Translations

ROYAL BELGIAN SOCIETY FOR PLASTIC SURGERY

Cosmetic Medicine

Spreading Pattern and Tissue Response to Hyaluronic Acid Gel Injections in the Subcutis: Ultrasound Videos, Ultrasound Measurements, and Histology

Joan Vandeputte, MD, FCCP, FEBOPRAS; Gaëlle Leemans, MD; Karl Dhaene, MD, PhD; Ramses Forsyth, MD, PhD, MBA, IFCAP, FRSM; Jurgen Vanslembrouck, MD; Frank Hatem, MD; and Patrick Micheels, MD

Aesthetic Surgery Journal
2021, Vol 41(2) 224–241

DOI: 10.1093/asj/sjaa007
www.aestheticsurgeryjournal.com

OXFORD UNIVERSITY PRESS

Abstract

Background: Despite the popularity of hyaluronic acid (HA) filler treatments, few publications focus on their effects on adipose tissue.

Objectives: The authors assessed the deposition pattern in the subcutis of injected HA, the tissue response at short and intermediate term, and the effects of remodeling the filler by strong finger pressure immediately after the treatment.

Methods: Two brands, specifically developed by the industry for deep injection, were compared. The gels were injected subcutaneously in 5 candidates for abdominoplasty or breast reduction, in the area of excision, 6 to 98 days before surgery. Ultrasound measurements and films were compared with postoperative histological findings. Tissue response was scored semi-quantitatively.

Results: Real-time ultrasound showed a slightly different deposition pattern of the 2 brands. Histologically, both were present in large pools of the same magnitude and looked the same. Linear retrograde injection sometimes resulted in a globular deposit due to elastic recoil of septae. After remodeling and over time, HA deposits became difficult to detect by ultrasound. Firm remodeling of the tissue immediately after injection or time had no significant effect on filler spread or tissue response. Except for 1 zone of granuloma formation, tolerance for both fillers was good.

Conclusions: HA deposition in adipose tissue occurs in much larger pools than in the dermis. Ultrasound examination is useful during and immediately after the injection but less reliable after filler remodeling or over time. Filler deposition can be less precise, and reshaping by finger pressure can have less effect than expected.

Resumen

Antecedentes

A pesar de la popularidad de los tratamientos de relleno con ácido hialurónico (HA), pocas publicaciones se enfocan en sus efectos sobre el tejido adiposo.

Dr Vandeputte is a plastic surgeon in private practice, Algemeen Ziekenhuis Oudenaarde. Dr Leemans is a Resident in Dermatology and Dr Dhaene is a Clinical Professor of Histopathology, Department of Histopathology, Universitair Ziekenhuis Brussel, Belgium. Dr Forsyth is a Professor of Pathology, Department of Pathology, Universitair Ziekenhuis Brussel, Belgium; and Department of Experimental Pathology, Vrije Universiteit Brussel, Belgium.

Dr Vanslembrouck is a radiologist in private practice in Sluis, the Netherlands. Dr Hatem is a radiologist, Algemeen Ziekenhuis Oudenaarde, Belgium. Dr Micheels is a general practitioner in private practice in Geneva, Switzerland.

Corresponding Author:
Dr Joan Vandeputte, Meerspoort 31, B-9700 Oudenaarde, Belgium.
E-mail: info@jvdp.be.

Objetivos
Los autores evaluaron el patrón de depósito en el tejido subcutáneo del HA inyectado, la respuesta del tejido a corto y mediano plazo y los efectos de la restauración del relleno mediante una fuerte presión con los dedos inmediatamente después del tratamiento.
Métodos
Se compararon dos marcas, desarrolladas específicamente por la industria para inyección profunda. Los geles se inyectaron por vía subcutánea en 5 candidatas a abdominoplastia o reducción de senos, en la zona de escisión, de 6 a 98 días antes de la cirugía. Las mediciones con ultrasonido y películas se compararon con los hallazgos histológicos posoperatorios. La respuesta del tejido se calificó de forma semi-cuantitativa.
Resultados
El ultrasonido en tiempo real mostró un patrón de depósito ligeramente diferente de las 2 marcas. Histológicamente, ambos estaban presentes en grandes acumulaciones de la misma magnitud y tenían el mismo aspecto. A veces, la inyección lineal retrógrada dio como resultado un depósito globular debido al retroceso elástico de los tabiques. Después de la restauración y con el tiempo, los depósitos de HA se volvieron difíciles de detectar con ultrasonido. La restauración firme del tejido inmediatamente después de la inyección o el tiempo no tuvo un efecto significativo sobre la expansión del relleno o la respuesta del tejido. Excepto por una zona de formación de granulomas, la tolerancia para ambos rellenos fue buena.
Conclusiones
El depósito de HA en el tejido adiposo se produce en depósitos mucho más grandes que en la dermis. El examen por ultrasonido es útil durante e inmediatamente después de la inyección, pero menos confiable después de la restauración del relleno o con el tiempo. El depósito de relleno puede ser menos preciso, y volver a dar forma mediante la presión con los dedos puede tener un menor efecto de lo esperado.

Level of Evidence: 4

Editorial Decision date: January 7, 2020; online publish-ahead-of-print January 17, 2020.

A variety of hyaluronic acid (HA) gels are injected worldwide in millions of patients. In the United States alone, it has been estimated that more than 2.1 million patients received HA injections in 2018.[1]

Some experimental ultrasound (US)[2,3] or histological[3-9] work has been published on HA in the dermis. Some clinical articles describe US[10-14] or histological findings in the subcutis in patients presenting with nodules or other complications. However, outside the context of complications, remarkably little has been published on US or histology of HA in the subcutis.[15,16] Some studies include the subcutis, but the specifics of the HA or the number of cases among other resorbable fillers are not mentioned.[10,11,17] Some ex vivo experiments on tissue samples have been reported.[11,18]

Because fat tissue is much softer than dermis, it is expected that injected HA spreads in a different way. The tissue response or the absorption rate may be different. Noninvasive follow-up by US could be a clinical point of interest. Any detected migration of filler would be another good reason for further study.

This single-center, single-blind, pilot study was undertaken to compare the effects, in vivo, on adipose tissue of 2 commonly employed HA fillers designed specifically for deep injection. The study examined:

- Immediate and intermediate-term measurement of filler deposition pattern and spread by US examination.
- Description of immediate and intermediate-term US characteristics.
- Short-term and intermediate-term histological measurement of filler pools and filler spread and comparison with the US measurements.
- Short-term and intermediate-term histological response of adipose tissue to the injections.
- US and histological evaluation of the effect of digital remodeling of the filler after injection.
- Acquiring knowledge for more efficient set-up of US and histological studies after injection of fat tissue with HA gels in more patients.

METHODS

The study was conducted after institutional (Algemeen Ziekenhuis Oudenaarde) and national ethical committee (Universitair Ziekenhuis Brussel) approval and in

Table 1. Study Population and Injection Sessions

Patients	Age at injection (y)	Anatomic area	Time between injection 1 and surgery (d)	Time between injection 2 and surgery (d)
1	46	Abdomen	16	6
2	47	Abdomen	Cancelled	Cancelled
3	34	Abdomen	29	8
4	45	Breasts	98	7
5	55	Breasts	85	15
6	48	Breasts	43	8

accordance with the 1964 Declaration of Helsinki and subsequent amendments.[19] Prior written informed consent was obtained from each participant.

Patients scheduled for abdominoplasty or breast reduction were injected subcutaneously in tissue planned to be surgically removed during their operation. Patients were blinded for the product brands. Of each brand, 0.5 to 0.55 mL was injected in a linear retrograde fashion over 4 cm with a 22 G cannula. Immediately afterwards, one-half of each injected area was remodeled by finger pressure. All injections were monitored by US. The first session was planned at the earliest convenience after enrollment in the study (16-98 days before the operation). At 6 to 15 days before the surgery, the injected areas were reexamined by US, and repeat injections were performed on the other side across the midline. The specimens were excised at the beginning of the operation. The histopathologists were blinded for product brands until tissue sampling and examination were completed. The study was conducted between March 27, 2017 (first injection) and April 11, 2018 (last surgery).

Study Participants

Three female patients scheduled for abdominoplasty and 3 patients scheduled for a bilateral breast reduction with a Wise pattern skin excision were initially recruited. One abdominoplasty patient cancelled her operation after the first filler injection session for reasons unrelated to the study.

Inclusion and Exclusion Criteria

Inclusion criteria included age 18 to 65 years and able to give consent without assistance. Exclusion criteria included patients prone to hypertrophic scarring or keloids; hypersensitivity to HA; hypersensitivity to lidocaine or amide-type local anesthetics; autoimmune disease; severe, multiple allergies or anaphylactic shock; epilepsy; heart rhythm disorders; porphyria; congenital methemoglobinemia; glucose-6 phosphate dehydrogenase deficiencies; treatment with methemoglobin-inducing substances; previous streptococcal disease (acute rheumatic fever with or without heart valve involvement); taking medication that slows hepatic metabolism (such as cimetidine, beta-blockers); diabetes requiring insulin treatment; major system disease; coagulation disorders; anticoagulation therapy; chronic utilization of acetylsalicylic acid; previous surgery in the areas eligible for injections; and contraindications to their surgical treatment (such as pregnancy or lactation).

A summary of the study population is given in Table 1.

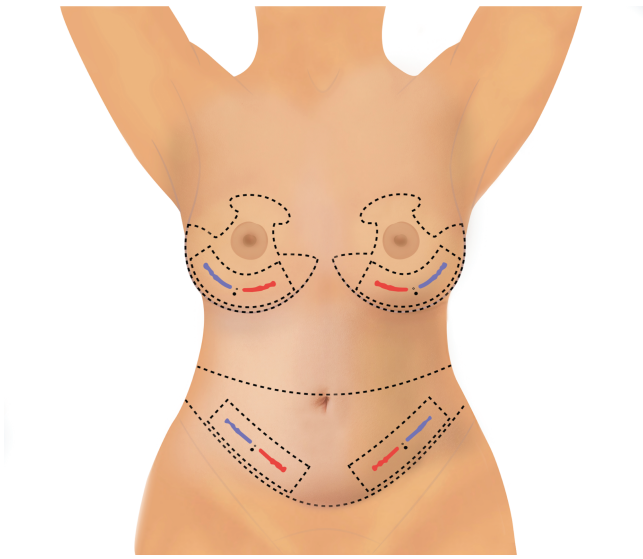


Figure 1. Injection sites. Injections were performed in the subcutis of either the breasts or the abdomen. A punctiform Chinese ink tattoo was made 5 mm below the entrance point. In the first session, linear retrograde injections with a 22 G 7-cm cannula were performed on the right side of the patient in the second session on the left. Injections began at a distance of 5 cm and ended at 1 cm from the entrance point. Product 1 was injected laterally and product 2 medially. The filler deposits and the remodeling in the first session were mirrored across the midline in the second session.

Studied HA Gels

Each study participant was injected subcutaneously with 2 CE-marked HA gels designed for deep injections and both registered as a medical device in Belgium.

Juvéderm Voluma with lidocaine (Allergan, Pringy, France) is a nonparticulate, monodensified HA gel. It is formulated from a mixture of low- and high-molecular-weight HA from bacterial origin (20 mg/mL) and contains 0.3% lidocaine in phosphate buffered saline. It is crosslinked with 1.4-butanediol diglycidyl ether. The cross-linking technology is registered as Vycross. It is packaged in 1-mL syringes.

Belotero Volume Lidocaine (Merz, Geneva, Switzerland) is a nonparticulate, polydensified HA gel. It is formulated from a high-molecular-weight sodium hyaluronate from bacterial origin (26 mg/mL) and contains 0.3% lidocaine in phosphate buffered saline. It is crosslinked with 1.4-butanediol diglycidyl ether. The cross-linking technology is registered as Cohesive Polydensified Matrix. It is packaged in 1-mL syringes.

There are no generic names uniquely identifying either gel. We therefore utilized their trade names in this article.

Injection Procedure and Ultrasound Examination

The US assessments were performed with a Philips IU22 device (Amsterdam, Netherlands) with a linear, 12-MHz probe.

Session 1: Right Breast or Right Side of Abdomen

The histopathologists were blinded for product brand until their evaluation was completed. The identification of the brands as product 1 or product 2 was determined by coin toss for each subject separately. The injection sites are shown in Figure 1.

The skin was prepped with chlorhexidine 5% in alcohol 70%. Local anesthesia was performed with 0.5 mL of lidocaine 2% with adrenaline 1/200,000 at a single, central injection point on the lower pole of the right breast or on the right half of the lower hemi-abdomen.

The injection trajectories were marked on the skin with a sterile, surgical marker. A single point tattoo was performed with Chinese ink and a sterile 23 G needle at 5 mm caudal to the intended injection point.

A single entrance point in the skin was created by puncture with a sterile 21 G needle. A 22 G (0.7 mm) 7-cm (2 ¾-inch) blunt cannula (TSK Steriglide, Emergo Europe, The Hague, the Netherlands) was connected to the syringe of product 1 and primed. The cannula was inserted in the entrance point and advanced laterally, parallel to the dermis but without direct contact, over 5 cm. Gel was applied to the skin, and the cannula was visualized longitudinally by US by a radiologist. The distance between the surface of the skin and the tip of the cannula was measured.

In a single pass, the cannula was slowly retracted to 1 cm of the entrance point, while 0.5 to 0.6 mL of product 1 was injected by hand, as uniformly as possible, in a linear retrograde manner. The pattern of spread into the tissues and the US characteristics of the gel were noted. Measurements were made of the widest laterolateral, craniocaudal, and anteroposterior diameters of the gel deposit.

Subsequently, product 2 was injected from the same entrance point in the same manner but directed medially.

The skin and subcutaneous layer at the lateral half of the lateral injection and also at the medial half of the medial injection was taken between the main investigator's fingers and firmly pinched and rolled in an attempt to remodel and further spread the gel. New US measurements were performed of the remodeled and unremodeled parts of the HA deposits.

Session 2: US Reassessment of First Session and Injection of Left Breast or Left Side of Abdomen

Using the skin tattoo point as a reference, the areas injected in the first session were reexamined by US. The injection procedure of the first session was mirrored across the midline in the left breast or left side of the abdomen.

Surgery

After marking, prepping, and draping, the principal investigator marked the injected areas and the area to be surgically removed for the study, with the tattoo as a reference. Per side, a single specimen was excised, with 1.5-cm peripheral margins around the injection trajectories and a deep margin of approximately 2 cm under the skin. The lateral side of each specimen was marked with a long suture, and the middle of the superior sides was marked with a short suture. The specimens were fixed in formaldehyde.

Infiltration with local anesthetics and adrenaline for the therapeutic part of the operation was always performed after removal of the study specimen so as not to interfere with the histology. Blood loss due to the removal of the study specimens was estimated to be between 10 and 20 mL.

Histology

Each operation provided 1 right and 1 left rectangular specimen corresponding to session 1 (Supplemental Figure 1A, available online at www.aestheticsurgeryjournal.com) and session 2 (Supplemental Figure 1B, available online at www.aestheticsurgeryjournal.com), respectively. Each specimen contained the 2 fillers. Each filler deposit had a remodeled part adjacent to the short margins of the specimen and a nonremodeled part closer to the center. This resulted in 8 tissue zones to be examined.

The specimen was inspected macroscopically describing location, color, and distribution pattern of the filler. Microscopy was performed on 3 samples of each zone taken from sections made every 5 mm. Acute and chronic inflammation, necrosis, granuloma formation, scar tissue, and collagen formation were assessed

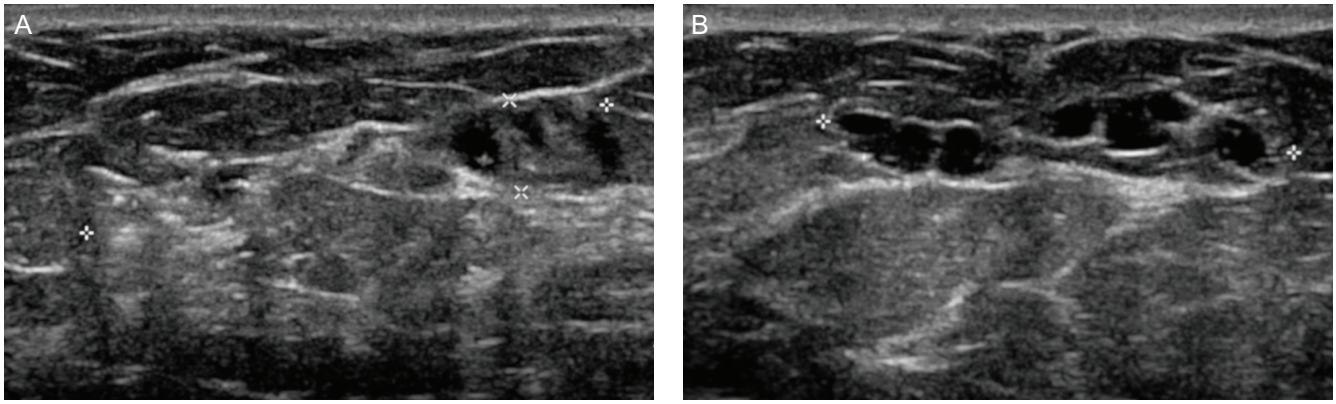
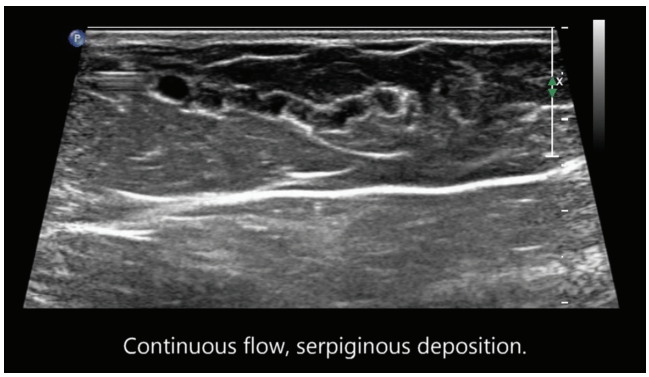
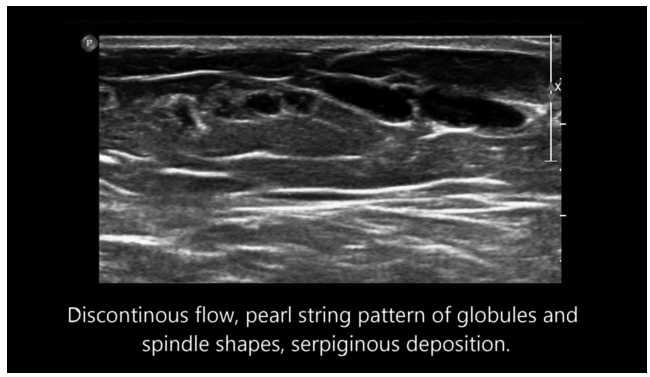


Figure 2. Representative ultrasound images of hyaluronic acid in breast subcutis (patient 4, session 1, view along the cannula trajectory). (A) Serpiginous deposit of Juvéderm Voluma (38.9 mm between "+" marks by 6.7 mm between "x" marks), poorly delineated, of heterogeneous echogenicity. (B) Serpiginous deposit of Belotero Volume (5.35 mm between "+" marks), sharply delineated and presenting as an agglomerate of anechogenous globules and spindle shapes (a pearl-string).



Video 1. Watch now at http://academic.oup.com/asj/article-lookup/doi/10.1093/asj/sjaa007



Video 2. Watch now at http://academic.oup.com/asj/article-lookup/doi/10.1093/asj/sjaa007

employing conventional hematoxylin and eosin staining and graded semi-quantitatively (0, 1+, 2+, 3+). The height and the width of filler pools and the height and width of the total filler spread were measured in 1-mm steps employing an ocular measurement device, and the largest measurements of each set were utilized for analysis. Additionally, the presence of macrophages was confirmed by immunohistochemistry utilizing an anti-CD68 antibody on a Ventana automate.

Analysis

After all histological samples were processed and described, the principal investigator disclosed the name of the brands per injected zone to the histopathologists.

Gradings and measurements were analyzed by a biostatistician. Descriptive results were calculated as means, medians, and quartiles. Due to the ordinal nature of the scores and the small sample size, nonparametric statistical methods were employed to assess differences between the 2 products (Wilcoxon[20]). All analysis was performed utilizing R (https://www.r-project.org/).

RESULTS

The study population is described in Table 1. The mean age of the participants who completed the study was 45.6 years (range, 34–55 years). The specimens were harvested from the abdomen in 2 patients and from the breasts in 3 patients.

Symptoms, Signs, and Clinical Findings

Apart from local tenderness on the day of treatment and minor bruising, all injections were uneventful. The punctiform tattoos were easy to find at session 2 and before the operation. None of the patients presented with a palpable mass, induration, or change in contour, skin color, or texture. The surgical section plane had a normal

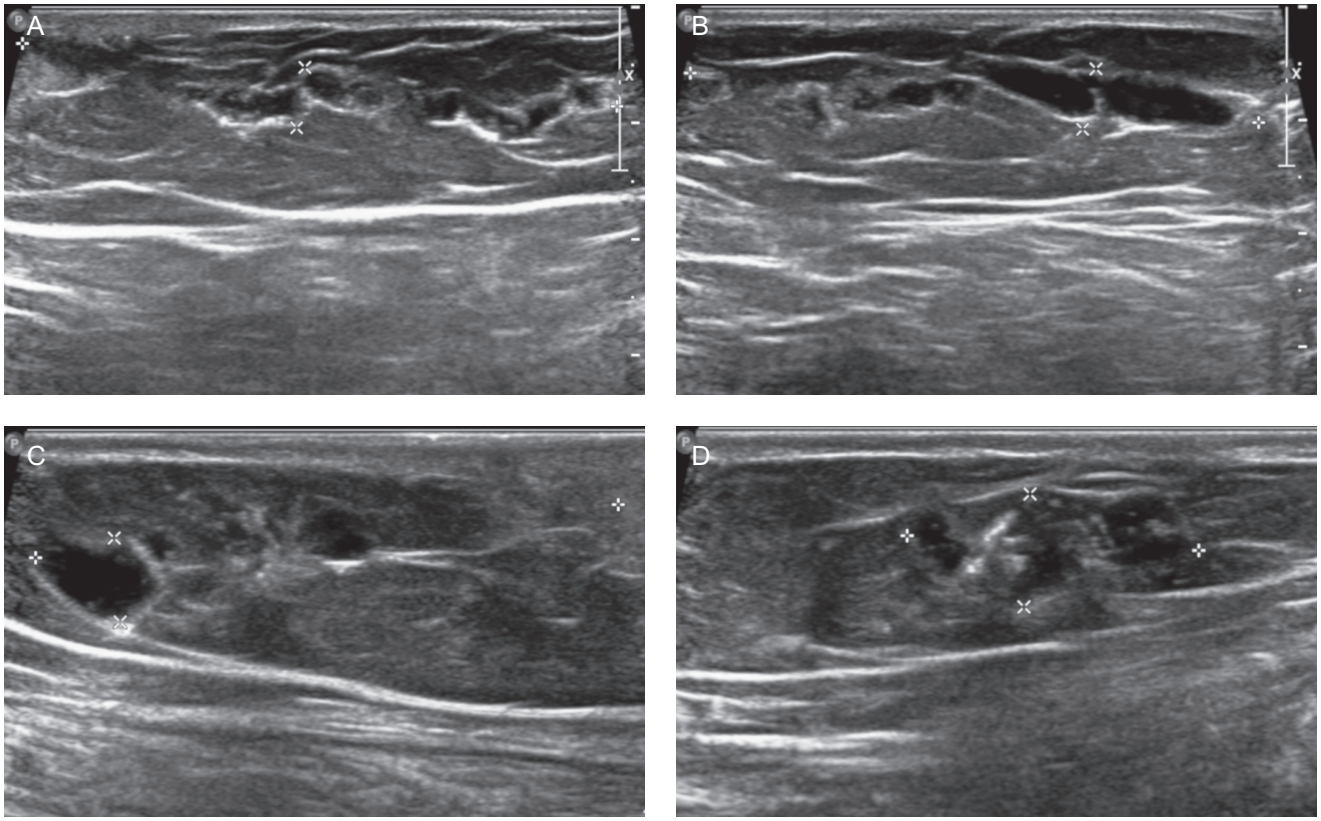


Figure 3. (A) Measurements after the accurate, linear retrograde injection of Juvéderm Voluma, shown in Video 1 (patient 1, abdomen). Markings: +, laterolateral diameter, 5.16 cm; x, anteroposterior diameter, 0.53 cm. (B) Measurements after the accurate, linear retrograde injection of Belotero Volume, shown in Video 2 (patient 1, abdomen). Markings: +, laterolateral diameter, 5.04 cm; x, anteroposterior diameter, 0.55 cm. (C) Measurements after inadvertent deposition of bolus of Juvéderm Voluma, shown in Video 3 (patient 2, abdomen). The tip of the cannula had been distending tissue that prevented spread of gel distal to the cannula. During cannula retraction, the accumulated bolus moved back with the surrounding tissue. Markings: +, laterolateral diameter, 4.56 cm; x, anteroposterior diameter, 0.65 cm. (D) Measurements after inadvertent deposition of bolus of Belotero Volume, shown in Video 4 (patient 2, abdomen), with significant shortening of the total injection trajectory. Markings: +, laterolateral diameter, 2.24 cm; x, anteroposterior diameter, 0.87 cm.

appearance in all patients and no trace of filler was visible during or after resection.

Ultrasound

Because of 1 drop-out after the first session (patient 2), both fillers were injected 11 times under continuous US examination (session 1: 6 times and session 2: 5 times).

The deposition of both HA fillers in the tissues during injection was immediate and fluent in all cases without any further spread after the end of injection.

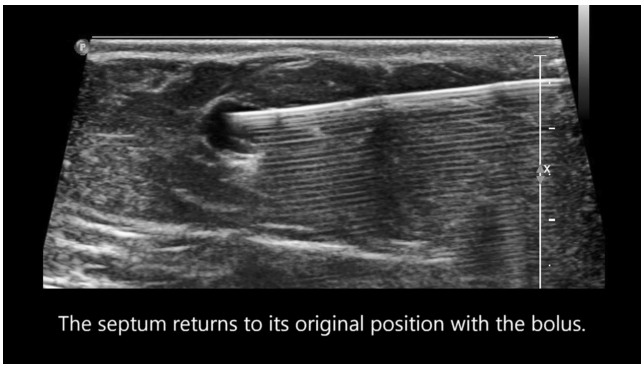
Differences between the 2 brands were observed every time. The typical finding for Juvéderm Voluma in the subcutis of the breast (Figure 2A) and the abdomen (Supplemental Figure 2A, available online at www.aestheticsurgeryjournal.com) was a serpiginous deposition with locally sharp but mostly poor delineation. The gel was locally hyperreflective. It was partially heterogeneous, ranging from hypo over hyperechogenous to strongly hyperechogenous. The typical finding for Belotero Volume in the breast (Figure 2B) and the abdomen (Supplemental Figure 2B, available online at www.aestheticsurgeryjournal.com) was a serpiginous deposition with sharply delineated lobules and spindle shapes. The characteristics varied from almost completely anechogenous to slightly heterogeneous.

In most cases, linear retrograde injection of Juvéderm Voluma (Video 1, available online at www.aestheticsurgeryjournal.com; Figure 3A) and Belotero Volume (Video 2, available online at www.aestheticsurgeryjournal.com; Figure 3B) effectively yielded a linear deposit. Occasionally, inadvertent formation of a bolus occurred for both Juvéderm Voluma (Video 3, available online at www.aestheticsurgeryjournal.com; Figure 3C) and Belotero Volume (Video 4, available online at www.aestheticsurgeryjournal.com; Figure 3D). When the cannula

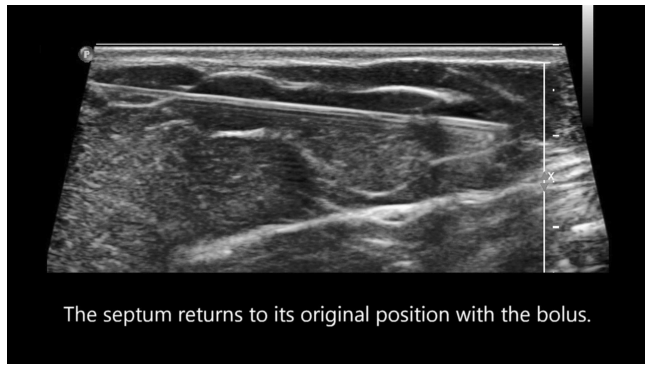
Table 2. Ultrasound Measurements of Gel Deposits Immediately After Injection

S	A	P	Session 1					Session 2				
			Quantity (mL)	Depth of tip (mm)	LL (mm)	CC (mm)	AP (mm)	Quantity (mL)	Depth of tip (mm)	LL (mm)	CC (mm)	AP (mm)
1	Abd	Bel	0.6	15	54	9	6	0.6	8	22	7	9
		Juv	0.6	12	50	6	8	0.6	10	46	5	7
2	Abd	Bel	0.5	6	50	5	12	N/A	N/A	N/A	N/A	N/A
		Juv	0.5	9	52	5	7	N/A	N/A	N/A	N/A	N/A
3	Abd	Bel	0.5	15	40	8	7	0.5	9	41	9	6
		Juv	0.5	7	38	7	11	0.5	9	38	7	5
4	Br	Juv	0.5	13	40	7	6	0.5	8	43	5	5
		Bel	0.5	1	35	5	8	0.5	18	46	7	7
5	Br	Bel	0.5	8	37	8	6	0.5	14	50	4	6
		Juv	0.5	6	37	7	4	0.5	13	44	4	5
6	Br	Juv	0.5	11	41	5	1	0.5	9	49	7	8
		Bel	0.5	7	43	1	5	0.5	14	46	7	8

A, area; Abd, abdomen; AP, anteroposterior; Bel, Belotero Volume; Br, breasts; CC, craniocaudal; Juv, Juvéderm Voluma; Depth of tip, depth of the tip of the cannula relative to epidermis after full insertion and before injection; LL, laterolateral; N/A, not applicable (surgery cancelled, subject withdrew from study); P, product; S, subjects.



Video 3. Watch now at http://academic.oup.com/asj/article-lookup/doi/10.1093/asj/sjaa007



Video 4. Watch now at http://academic.oup.com/asj/article-lookup/doi/10.1093/asj/sjaa007

compressed and distended tissue septa, filler deposition occurred strictly proximal to the tip. Upon retraction, the elastic recoil of the septa pushed back the gel while it was accumulating into a bolus.

Measurements of the largest laterolateral, craniocaudal, and anteroposterior dimensions of the gel deposits, immediately after injection, are represented in Table 2. The spread between fillers did not statistically significantly differ. Only for the anteroposterior dimension in session 2 was there a trend towards significance ($P = 0.0975$) for Belotero Volume spreading wider.

After remodeling one-half of each injection trajectory by finger pressure, US measurements of craniocaudal and anteroposterior dimensions were repeated. A third US was performed at session 2, 10 to 91 days later (Table 3).

In session 1, in all but 1 case per product, remodeling made the fillers undetectable by US when examined immediately afterwards (Supplemental Figure 3, available online at www.aestheticsurgeryjournal.com). Strikingly, after 10 and 21 days, respectively, the fillers reappeared on US in the 2 abdominoplasty patients (patients 1 and 3 in Table 3). There was little change in dimensions of either

Table 3. Ultrasound Measurements After Firm Remodeling of Lateral Half of Gel Deposits

S	A	P	Session 1 deposits										Session 2 deposits			
			Immediately after remodeling				Time gap (d)	At session 2					Immediately after remodeling			
			Remod		Nonremod			All	Remod		Nonremod		Remod		Nonremod	
			CC	AP	CC	AP		LL	CC	AP	CC	AP	CC	AP	CC	AP
1	Abd	Bel	—	—	8	9	10	52	7	6	6	6	—	—	7	10
		Juv	—	—	5	6		52	9	7	7	7	4	4	5	3
2	Abd	Bel	—	—	—	—	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
		Juv	—	—	—	—	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
3	Abd	Bel	—	—	18	8	21	53	7	5	8	8	—	—	8	4
		Juv	—	—	28	8		46	9	6	4	6	5	4	5	4
4	Br	Juv	—	—	6	6	91	4	—	—	3	5	—	—	—	—
		Bel	4	5	5	5		—	—	—	—	—	6	6	5	8
5	Br	Bel	—	—	9	5	70	—	—	—	—	—	4	4	3	3
		Juv	—	—	4	5		—	—	—	—	—	3	3	3	3
6	Br	Juv	5	4	—	—	35	—	—	—	—	—	6	6	3	5
		Bel	—	—	—	—		—	—	—	—	—	—	—	8	5

—, filler cannot be visualized; A, area; Abd, abdomen; AP, anteroposterior (mm); Br, breasts; Bel, Belotero Volume; CC, craniocaudal (mm); Juv, Juvéderm Voluma; Remod, lateral, remodeled part of the gel deposit; Nonremod, medial, nonremodeled part of the gel deposit; LL, laterolateral (mm); N/A, not applicable (surgery cancelled, subject withdrew from study); P, product; S, subjects.

Figure 4. Macroscopic image of a histological specimen, showing Juvéderm Voluma in the superficial part of subcutaneous abdominal fat 29 days after injection and remodeling by firm finger pressure (patient 3).

remodeled or unremodeled zones compared with the original situation.

US of the "unremodeled areas" changed little in 3 patients after the manipulation. However, in 2 cases, all supposedly unremodeled gel became undetectable, and both fillers spread more in the craniocaudal dimension in 1 patient (3). This shows that it was not possible to consistently remodel one half of the gel deposit without interfering with the other half.

In the 3 breast reduction patients, after 35, 70, and 91 days, all filler deposits had become undetectable by US except for small traces of Juvéderm Voluma in patient 4 (91 days).

In session 2, 1 out of 2 fillers in 4 cases became undetectable by US in the remodeled zones. There was less influence on the unremodeled zones than in the first session.

Overall, remodeling the fillers decreased visibility under US examination, but when visible, there was no statistical significance in dimensions before or after remodeling nor between fillers. The histology demonstrated that the disappearance of detectability by US was a false negative result.

Histology

Results were obtained from the 5 participants who completed the study. A right and a left specimen each contained deposits of the 2 fillers. For each deposit, 3 samples were prepared from both a remodeled and an unremodeled part.

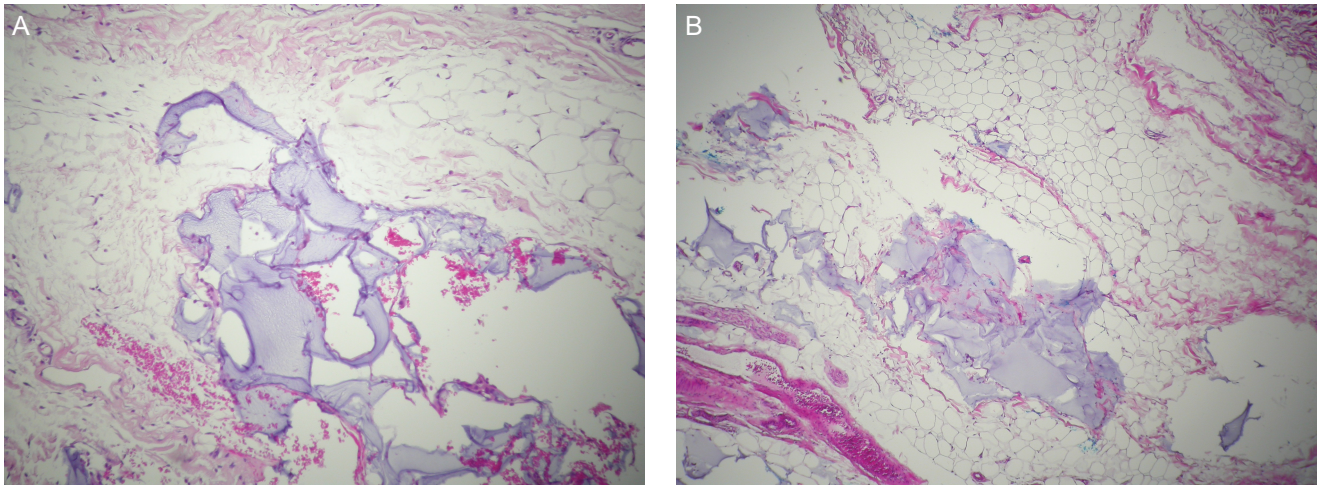


Figure 5. Remodeled filler, injected in subcutaneous breast fat, hematoxylin and eosin staining. (A) Juvéderm Voluma after 7 days (patient 4): scar 0. (B) Belotero Volume after 8 days (patient 6): scar 1+. Both: acute inflammation 0, chronic inflammation 0, necrosis 0, granuloma 0.

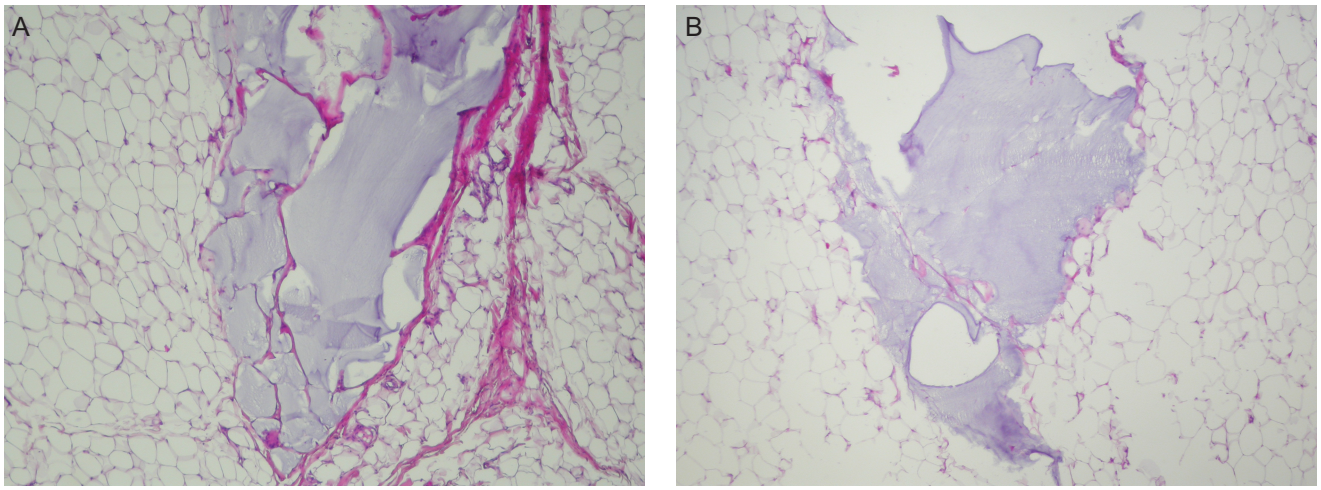


Figure 6. Unremodeled filler 29 days after injection in subcutaneous abdominal fat (patient 3), hematoxylin and eosin staining. (A) Juvéderm Voluma. (B) Belotero Volume. Both: acute inflammation 0, chronic inflammation 0, necrosis 0, granuloma 0, scar 0.

One sampling error occurred. In patient 4, one area of unremodeled Belotero volume, injected 7 days before the operation in session 2, clearly demonstrated by US, could not be found in the surgical specimen. The surgical section plane may have been superficial to the deposit. The position of the tip of the cannula before injection was 18 mm, the deepest of the study (Table 2).

In the remodeled and unremodeled tissue, filler was located geographically in the hypodermis and visible as amorphous amphophilic-staining material. Figure 4 is a macroscopic image of a section on the carrier glass showing the deposition of filler in the superficial part of the subcutaneous fat.

Figures 5A, 6A, and 7A show histology on Juvéderm Voluma, remodeled and unremodeled, at various intervals between the injection and the surgery. Figures 5B, 6B, and 7B are representative images for Belotero Volume.

No volume effect—as one could deduce from expansile pushing on or compressing of septae, individual collagen fibers, fat cells, or entire lobules—was discernible.

Numerical measurements concerning filler distribution were maximum height (skin fascia) and width (parallel to skin) of filler pools in the adipose tissue, and maximum height (skin fascia) and width (parallel to skin) of filler spread in the tissue for each zone.

The measurements from the tissue, injected in sessions 1 and 2, are represented in Table 4. The average dimensions of both filler pools and filler spread (Table 5) were lower for Juvéderm Voluma than for Belotero Volume.

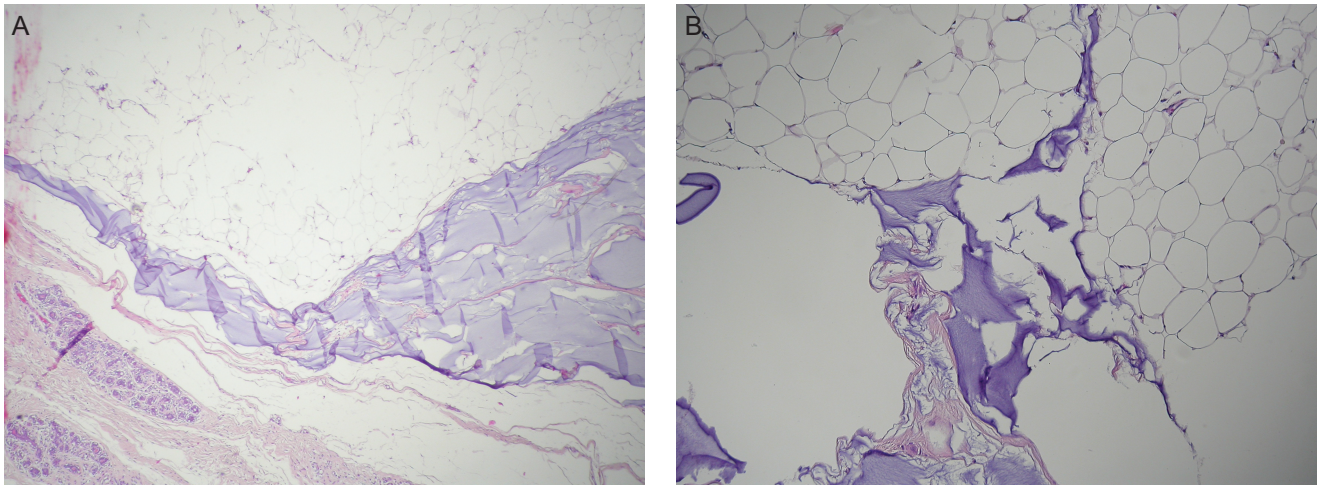


Figure 7. Remodeled filler 98 days after injection in subcutaneous breast fat (patient 4), hematoxylin and eosin staining. (A) Juvéderm Voluma. (B) Belotero Volume. Both: acute inflammation 0, chronic inflammation 0, necrosis 0, granuloma 0, scar 0.

Table 4. Histological Measurements of Filler Pools and Filler Spread

S	A	P	Rem	Injected at session 1						Injected at session 2					
				Z	Time gap (d)	Pools (mm)		Spread (mm)		Z	Time gap (d)	Pools (mm)		Spread (mm)	
						CC	AP	CC	AP			CC	AP	CC	AP
1	Abd	Bel	Rem	A	16	5	9	5	9	H	6	4	4	4	4
		Bel	Not rem	B	16	3	6	10	10	G	6	5	5	10	20
		Juv	Not rem	C	16	3	15	3	15	F	6	2	2	2	2
		Juv	Rem	D	16	3	9	3	9	E	6	5	5	15	10
3	Abd	Bel	Rem	A	29	11	9	11	9	H	8	7	7	7	14
		Bel	Not rem	B	29	2	4	5	7	G	8	5	12	5	12
		Juv	Not rem	C	29	7	7	7	7	F	8	5	4	5	8
		Juv	Rem	D	29	4	8	11	9	E	8	4	6	4	10
4	Br	Juv	Rem	A	98	6	9	6	12	H	7	3	7	3	7
		Juv	Not rem	B	98	2	4	8	7	G	7	1	2	9	6
		Bel	Not rem	C	98	5	5	5	5	F	7	0	0	0	0
		Bel	Rem	D	98	2	8	14	8	E	7	3	5	3	23
5	Br	Bel	Rem	A	85	4	7	4	7	H	15	6	13	6	13
		Bel	Not rem	B	85	5	13	7	19	G	15	9	18	11	25
		Juv	Not rem	C	85	3	6	3	6	F	15	2	6	2	6
		Juv	Rem	D	85	12	15	15	15	E	15	5	7	7	12
6	Br	Juv	Rem	A	43	1	4	1	4	H	8	2	2	10	15
		Juv	Not rem	B	43	6	8	6	12	G	8	3	2	6	6
		Bel	Not rem	C	43	5	12	5	15	F	8	6	5	11	19
		Bel	Rem	D	43	4	9	9	12	E	8	3	2	5	18

A, area; Abd, abdomen; AP, anteroposterior (height); Br, breasts; Bel, Belotero Volume; CC, craniocaudal (width); Juv, Juvéderm Voluma; Not rem, not remodeled; P, product; Rem, remodeled; S, subjects; Time gap, time between injection and collection of specimen; Z, injected zone.

Table 5. Analysis of Histological Dimensions of Filler Pools and Filler Spread

	Filler pools						Filler spread					
	Craniocaudal (width, mm)			Anteroposterior (height, mm)			Craniocaudal (width, mm)			Anteroposterior (height, mm)		
	Bel	Juv	B – J	Bel	Juv	B – J	Bel	Juv	B – J	Bel	Juv	B – J
Mean	4.70	4.10	0.60	7.65	6.40	1.25	6.85	6.30	0.55	12.45	8.90	3.55
Median	3.50	0.50	12.00	7.00	6.00	1.00	5.50	6.00	0.00	12.00	8.50	2.50
Q 1	2.75	−0.25	7.75	5.00	4.00	−1.25	5.00	3.00	−2.25	7.75	6.00	−0.50
Q 3	5.00	2.25	18.25	9.75	8.00	4.25	10.00	8.25	4.25	18.25	12.00	8.50
Wilcoxon *P* value			0.35			0.29			0.64			0.09

Bel, Belotero Volume; Juv, Juvéderm Voluma; Q, Quartile.

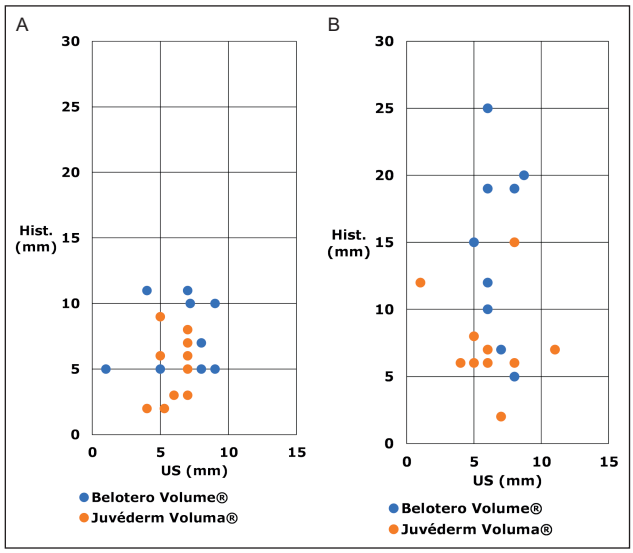


Figure 8. Graph of unremodeled filler spread as measured by ultrasound (US) and histological examination (Hist.), data from sessions 1 and 2 pooled. (A) Width (parallel to skin). Both measurements are in the same order of magnitude. (B) Height (skin fascia). Histological measurements tend to be higher than ultrasound measurements. This effect is more pronounced for Belotero Volume than for Juvéderm Voluma ($P < 0.001$).

Although these differences were not statistically significant at $P < 0.05$, data for height of spread suggest that Belotero Volume results in higher value ($P = 0.09$).

Remodeling had no significant effect on either of these dimensions (Wilcoxon *P* value = 0.51 for pools and 1 for spread).

It is noteworthy that the US measurements of width (parallel to skin, craniocaudal) of filler spread before remodeling are in the same order of magnitude as histological measurements (Figure 8A). No correlation can be demonstrated at this small sample size. To the contrary, height (skin fascia, anteroposterior) of filler spread is higher on histological measurement than measured by US (Figure 8B). This difference is significantly higher for Belotero Volume than for Juvéderm Voluma (nonparametric test of no difference between 2 matched samples: $P < 0.001$).

There were no signs of acute inflammation and there was no necrosis in any of the samples. Scar tissue was graded as 0 in all but the following occurrences. Only in 1 patient (1) it was graded as 2 for both products, but only in the remodeled zones of injection session 2. It was graded as 1 in 1 other patient (6) for the remodeled and unremodeled Juvéderm Voluma from session 2, and for the remodeled and unremodeled Belotero Volume from session 1.

The extent of the reaction and the amount of macrophages were further detailed employing anti-CD68 immunohistochemistry. Table 6 represents chronic inflammation and CD68 positive macrophages in the specimens from injection sessions 1 and 2, respectively. Gradings vary from 0 to 3. There was no statistically significant difference between the fillers for either parameter, although there is a tendency towards significance for a higher number of CD68 positive macrophages in response to Belotero Volume ($P = 0.08$) (Table 7). Remodeling had no statistically significant effect on either parameter.

On 1 occasion, granulomas were found in the remodeled part of the area injected with Juvéderm Voluma 29 days before surgery (patient 3, session 1). They were graded 3+. Figure 9A and B shows the histological picture. Clinically, there had not been any adverse event. It is noteworthy that neither the unremodeled area of the same filler nor the remodeled or unremodeled zones injected 8 days before surgery (session 2) were affected. In comparison, Figure 10 shows a +1 grading of CD68 positive macrophages 29 days after injection and remodeling of Belotero Volume in the same patient.

Table 6. Chronic Inflammation and CD68 Positive Macrophages

S	A	P	Rem	Injected at session 1				Injected at session 2			
				Z	TG (d)	Chronic inflammation	CD68 + macrophages	Z	TG (d)	Chronic inflammation	CD68 + macrophages
1	Abd	Bel	Rem	A	16	1	1	H	6	1	1
		Bel	Not rem	B	16	2	2	G	6	1	2
		Juv	Not rem	C	16	0	0	F	6	0	0
		Juv	Rem	D	16	1	0	E	6	1	0
3	Abd	Bel	Rem	A	29	0	1	H	8	0	1
		Bel	Not rem	B	29	0	1	G	8	0	2
		Juv	Not rem	C	29	0	1	F	8	0	0
		Juv	Rem	D	29	2	3	E	8	1	2
4	Br	Juv	Rem	A	98	0	2	H	7	0	2
		Juv	Not rem	B	98	0	0	G	7	0	1
		Bel	Not rem	C	98	0	1	F	7	0	0
		Bel	Rem	D	98	0	3	E	7	0	2
5	Br	Bel	Rem	A	85	0	2	H	15	0	1
		Bel	Not rem	B	85	0	0	G	15	1	3
		Juv	Not rem	C	85	1	1	F	15	0	0
		Juv	Rem	D	85	0	2	E	15	1	0
6	Br	Juv	Rem	A	43	0	0	H	8	1	0
		Juv	Not rem	B	43	0	0	G	8	1	0
		Bel	Not rem	C	43	1	1	F	8	0	0
		Bel	Rem	D	43	1	2	E	8	0	0

A, area; Abd, abdomen; Bel, Belotero Volume; Br, breasts; Juv, Juvéderm Voluma; Not rem, not remodeled; P, product; Rem, remodeled; S, subjects; TG, time gap, time between injection and collection of specimen; Z, injected zone.

Table 7. Analysis of Difference in Chronic Inflammation and CD68 Positive Macrophages Between Fillers

	Chronic inflammation			CD68 positive macrophages		
	Bel	Juv	B − J	Bel	Juv	B − J
Mean	0.40	0.45	−.05	1.30	0.75	0.55
Median	0.00	0.00	0.00	1.00	0.00	1.00
Q 1	0.00	0.00	−1.00	1.00	0.00	−0.25
Q 3	1.00	1.00	0.25	2.00	1.25	1.25
Wilcoxon *P* value			0.85			0.08

Bel, Belotero Volume; Juv, Juvéderm Voluma; Q, Quartile.

After collection of the results and unblinding the histopathologists, the samples were reexamined to ascertain if any difference in pattern of filler distribution in the tissues could be detected. There was no clear difference. The morphology of the interface between filler and tissue, which may play a part in the diminished visibility on US after remodeling and over time, was also reexamined, but no differences could be demonstrated.

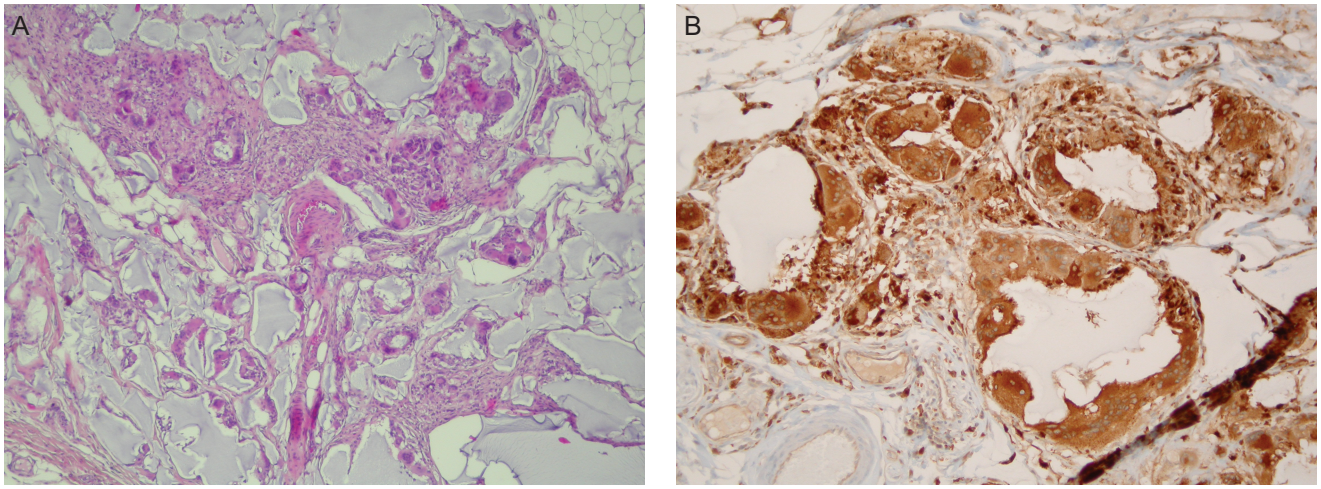


Figure 9. Remodeled Juvéderm Voluma 29 days after injection in subcutaneous abdominal fat (patient 3). (A) Hematoxylin and eosin staining. Acute inflammation 0, chronic inflammation 2+, necrosis 0, granuloma 3+, scar 0. (B) CD68 immunohistochemistry. CD68 positive macrophages: 3+, granuloma 3+.

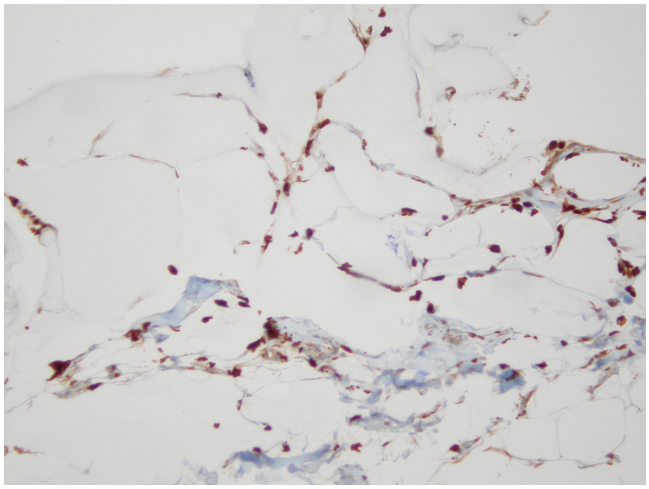


Figure 10. Remodeled Belotero Volume 29 days after injection in subcutaneous abdominal fat (patient 3), CD68 immunohistochemistry. CD68 positive macrophages:1+, granuloma 0.

DISCUSSION

Experimental and clinical research and reports on filler complications show that HA gels, injected as "dermal fillers," are frequently partially or completely located in the subcutis.[3,5,6,11,21,22] Other HA gels, like the 2 brands studied in this article, were specifically designed by the industry for injection of deeper layers, where they may persist for several years.[23] The paucity of publications on histology and US findings after injection of HA in periosteum, areolar connective tissue, muscle, and deep and subcutaneous fat compartments warrants further research on filler behavior and tissue response.

The structure and physiology of abdominal or breast fat differ from deep or subcutaneous facial fat. Nevertheless, breast reduction and abdominoplasty offer the opportunity to study the entire trajectory of linear retrograde or bolus filler injections and short- and mid-term tissue response in healthy tissue that otherwise would be discarded.

Ultrasound Video

In our experiment, real-time US of injections gave a clear image of the spreading of the fillers, mostly in a pearl-string manner rather than as a continuous deposit but more so for Belotero Volume than for Juvéderm Voluma.

The occasional finding of inadvertent deposition of a bolus during linear retrograde injection (Videos 3 and 4, available online at www.aestheticsurgeryjournal.com; Figure 3C and D) may have clinical relevance. In case elastic resistance is met at the end of the trajectory of the cannula, a distended septum may be preventing the filler from spreading distal to the tip. When the tissue recoils into its original position, the filler deposit is moving backwards with it. As will be discussed below, it is unlikely that this can be corrected after the injection by external pressure or pinching. For linear retrograde injections, it is recommended to utilize a cannula that is longer than the intended length of a filler deposit. In case any resistance is met at the end of the trajectory, a choice is to be made between perforating this tissue or accepting a shorter deposit before initiating injection.

Ultrasound Measurements

Immediately after injection, measuring the filler deposits by US was straightforward in all cases. Histology confirmed that the width of filler spread (parallel to skin, craniocaudal) was in the same range of magnitude as

measured by US (Figure 8A), although no correlation could be found in this small study group. Filler spread height (skin fascia, anteroposterior) was in a higher range histologically than measured by US (Figure 8B). This may be caused by the compression of the tissues by the US probe. This effect was significantly stronger for Belotero Volume than for Juvéderm Voluma ($P < 0.001$). The difference could theoretically be due to different product elasticity. However, laboratory research shows Belotero Volume with lidocaine to have a higher elasticity modulus (E') than Juvéderm Voluma (without lidocaine).[24] This means that a higher force is needed to compress the first product to reach the same amount of deformation between the test plates. The normal force, measured when a given amount of product is compressed down to a defined thickness, is also higher for Belotero Volume with lidocaine than for Juvéderm Voluma.[24] This raises more questions: is the range of pressure on the gel in vivo and the oscillation frequency of compression by the radiologist similar to laboratory tests between metal plates? What is the cumulative elastic behavior of injected tissues? Was our finding the result of different US characteristics, with some parts of the gel deposits being undetectable by US?

Remodeling parts of the injected areas reduced the US visibility of both brands or even reduced it to zero, whereas histology nevertheless confirmed the presence of the gel.

Filler that became undetectable by US after remodeling in the first session reappeared at the second session in the 2 abdominal specimens (Table 3). There was no typical histological difference between remodeled and unremodeled zones that could explain this phenomenon. It could be argued that filler deposits can be flattened so much by external pressure that they become invisible and later return to their previous shape.

In all 3 breast cases, the gel injected during session 1 could not be detected by US at session 2 except for 1 unremodeled zone in 1 patient. Again, histology confirmed that the filler had not disappeared.

We suggest that US is valuable to measure HA deposits immediately after injection but unreliable to localize and measure HA fillers after manipulation of an injected area or over time. MRI may be a better option for future studies.[23] The situation is very different in clinical cases of suspected granuloma and capsule formation. Hypoechoic nodules may be present and US assessment is a highly recommended part of the work-up.[11,12]

Filler Distribution

The deposition of Juvéderm Voluma (Video 1 and Video 3) in adipose tissue seems to happen in a slightly different way than for Belotero Volume (Video 2 and Video 4). During continuous injection, Belotero Volume is mainly deposited as a series of globules. Juvederm Voluma flows in a more continuous manner. This is probably due to the different rheologic character of the gels, and it concurs with laboratory tests showing Belotero Volume to be more cohesive.[24,25] Laboratory tests may correlate better with the behavior of filler gels while flowing through the needle and into the tissues than with the consequences of external forces applied to injected tissues.

Despite the different flow pattern, the histology of the 2 fillers was very similar. Neither the fillers nor the anatomy of tissue integration could be distinguished under the microscope after hematoxylin-eosin staining.

The filler separated the tissue and formed pools with dimensions in the order of millimeters (2-18) as shown in Tables 6 and 7. No flattening of surrounding fat cells or any distorted appearance of the fibrous septae could be detected.

This is very different from the spread of HA gels in the dermis as described in the literature.[2,5-7] Filler pools in the dermis have dimensions of tens to hundreds of microns. Particle size in particulate gels and cross-linking characteristics of nonparticulate gels can make a difference. Some gels remain confined to the slightly larger spaces in the collagen fiber maze of the mid- and deep dermis and at times deform the collagen bundles. Others can spread into the superficial dermis. This has clinical relevance with respect to injection technique and avoidance of complications (filler visibility, blue-gray dyschromia[26]).[5-7] It seems that filler pools in adipose tissue are much larger.

It is noteworthy that over time the spread of the fillers did not change significantly. The question is sometimes raised in clinical practice if loss of definition of a volumetric correction is due to resorption of the filler, to migration, or to reactive edema of the surrounding tissues. We found no traces of migration of either brand.

There was no difference in aspects of the filler deposits over time that could indicate water absorption, fragmentation, or migration.

Effect of Remodeling

A most remarkable finding was that remodeling of the filler after injection, by firm squeezing and rolling of tissues between the fingers of the injector, did not have any significant effect on the size of filler pools, on filler spread, or on tissue response. In clinical practice, a filler is often remodeled by pressure immediately after injection. We may be only changing the shape of filler deposits in fat compartments rather than spreading the gel any further. It is recommended to inject fillers as precisely as possible to minimize the need for remodeling.

The elastic behavior of fillers as measured in the laboratory between test plates—either by back and forth (oscillatory) rotation or by compression of the filler—can be described by the elastic modulus G' for shear stress and E' for compression. Although elasticity may help to maintain projection obtained with volumizing fillers, the amount of force needed per amount of reversible deformation (G' and E') may be less important for a good result than the ability for the gel pools to return to their original shape after compression. At present, we lack scientific data about the contribution of elastic tissue recoil to maintenance or loss of shape after filler injection.

Low values of loss moduli G'' (shear stress) and E'' (compression) implicate that the gel behaves more like a soft solid under compression than a viscous fluid, indeed an interesting property for the art of reshaping. A low dissipation factor tan δ (G''/G') reflects more elastic than viscous behavior under shear stress. It is lower for Juvéderm Voluma than for Belotero Volume.[24] A low dissipation factor tan δc (E''/E') reflects more elastic than viscous behavior under compression. That parameter was found to be lower for Belotero Volume than for Juvéderm Voluma.[24] An optimal description of filler characteristics is laudable, but the relevance of these parameters for good clinical results is unclear.

Further research is warranted about the relationship between externally applied pressure and interstitial pressure in the subcutis or deeper tissues. If fibrous septae prevent fillers from spreading under any tolerable pressure, there must be other reasons for loss of projection before total resorption of fillers, such as cumulative changes in shape of filler pools, adaptation of the injected tissue, or locoregional reactive edema.

The relative protection from mechanical, hydrodynamical breakdown by surrounding fat may contribute to the relatively longer lifespan of HA after deep injection compared with intradermal injection.

Nevertheless, it may be possible to exert more pressure on fillers in the face than in abdominal subcutaneous fat because of the smaller size of facial fat lobules (except in the buccal fat pad) and the vicinity of the bone. Further research is needed to ascertain if facial fillers can be displaced by external force.

The term "volumizing capacity" in publications on fillers[24,27] can cause confusion. Whereas it suggests a physical measure of volume increase, it is utilized to express a more or less subjective appreciation. What is usually meant with "high volumizing capacity" is that a brand is good at maintaining shape and projection or receiving high gradings of aesthetic results by patients or neutral assessors or that the duration of the effect is favorable. If not measurable on the test bench, these properties can still be described in more accurate terms, such as "effectiveness."[27]

Tissue Response

Discussing natural HA degradation, Fraser et al state that only 20% to 30% is metabolized in situ. The rest is taken up in lymphatic flow and further in the bloodstream to be metabolized in the lymph nodes and the liver and kidneys, respectively. Only 1% to 2% is excreted in the urine. Lymph contains very large polymers similar to those in the tissues, which suggests that they are displaced from the tissues hydrodynamically rather than by diffusion.[28] They state that natural hyaluronan is catabolized by receptor-mediated endocytosis and lysosomal degradation either locally or after transport. Our findings show chronic inflammation and CD68+ macrophages ranging from 0 to 3+. For both tests, 0 and 1+ were the most common findings. Scar formation was rare.

Both fillers were well tolerated by the tissues, with the exception of granuloma formation in 1 remodeled zone injected with Juvéderm Voluma 29 days before surgery. This finding was purely histological. There were no clinical indications and no abnormality was detected by US 21 days after injection.

Shah et al describe granulomatous inflammation as a distinctive form of chronic inflammation produced in response to various infectious, autoimmune, toxic, allergic, and neoplastic conditions. "It is defined by the presence of mononuclear leukocytes, specifically histiocytes (macrophages), which respond to various chemical mediators of cell injury. This pattern of injury response occurs in all age groups and within all tissue sites. Through light microscopy, the activated histiocytes appear as epithelioid cells with round to oval nuclei, often with irregular contours and abundant granular eosinophilic cytoplasm with indistinct cell borders. They may also coalesce to form multinucleated giant cells."[29] They categorize several patterns of granulomatous inflammation: foreign body (including in response to HA), necrotizing, nonnecrotizing, and suppurative. Histiocytic response may occur without granulomas.

Granulomas have been described as a complication of many fillers. HA is not an exception, but its track record is better than nonresorbable fillers.[12,30-32] To our knowledge, there are no reports of a granuloma caused by Belotero Volume at the time of submission of this paper. There is 1 case report on a reaction to Belotero Balance, an HA filler designed for intradermal injection with a different version of the proprietary cohesive polydensied matrix production technology. There was a strong clinical indication of a granulomatous reaction but no histological confirmation.[33] Beleznay et al discuss 23 patients out of 4702 treatments (0.5%) with Juvéderm Voluma who developed late-onset nodules, but none of the lesions was biopsied for histology.[34]

The fact that the subclinical granuloma in our case developed only on the right side, 29 days after injection, may

be compatible with a late-onset reaction. It is remarkable, however, that the unremodeled part of the filler did not show any granulomatous reaction.

Limitations

This pilot study was a repeated experiment (2 sessions) utilizing 2 similar products in 5 patients. Because of the small sample size, it was unlikely that statistically significant differences would be found.

The results of injections in 2 regions with different anatomy were pooled. The diameter of fat lobules in the breast is smaller than in the abdomen. Due to the variance of depth of injection, some of the filler was injected below the superficial fascia and some above. In the deep part of the subcutis, fat lobules are larger, whereas more septa are met in a superficial trajectory. The manual injections were not standardized for injection pressure or speed.[35] There was a light variance in quantity injected (0.5-0.6 mL).

The time intervals between the injection and the operation varied from 16 to 98 days for the first session and from 6 to 15 days for the second session.

Histological measurements of filler spread relied on the largest measurements out of 3 cross-section samples of the linear deposits. For US measurements the entire deposit was visualized. This may have contributed to the absence of correlation between the 2 sets of measurements.

One-half of the length of the linear deposits was remodeled by finger pressure, but this also influenced US measurements of the nonremodeled zones. Bias on the histology of these adjacent zones cannot be excluded. The laterolateral diameter spanned the unremodeled and remodeled areas. We could therefore only compare cross-sections but not volume or 3-dimensional shape.

CONCLUSIONS

Literature on HA gels injected in the subcutis is scarce. Invasive clinical research without potential direct health benefit for the volunteers is ethically and operationally complex. Therefore, a pilot study was conducted on a small number of patients. The study was intended to collect descriptive data, which could also be helpful for the design of studies on larger population samples.

US examination was helpful to study injection of Juvéderm Voluma with lidocaine and Belotero Volume with lidocaine in fat tissue in real time. During linear retrograde injection with a cannula 22 G, the deposits were clearly visualized as serpiginous strings of globule and spindle shapes.

Filler pool and total filler spread dimensions were in the order of millimeters. This is much larger than HA pools in the dermis, as described in the literature, with diameters of tens to a few hundreds of microns.

It was visualized how subcutaneous septae can prevent filler from spreading distal to the tip of a blunt cannula. Injection techniques can be adapted to overcome this impediment.

Remodeling the injected area by pinching decreased the visibility of the gel on US or reduced it to zero, whereas histology later proved that it was still present. Over time, both the remodeled and unremodeled zones were more difficult or impossible to find by US, also in contradiction with the histology.

US may be unreliable to study the dimensions of uncomplicated HA filler deposits over time, leaving MRI as the better option.

Histology showed that both HA gels were tolerated well by fat tissue. There was no capsule formation and no or only a mild histiocytic response, with 1 exception. A granulomatous reaction on Juvéderm Voluma with lidocaine was detected in the remodeled part of 1 deposit, whereas the corresponding site across the midline and the unremodeled zones remained unaffected.

For research purposes, it can be considered that linear deposits have an advantage over boli: they can be sampled repetitively by a series of cross-sections that potentially have the same diameter.

Neither the quantified measurements nor a review of the descriptive histology after unblinding showed any obvious difference between the samples injected in the first vs the second session in any of the patients. For future studies on the evolution of HA in the subcutis over time, time gaps of more than 3 months are recommended.

Remodeling one-half of the length of the linear filler deposits allowed us to study more variables at an acceptable burden for the patients. However, it cannot be excluded that the “unremodeled” zones were in fact influenced by the squeezing nearby. For future research, it is recommended to study unremodeled filler deposits and their remodeled counterparts separately.

Supplemental Material

This article contains supplemental material located online at www.aestheticsurgeryjournal.com.

Acknowledgments

Statistical analysis was performed by Donna F. Stroup, PhD, MSc (Data for Solutions, Inc., Decatur, GA).

Disclosures

Dr Vandeputte is a consultant for Merz Aesthetics (Frankfurt, Germany) and Advanced Aesthetic Technologies (AAT; Brookline, MA) and holds minor stock options in AAT. Dr Micheels is a consultant or trainer for Merz Aesthetics, Allergan (Dublin, Ireland), Galderma (Lausanne, Switzerland), Vivacy (Paris, France), and Teoxane (Geneva, Switzerland). The other authors declared no potential conflicts of interest with respect to the research, authorship, and publication of this article.

Funding

Merz Pharmaceuticals GmbH (Frankfurt am Main, Germany) provided a restricted grant to support data collection and analysis. Merz Pharmaceuticals did not participate in the design of the study or in the collection, analysis, or interpretation of the data. Merz did not provide editorial support for this publication other than recommending the biostatistician.

REFERENCES

1. The Aesthetic Society's Cosmetic Surgery National Data Bank: Statistics 2018. *Aesthet Surg J*. 2019;39(Supplement_4):1-27.
2. Micheels P, Besse S, Sarazin D. Two crosslinking technologies for superficial reticular dermis injection: a comparative ultrasound and histologic study. *J Clin Aesthet Dermatol*. 2017;10(1):29-36.
3. Micheels P, Sarazin D, Besse S, Sundaram H, Flynn TC. A blanching technique for intradermal injection of the hyaluronic acid Belotero. *Plast Reconstr Surg*. 2013;132(4 Suppl 2):59S-68S.
4. Duranti F, Salti G, Bovani B, Calandra M, Rosati ML. Injectable hyaluronic acid gel for soft tissue augmentation. A clinical and histological study. *Dermatol Surg*. 1998;24(12):1317-1325.
5. Flynn TC, Sarazin D, Bezzola A, Terrani C, Micheels P. Comparative histology of intradermal implantation of mono and biphasic hyaluronic acid fillers. *Dermatol Surg*. 2011;37(5):637-643.
6. Tran C, Carraux P, Micheels P, Kaya G, Salomon D. In vivo bio-integration of three hyaluronic acid fillers in human skin: a histological study. *Dermatology*. 2014;228(1):47-54.
7. Dugaret AS, Bertino B, Gauthier B, et al. An innovative method to quantitate tissue integration of hyaluronic acid-based dermal fillers. *Skin Res Technol*. 2018;24(3):423-431.
8. da Costa A, Biccigo DGZ, de Souza Weimann ET, et al. Durability of three different types of hyaluronic acid fillers in skin: are there differences among biphasic, monophasic monodensified, and monophasic polydensified products? *Aesthet Surg J*. 2017;37(5):573-581.
9. Wanick FBF, Issa MCA, Luiz RR, Filho PJS, Olej B. Skin remodeling using hyaluronic acid filler injections in photoaged faces. *Dermatol Surg*. 2016;42(3):352-359.
10. Grippaudo FR, Mattei M. The utility of high-frequency ultrasound in dermal filler evaluation. *Ann Plast Surg*. 2011;67(5):469-473.
11. Wortsman X, Wortsman J, Orlandi C, Cardenas G, Sazunic I, Jemec GB. Ultrasound detection and identification of cosmetic fillers in the skin. *J Eur Acad Dermatol Venereol*. 2012;26(3):292-301.
12. Wortsman X. Identification and complications of cosmetic fillers: sonography first. *J Ultrasound Med*. 2015;34(7):1163-1172.
13. Mlosek RK, Skrzypek E, Skrzypek DM, Malinowska S. High-frequency ultrasound-based differentiation between nodular dermal filler deposits and foreign body granulomas. *Skin Res Technol*. 2018;24(3):417-422.
14. Pienaar WE, McWilliams S, Wilding LJ, Perera IT. The imaging features of MACROLANE™ in breast augmentation. *Clin Radiol*. 2011;66(10):977-983.
15. Vent J, Lefarth F, Massing T, Angerstein W. Do you know where your fillers go? An ultrastructural investigation of the lips. *Clin Cosmet Investig Dermatol*. 2014;7:191-199.
16. Micheels P, Besse S, Sarazin D, et al. Ultrasound and histologic examination after subcutaneous injection of two volumizing hyaluronic acid fillers: a preliminary study. *Plast Reconstr Surg Glob Open*. 2017;5(2):e1222.
17. Young SR, Bolton PA, Downie J. Use of high-frequency ultrasound in the assessment of injectable dermal fillers. *Skin Res Technol*. 2008;14(3):320-323.
18. Santer V, Molliard SG, Micheels P, et al. Hyaluronic acid after subcutaneous injection-an objective assessment. *Dermatol Surg*. 2019;45(1):108-116.
19. World Medical Association. WMA declaration of Helsinki – ethical principles for medical research involving human subjects. https://www.wma.net/wp-content/uploads/2016/11/DoH-Oct2013-JAMA.pdf. Accessed March 1, 2020.
20. Wilcoxon F. Individual comparisons by ranking methods. *Biometrics Bull*. 1945;1(6):80-83.
21. Arlette JP, Trotter MJ. Anatomic location of hyaluronic acid filler material injected into nasolabial fold: a histologic study. *Dermatol Surg*. 2008;34(Suppl 1):56-63.
22. Chopra K, Calva D, Sosin M, et al. A comprehensive examination of topographic thickness of skin in the human face. *Aesthet Surg J*. 2015;35(8):1007-1013.
23. Micheels P, Besse S, Vandeputte J. Cohesive Polydensified Matrix® cross-linked hyaluronic acid volumizing gel: a magnetic resonance imaging and computed tomography study. *Clin Cosmet Investig Dermatol*. 2019;12:1-10.
24. Gavard Molliard S, Albert S, Mondon K. Key importance of compression properties in the biophysical characteristics of hyaluronic acid soft-tissue fillers. *J Mech Behav Biomed Mater*. 2016;61:290-298.
25. Sundaram H, Rohrich RJ, Liew S, et al. Cohesivity of hyaluronic acid fillers: development and clinical implications of a novel assay, pilot validation with a five-point grading scale, and evaluation of six U.S. food and drug administration-approved fillers. *Plast Reconstr Surg*. 2015;136(4):678-686.
26. Rootman DB, Lin JL, Goldberg R. Does the Tyndall effect describe the blue hue periodically observed in subdermal hyaluronic acid gel placement? *Ophthalmic Plast Reconstr Surg*. 2014;30(6):524-527.
27. Kerscher M, Agsten K, Kravtsov M, Prager W. Effectiveness evaluation of two volumizing hyaluronic acid dermal fillers in a controlled, randomized, double-blind, split-face clinical study. *Clin Cosmet Investig Dermatol*. 2017;10:239-247.
28. Fraser JR, Laurent TC, Laurent UB. Hyaluronan: its nature, distribution, functions and turnover. *J Intern Med*. 1997;242(1):27-33.
29. Shah KK, Pritt BS, Alexander MP. Histopathologic review of granulomatous inflammation. *J Clin Tuberc Other Mycobact Dis*. 2017;7:1-12.

30. El-Khalawany M, Fawzy S, Saied A, Al Said M, Amer A, Eassa B. Dermal filler complications: a clinicopathologic study with a spectrum of histologic reaction patterns. *Ann Diagn Pathol*. 2015;19(1):10-15.
31. Dadzie OE, Mahalingam M, Parada M, El Helou T, Philips T, Bhawan J. Adverse cutaneous reactions to soft tissue fillers–a review of the histological features. *J Cutan Pathol*. 2008;35(6):536-548.
32. Ozturk CN, Li Y, Tung R, Parker L, Piliang MP, Zins JE. Complications following injection of soft-tissue fillers. *Aesthet Surg J*. 2013;33(6):862-877.
33. Gandy J, Bierman D, Zachary C. Granulomatous reaction to belotero balance: a case study. *J Cosmet Laser Ther*. 2017;19(5):307-309.
34. Beleznay K, Carruthers JD, Carruthers A, Mummert ME, Humphrey S. Delayed-onset nodules secondary to a smooth cohesive 20 mg/mL hyaluronic acid filler: cause and management. *Dermatol Surg*. 2015;41(8):929-939.
35. Lorenc ZP, Bruce S, Werschler WP. Safety and efficacy of a continuous-flow, injection-assisted device in delivery of dermal fillers. *Aesthet Surg J*. 2013;33(5):705-712.