Nonanimal Stabilized Hyaluronic Acid for Lip Augmentation and Facial Rhytid Ablation

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Objective: To evaluate the effectiveness of nonanimal stabilized hyaluronic acid as an injectable filling agent.

Design: Nonrandomized, retrospective, interventional case series.

Results: A total of 1446 consecutive patients (1029 women and 417 men) underwent intradermal injection of commercially available nonanimal stabilized hyaluronic acid (2242 treatments) for the enhancement of lip volume and contour and the reduction of visible facial rhytids. Almost 61% of all patients remained satisfied with their results after 9 months. The effect was longest in the glabellar and nasolabial fold areas. Minimal transient sequelae were noted.

Conclusions: Nonanimal stabilized hyaluronic acid is an effective and safe facial soft tissue expander. Its duration varies with each facial area treated.


Hyaluronic Acid is a natural high-viscosity mucopolysaccharide in the human body. It is found in the umbilical cord, vitreous humor, synovial fluid, and pathologic joints and is a universal component of the spaces between the cells of body tissues. Pure hyaluronic acid is inherently biocompatible. It has the same chemical structure in all species and all tissues. Although the highest concentrations of hyaluronic acid are found in connective tissues, most hyaluronic acid is found in the skin.

Hyaluronic acid used as an intraocular filling agent has kept globes from collapsing during intraocular surgery since the 1970s. Cross-linked hyaluronic acid biocompatibility with dermal tissue was investigated in 1993. A hylan B gel molecule, isolated from rooster combs, was cross-linked to make it more viscous and longer lasting. When used to reconstruct traumatic superior sulcus deformities in monkeys, this gel maintained volume for more than 2 years (S.B., unpublished data, 1990-1995). Subsequently, it was used to reconstruct micro-ophthalmic and anophthalmic sockets (S.B., unpublished data, 1990-1995).

In 1996, a nonanimal stabilized hyaluronic acid (NASHA) gel became available in Europe. It is the product of fermentation and filtration, which yields a more purified hyaluronic acid. Use of this gel reduces the possibility of impurities and thus the incidence of sensitivity and allergic reactions.

We present our 6-year clinical experience in an oculoplastic clinic in Brazil using a commercially available NASHA gel product (Restylane; Q-Med AB, Uppsala, Sweden) as a temporary facial soft tissue expander and filling agent for the correction or amelioration of facial wrinkles and furrows and for the augmentation of lips.

Methods

Study participants were selected consecutively from a large pool of patients from an oculoplastic clinic (Oftalmoclinica Botafogo) in Rio de Janeiro. Before participating in the protocol, the patients were asked to sign an informed consent form permitting the investigators to perform the procedure and to photographically record the results. A detailed explanation of the potential risks, complications, and limitations of the proposed procedures was provided to each patient. There was no requirement for institutional review board approval for the study.

A total of 1466 consecutive patients (1029 women and 417 men) were selected to participate in the study. The mean ± SD patient age was 50.46 ± 10.23 years. No participants were treated previously with any permanent injectable filler. Having a history of previous facial surgery involving the areas of treatment or previous placement of permanent alloplastic facial implants...
was an exclusion criterion for participation in the protocol. Skin type based on the Fitzpatrick classification was recorded for each participant. Patients of each skin type were treated in a similar manner. Skin pretesting was not performed on the participants. The NASHA gel (Restylane) used for tissue augmentation consisted of NASHA, 20 mg/mL, in physiologic sodium chloride solution buffered to pH 7 in a gel form (250-mg/mL gel bead size and 100,000 U/mL) preloaded in a 0.7-mL glass syringe with a Luer-Lok fitting. A 30-gauge, 1.27-cm needle was supplied in the package.

Before treatment, a topical anesthetic containing tetracaine and lidocaine (Photocaine; University Pharmacy, Salt Lake City, Utah) was applied, unoccluded, for 20 minutes to the area to be treated. Localized ice compresses were applied after removal of the anesthetic cream.

The NASHA gel was injected into the superficial to middle dermal layer with the needle inserted bevel up. For linear depressions, serial punctures were combined with a linear threading technique. For broader depressions, fanning or cross-hatching techniques were used, supplemented with serial punctures. Rhytids were corrected to 100% of their depth, but without overcorrection. The maximum amount of “filler” used in a single area was 1.4 cm³. For lip augmentation, 1 or 2 techniques were used, depending on the patient’s desires. Linear threading was used to augment the lip vermilion border. The NASHA gel was then injected directly into the “body” of the lip in preselected locations to either change the shape and contour of the lip or to augment the volume of selected sections. The maximum amount of NASHA used in the lips was 0.7 cm³ per treatment session.

Immediately after the injections, to minimize the potential for ecchymosis, direct, constant pressure with ice compresses was applied to the injection sites until there was no evidence of bleeding. Massaging of the injected area was performed when necessary to attain the desired contour.

Participants were allowed to have treatment of more than 1 area of the face. A total of 2242 treatments of facial tissue augmentation were performed in 4 anatomic areas. Four groups were created on the basis of treatment location. The first group included 185 injections in the glabellar area for the reduction of visible static rhytids. This group differed from the other groups in that the participants were pretreated with the chemodenervation agent botulinum toxin type A (Botox; Allergan Inc, Irvine, Calif). The second group included 1020 treatments to reduce the depth of the nasolabial folds. In this group, 532 individuals had their lips and 264 had their oral commissures injected during the same treatment sessions. The third group included 352 oral commissure (“marionette line”) treatments (88 oral commissures were treated alone and 264 were treated with the nasolabial folds). The fourth group included 685 treatments for augmentation of the lips (153 lips were treated alone and 532 were treated with the nasolabial folds). The treatments and subsequent follow-up visits were performed by the same physician-investigators (S.B. and M.C.Z.).

Participants were evaluated 24 hours after the treatments, again after 1 and 2 weeks, and then at 3-month intervals for 9 months. During the first 2 weeks, touch-up injections were performed.

Evaluation of the results was based on investigator and participant input. To evaluate the results, 2 separate rating scales were implemented. Participants were asked to rate the results on a 3-level scale based on degree of satisfaction (unsatisfied, satisfied, and very satisfied). Investigator evaluation was based on clinical observation and comparison of pretreatment and posttreatment photographs of the areas of interest at each follow-up visit.

The physician rating scale for the glabellar, nasolabial fold, and oral commissure areas was based on 4 degrees of rhytid reduction ranging from 0 to 3 (0 indicates no improvement from the pretreatment condition; 1, minimal improvement; 2, moderate improvement; and 3, complete correction). For lip augmentation results, the evaluation was based on a pretreatment and posttreatment photographic comparison of volume and contour characteristics using a scale from 0 to 3 (0 indicates no effect; 1, minimal effect; 2, moderate effect; and 3, full volume and contour preservation).

An acceptable tissue volume-enhancing result was predefined as “satisfied” or “very satisfied” on the patient evaluation scale and as a score of 2 or 3 on the investigator scale.

The outcomes of 2242 NASHA treatments performed on 4 facial locations of 1446 patients were reviewed retrospectively. After initial touch-up sessions during the first 2 weeks after treatment, patients were evaluated by the investigators every 3 months for 9 months. The participants also provided their feedback regarding their degree of satisfaction.

After 3 months, 87.88% of participants were satisfied or very satisfied with their treatment result. After 6 and 9 months, 72.73% and 60.67% of participants, respectively, were satisfied or very satisfied (Table 1).

The physician evaluation of acceptable rhytid and furrow filling (score of 2 or 3 on the investigator scale) for all 4 groups was 95.40% at 3 months, 68.12% at 6 months, and 53.95% at 9 months. Complete correction (score of 3) at 3, 6, and 9 months was 58.24%, 36.41%, and 32.29%, respectively (Table 1).

The data were further analyzed by area treated (Figure 1 and Table 2). For the glabellar area, the patient satisfaction rates (satisfied and very satisfied) were 99.46%, 97.29%, and 88.11% at 3, 6, and 9 months, respectively. The investigator evaluation rates of acceptable effect (score of 2 or 3) were 100% at 3, 6, and 9 months. Full effect (score of 3) was 87.57%, 84.86%, and 76.22% at 3, 6, and 9 months, respectively (Figure 2).

For the nasolabial fold group, the rates of patient satisfaction (satisfied and very satisfied) were 99.81%, 97.35%, and 92.94% at 3, 6, and 9 months, respectively. The investigator evaluation rates of acceptable effect (score of 2 or 3) were 100%, 98.24%, and 93.63% at 3, 6, and 9 months, respectively. Full effect (score of 3) was 38.92%, 55.10%, and 52.94% at 3, 6, and 9 months, respectively (Figure 3).

### Table 1. Overall Physician Evaluation Scores and Patient Satisfaction Ratings After Nonanimal Stabilized Hyaluronic Acid Injections

<table>
<thead>
<tr>
<th>Variable</th>
<th>3 mo</th>
<th>6 mo</th>
<th>9 mo</th>
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<tr>
<td>Physician evaluation score*</td>
<td>0</td>
<td>9.93</td>
<td>19.34</td>
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<tr>
<td>1</td>
<td>4.60</td>
<td>21.95</td>
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<td>2</td>
<td>37.16</td>
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<td>3</td>
<td>58.24</td>
<td>36.41</td>
<td>32.29</td>
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<tr>
<td>Patient satisfaction rating</td>
<td>Unsat.</td>
<td>12.12</td>
<td>27.27</td>
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<tr>
<td>Satisfied</td>
<td>32.58</td>
<td>30.23</td>
<td>29.00</td>
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<tr>
<td>Very satisfied</td>
<td>55.30</td>
<td>42.50</td>
<td>31.67</td>
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*0 indicates no improvement/effect; 1, minimal improvement/effect; 2, moderate improvement/effect; and 3, complete improvement/effect.
Figure 1. Physician evaluation scores of nonanimal stabilized hyaluronic acid effect by treated facial area 3, 6, and 9 months after treatment. 0 Indicates no improvement/effect; 1, minimal improvement/effect; 2, moderate improvement/effect; and 3, complete improvement/effect.

Table 2. Physician Evaluation Scores and Patient Satisfaction Ratings After Nonanimal Stabilized Hyaluronic Acid Injections, by Area Treated

<table>
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<tr>
<th>Variable</th>
<th>Patients, No. (%)</th>
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<td><strong>Physician evaluation score</strong></td>
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<td>12 (1.18)</td>
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<td>1</td>
<td>5 (2.79)</td>
<td>22 (11.89)</td>
<td>0</td>
<td>18 (1.76)</td>
<td>53 (5.20)</td>
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<td>2</td>
<td>26 (14.05)</td>
<td>15 (8.11)</td>
<td>394 (38.63)</td>
<td>416 (40.78)</td>
<td>560 (54.90)</td>
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<td>3</td>
<td>158 (85.41)</td>
<td>148 (80.00)</td>
<td>624 (61.18)</td>
<td>577 (56.57)</td>
<td>388 (38.04)</td>
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<td>Unsatisfied</td>
<td>4 (1.14)</td>
<td>31 (8.80)</td>
<td>100 (28.57)</td>
<td>100 (28.57)</td>
<td>100 (28.57)</td>
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<td>Satisfied</td>
<td>266 (74.32)</td>
<td>106 (29.57)</td>
<td>272 (77.14)</td>
<td>272 (77.14)</td>
<td>272 (77.14)</td>
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<tr>
<td>Very satisfied</td>
<td>190 (51.65)</td>
<td>114 (32.05)</td>
<td>290 (82.35)</td>
<td>290 (82.35)</td>
<td>290 (82.35)</td>
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<td>Total</td>
<td>352 (100)</td>
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*0 Indicates no improvement/effect; 1, minimal improvement/effect; 2, moderate improvement/effect; and 3, complete improvement/effect.*
For the lip group, the patient satisfaction rates (satisfied or very satisfied) at 3, 6, and 9 months were 77.81%, 50.80%, and 36.64%, respectively. The investigator evaluation rates of acceptable effect (score of 2 or 3) were 96.65%, 40.43%, and 16.79% at 3, 6, and 9 months, respectively. Full effect (score of 3) was 76.50%, 5.69%, and 0% at 3, 6, and 9 months, respectively (Figure 4).

For the oral commissure group, the rates of patient satisfaction (satisfied or very satisfied) were 74.43%, 45.43%, and 25.00% at 3, 6, and 9 months, respectively. The investigator evaluation rates of full effect (score of 3) were 9.94%, 0%, and 0% at 3, 6, and 9 months, respectively. The rates for the combined scores of 2 and 3 were 84.94%, 33.81%, and 5.40% at 3, 6, and 9 months, respectively (Figure 5).

Figure 2. The appearance of the glabellar area in a 42-year-old woman. A, The patient had prominent glabellar furrows that improved but persisted after botulinum toxin type A injection. B, The patient experienced further improvement after furrow “filling” with nonanimal stabilized hyaluronic acid.

Figure 3. The appearance of the nasolabial fold area in a 39-year-old woman. A, The patient had moderate nasolabial fold depressions before treatment. B, The nasolabial folds were diminished after nonanimal stabilized hyaluronic acid injections.

Figure 4. The appearance of the lips in a 41-year-old patient. A, The patient had thin lips before treatment. B, Significant lip enhancement is evident after nonanimal stabilized hyaluronic acid injections.

Figure 5. The appearance of the oral commissure area in a 44-year-old woman. A, The patient had mild oral commissure depression and a prominent supramental crease before treatment. B, The oral commissures and supramental crease were improved after nonanimal stabilized hyaluronic acid augmentation.
The extent and duration of the filling effect and patient satisfaction with the result varied for each facial area treated. The filling effect was notably longer in the areas less affected by animation. Patients who had their glabellar areas treated attained the highest percentages of satisfaction and the longest-lasting satisfaction. The degree and duration of the correction in this area exceeded the duration of the botulinum toxin type A effect. The levels of correction in the other areas were, in decreasing order, the nasolabial folds, lips, and oral commissures. These observations highlight some related factors. The glabellar area was pre-treated with botulinum toxin type A injections to allow the implanted NASHA to remain in place without being displaced by the squeezing of the corrugator and procerus muscles and perhaps to encourage collagen remodeling. In contrast, high levels of patient satisfaction were obtained with NASHA injections to the lips; however, because of the continuous movement of this area, longevity was considerably less. Although NASHA injections to the oral commissures resulted in substantial cosmetic improvement, the levels of satisfaction were not as high as in other areas treated, perhaps because of the complex, multicontoured anatomy, muscle interaction, and movement of this area. In the future, combining NASHA filling with other treatment modalities in this area may increase effectiveness. Nevertheless, the overall effect of NASHA injection is longer lasting than that of injectable bovine collagen, which has a reported clinical effect of 4 to 6 months.6-12

In Europe, NASHA has been used as a temporary soft tissue expander since 1996 with satisfactory results.13-18 Until now, there has been limited published scientific literature about its recommended use and safety. The most common adverse effects reported to date are transient erythema and mild induration. There is 1 report of arterial embolism19 and 3 reports of granulomatous foreign body reaction20-22 after injection of hyaluronic acid. Friedman et al,23 in 2002, reviewed a large series of patients (an estimated 144,000 patients) treated with NASHA and reported a 0.06% incidence of adverse reactions resulting from its use, mainly localized hypersensitivity reaction. This reaction was believed to be secondary to trace amounts of proteins in the hyaluronic acid raw material.23

In our study, aside from transient erythema at the injection sites and infrequent bruising, these patients were free of adverse effects. One patient in 1996 and 1 patient in 1997 developed induration and sterile suppuration at injection sites 10 to 14 days after treatment. The first patient required drainage of the site, and the second patient was self-limited with warm compresses and topical corticosteroid cream.

The duration of the NASHA filling effect combined with the lack of substantial complications or skin pretesting makes NASHA an effective alternative to other temporary injectable fillers. We presented our experience with 1446 consecutive patients (2242 treated areas) treated with NASHA and documented the effectiveness and duration of its effect as a facial temporary soft tissue filling agent. At the end of 9 months, 33.95% of patients on the physician rating scale and 60.67% of patients on the patient rating scale noted a satisfactory effect. After 9 months, patient satisfaction was greater than 88% for the glabellar area and almost 93% for the nasolabial folds. The comparison of the effect of injectable NASHA vs other temporary injectable fillers by anatomic area is a subject for investigation by future studies.

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REFERENCES

15. Carruthers J, Carruthers A. A prospective, randomized, parallel group study analyzing the effect of BTX-A (Botox) and nonanimal sourced hyaluronic acid (NASHA, Restylane) in combination compared with NASHA (Restylane) alone in severe glabellar rhytides in adult female subjects: treatment of severe glabellar rhytides with a hyaluronic acid derivative compared with the derivative and BTX-A. Dermatol Surg. 2003;29:802-809.