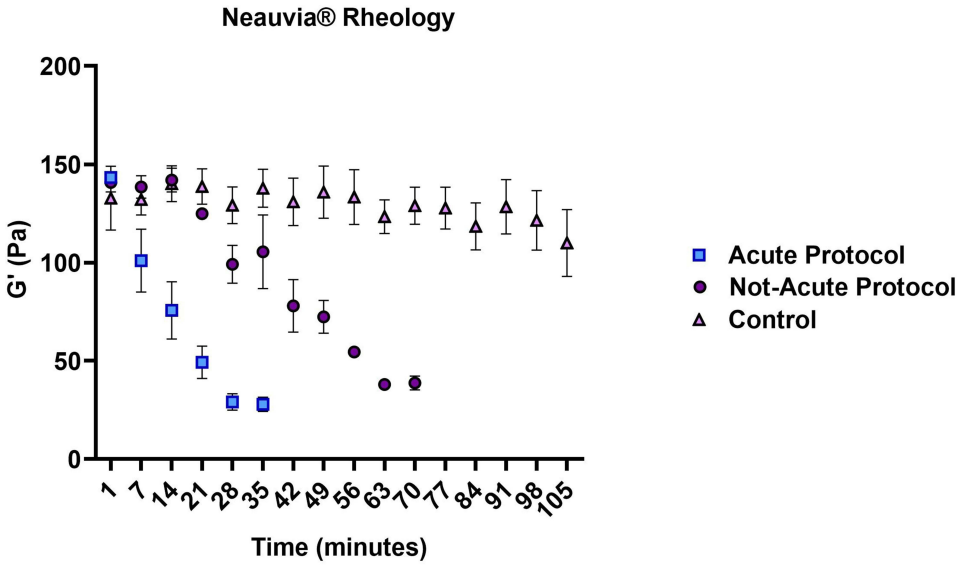

Figure 3 Graphical trend of the Neauvia® Intense Rheology degradation in acute protocol (square), not-acute protocol (circle) and in control condition (triangle, added with NaCl 0.9%).

additions, with a 15.74% of maximum variability of data obtained from triplicate, in 32 minutes in the acute reaction simulation and in 75 minutes in the non-acute adverse reaction simulation with an RSD between data of triplicate at most of 11.52%. In both experimental conditions, the percentage of degradation was approximately 79%. The intrinsic variability of the sample influences the repeatability of the results obtained.

As shown in Tables 5–7, the progressive decrease in G′, with the addition of hyaluronidase, is statistically significant (***) for each product analyzed compared to the control when G′ becomes lower than 30 Pa, both in Acute and Not-Acute protocol.

Table 5 Results Obtained for the Neauvia® Stimulate Man Hyaluronic Acid Dermal Filler

	Acute Protocol	Not-Acute Protocol	Control (NaCl 0.9%)
G′ (Pa) T0	215.14	208.76	233.62
RSD (%)	9.90	2.42	0.79
G′ (Pa) final	25.28	30.28	230.33
RSD (%)	6.40	15.05	5.65
Degradation (%)	88.25	85.50	1.41
Time (min)	42	103	103

Notes: G′ (Pa) is reported at the start and at the end of the experiment with its relative standard deviation percentage (RSD). Table presents also the percentage of degradation and the time needed to reach 30 Pa.

Table 6 Results Obtained for the Intense Hyaluronic Acid Dermal Filler

	Acute Protocol	Not-Acute Protocol	Control (NaCl 0.9%)
G′ (Pa) T0	165.05	179.82	176.08
RSD (%)	6.74	9.87	4.28
G′ (Pa) final	30.18	30.48	155.63

(*Continued*)

Table 6 (Continued).

	Acute Protocol	Not-Acute Protocol	Control (NaCl 0.9%)
RSD (%)	4.41	10.08	6.01
Degradation (%)	81.71	83.05	11.61
Time (min)	34	109	109

Notes: G′ (Pa) is reported at the start and at the end of the experiment with its relative standard deviation percentage (RSD). Table also presents the percentage of degradation and the time needed to reach 30 Pa.

Table 7 Results Obtained for the Neauvia® Intense Rheology Hyaluronic Acid Dermal Filler

	Acute Protocol	Not-Acute protocol	Control (NaCl 0.9%)
G′ (Pa) T0	145.71	142.76	134.37
RSD (%)	1.49	3.58	9.88
G′ (Pa) final	30.41	30.45	109.15
RSD (%)	11.35	7.44	8.07
Degradation (%)	79.13	78.67	18.77
Time (min)	32	75	106

Notes: G′ (Pa) is reported at the start and at the end of the experiment with its relative standard deviation percentage (RSD). Table presents also the percentage of degradation and the time needed to reach 30 Pa.

Discussion

Despite the expanding variety of injectable fillers with different innovative compounds, HA-based dermal fillers continue to be the most popular choice for facial rejuvenation management.[22] Unlike other aesthetic practices, dermal fillers achieve tissue volume augmentation through non-invasive procedures using biodegradable polymers such as HA. Scientists and physicians in the field of aesthetic medicine are continuously seeking the "ideal" dermal filler. Such a product should be safe, effective, biocompatible, non-immunogenic, easy to distribute and store, cost-effective, have an acceptable persistence, and be easily removable if required.[23]

Biodegradability is a salient safety aspect for this class of injectable products. We emphasize the importance of examining the degradability of HA fillers with hyaluronidase, a topic that has received limited attention in previous studies. Most existing investigations rely on colorimetric determination of N-acetyl-d-glucosamine or the analysis of degradation products using size-exclusion chromatography (SEC).[24] Both methods necessitate specific and costly instruments, specific sample preparation and long wait times for results. Moreover, these techniques do not directly assess the rheological properties of the hydrogel, which are crucial in a clinical context.

Considering these considerations, our study is geared towards systematically analyzing the degradability of three HA-fillers by bovine testes hyaluronidase with a time-dependent approach, employing a standardized rheological in vitro method. In our preliminary investigation, we selected three dermal fillers with varying concentrations of HA and the presence or absence of CaHA microparticles (Neauvia® Stimulate Man, Neauvia® Intense, and Neauvia® Intense Rheology provided by Matex Lab S.p.a., Brindisi, Italy). Our focus was on determining the LVER through an Amplitude Sweep test, ensuring that we work on the sample without altering its inner structure. At the end of LVER, the material's original shape cannot be recovered due to the significant deformation.[25] The data obtained from our investigation were utilized to develop a rheological protocol for studying the degradability of HA dermal fillers in two different event scenarios post-administration. To mimic in-vivo frequency and stress conditions, we established customized sequences that best suited the sample type and the desired experimental conditions, allowing us to simulate in vivo rheology-based degradation tests with real-time monitoring of elastic modulus (G′). Although not entirely representative

of the gel's physicochemical properties, our product characterization has primarily relied on G′ due to its incorporation of numerous factors affecting gel strength (eg, degree of chemical crosslinking/chain entanglements and total HA concentration).[18,26] A control (NaCl 0.9%) was included in the experimental plan to demonstrate that the decrease in elastic modulus (G′) resulted from the use of the hyaluronidase enzyme rather than by sample dilution. The data collected revealed that in all products gel degradation does not reach a plateau, but progressively decreases according to the enzyme additions, while high values of G′ are maintained in the control condition (NaCl 0.9%). Multi-dose injections of smaller hyaluronidase volumes slow down the complete degradation of the filler regardless of the initial HA concentration of the product, suggesting this approach could be used for mild reactions. Faster effects are achieved for all types of fillers with a multi-dose approach, with higher enzyme concentrations leading to faster intervention in severe reactions, regardless of low or high HA concentrations and the presence or absence of CaHA microspheres.

Conclusion

This study introduces a different approach for investigating the degradability of HA-based dermal fillers using rheology, which better reflect the degradation parameters relevant for future in vivo studies. Unlike conventional methods, our approach eliminates the need for hazardous solvents and multiple instruments, presenting a faster, cost-effective, and precise alternative that better reflects the real-use environment. By utilizing hyaluronidase from bovine testes, we achieved real-time kinetic monitoring of filler degradation. In conclusion, we were able to assess that with this method all PEGDE-based gels examined achieved a sufficient degradation rate.

Future studies will consider incorporating the use of commercial and clinical hyaluronidase solutions, to evaluate their effect on different type of dermal filler in terms of crosslinking and formulation and to compare the results with other well-established method for the study of HA gels degradation.

Disclosure

Prof. Dr. Nicola Zerbinati reports personal fees from matexlab, during the conduct of the study. The authors report no conflicts of interest in this work.

References

1. Brandt FS, Cazzaniga A. Hyaluronic acid gel fillers in the management of facial aging. *Clin Interv Aging*. 2008;3:153–159. doi:10.2147/CIA.S2135
2. Stojanovič L, Majdič N. Effectiveness and safety of hyaluronic acid fillers used to enhance overall lip fullness: a systematic review of clinical studies. *J Cosmet Dermatol*. 2019;18(2):436–443. doi:10.1111/jocd.12861
3. Zerbinati N, Sommatis S, Maccario C, et al. Toward physicochemical and rheological characterization of different injectable hyaluronic acid dermal fillers cross-linked with polyethylene glycol diglycidyl ether. *Polymers*. 2021;13:948. doi:10.3390/polym13060948
4. Wang C, Sun T, Li H, Li Z, Wang X. Hypersensitivity caused by cosmetic injection: systematic review and case report. *Aesthet Plast Surg*. 2021;45:263–272. doi:10.1007/s00266-020-01684-4
5. Abduljabbar MH, Basendwh MA. Complications of hyaluronic acid fillers and their managements. *J Dermatol Surg*. 2016;20:100–106.
6. Zerbinati N, Mocchi R, Galadari H, et al. In vitro evaluation of the biological availability of hyaluronic acid polyethylene glycols-cross-linked hydrogels to bovine testes hyaluronidase. *Biomed Res Int*. 2019;2019:3196723. doi:10.1155/2019/3196723
7. Owczarczyk-Saczonek A, Zdanowska N, Wygonowska E, Placek W. The immunogenicity of hyaluronic fillers and its consequences. *Clin Cosmet Invest Dermatol*. 2021;14:921–934. doi:10.2147/CCID.S316352
8. DeLorenzi C. Transarterial degradation of hyaluronic acid filler by hyaluronidase. *Dermatol Surg*. 2014;40:832–841. doi:10.1097/DSS.0000000000000062
9. Jung H. Hyaluronidase: an overview of its properties, applications, and side effects. *Arch Plast Surg*. 2020;47(4):297–300. doi:10.5999/aps.2020.00752
10. Žádníková P, Šínová R, Pavlík V, et al. The degradation of hyaluronan in the skin. *Biomolecules*. 2022;12:251. doi:10.3390/biom12020251
11. Cavallini M, Gazzola R, Metalla M, Vaienti L. The role of hyaluronidase in the treatment of complications from hyaluronic acid dermal fillers. *Aesthet Surg J*. 2013;33(8):1167–1174. doi:10.1177/1090820X13511970
12. Colon J, Mirkin S, Hardigan P, Elias MJ, Jacobs RJ. Adverse events reported from hyaluronic acid dermal filler injections to the facial region: a systematic review and meta-analysis. *Cureus*. 2023;15(4):e38286. doi:10.7759/cureus.38286
13. Soparkar CN, Patrinely JR, Tschen J. Erasing restylane. *Ophthalmic Plast Reconstr Surg*. 2004;20(4):317–318. doi:10.1097/01.IOP.0000132164.44343.1A
14. Shumate GT, Chopra R, Jones D, Messina DJ, Hee CK. In vivo degradation of crosslinked hyaluronic acid fillers by exogenous hyaluronidases. *Dermatol Surg*. 2018;44(8):1075–1083. doi:10.1097/DSS.0000000000001525
15. 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(6):NP402–NP410. doi:10.1093/asj/sjae032

16. De la Guardia C, Virno A, Musumeci M, Bernardin A, Silberberg MB. Rheologic and physicochemical characteristics of hyaluronic acid fillers: overview and relationship to product performance. *Facial Plast Surg*. 2022;38(2):116–123. doi:10.1055/s-0041-1741560
17. Lorenc ZP, Öhrlund Å, Edsman K. Factors affecting the rheological measurement of hyaluronic acid gel fillers. *J Drugs Dermatol*. 2017;16 (9):876–882.
18. Flégeau K, Jing J, Brusini R, et al. Multidose hyaluronidase administration as an optimal procedure to degrade resilient hyaluronic acid soft tissue fillers. *Molecules*. 2023;28:1003. doi:10.3390/molecules28031003
19. Sundaram H, Voigts B, Beer K, Meland M. Comparison of the rheological properties of viscosity and elasticity in two categories of soft tissue fillers: calcium hydroxylapatite and hyaluronic acid. *Dermatol Surg*. 2010;36(3):1859–1865. doi:10.1111/j.1524-4725.2010.01743.x
20. Lee A, Grummer SE, Kriegel D, Marmur E. Hyaluronidase. *Dermatol Surg*. 2010;36(7):1071–1077. doi:10.1111/j.1524-4725.2010.01585.x
21. Brusini R, Iehl J, Clerc E, Gallet M, Bourdon F, Faivre J. Comparative preclinical study of lidocaine and mepivacaine in resilient hyaluronic acid fillers. *Pharmaceutics*. 2022;14:1553. doi:10.3390/pharmaceutics14081553
22. Lupo MP. Hyaluronic acid fillers in facial rejuvenation. *Semin Cutan Med Surg*. 2006;25:122–126.
23. Duranti F, Salti G, Bovani B. Injectable hyaluronic acid gel for soft tissue augmentation, a clinical and histological study. *Dermatol Surg*. 1998;24:1317–1325. doi:10.1111/j.1524-4725.1998.tb00007.x
24. Buhren BA, Schrumpf 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(1):37. doi:10.1186/s40001-018-0334-9
25. Öhrlund Å. Evaluation of rheometry amplitude sweep cross-over point as an index of flexibility for HA fillers. *J Cosmet Dermatological Sci Appl*. 2018;08:47–54. doi:10.4236/jcdsa.2018.82008
26. Fagien S, Bertucci V, Von Grote E, Mashburn JH. Rheologic and physicochemical properties used to differentiate injectable hyaluronic acid filler products. *Plast Reconstr Surg*. 2019;143(4):707e–720. doi:10.1097/PRS.0000000000005429