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Evaluation of changes in skin biophysical parameters and appearance after pneumatic injections of non-cross-linked hyaluronic acid in the face

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ABSTRACT

Background: Pneumatic injections of non-cross-linked hyaluronic acid are effective in skin rejuvenation, however, the associated biophysical parameters and appearance have not been evaluated. **Objectives**: To determine the changes in skin biophysical parameters after facial pneumatic injections of non-cross-linked hyaluronic acid. **Patients and methods**: Twenty-eight healthy female volunteers received pneumatic injections of non-cross-linked hyaluronic acid into the face for consecutive 5 weeks. Skin biophysical parameter assessment and clinical evaluation were performed using the CK Multi-Probe Adapter and Visia system. Five of the volunteers also underwent retroauricular skin biopsy before and after the last treatment. The skin tissues were all stained with Masson-trichrome, Verhoeff-van Gieson stain, and hematoxylin-eosin to evaluate the changes in collagen, elastic fibers, and the epidermis, before and after the last treatment. **Results**: Transepidermal water loss was significantly lower in week 4 (18.46 ± 4.70 g/h/m^2) than at the baseline (22.03 ± 7.15 g/h/m^2, $p < 0.05$). Skin texture was better in week 4 (599.29 ± 354.32) than at the baseline (668.43 ± 342.55, $p < 0.05$). Skin pores also improved significantly at week 4 (934.07 ± 458.78) compared to the baseline (1024.57 ± 415.31, $p < 0.05$). Skin wrinkles were improved at the 3-month follow-up (29.29 ± 11.11) compared to the baseline (35.83 ± 16.05, $p < 0.05$). **Conclusion**: Pneumatic injections of non-cross-linked hyaluronic acid improved skin TEWL, texture, pores, and wrinkles.

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KEYWORDS
Non-cross-linked hyaluronic acid; pneumatic injection; facial rejuvenation; biophysical parameters

Introduction

Photoaging of the skin on the face caused by ultraviolet radiation is manifested as facial sagging, decreased skin elasticity, uneven pigmentation, and the development of erythema, brown spots, and a leathery appearance (1). Several methods have been used to ameliorate skin photoaging, such as intense pulsed light and fractional laser treatments, which promote collagen and elastin formation (2,3). Subcutaneous injections of fillers such as stabilized hyaluronic acid of non-animal origin and autologous fat, stromal, and stem cells, and platelet-rich plasma have also been used to effectively improve skin photoaging (4–6).

Due to its high biocompatibility, reversibility, longevity, and high hydrophilicity, hyaluronic acid is the most widely used dermal filler (7,8). By altering its particle size, cross-link density, and viscosity, hyaluronic acid can be rendered suitable for injection in different skin layers. Usually, low-density, less-viscous hyaluronic acid is used for the superficial dermis; small gel particles with medium density are used for the mid-dermis; and large particles with high density are used for the deep dermal, subcutaneous, and even supraperiosteal layers (7). Hyaluronic acid is commonly injected using manual syringes or stamp-type multineedle injectors.

JetPeel-3V is a pneumatic device that injects cosmetic products into the skin with high-pressure air flux at speeds of up to 200 m/s while keeping the epidermis intact (9,10). The pneumatic device has been used to inject drugs and vaccines to a controlled depth into the skin, in a minimally invasive and pain-free manner (11). Since the technique was discovered to promote wound healing and induce collagen remodeling, it has been widely used for the cosmetic application of hyaluronic acid, botulinum, vitamins, etc., to minimize acne scars, depressed scars, keloids, and wrinkles (12–16). However, the associated skin biophysical parameters and appearance after such injections have not yet been investigated. Therefore, in this study, we aimed to determine the alterations that occur in skin biophysical parameters and appearance after hyaluronic acid injections. To this end, we evaluated the changes in skin biophysical parameters in 28 healthy female volunteers who received pneumatic injections of non-cross-linked hyaluronic acid once a week for 5 weeks and followed up at 1 month and 3 month later.

Materials and methods

Materials

Non-cross-linked hyaluronic acid (Biohyalux, Bloomage BioTechnology Corporation Limited, Jinan, China; molecular weight, 3000–10,000 D) was used as the rejuvenation material.

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This drug was supplied in the form of a colorless transparent liquid, sealed in an ampoule. The face was cleaned with normal saline before the injection of hyaluronic acid with the JetPeel-3V device (Tav-Tech, Israel). The CK Multi-Probe Adapter 580 (Courage+Khazaka Electronic GmbH, Germany) was used to measure skin hydration, transepidermal water loss (TEWL), elasticity, melanin index, and erythema index. The VISIA facial imaging system (Canfield Company, USA) was used to assess skin texture, wrinkles, spots, and pores.

Subjects and study design

This was a single-center, evaluator-blinded, historical control trial. Between November, 2015 and July 2016, 28 healthy female volunteers (age range, 35–65 years) were given pneumatic injections of non-cross-linked hyaluronic acid once a week for 5 weeks. All participants were Asian, with skin types III–V according to the Fitzpatrick classification. The study was approved by the institutional review board of Beijing Friendship Hospital Ethics Committee (NO. 2015-P2-112–01). All volunteers were provided written informed consent before participating in the study as well as permission to publish their photographs.

Selection criteria

Volunteers were excluded if they were aged below 35 years or above 65 years, or had hypersensitivity to hyaluronic acid or an active skin disease. Participants were also excluded if they had received oral steroids, collagen powder/liquid, or topical hyaluronic acid in the last three months, had undergone a skin-rejuvenation procedure in the last three months or within three months after the last study treatment, were pregnant or breastfeeding, or had severe cardiovascular, cerebrovascular, renal, or hepatic disease.

Treatment protocol

Before the treatment, the volunteers cleaned their faces and rested in a quiet environment for 30 min. Then, analyses with the VISIA and CK systems were performed. In the case of the CK system, measurements were taken at a site 2 cm below the lateral canthus of each eye in a temperature-controlled room maintained at 25°C and 50% humidity. The VISIA images and counts were taken in the front side range which includes forehead, cheeks, and were drawn and counted by the device itself.

With the participant lying supine on the bed, we used JetPeel-3V to clean the whole face with normal saline. The air–fluid mixture spouting from the nozzle of the device was directed against the skin surface, and the distance could be controlled within 5–7 cm. After the cleaning, normal saline was replaced with 5 mL hyaluronic acid to complete the rejuvenation procedure; this time, the distance between the nozzle and the skin surface was decreased to 1 cm. All five treatment sessions in each volunteer were performed by the same doctor.

Assessments

Changes in skin biophysical parameters were evaluated before each of the five treatments, and at 1 and 3 months after the last treatment. The CK multi-probe adapter was used to measure skin hydration, TEWL, skin elasticity, and the melanin and erythema indices. The VISIA facial imaging system was used to assess skin texture, wrinkles, spots, and pores. In addition, each participant was asked about their subjective satisfaction with skin smoothness, skin moisture, skin brightness, pore size, skin spots, and skin redness after the last treatment. Finally, the Global Aesthetic Improvement Scale (GAIS) was used to evaluate the participant and dermatologist satisfaction with the treatment outcomes. The GAIS is a five-point scale that ranks treatment outcomes as follows: 1, highly improved; 2, much improved; 3, improved; 4, no change; and 5, worse than before. Three dermatologists, who were not involved in the administration of the skin-rejuvenation treatments, assessed and scored photographs of the subjects taken before the first treatment and after the last treatment, in a blinded manner. Similarly, the participants also scored their own photographs.

Histologic examination

Before treatment, left retroauricular skin tissue was harvested from five volunteers, who received the same treatment on the right retroauricular skin as that given on the face. After the last treatment, symmetrical right retroauricular skin tissue samples were obtained from these volunteers. The skin tissue samples were fixed in formalin, dehydrated, embedded in paraffin, cut into 4-μm-thick slices, and dewaxed. The samples were then stained with Masson-trichrome, Verhoeff-van Gieson (EVG), and hematoxylin-eosin stains to evaluate the changes in collagen fibers, elastic fibers, and epidermal thickness, respectively, before and after the last treatment.

Statistical analyses

The SPSS 17.0 software was used to perform statistical analyses. Repeated-measures analysis of variance was used to compare the results among the five treatments. The independent-samples t-test was used to compare the results between the baseline and follow-up examinations. $p < 0.05$ was considered statistically significant.

Results

General information

All 28 volunteers (median age, 42 years) completed the five-session treatment protocol, and we compared the results among all five treatments. However, only 26 volunteers attended the 1-month follow-up, and 24 attended the 3-month follow-up; thus, the follow-up results were compared with the baseline.

Changes in skin biophysical parameters

Comparisons of skin biophysical parameters between each treatment, and between the baseline and follow-up examinations are presented in Tables 1–3. After three treatment

Table 1. Changes in biophysical parameters and appearance after five sessions of treatment with pneumatic hyaluronic acid injections, as assessed using the CK and VISIA systems, in the 28 volunteers in our study.

		Week 2		Week 3		Week 4		Week 5	
Parameter	Week 1	Mean ± SD	*p*	Mean ± SD	*P*	Mean ± SD	*p*	Mean ± SD	*p*
Skin hydration	75.99 ± 8.77	75.31 ± 11.09	0.73	77.47 ± 10.89	0.49	75.75 ± 13.19	0.91	77.00 ± 11.30	0.57
TEWL (g/h/m^2)	22.03 ± 7.15	19.82 ± 5.58	0.10	19.94 ± 5.43	0.10	18.46 ± 4.70	0.00	18.51 ± 5.12	0.00
Melanin index	137.11 ± 21.20	135.07 ± 21.13	0.43	137.00 ± 19.53	0.96	136.63 ± 19.59	0.88	139.50 ± 23.41	0.40
Erythema index	316.75 ± 82.13	311.80 ± 87.06	0.62	303.29 ± 74.62	0.06	295.23 ± 68.78	**0.02**	313.96 ± 80.93	0.77
R^2	0.54 ± 0.11	0.53 ± 0.09	0.67	0.51 ± 0.10	0.32	0.52 ± 0.09	0.58	0.52 ± 0.09	0.48
Texture	668.43 ± 342.55	647.79 ± 420.21	0.49	612.36 ± 385.53	0.11	599.29 ± 354.32	**0.02**	579.71 ± 361.48	**0.00**
Wrinkles	36.93 ± 15.27	34.64 ± 18.33	0.51	36.64 ± 14.13	0.93	36.50 ± 12.52	0.88	31.46 ± 13.65	0.11
Spots	231.61 ± 114.22	208.46 ± 44.69	0.23	205.04 ± 43.24	0.22	203.96 ± 46.68	0.16	200.11 ± 45.42	0.11
Pores	1024.57 ± 415.31	982.93 ± 454.66	0.13	965.00 ± 455.39	0.06	934.07 ± 458.78	**0.00**	927.00 ± 442.45	**0.00**

Values are expressed as mean and standard deviations.
R^2 values indicate skin elasticity. The closer the value is to 1, the better the skin elasticity.
All *p*-values are for comparisons with the results obtained in week 1. *p*-values indicating significant differences (i.e., $p < 0.05$) from the week-1 results are shown in bold.

Table 2. Differences in biophysical parameters and appearance between week 1 and the 1-month follow-up, as measured using the CK and VISIA systems ($n = 26$).

Parameter	Week 1	Follow-up at 1 month	*p* value
Hydration	75.98 ± 8.76	78.35 ± 9.49	0.23
TEWL (g/h/m^2)	22.28 ± 7.34	17.59 ± 5.94	**0.00**
Melanin index	137.67 ± 21.92	143.35 ± 30.29	0.15
Erythema index	321.04 ± 83.51	316.90 ± 77.90	0.67
R^2	0.54 ± 0.12	0.49 ± 0.09	0.06
Texture	674.96 ± 355.05	618.58 ± 308.42	**0.03**
Wrinkles	37.50 ± 15.54	32.88 ± 12.08	0.14
Spots	234.38 ± 118.22	206.46 ± 43.19	0.19
Pores	1026.31 ± 415.36	951.19 ± 432.10	0.01

The above results were obtained from 26 volunteers who attended all five treatment sessions as well as the 1-month follow-up assessment.
R^2 values indicate skin elasticity. The closer the value is to 1, the better the skin elasticity.
Values are expressed as mean and standard deviation. Significant differences ($p < 0.05$) are shown in bold.
TEWL, transepidermal water loss

Table 3. Differences in biophysical parameters and appearance between week 1 and the 3-month follow-up, as assessed using the CK and VISIA systems ($n = 24$).

Parameter	Week 1	Follow-up at 3 months	*p* value
Hydration	76.03 ± 9.10	83.07 ± 11.13	**0.00**
TEWL (g/h/m^2)	21.78 ± 7.61	15.08 ± 3.03	**0.00**
Melanin index	136.83 ± 22.46	142.21 ± 26.89	0.13
Erythema index	317.92 ± 88.09	324.21 ± 80.74	0.43
R^2	0.54 ± 0.11	0.55 ± 0.05	0.90
Texture	672.04 ± 365.46	707.63 ± 433.20	0.31
Wrinkles	35.83 ± 16.05	29.29 ± 11.11	**0.03**
Spots	239.37 ± 121.78	212.04 ± 45.74	0.25
Pores	1005.71 ± 436.68	943.92 ± 435.01	**0.03**

The above results were obtained from 24 volunteers who attended all five treatment sessions as well as the 1- and 3-month follow-up assessments.
R^2 values indicate skin elasticity. The closer the value is to 1, the better the skin elasticity.
Values are expressed as mean and standard deviation. Significant differences ($p < 0.05$) are shown in bold.
TEWL, transepidermal water loss

sessions, TEWL significantly decreased from 22.03 g/hm^2 in week 1 to 18.46 g/hm^2 in week 4 ($p = 0.00$). Moreover, at 1 month (17.59 ± 5.94 g/hm^2) and 3 months (15.08 ± 3.03 g/hm^2) after the last treatment, the reduction in the TEWL value remained significant ($p < 0.05$). There was no significant improvement in skin hydration until 3 months after the last treatment. At the 3-month follow-up, skin hydration significantly increased from 76.03 ± 9.10 in week 1 to 83.07 ± 11.13 ($p = 0.00$).

The melanin and erythema indices remained the same throughout the study.

Skin elasticity values also did not significantly differ during the study.

Changes in skin appearance

Comparisons of skin pores, texture, wrinkles, and spots between each treatment, and between the baseline and follow-up examinations are presented in Tables 1–3. The skin pore value, which was assessed using the VISIA system, decreased after three treatment sessions. The value in week 1 was 1024.57 ± 415.31, whereas as that in week 4 was 934.07 ± 458.78; the difference was statistically significant ($p < 0.05$). At 1 month (951.19 ± 432.10) and 3 months (943.92 ± 435.01) after the last treatment, the difference from the week-1 value remained statistically significant ($p < 0.05$; Figure 1). The wrinkle value, as

Figure 1. Improvement in the appearance of skin pores after five treatment sessions.

Figure 2. Improvement in fine skin wrinkles after five treatment sessions.

Figure 3. Minimal fading of facial spots after five treatment sessions.

determined using the VISIA system, at 3 months after the last treatment (29.29 ± 11.11) was significantly lower than that measured in week 1 (35.83 ± 16.05, $p < 0.05$; Figure 2).

The skin texture value, as determined using the VISIA system, decreased after three treatment sessions. The values in week 4 (599.29 ± 354.32) and week 5 (579.71 ± 361.48) were significantly lower than that in week 1 (668.43 ± 342.55; $p < 0.05$). However, this value had returned to the baseline (week 1) by the 1- and 3-month follow-up assessments. The spots value, determined using the VISIA system, remained the same throughout the study (Figure 3).

Satisfaction with treatment outcomes

The GAIS was used to evaluate the participants and dermatologists satisfaction with the treatment outcomes. In week 5, 12 (42.86%) participants assessed their photographs as "improved," while 16 (57.14%) assessed their photographs as "much improved." The dermatologist-determined GAIS scores were the same as the volunteers' self-assessed scores.

After the five treatment sessions, 27 (96.43%) volunteers felt that there was an improvement in skin smoothness and moisture, while 18 (64.29%) volunteers felt that there was an improvement in pore size (Figure 1) and skin brightness (Figure 4). Only 3 (10.71%) volunteers thought that the

Figure 4. Improvement in skin brightness after five treatment sessions.

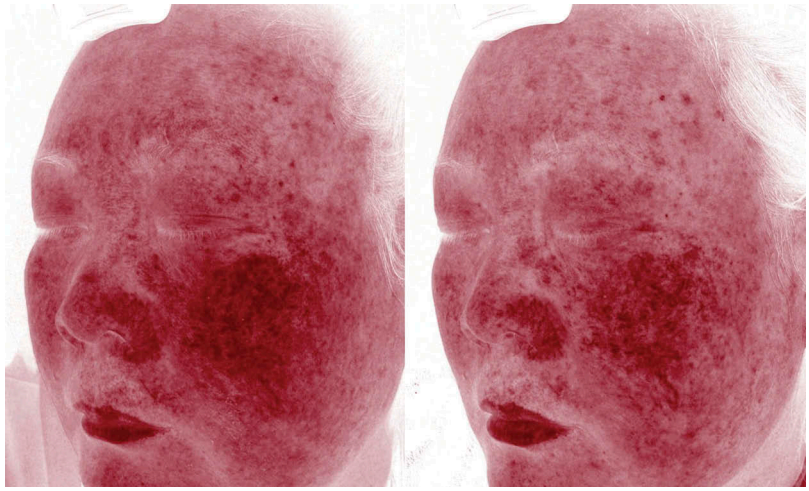

Figure 5. Improvement in skin redness after five treatment sessions.

therapy had faded their facial spots (Figure 3) and decreased facial redness (angiotelectasis; Figure 5).

Adverse effects

None of the volunteers developed any adverse effects during the study period. Since no topical anesthesia was used before the treatment, some participants reported feeling a slight and bearable stinging sensation in some areas of the face. No erythema, lump formation, or bleeding occurred during the procedure. There was no downtime due to the treatment.

Histologic examination

After the treatments, the density and quantity of the collagen fibers increased (Fig. 6), the thickness, length, and arrangement of the elastic fibers improved (Fig. 7), and the epidermis became thicker (Fig. 8).

Discussion

The findings of this study indicated that pneumatic injections of non–cross-linked hyaluronic acid reduced TEWL and pore size, and better the appearance of skin texture and fine wrinkles. The treatment was not associated with any complications or downtime, and produced satisfactory results.

According to the American Society for Aesthetic and Plastic Surgery, nearly eleven million non-surgical and two million surgical cosmetic procedures were performed in 2015 (17) in the USA. Thus, non-surgical procedures are over five times as common as surgical procedures, suggesting that people greatly prefer the former. The second-most common

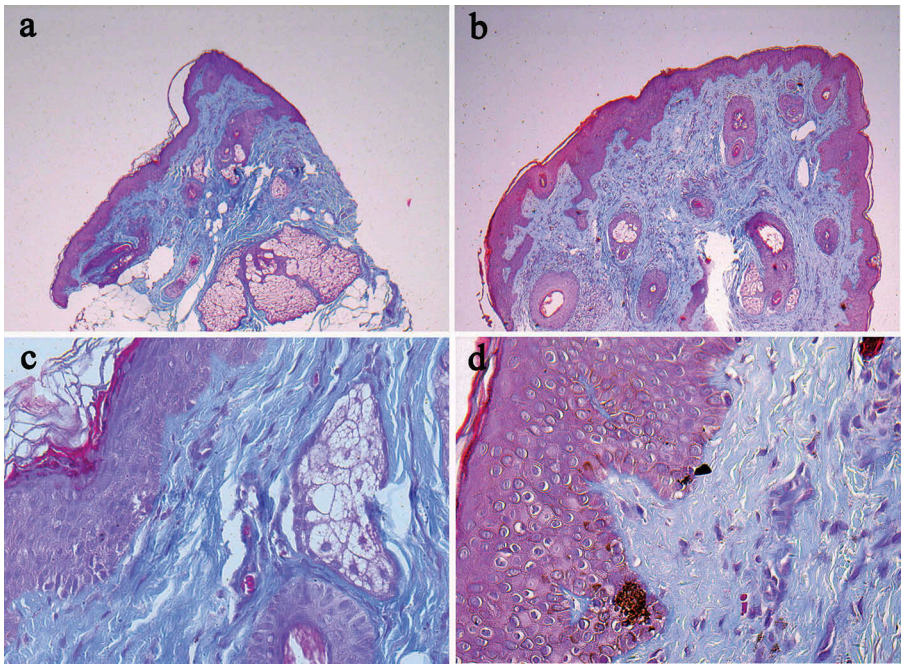

Figure 6. a(×40) c(×400), skin biopsy with Masson-trichrome stains before the treatment; b(×40) d(×400), skin biopsy with Masson-trichrome stains after the last treatment from same volunteer.

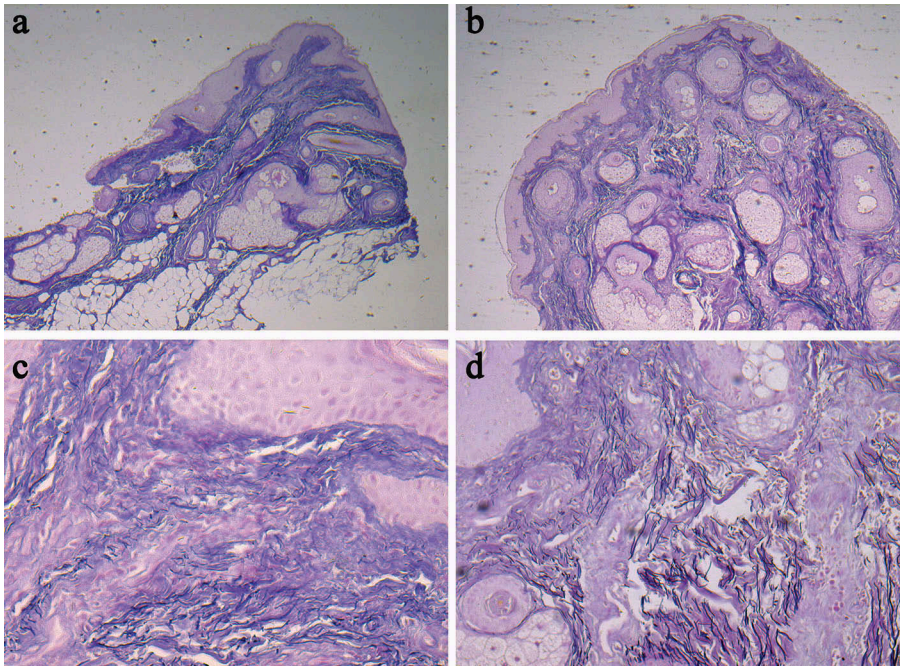

Figure 7. a(×40) c(×400), skin biopsy with Verhoeff-van Gieson stains before the treatment; b(×40) d(×400), skin biopsy with Verhoeff-van Gieson stain after the last treatment from same volunteer.

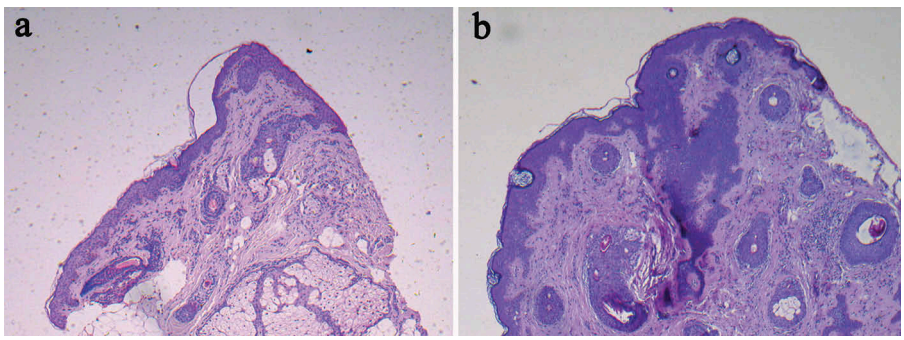

Figure 8. a(×40), skin biopsy with hematoxylin-eosin stains before the treatment; b(×40), skin biopsy with hematoxylin-eosin stains after the last treatment from same volunteer.

non-surgical cosmetic procedure performed in 2015 was hyaluronic acid injection, with over two million procedures performed. Hyaluronic acid filler injections are primarily used for facial, hand, and neck correction or augmentation. These injections are typically administered with an automatic stamp-type microneedle intradermal injector, owing to its effectiveness and painless application (18). However, topical anesthesia is still needed, and the side effects of this injection method include mild erythema, transient lumps, mild pain, and occasional bruising (19). A more time-saving, pain-free, side effect–free, and effective technique of administering hyaluronic acid is pneumatic injection. In this study, we evaluated the changes in skin biophysical parameters after five consecutive facial pneumatic injections of non–cross-linked hyaluronic acid.

Our results showed that the TEWL decreased after three treatment sessions and remained low at the 1- and 3-month follow-up assessments. TEWL is an indicator of the skin barrier function; the lower the value of TEWL, the more optimal the barrier function of the skin. Thus, our results indicate that the barrier function of the skin improved after three treatment sessions. Two factors might have contributed to this finding: firstly, injection of 5 mL hyaluronic acid solution into the skin would have increased the skin density because of increased collagen formation, and thus, less water could pass through the epidermal layer. Secondly, hyaluronic acid has been reported to induce mechanical stretching of the fibroblasts, which also promotes collagen formation (20).

Stratum corneum hydration level did not increase until 3 months after the last treatment, which is different from the findings of other studies (17,19). Since the most prominent trait of hyaluronic acid is its high hydrophilicity, we carefully analyzed the baseline hydration level, which at 75.99 ± 8.77, was obviously higher than that in other studies (18,21). We speculate that because the baseline hydration level was already high, this value did not appreciably increase after the administration of hyaluronic acid. The increase in this value at the 3-month follow-up was, we believe, attributable to

seasonal changes; the first treatment session was conducted during winter, while the 3-month follow-up fell in the summer. Although the temperature and humidity in the testing room was kept constant, the difference in weather conditions could have slightly increased the hydration level (22).

The melanin and erythema indices did not significantly differ during our study. These results are consistent with those reported by Roh et al. (19). In fact, thus far, no study has shown that hyaluronic acid injections damage melanocytes, capillaries, or hemoglobin, and thus, the melanin and erythema indices were not expected to change after the treatment. No significant difference in skin elasticity was found in our study; however, skin wrinkles were found to be significantly reduced at the 3-month follow-up. Deglesne et al. (23) found that hyaluronic acid injecting result in the increase of type I collagen and elastin. Cameli et al. (24) found that although hyaluronic acid filler improved the appearance of the nasolabial folds, it did not improve biophysical parameters such as skin hydration and elasticity, which is consistent with our study. We think that the pneumatic injections administered in this study activated skin fibroblasts through mechanical stretching, which helped increase the dermal volume (Fig 6). It should be noted that in our study, the R^2 value was assessed on the cheek, where volume loss is minimal, and therefore, the measurement may not have been sensitive enough to detect any difference. We found that the reduction in skin wrinkles mainly applied to the fine periocular wrinkles, where volume loss was relatively severe in VISIA images. The biosynthesis of new dermal components probably led to the observed decrease in skin wrinkles.

The appearance of skin pores improved after three treatment sessions, and the improvement was maintained at the 3-month follow-up. According to Lee et al. (25), pore size is related to three factors: sebum secretion, elasticity of the skin around the pores, and hair follicle volume. When the elasticity of the skin around the pores increases, the pore size decreases. Since elasticity is relative to the volume of collagen and elastic fibers, we believe that the increase in collagen and elastic fibers resulting from the mechanical stimulation induced by exogenous HA led to an improvement in pore size.

Skin texture refers to the smoothness of the skin. After three treatment sessions, there was a marked decrease in the skin texture value, which indicated that the roughness of the skin had decreased. Lee et al. (26) found that after three intradermal injections of hyaluronic acid, skin roughness was significantly improved. Succi et al. (27) also noted an improvement in skin texture, brightness, and fine wrinkles after the micropuncture injection of non-cross-linked hyaluronic acid. We consider that the improvement in skin texture was related to the reduction in pore size and improvement in dermal volume. However, by the 3-month follow-up, the skin texture value had increased, possibly due to degradation of the hyaluronic acid. Thus, it might be appropriate to repeat the treatment after 3 months. No improvement in skin spots was observed in our study. This is as expected since hyaluronic acid does not break down melanin.

In conclusion, the pneumatic injection of non-cross-linked hyaluronic acid can effectively improve skin TEWL by boosting the barrier function of the skin. The treatment also improved the appearance of the skin by reducing pore size and softening the appearance of skin texture and fine wrinkles. Additionally, treatment was not associated with any complications or downtime, and produced satisfactory results. However, further studies with larger sample sizes are needed to evaluate the effectiveness of this treatment.

Conflict of Interest

The authors declare that they have no conflict of interest.

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