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ORIGINAL ARTICLE

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Comparison of Two Swiss-Designed Hyaluronic Acid Gels: Six-Month Clinical Follow-Up

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ABSTRACT

The aim of this paper is to compare 2 hyaluronic acid gel fillers from the same Swiss manufacturer and with the same indications: filling of line wrinkles and folds. The products differ by their cross-linking process. With very simple easy-to-reproduce tests, cohesivity and resistance to traction forces were examined. Also, both gels were injected under ultrasound control in the mid reticular dermis of three subjects. The papules were controlled under ultrasound and biopsies at D0 and D15. Results showed significant differences between the 2 gels in all the tests. The new gel, manufactured with a lower-crosslinking density, seems to benefit from better integration in the tissue of the mid reticular dermis and to have a more cohesive nature than its comparator from a previous crosslinking technology. Under clinical observation, the range of new products present excellent tissue integration properties.

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INTRODUCTION

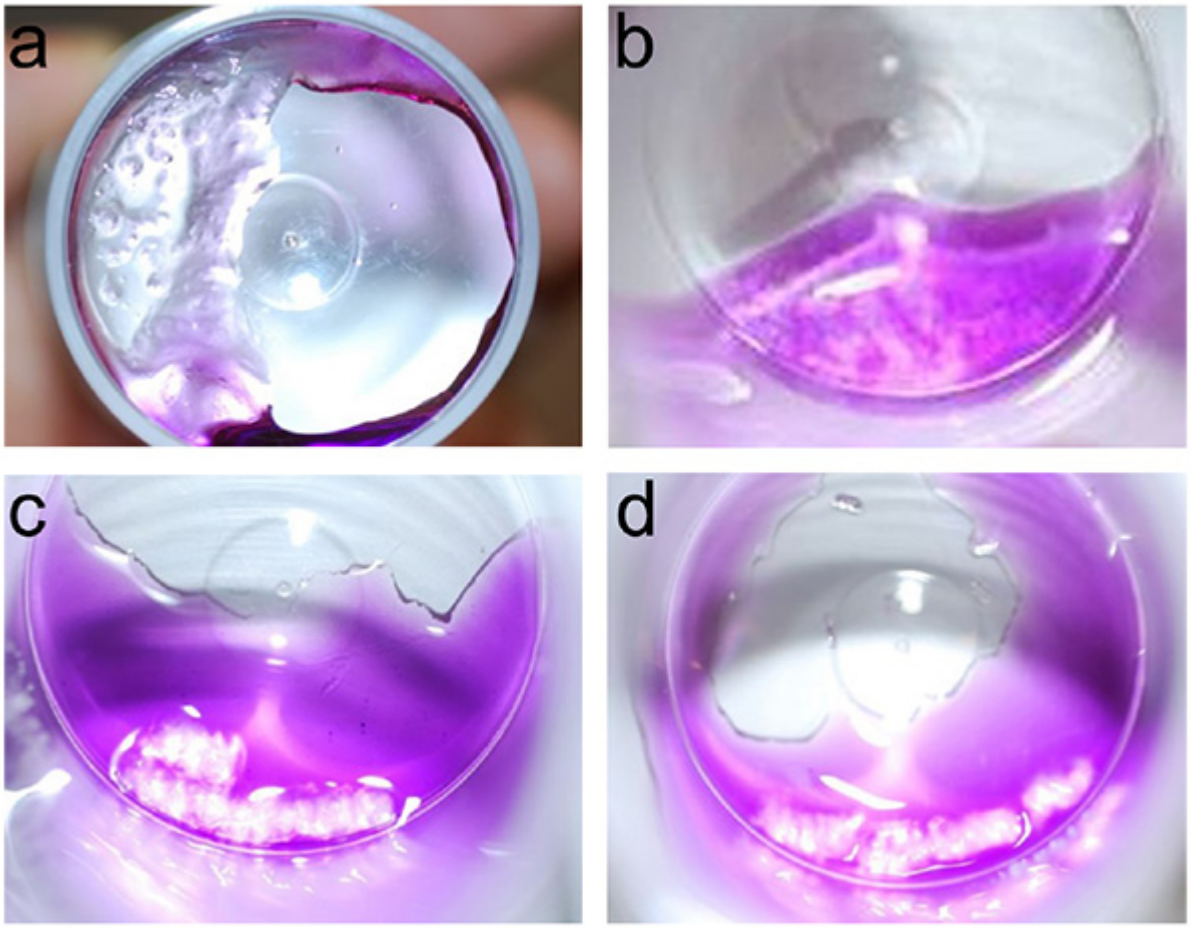
Over the past two decades, hyaluronic acid (HA) has gradually become the benchmark product for wrinkle-filling and face volumetry.[1,2] With the market booming, HA is one of the most in-demand beauty treatments in the United States, Europe, and Asia. Considering this development, many laboratories now manufacture HA for the purposes of beauty treatments. For some years now, several gels benefit from the FDA approval.Others, including the ones presented here, are currently in the process of being registered. Few manufacturers offer more than one range of dermal fillers, meaning that they offer different products with the same indication for use. The rationale behind this is often not very clear since the manufacturers usually avoid to present comparative data between dermal fillers from the different ranges.We have selected two dermal fillers from the same manufacturer based in Geneva, Switzerland, with the same indication for use: “filling of line wrinkles on the face, damaged skin such as mild or moderate nasolabial folds, peribuccal, and glabella wrinkles.” Interestingly, the manufacturer states that both products are made from the same type of high-molecular weight HA crosslinked with butane diglycidyl ether (BDDE). The new product includes less BDDE cross-linker than the previous one. To assess if there was a real advantage of the newer product (RHA 2) compared to the existing range (PS-GA), the two products were submitted to various laboratory tests for cohesivity, resistance to stretching, and spreading, as well as to histology and ultrasound monitoring. In a second part of the article, the personal experience of the first author with the new range of products (RHA 1-4) is described based on the 6-month follow-up of 27 treated patients. Data about PS-GA are subject of a poster.[3] This article also acts as a supplement to previously published articles.[4-6]

MATERIALS

Examined Gels Butane diglycidyl ether is the crosslinking agent for both gels. Both are manufactured with

0.3% lidocaine.*Teosyal® PureSense Global Action (PS-GA)*: batch number: TS30L134103C (TEOXANE). The gel was examined in a previous comparative study.4-6 The gel HA concentration is 25 mg/mL. The crosslinking process aims to yield an "homogeneous crosslinking network" (isotropic distribution of crosslinking bridges; Personal communication, S. Meunier, TEOXANE).*Teosyal® RHA 2 (RHA 2)*: batch number: TP30L-143601B (ultrasound monitoring), TP30L-151705B (histological tests). This gel HA concentration is 23 mg/mL. The crosslinking technology aims to minimize the degradation of the hyaluronic acid chains during the manufacturing process and also to reduce the crosslinking degree of HA in the final product (Personal communication, S. Meunier, TEOXANE).**Instruments and Products**For laboratory testing: Petri dish, jar for urine samples, tape measure, NaCl 0.9% in water (B. Braun), colorant: Royal Talens

FIGURE 1. Cohesivity test before and after addition of 2 drops of ethanol: PS-GA (A, B) and RHA 2 (C, D).



Blu e Violet Ecoline® (number 548), Adson plyer, 1 mL syringes and Omnican® 50 syringes with atraumatic 30G½ needle, 70% ethanol. Nikon D40x digital camera, AF system Micro Nikkor 60 mm 1:2.8 D, to take photographs for cohesivity and resistance to stretching testings.[4-6]At the Viollier SA laboratory, Geneva, Switzerland, toluidine blue (0.069% concentration), microscope slides, cover slips, and double-distilled water to rinse the slides before each examination with the microscope were used.[4-6] The preparations were examined under an: Olympus SC100 microscope.**Histology and Ultrasound Monitoring***Ultrasound Monitoring*[7]MedImage radiological institute, Geneva, Switzerland, performed the ultrasound-monitoring of injections in the mid reticular dermis: LOGIQ E9 with L8-18i-D transducer (General Electric Company, Fairfield, CT), at a frequency of 17 MHz. To ensure image good quality, a solid gelled interface by Geistlich Pharma AG, Wolhusen, Switzerland, batch number: 1000353, was placed on the surface to be injected.*Histological Tests*To perform the histological tests, anesthetic lidocaine 1% (without epinephrine) was used as well as a 4 mm round punch and a 4/0 thread for stitching. The preparations were examined at Viollier SA, Geneva, Switzerland.

METHODS

Simple Cohesivity Test[4-6]A sample of 0.6 ml of saline solution was colored with 2 drops of Ecoline®. Using the tip of the original syringe, 0.2 ml of the test gel were added. The preparation was stirred Eliasmanually for few seconds and then photographed. Two drops of ethanol were added and the mixture was stirred manually for few seconds and then photographed a second time.**Resistance to Stretching**As previously described, 0.2 ml of gel was placed into the center of a Petri dish for testing.[4-6] Using Adson plyer, the gel was stretched to obtain the longest possible filament.**Spreading the Gels**The gels were spread for testing over a microscope slide.[4-6] Two drops of toluidine blue (0.069% concentration) were applied and left for 30 seconds. The gel was rinsed with 2 ml of double-distilled water before observation under a binocular microscope.**Histology and Ultrasound Monitoring**Having provided informed consent and in accordance with the Declaration of Helsinki, 3 subjects accepted to be given an ultrasound-monitored injection, after which, a biopsy would be performed on her gluteal region on D0 and D15.0.2 ml of gel was injected under ultrasound-monitoring into the mid dermis of the left and right buttock. Prior to injection, the position of the needle was verified and photographed. The gel was then injected under ultrasound monitoring. To calculate the injection depth, a measurement was taken of the needle's penetration angle as well as the length of inserted needle.[7] On D0, and under local anesthesia, a 4mm biopsy was performed on the right buttock. On D15, after performing an ultrasound image on the left buttock papule, the same anesthesia and biopsy were performed.

RESULTS

Simple Cohesivity Test After a few seconds, the PS-GA gel separated into several clusters; a phenomenon accelerated by the addition of ethanol (Figure 1). Under the same conditions, RHA 2 gel stayed on a sausage-like form even after addition of ethanol, showing a higher cohesivity.

FIGURE 2. Resistance to stretching: (A) PS-GA 0.5 cm, (B) RHA 2, 1.0 to 1.5 cm.

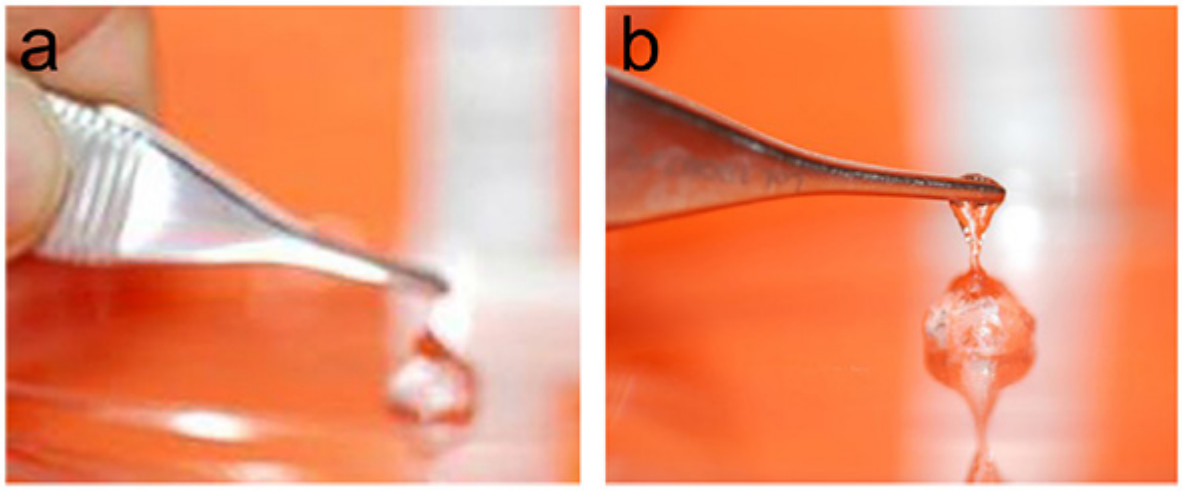
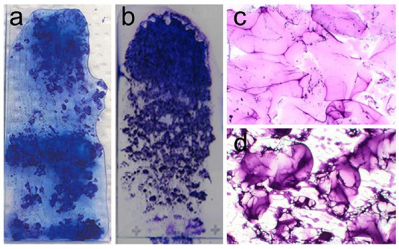


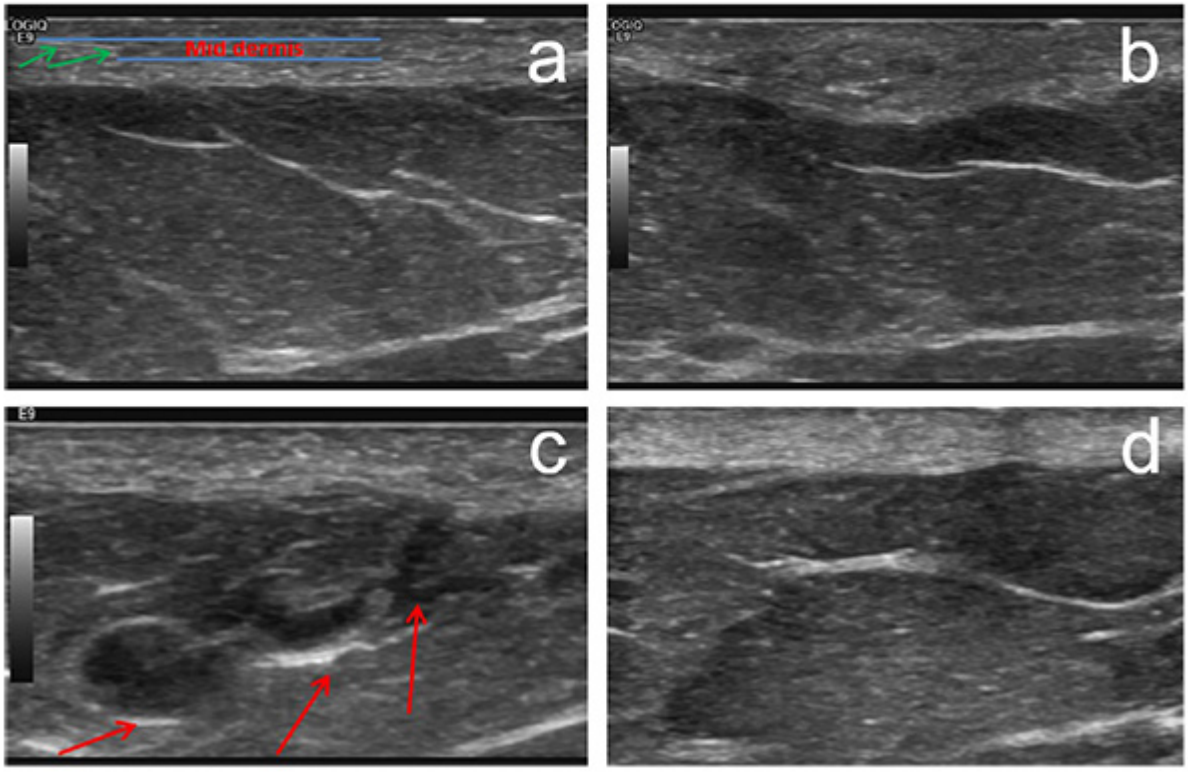
FIGURE 3. Spreading the gels macroscopic and microscopic observations (x12.5): PS-GA (A, C) and RHA 2 (B, D).



Resistance to Stretching The PS-GA gel could not be stretched over 0.5 cm (Figure 2). RHA 2 gel mainly remained a compact mass, and could be stretched to a maximum of 1.0 to 1.5 cm.**Spreading the Gels**PS-GA appeared very viscous and therefore resistant to spreading (Figure 3). Under the microscope magnification, the preparation appeared to be well spread and contained large masses

of particles.[4-6]RHA 2 behaved in a very similar manner. Once spread onto the slide, it demonstrated medium viscosity and remained relatively adhesive. Under the microscope, RHA 2 appeared similar to PS-GA, with smaller particles.In this test, both gels had a partially cohesive nature.

FIGURE 4. Ultrasound monitoring: (A) before injection of RHA 2, the needle is placed in the mid reticular dermis (green arrows). (B) right after injection and needle withdrawal. (C) D0 at the right injection site, leakage of the product in the hypodermis (red arrows). (D) D15, at the left injection site, the product is homogeneous and isoechoic to the nearby dermis.



Ultrasound Monitoring Prior to injection, the position of the needle was verified under ultrasound (Figure 4a). From the angle and length of the inserted portion of the needle are calculated the depths of injection. which corresponded to the mid reticular dermis of the three subjects (Table 1).[7]Eight injections on two subjects were performed with PS-GA, 4 in the superficial dermis and 4 in the mid dermis.3 In 6 cases, a leak in the subcutis was observed from light to very important. 3 The papules had an isoechogeneous (5/8 injections) or a hypoechogeneous (3/8 injections) aspect, compared to the non-injected surrounding dermis. There was only one cone of shadow observed and 3 times a posterior reinforcement. At the D15 follow up, the ultrasound observation on the papules indicated that the thickening of the dermis was unchanged since D0.Upon injection of RHA 2 into the mid dermis of subject C, no leakage into the hypodermis was observed on the left injected site (Figure 4b). On the right site, there was a significant leak into the hypodermis (Figure 4c) that resulted from a marginally deeper injection on the right than on the left (1.2 mm as opposed to 1.0 mm). In both cases, the RHA 2 gel appeared as a homogeneous, isoechoic papule in comparison with the nearby, uninjected dermis. No acoustic shadowing or acoustic enhancement was observed. At D15, the ultrasound examination showed a smaller papule than at D0 (Figure 4d). The dermis very slightly increased in thickness, despite marginally decreasing in size with regards to the first measurements taken immediately after injection. It grew from 2.7 mm before injection to 6.6 mm just after on D0, and measured 3.0 mm thick on D15 (only the left injection site was considered).**Histological Monitoring**The histological analysis right after injection of PS-GA showed in both subjects small to large pools of material in the superficial and mid reticular dermis (Table 2; Figure 5a).[3] For subject B, the

gel diffused between the collagen fibers and elastic fibers. No inflammatory reaction or fibrosis was observed on D0. The D15 biopsy showed that the PS-GA gel had the same location and distribution as on D0. For subject A at D15, no inflammatory reaction or fibrosis was observed. Subject B presented a very minor inflammatory lymphocyte and reaction that was accompanied by rare eosinophils (Figure 5b).The histological analysis right after injection of RHA 2 showed a diffuse profile of the implant in the mid and deep reticular dermis (Table 2; Figure 5c). No inflammatory reaction or fibrosis was observed. The D15 biospy showed identical profile and distribution of the implant in the dermis as on D0. Also in this case, a slight decrease in the thickness of the whole dermis was observed compared with D0 measurements (Figure 5d).**Personal Experience With the RHA 1-4 Range**The 4 products from RHA range (RHA 1 to 4) were tested by the first author as part of his regular practice. Within a couple of months, 27 is not specified in the product leaflet, RHA 2 and 3 were injected twice to redefine the vermilion border or plump up the lips.The injector was surprised at how easily the 4 variations of gel can be injected. The RHA 1, 2, and 3 gels are supple, fluid, and blend extremely well into the dermis. RHA 4 was only injected female patients were treated with an average age of 61.3 years (45 to 74 years). Table 3 provides a list of the indications and the tested gels. Table 4 lists the patients, their age, the various areas treated, injection technique, and the needle or micro-cannula used. As this

FIGURE 5. Biopsies performed at D0 and D15 after injection of PS-GA (A, B) x12.5 and RHA 2 (C, D) x25.

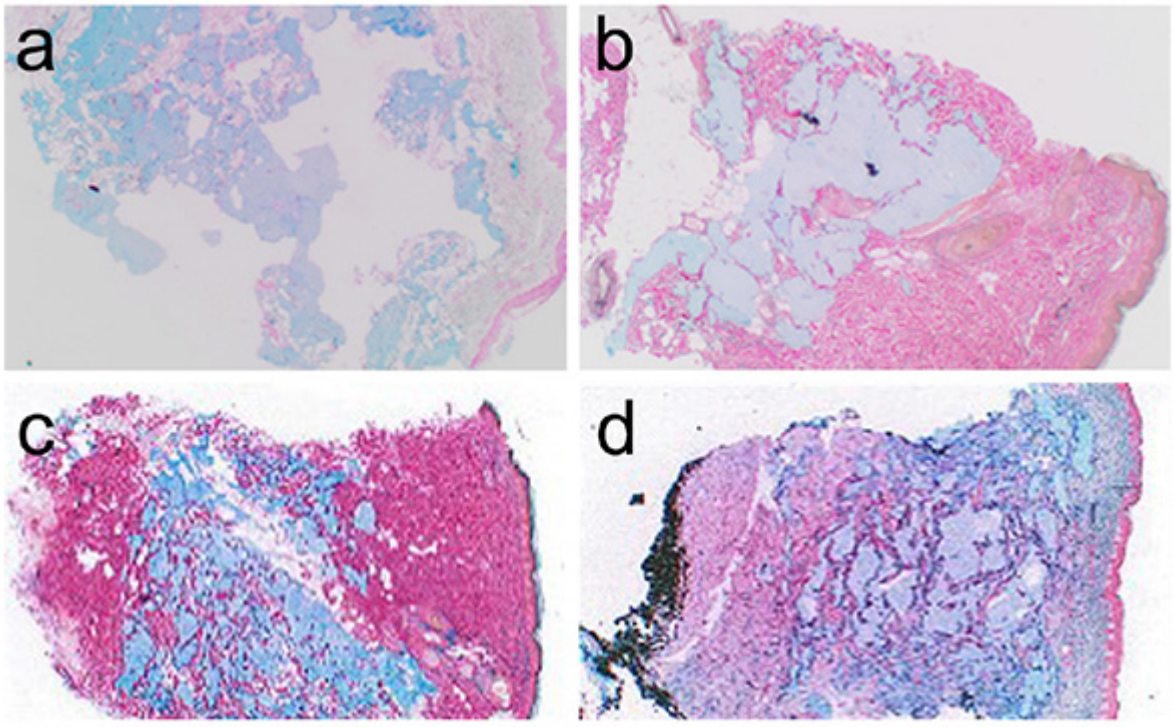


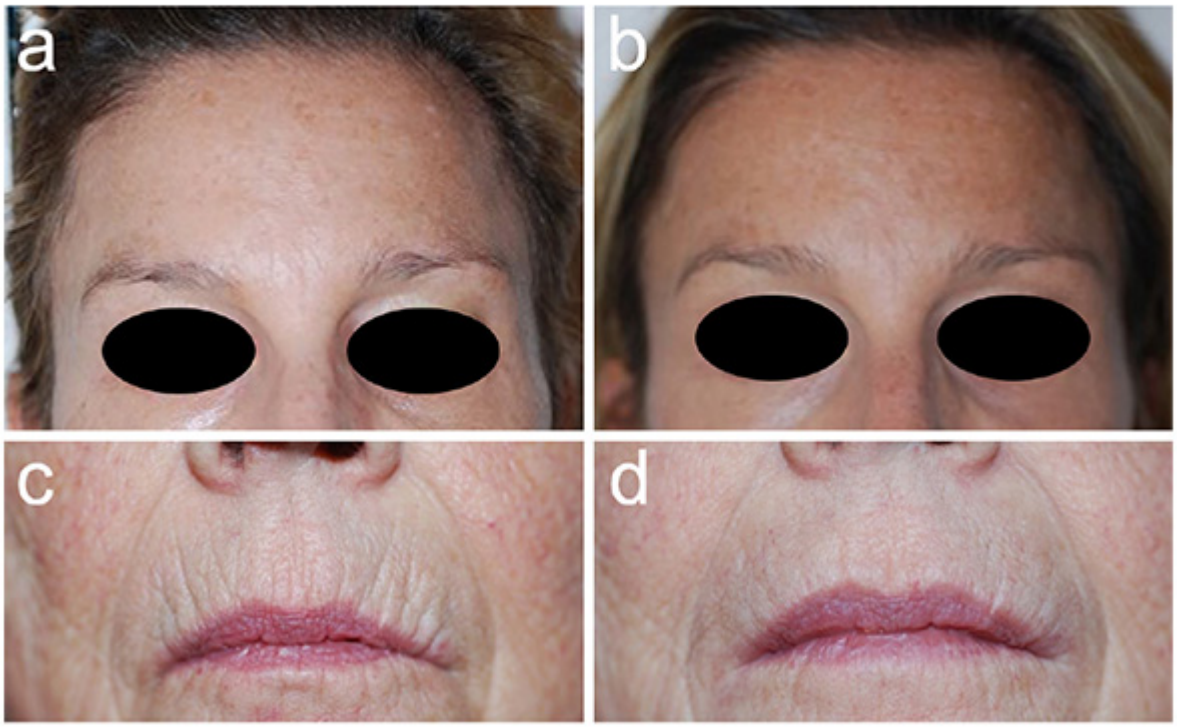
TABLE 3.
Zones Treated (listed in alphabetical order) and Percentage of Patients Treated According to Zone and Type of Product (total number of patients: 27)

Treated zones	Number of participants (%)			
	RHA 1	RHA 2	RHA 3	RHA 4
Zones treated				
Marionette lines	4 (14.8%)	6 (22.2%)	4 (14.8%)	
Lower part of NLF	2 (7.4%)	3 (11.1%)		
Labial commissure				2 (7.4%)
Cheek hollows				2 (7.4%)
Chin				1 (3.7%)
Temporal fossa				6 (22.2%)
Forehead lines	1 (3.7%)			
Glabella	2 (7.4%)	1 (3.7%)		
Upper part of NLF	1 (3.7%)	1 (3.7%)	3 (11.1%)	1 (3.7%)
White lip	1 (3.7%)	1 (3.7%)	1 (3.7%)	
Full NLF		3 (11.1%)	1 (3.7%)	
Oval of the face				1 (3.7%)
Posterior cheeks				1 (3.7%)
Orbital rim				1 (3.7%)
Procerus wrinkle		1 (3.7%)		
Perioral lines	5 (18.5%)	7 (25.9%)	1 (3.7%)	
Chin lines	1 (3.7%)	1 (3.7%)	1 (3.7%)	
Tear trough				2 (7.4%)

sub-periosteally, it is supple and fluid, and injects very easily without the need to apply too much pressure on the plunger.On a monitoring basis conducted over more than 6 months, it can be concluded that the gels from the RHA range blend into wrinkles and folds with excellent tissue integration. The results were satisfactory in terms of wrinkle filling and face volumetry and lasted for at least six months

(duration of the follow-up, Figure 6a and b). Treatments by very superficial injection technique known as "blanching technique" were successfully performed on 6 cases with the gels RHA 1 and RHA 2 with no Tyndall effects or appearance of visible or substantial cords (Figure 6c,d and 7).At no point were a nodule or inflammatory reaction observed. The treated area immediately appeared and remained supple and natural-looking for the duration of the follow-up, even for volumetry treatment with RHA 4.To our knowledge, the most effective gel for correcting the vermilion border is RHA 2. RHA 3 seemed to be the most effective for plumping up the lips.

FIGURE 6. Patient 17, injection of RHA 4 in the temple fossa (A) immediately and (B) 6 months after injection. Patient 22, injection of RHA 3 in the vermillion border and RHA 2 in the perioral area by the blanching technique (C) before and (D) 6 months after injection.



Regarding safety evaluation, after more than six months of monitoring, no immediate, intermediary, or late-onset inflammatory reaction of the skin, of whatever type, was observed following injection of the RHA range of products. Only injection site reactions were noted as mild ecchymosis. No hematoma was observed.

DISCUSSION

The RHA 2 gel ultrasound image appeared more systematized than that of PS-GA gel right after injection at D0. The gel could be described as being of better quality, as in ultrasound monitoring it presented an isoechoic profile in comparison with the nearby, uninjected dermis. Additionally, the papule had a homogeneous profile with no hypoechoic or hyperechoic area within it. The leakage of RHA 2 gel into the hypodermis for one of the two injection sites could be the result of an injection that was slightly deeper than the other; alternatively, it could be that a slightly greater amount of pressure was applied to the syringe plunger rod. In order to confirm or disprove this supposition, it would be interesting to conduct a similar study with injections performed manually on one group and on the other using a motorized injection system, whereby constant pressure is applied to the plunger rod.

FIGURE 7. Patient 13, injection of RHA 1 in the perioral area with the blanching technique, (A) immediately and (B) 7 minutes after injection.

The remaining products in the RHA range were found to be excellently suited to the indications described on their instructions for use. Each type of gel demonstrates excellent tissue integration in the superficial and mid reticular dermis, including when injected following the “blanching technique”. Caution must still be observed when injecting per the “blanching technique”, as very few patients were injected in such a manner.

CONCLUSION

The fact that one manufacturer supplies different ranges of products with similar indications can be a source of confusion for the injectors. In this study, 2 dermal fillers produced by the same manufacturer with the same approved indications were compared, one from an older product range and one from a newer product range.Laboratory testing as well as ultrasound monitoring and histological examinations succeeded in showing obvious differences between the 2 formulations. The newest formulation being repeatedly assessed as better quality.To our knowledge, this paper contains the first clinical evaluation in real practice conditions of the gels from the RHA range of products. With more than 20 years of experience with various HA gels marketed over this long period, the authors can say that under clinical observation over 6 months, RHA gels 1, 2, 3, and 4 present excellent tissue integration properties. The gels are very well adapted to their indications, with none demonstrating a cordon effect or Tyndall effect.

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DISCLOSURES

TEOXANE, located in Geneva, Switzerland, has exclusivelysponsored the work on the product Teosyal® RHA 2 upon requestby Dr. P. Micheels.

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