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Long-Term Follow-Up of Longevity and Diffusion Pattern of Hyaluronic Acid in Nasolabial Fold Correction through High-Frequency Ultrasound

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Background: Injectable hyaluronic acid fillers have been widely applied in the clinical treatment of facial wrinkles. However, further information and clinical evidence concerning dermal changes and hyaluronic acid filler longevity after injection and diffusion pattern are limited.

Methods: The authors evaluated the longevity and diffusion pattern of two hyaluronic acid fillers generated by different cross-linking technologies used in the treatment of nasolabial folds using high-frequency ultrasound. Forty-one subjects were treated with Restylane 2 and the remaining 41 were treated with Dermalax DEEP. Wrinkle severity rating scale score and high-frequency ultrasound evaluation of nasolabial folds were performed before and after the injection of hyaluronic acid filler. The ultrasound images were acquired and analyzed to determine dermal thickness and the shape and distribution of hyaluronic acid filler.

Results: At 2 and 24 weeks from baseline, increased dermal thickness induced by hyaluronic acid filler treatment was not significantly different between groups. At 48 weeks after injection, increased dermal thicknesses of the Restylane 2 group (0.14 ± 0.12 mm) were much lower than those of the Dermalax DEEP group (0.20 ± 0.13 mm). Ultrasound examination revealed that hyaluronic acid materials form well-demarcated and hypoechogenic areas. Restylane 2 tended to form a more diffuse pattern, with multiple smaller bubbles, whereas Dermalax DEEP developed into a more localized configuration, with larger clumps.

Conclusions: This study is the first long-term assessment of nasolabial fold correction that reveals the performance of different hyaluronic acid materials in vivo and validates high-frequency ultrasound as a simple and rapid modality. Hyaluronic acid fillers generated by different cross-linking technologies display differential diffusion patterns in skin tissues. (*Plast. Reconstr. Surg.* 144: 189e, 2019.)

CLINICAL QUESTION/LEVEL OF EVIDENCE: Therapeutic, II.

Skin aging is associated with intrinsic, hormonal, and environmental factors in addition to chronic ultraviolet radiation. The

mechanical and morphologic appearance of the skin hinges largely on the connective tissue components, including collagen, fibroblasts, and elastic fibers. Accompanying the aging of skin, the dermal extracellular matrix is rearranged into a type of more sparsely interlinked collagen network with reduced connective tissue components.¹

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As an endogenous glycosaminoglycan, hyaluronic acid is an essential component of the skin and connective tissues. With high affinity for water, hyaluronic acid polymers play a critical role in mediating the hydration and flexibility of the skin. Recent study suggested that hyaluronic acid could promote fibroblast proliferation and collagen synthesis.² Accordingly, skin aging is naturally accompanied by hyaluronic acid reduction, which could accelerate the dehydration, loss of elasticity, and wrinkling of the skin.³ In the past few decades, injectable hyaluronic acid fillers have been widely applied in the treatment of facial wrinkles.⁴ Indeed, the cosmetic outcome is strongly associated with the longevity and distribution of hyaluronic acid products in skin tissues.⁵ However, there are limited details and clinical evidence regarding postinjection dermal changes in addition to the longevity⁶ and diffusion pattern of hyaluronic acid filler over time.

High-frequency ultrasound is a noninvasive soft-tissue imaging technology that can detect various echogenicities because of different tissue densities.⁷ With the ability to acquire high-resolution images and visualize changes of the skin and subcutaneous tissues, ultrasonographic evaluation is well applied in the assessment of the efficacy of injectable dermal fillers.⁸ Our current study is aimed at evaluating the longevity and diffusion of two hyaluronic acid fillers, Restylane 2 and Dermalax DEEP, generated by different hyaluronic acid cross-linking technologies in the treatment of nasolabial folds, and also to assess the effectiveness of high-frequency ultrasound.

PATIENTS AND METHODS

This study was designed to be randomized and patient and evaluator blinded, and the protocol was approved by the institutional review board. The ultrasonographic examinations were all performed at Peking Union Medical College Hospital (Beijing, People's Republic of China). A total of 84 subjects were enrolled in this study, all with written informed consent. For the inclusion criteria of the enrollment, subjects were healthy, with (1) age ranging from 25 to 65 years, (2) moderate to severe nasolabial folds, and (3) a Wrinkle Severity Rating Scale score of 3 or 4 (Table 1). Subjects with one of the following were excluded from the study: (1) history of allergy or hypersensitivity to filler materials, including hyaluronic acid and streptococcal protein; (2) dermatologic disease, including active infection or unhealed wound on the face; (3) the use of topical corticosteroid or

Table 1. Wrinkle Severity Rating Scale for Nasolabial Folds

Grade	Severity
1	Absent: no visible NLFs; continuous skin line
2	Mild: shallow but visible NLFs with a slight indentation
3	Moderate: moderately deep NLFs; visible at normal appearance but not when stretched
4	Severe: very long and deep NLFs; <2-mm visible NLFs when stretched; prominent facial features
5	Extreme: extremely deep and long NLFs; 2- to 4-mm V-shaped folds when stretched; detrimental to facial appearance

NLFs, nasolabial folds.

retinoic acid at the nasolabial folds within 4 weeks; (4) history of facial botulinum toxin injection, laser resurfacing, face-lift procedures, tissue augmentation, or chemical peels within 6 months; (5) facial cosmetic surgery or hyaluronic acid injection within 12 months before inclusion; (6) history of bleeding disorder or use of aspirin, nonsteroidal antiinflammatory drugs, warfarin, or other drugs that can prolong the coagulation time; (7) history of vitiligo or psoriasis; (8) systemic disease; (9) infectious disease including syphilis and acquired immune deficiency syndrome; (10) pregnancy or breast feeding; or (11) history of drug or alcohol abuse.

At the beginning, the enrolled subjects were randomly separated into two groups: (1) 42 subjects treated with Restylane 2 (Q-MED AB, Uppsala, Sweden) using a 30-gauge needle; and (2) 42 subjects treated with Dermalax DEEP (ACROSS Co., Seoul, Republic of Korea) using a 27-gauge needle. Dermalax DEEP had not yet been registered in China at the beginning of the study. A topical 5% lidocaine cream was applied to each side of nasolabial folds 1 hour before each injection. Hyaluronic acid filler was injected into each subject at the nasolabial folds by the same experienced dermatologist. The volume of hyaluronic acid filler that was injected into each side of the nasolabial folds ranged from 0.5 to 1 ml at a concentration of 20 mg/ml.

Wrinkle Severity Rating Scale scores of all subjects were evaluated (Table 1) by well-trained investigators before inclusion and at 2, 8, 16, 24, 36, and 48 weeks after injection. Photographs of nasolabial folds of all subjects were taken before injection and at 2, 8, 16, 24, 36, and 48 weeks after injection, and with shutting the mouth and with the same posture under constant environmental conditions by the same investigator and equipment. For ultrasound analysis, high-frequency ultrasound evaluation (3XSKIN; MEDA, Tianjin,

People's Republic of China) of nasolabial folds was performed at the 20 MHz before injection and at 2, 24, and 48 weeks after injection by the same investigator. The probe was placed perpendicular to the nasolabial folds with the subject in the lying position. The ultrasound images of each side of nasolabial folds were acquired and reviewed to determine the dermal thickness and the shape and distribution of hyaluronic acid filler. All data were analyzed by repeated measures analysis of variance using SAS 9.3 (SAS Institute, Inc., Cary, N.C.), and a value of $p < 0.05$ was considered to be statistically significant.

RESULTS

From October of 2016 to March of 2018, 82 of 84 enrolled subjects successfully completed the study. Forty-one subjects were treated with Restylane 2, whereas the other 41 subjects were treated with Dermalax DEEP. The mean age of the subjects was 44.99 years (range, 26 to 60 years). The mean age of patients in the Dermalax DEEP

group was 45.14 years (range, 26 to 60 years). The mean age of patients in the Restylane 2 group was 44.83 years (range, 28 to 60 years). In the Restylane 2 group, 21 subjects were defined as having a Wrinkle Severity Rating Scale grade of 3 and 20 were grade 4; in the Dermalax DEEP group, 24 subjects were defined as having a Wrinkle Severity Rating Scale grade of 3 and 17 were grade 4. For hyaluronic acid injection, the average volume into each side of nasolabial folds was 0.76 ml of Restylane 2 and 0.68 ml of Dermalax DEEP. The two groups did not display any significant differences in the Wrinkle Severity Rating Scale grade and hyaluronic acid volumes.

As expected, the skin exhibited a three-layer structure by analysis with high-frequency ultrasound (Fig. 1, *above, left*). Interestingly, the epidermis presented as a bright hyperechogenic line with a mean thickness of 0.15 mm, and the dermis appeared to be a homogenous hypoechoic band with a hyperechogenic area beneath it. Two weeks after treatment, the dermis was obviously noted to be thicker, with less echogenicity (Fig. 1,

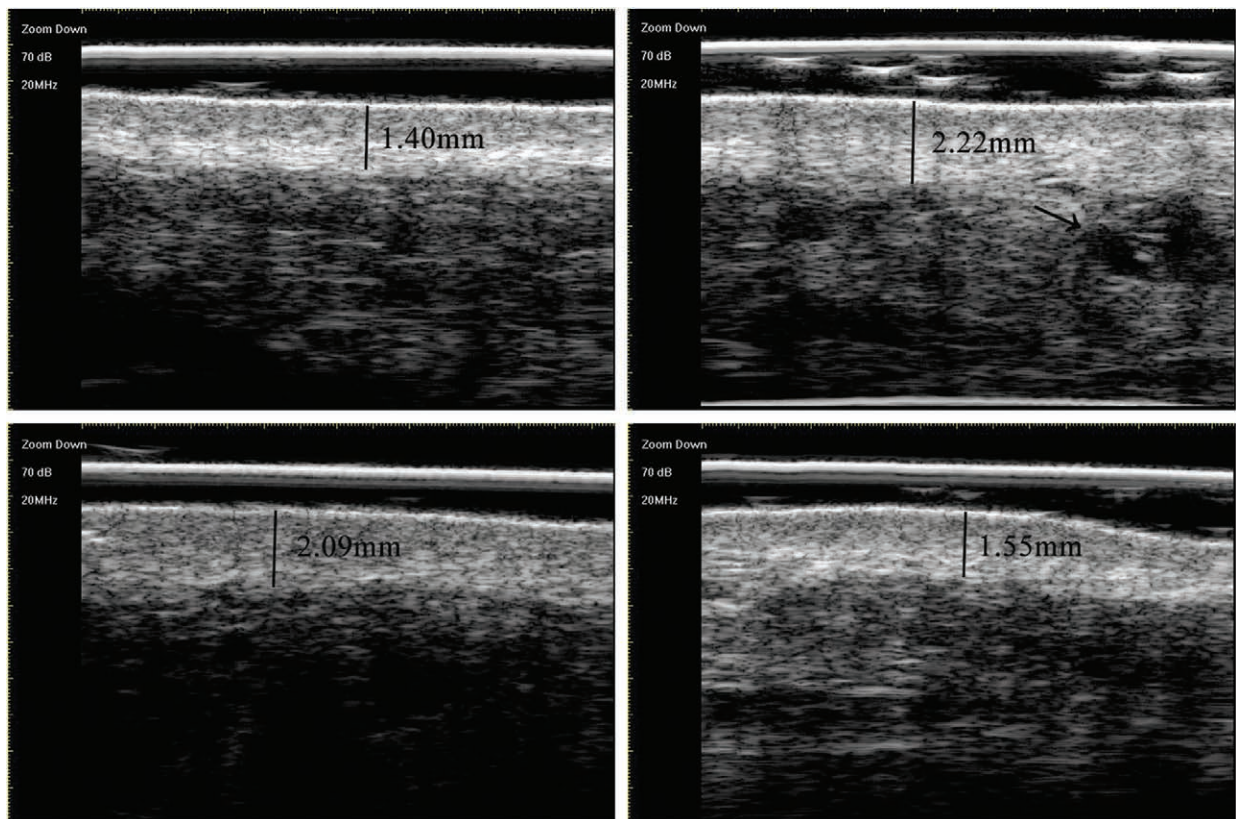


Fig. 1. Human skin was revealed as a three-layer structure by means high-frequency ultrasound imaging. (*Above, left*) Before treatment, the dermis appeared as a homogenous hypoechoic band with a hyperechogenic area beneath it and the epidermis as a bright hyperechogenic line. The dermis displayed increased in thickness with lower echogenicity, (*above, right*) two weeks, (*below, left*) 24 weeks, and (*below, right*) 48 weeks after treatment. Hyaluronic acid materials presented as well-demarcated, homogeneous, hypoechoic, or nonechogenic areas (*black arrow*).

above, right). Meanwhile, the results of Wrinkle Severity Rating Scale also revealed decreases of three grades in 22 subjects, two grades in 50 subjects, and one grade in 10 subjects 2 weeks after injection (Fig. 2). The data regarding dermal thickness are summarized and presented in Figure 3.

The dermal thickness of all subjects before treatment was 1.45 ± 0.16 mm (minimum, 1.03 mm; maximum, 1.84 mm). Impressively, significant elevations of dermal thickness were observed in all subjects after hyaluronic acid injection (Fig. 1, above, right, and below, left and right). The increase in dermal thickness from baseline was determined to be 40.29 percent at 2 weeks, 21.34 percent at 24 weeks, and 11.74 percent at 48 weeks after injection (Fig. 3, left). However, a dramatic reduction of dermal thickness was observed at 48 weeks after injection compared with that at 24 weeks. Repeated measures analysis of variance showed significant differences ($p < 0.001$) in all subjects at 2, 24, and 48 weeks after injection, and demonstrated a time effect in the increased dermal thickness.

At 2 and 24 weeks after injection, the increased dermal thicknesses were 0.57 ± 0.20 and $0.27 \pm$

0.18 mm in the Restylane 2 group and 0.59 ± 0.15 and 0.34 ± 0.14 mm in the Dermalax DEEP group, respectively (Fig. 3, right). Both Restylane 2 and Dermalax DEEP injection resulted in satisfactory cosmetic outcomes in nasolabial fold correction within 24 weeks after injection. At 48 weeks after injection, however, increased dermal thickness in the Restylane 2 group (0.14 ± 0.12 mm) was much less than that of the Dermalax DEEP group (0.20 ± 0.13 mm). Variation between groups was demonstrated to be statistically significant ($p < 0.01$), with a linear trend of time between groups ($p < 0.05$). These findings indicate that relative to Dermalax DEEP, Restylane 2 may be more prone to be diffused or degraded and displays much shorter longevity in vivo. Compared with 2 weeks after treatment, the average Wrinkle Severity Rating Scale scores of the Dermalax DEEP and Restylane 2 groups increased 0 and 0.107 at 24 weeks and 0.293 and 0.327 at 48 weeks, respectively. There was no significant difference on Wrinkle Severity Rating Scale scores at 24 and 48 weeks between the two groups. However, given that the Wrinkle Severity Rating Scale scores are not sufficiently detailed, the possibility of clinically significant differences may not be excluded.

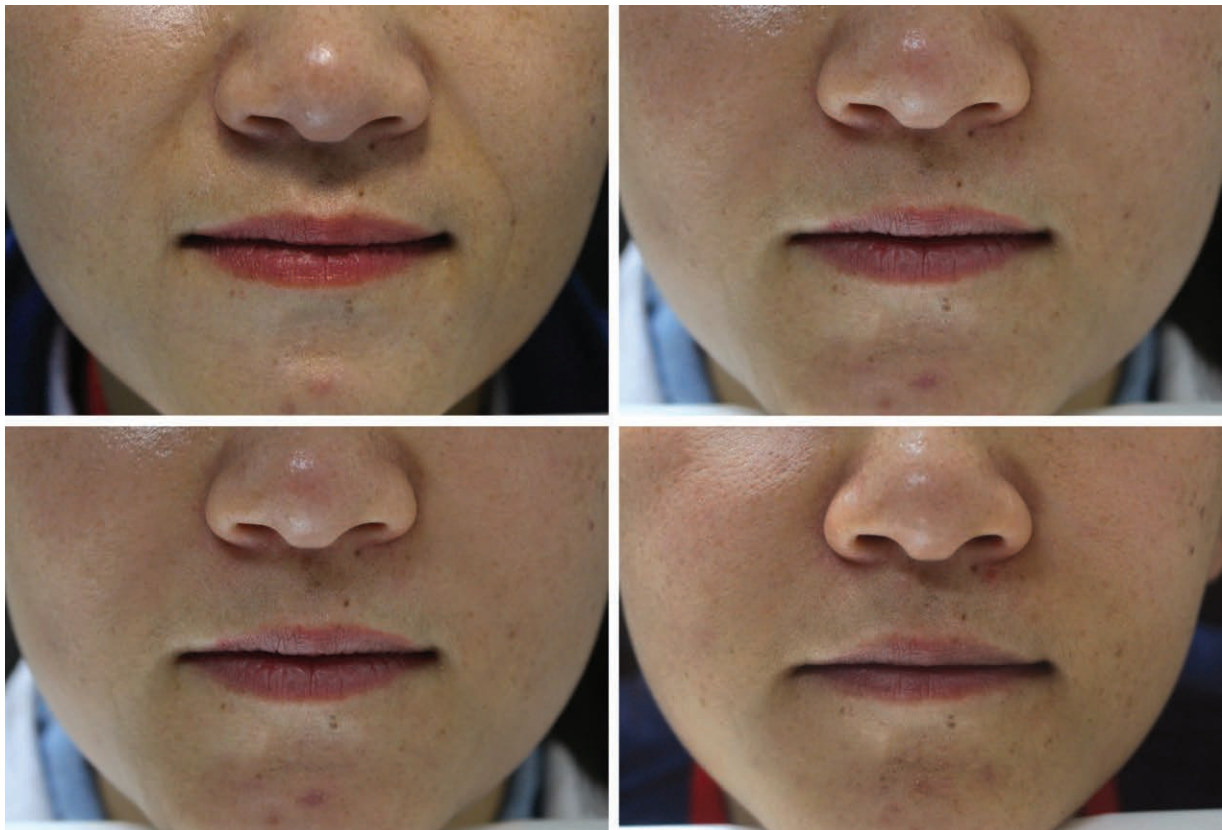


Fig. 2. Clinical representative images (above, left) before and (above, right) 2 weeks, (below, left) 24 weeks, and (below, right) 48 weeks after hyaluronic acid injection.

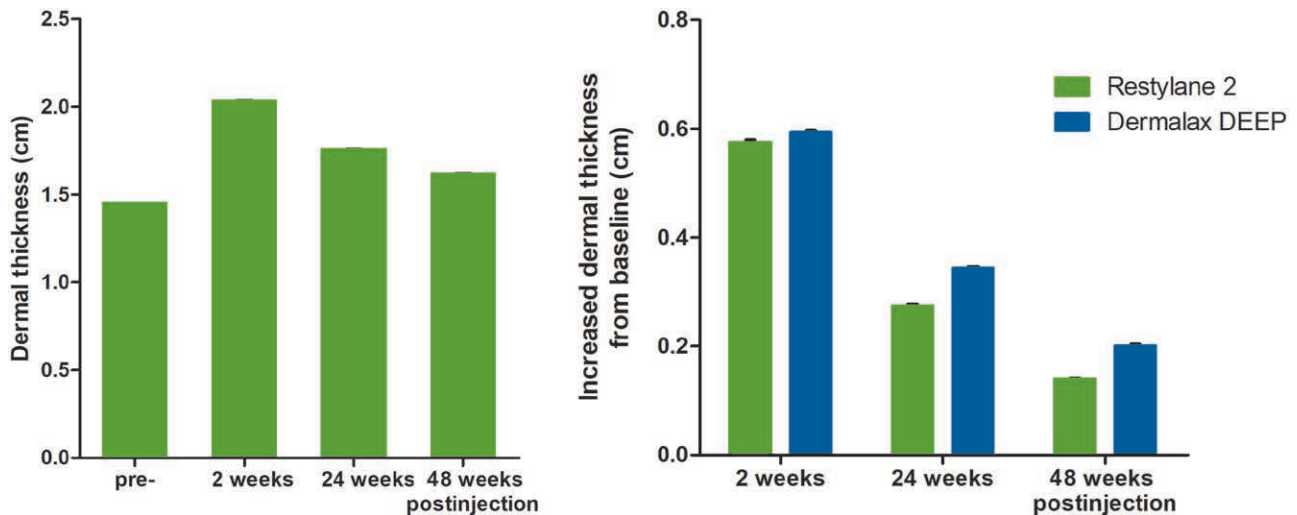


Fig. 3. The thickness (mean \pm SE) of dermis was measured using high-frequency ultrasound. (Left) The dermis displayed significantly increased thickness at 2, 24, and 48 weeks after hyaluronic acid injection. (Right) The dermal thickness of the two groups showed similar changes at 2 and 24 weeks. At 48 weeks after injection, the dermal thickness of the Dermalax DEEP group increased compared with the Restylane 2 group (repeated measures analysis of variance).

Because of different echogenicities and distinct boundaries along with the surrounding tissues, hyaluronic acid materials of both the Restylane 2 and Dermalax DEEP groups could be detected and visualized using ultrasound.⁹ In fact, hyaluronic acid materials were found to localize in the subcutaneous tissues and present as well-demarcated, homogeneous, hypoechogenic and nonechogenic areas (Fig. 1, *above, right*). Images obtained at 2, 24, and 48 weeks after injection clearly demonstrated the diffusion and distribution patterns of hyaluronic acid materials over time (Fig. 4). The results revealed that Dermalax DEEP developed into a localized configuration and broke up into hypoechogenic clumps with slightly degraded sizes over time. However, Restylane 2 tended to spread more widely and form a more diffuse pattern, with multiple smaller bubbles in the skin tissues compared with Dermalax DEEP.

DISCUSSION

Because it is a safe and natural substance, with a reversible procedure, hyaluronic acid filler injection is currently a widely used approach for facial wrinkle correction.¹⁰ However, hyaluronic acid usually exhibits a short lifespan, as the material is rapidly degraded in skin tissues.¹¹ Therefore, the longevity of hyaluronic acid products in skin tissues is considered the key factor that impacts cosmetic outcome. An increasing number of methods are applied to determine the longevity

of hyaluronic acid fillers in vivo, including histology, cytochemistry, magnetic resonance imaging, electron microscopy, and high-frequency ultrasound.¹² High-frequency ultrasound is a noninvasive approach for visualizing hyaluronic acid fillers and dermal modifications that can be performed without skin biopsies. Moreover, dermal changes over a long-term period can be monitored and quantified by the use of high-frequency ultrasound, in the event of cyst or granuloma induced by hyaluronic acid filler treatment. Furthermore, compared with magnetic resonance imaging, high-frequency ultrasound can display dynamics of hyaluronic acid diffusion beneath the skin surface, and is much more easy to perform.

The Wrinkle Severity Rating Scale is a validated five-point reference scale for classifying facial wrinkles, and it is widely used in a growing number of investigational studies. Thus, the Wrinkle Severity Rating Scale for nasolabial folds enables valid assessment and represents a clinically meaningful change in nasolabial fold severity. In the current study, the results of Wrinkle Severity Rating Scale scores confirmed satisfactory cosmetic outcomes in nasolabial fold correction and were also consistent with our observations by ultrasound examination.

Our high-frequency ultrasound examination data revealed that the skin displayed a three-layer structure, which is in accordance with previous study.¹³ Normally, the epidermis presented as a bright hyperechogenic line and the dermis appeared as a homogenous hypoechogenic band

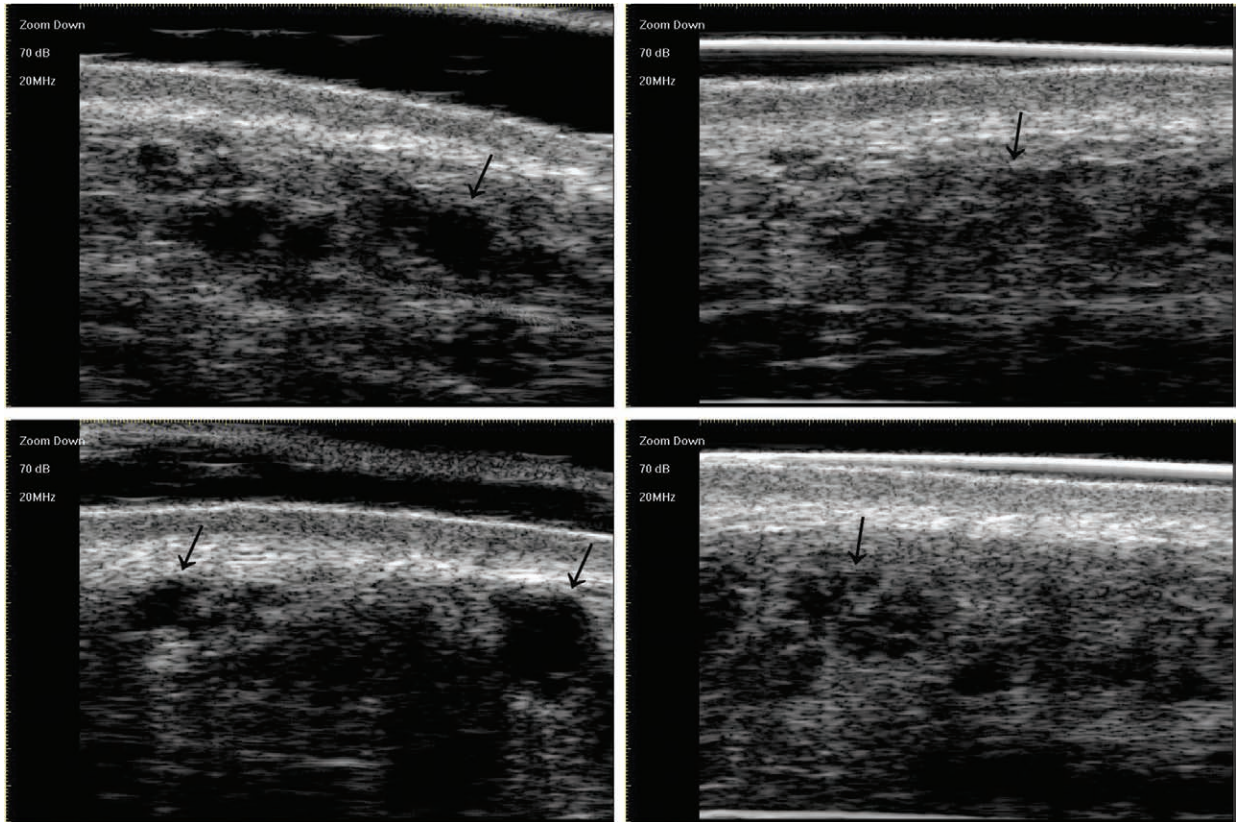


Fig. 4. Hyaluronic acid distribution in vivo was visualized by high-frequency ultrasound (*black arrow*). Representative high-frequency ultrasound images revealed that Restylane 2 tended to spread more widely and form a more diffuse pattern, with multiple smaller bubbles (*above, left, 2 weeks; above, right, 24 weeks* after injection); and Dermalax DEEP developed into a more localized configuration and broke up into hypoechoic clumps, with slightly degraded sizes (*below, left, 2 weeks; below, right, 24 weeks* after injection).

with a hyperechogenic area beneath it. After injection, the dermis became much thicker, with lower echogenicity in all subjects. It has been considered that hyaluronic acid fillers improve the aesthetic appearance by means of filling space.³ It is perfectly confirmed by our ultrasound examination, which identified injected hyaluronic acid materials as well-demarcated, homogeneous, hypoechoic or nonechogenic areas in skin tissues. The hydration effect and the induction of collagen synthesis¹⁴ by hyaluronic acid materials are also hypothesized to contribute to maintenance of cosmetic benefits. It is worth noticing that dermal thickness at 48 weeks after injection showed an obvious reduction in comparison with that at 24 weeks after injection, suggesting that injected hyaluronic acid fillers might be diffused, reabsorbed, degraded, or fragmented in skin tissues.

The longevities of two different hyaluronic acid fillers were determined in our current study. At 2 or 24 weeks after injection, the increased dermal thickness induced by Restylane 2 injection was not significantly different compared to Dermalax

DEEP, indicating that both hyaluronic acid fillers provided satisfactory cosmetic outcomes in nasolabial fold correction. However, the increased dermal thickness at 48 weeks after injection was significantly different between the two groups, suggesting that, relative to Restylane 2, Dermalax DEEP is not easy to diffuse or be degraded and has greater longevity in vivo.

By high-frequency ultrasound, we also evaluated the differences of diffusion patterns between Restylane 2 and Dermalax DEEP in vivo. Our data revealed that Restylane 2 tended to form a more diffuse pattern, with multiple smaller bubbles, whereas Dermalax DEEP developed into a more localized configuration, with larger clumps. These results are consistent with our clinical experience with hyaluronic acid filler injection. Restylane 2 is relatively smooth for correcting facial wrinkles and has better performance in superficial wrinkle correction. In contrast, Dermalax DEEP has a more powerful and long-lasting effect of physical volume occupation, and thus is more suitable for deep wrinkle improvement and facial contour

correction. In addition, Restylane 2 is much easier for injection and molding of fillers compared with Dermalax DEEP. In clinical practice, the selection of hyaluronic acid filler products usually depends on individual specific requirements. Our current study might provide more helpful clues for the selection and clinical application of hyaluronic acid products in padding treatment.

In consideration of the hyaluronic acid clumps on the ultrasound images, we observed palpable but invisible nodules in the nasolabial folds from both groups. There were four reports of palpable nodules from Dermalax DEEP, with three nodules regressing within 1 month and the other one remaining for 9 months. In the Restylane 2 group, one nodule was reported regressing in 1 week and another one remained for 6 months. In addition, high-frequency ultrasound could also be used in the clinical application for the differentiation of potential confused occurrences, including nodules, granulomas, or other adverse reactions.

The cross-linking technology and percentages have major influences on the longevity and diffusion pattern of hyaluronic acid fillers in vivo.¹⁵ Restylane 2 and Dermalax DEEP are both derived from nonanimal hyaluronic acid substances that are synthesized by *Streptococcus* species biological fermentation. The enhanced stability of hyaluronic acid could be generated when the hyaluronic acid molecules are cross-linked by means of 1,4-butanediol diglycidyl ether.¹⁶ Compared with Restylane 2, Dermalax DEEP has a greater cross-linking percentage and a larger particle size because of different 1,4-butanediol diglycidyl ether bonding technology. These differential properties result in variations of rheologic characteristics and thus clinical performance in Restylane 2 and Dermalax DEEP.¹⁷

The present study has some limitations. First, we could not confirm that the hyaluronic acid product was injected at the same depth in all subjects. However, we have three ways of reducing interference through the (1) resistance of injection, (2) outline of the needle, and (3) elevation of the skin. Moreover, during preexperiment study, we evaluated the depth of injection through high-frequency ultrasound immediately after injection, which revealed that all of the hyaluronic acid materials were in the middle and lower dermis. Second, the needles used and the average volume injected in the two groups were different. The hyaluronic acid volume injected into each side of nasolabial folds was scaled from 0.5 to 1 ml. The specific volume depended on satisfactory cosmetic outcomes in each subject.

Third, we did not include a more powerful product with a longer lasting effect or a softer and less robust product in this study. In addition, further studies are required to illustrate the utility of high-frequency ultrasound in the differentiation of potential confused occurrences, including nodules, granulomas, or other adverse reactions.

CONCLUSIONS

The current study is the first long-term assessment of nasolabial fold correction, which visualized hyaluronic acid material and its subsequent performance in vivo, and also validated high-frequency ultrasound as a simple and rapid modality for follow-up assessment. This large series illustrates our clinical and ultrasonographic observations with respect to the dermal changes and hyaluronic acid filler longevity and diffusion pattern, which complement previous studies. Injectable hyaluronic acid fillers generated by different cross-linking technologies displayed differential diffusion patterns in the skin tissues. This study would be of great assistance for the selection and clinical application of hyaluronic acid products in padding treatment.

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