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미세 집속 초음파의 일시적인 눈썹 리프팅과 미간 주름 개선 효과에 대한 연구

The effect of microfocused ultrasound on temporary eyebrow lifting and glabellar wrinkle reduction

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ABSTRACT

Background: Microfocused ultrasound (MFU) is a non-invasive treatment device widely used for skin tightening. We aimed to investigate the efficacy of a novel MFU device in eyebrow lifting as other MFU devices are approved for their use in improving skin laxity. We also evaluated the effect on reduction of glabellar wrinkles of the MFU device, as studies on the efficacy of MFU in alleviating wrinkles at the frown state are scarce. In addition, we tried to elucidate histological changes of skin collagens induced by MFU.

Methods: This clinical trial was a prospective, single-center, single-arm, and open-label study. Every patient was treated with one session of MFU at the forehead and both lateral eye areas. Efficacy was measured at week 4, week 8, week 12, and week 16 by calculating average eyebrow height and maximal eyebrow height, patient satisfaction score, and Glabellar Line Scale score. Also we conducted *in vivo* experiments with hairless rats to analyze any alterations of collagen induced by treatment with a MFU device

Results: In the clinical study, a total of 38 patients were enrolled. Average eyebrow height was elevated with a mean difference of 1.99 mm at week 4, and remained elevated at week 16, with a mean difference of 1.57 mm. The mean maximal eyebrow height was also increased by 2.21 mm at week 4 and by 1.63 mm at week 16. Glabellar wrinkles of some patients were temporarily alleviated. The histological examination of the dorsal skin of hairless rats revealed a mild increase of collagen in the dermis and collagen IV in the basement membrane.

Conclusion: This study suggests that MFU temporarily lifts eyebrows and reduces glabellar wrinkles effectively by affecting collagen in the basement membrane and the dermis.

Keywords: Ultrasound; skin aging; skin wrinkling; eyebrows; collagen

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INTRODUCTION

At present, skin aging characterized by skin laxity and wrinkles is a common aesthetic issue. While multiple treatment options are available, such as rhytidectomy, thread lift, fractional lasers, radiofrequency devices, and fillers, minimally invasive or noninvasive techniques are gaining more popularity owing to their advantages, including fast recovery due to minimal epidermal damage and no surgery-associated complications.[1-4]

Microfocused ultrasound (MFU) is a noninvasive energy-based device that creates thermal injury zones by heating the tissues, induces thermal collagen denaturation and tissue shrinkage, and increases dermal thickness and dermal collagen.[5-7] MFU was initially approved by the US Food and Drug Administration for use in eyebrow lifting in 2009 and has also been frequently studied for the improvement of wrinkles at facial areas and body sites, such as the neck, chest, upper arms, elbow, knees, and even medial thighs.[8-15]

We aimed to investigate the efficacy of a novel MFU device in eyebrow lifting because many previous studies have reported such an effect of other MFU devices.[8, 9, 16, 17] Meanwhile, studies on the efficacy of MFU in reducing glabellar wrinkles at the frown state are scarce. Thus, in this study, we evaluated the clinical improvement of eyebrow laxity and also reduction of glabellar wrinkles with the use of a new MFU device. In addition, we tried to elucidate the effect of MFU on skin collagens by analyzing histological changes induced by the treatment in hairless rats.

MATERIALS AND METHODS

Patient selection

This prospective, single-center, single-arm, open-label study enrolled patients aged 19 years and above with moderate or severe glabellar wrinkles who were willing to undergo eyebrow lift. The exclusion criteria were as follows: active local infection or skin conditions (e.g., severe acne, seborrheic dermatitis, psoriasis, vitiligo, systemic lupus erythematosus, scleroderma) that may affect recovery on the facial area where the medical device would be applied; open wound, keloid, or hypertrophic scar in the intended treatment areas; history of cosmetic treatment within 6 months, filler or botulinum toxin treatment within 6 months, radiation therapy or chemotherapy, or any malignancies; past medical history of infection, dermatitis, or herpes zoster induced by thermal stimulation; history of taking isotretinoin, other retinoids, or steroid in the last 4 weeks; pregnancy, breastfeeding, or planning for pregnancy; and history of participation in other clinical trials in the last 30 days before the study that may affect the efficacy and safety of the treatment.

Treatment protocol

At the screening visit, the patient's baseline demographics and eligibility were examined. All the patients underwent a single treatment with an MFU device (LinearFirm [ULTRAcel Q+], Jeisys Inc., Seoul, Korea). Baseline photographs of the patients were taken using a 3D Vectra H1-270 camera (Canfield Scientific Inc., Parsippany, NJ, USA) before the MFU treatment. No local anesthetic or oral analgesia was administered before the treatment. A thin layer of ultrasound gel was applied to the skin, and the MFU device was gently pressed to the skin surface.

The forehead was treated with two different linear-shot transducers. Three types of linear transducers were available: 7 MHz, 2.0-mm focal depth; 7 MHz, 3.0-mm focal depth; and 4 MHz, 4.5-mm focal depth. A trained physician selected the combination of a 2.0-mm and a 3.0-mm transducer or a 3.0-mm and a 4.5-mm transducer to target dual depths. The treatment energy ranged between 0.2 and 0.6 J; the highest energy that a patient could tolerate was applied. The shots were delivered with a spacing of 1-mm width to cover the forehead area. A single pass was applied with each transducer. 400 or more shots were delivered to the forehead

according to its size. After the treatment, the ultrasound gel was removed. No post-treatment care, such as ice pack placement, was given. The patients rated the residual pain using the visual analogue scale (VAS) 30 minutes after the treatment.

The patients were followed up at weeks 4, 8, 12, and 16 after the treatment. Facial photographs were taken using a 3D Vectra H1-270 camera (Canfield Scientific Inc., Parsippany, NJ, USA) at every follow-up visit. The patient satisfaction (PS), glabellar line scale (GLS), and VAS scores were measured at every visit. Adverse events were also evaluated.

Primary and exploratory endpoints

The primary outcomes were average eyebrow height (AEH), maximal eyebrow height (MEH), and PS score. The eyebrow heights were measured on 3D reconstructed photographs generated by the Vectra Analysis Module software (Canfield Scientific Inc., Parsippany, NJ, USA). AEH was calculated as the average distance between the eyebrow and the line connecting the medial canthus at five points per side with 8-mm intervals from the medial canthus. MEH was measured at the highest point of the eyebrow from the line connecting the medial canthus. (Figure 1) The measurements were performed by two independent evaluators, and the lower value between two raters was used. The PS score was measured using a 7-scale questionnaire: 0 = very dissatisfied, 2 = dissatisfied, 3 = somewhat dissatisfied, 4 = neutral, 5 = somewhat satisfied, 6 = satisfied, 7 = very satisfied. The percentage of patients whose glabellar wrinkles improved was set as an exploratory endpoint. Glabellar wrinkles at the maximal frown state were evaluated by a dermatologist using the GLS, a 4-point clinical severity score: 0 = none, 1 = mild, 2 = moderate, 3 = severe.[18] The VAS ranging from 0 to 100 was used to evaluate pain intensity associated with the treatment.

***In vivo* experiment protocol**

A total of 11 hairless rats were used for experiments. The rats were all 22 weeks old at the time of application of an MFU device (ULTIGHT, Medicon Inc., Gangwon-do, Korea) The dorsal skin of each rat was divided into four quadrants. The upper right quadrant served as a control, and the others were designated as experimental areas. In each quadrant, a circle with

a diameter of 3cm was defined as the target area. The MFU device was applied on the dorsal skin of the rats. A total of 60 shots were delivered on each target site; 20 shots each in horizontal, vertical, and diagonal directions. The power of the device could be set from steps 1 to 3; step 1 (0.17 J), step 2 (0.25 J), and step 3 (0.30 J). The upper left quadrant was set as a control, and the other quadrants were treated with the MFU device at power levels corresponding to step 1, step 2, and step 3, respectively in a counterclockwise manner. (Figure 2a) Ultrasound gel was applied previously to the treatment.

Hairless rats were divided into four groups (groups A – D). Group A comprised two rats, and groups B – D comprised three rats each. (Figure 2b) Groups A and B were treated once with the MFU device, while groups C and D were treated twice with a two-week interval between treatments. Groups A and C were sacrificed two weeks after the last treatment, whereas groups B and D were sacrificed four weeks after the treatment. (Figure 2c) Upon euthanasia, the dorsal skin of the rats was harvested. Half of the tissue samples were fixed in a 10% formaldehyde solution to create paraffin blocks, while the other half were stored at -80°C.

Histological analyses

Tissue specimens were stained with hematoxylin and eosin (H&E), Masson's Trichrome, and immunohistochemistry (IHC) stain. H&E staining was used to observe the overall tissue structure, and Masson's Trichrome was utilized to assess collagen in the dermis. IHC staining for collagen IV was performed to analyze the change in the basement membrane. Image J software (National Institute of Health, Bethesda, Maryland, USA) was used for quantifications of the histologic findings in tissues. The expression levels of collagen were measured as the average area fractions of collagen.

Data analyses

Statistical analysis was conducted using IBM SPSS (ver. 21.0; IBM Corp., Armonk, NY, USA). Paired-sample *t*-test or Wilcoxon signed-rank test was employed to evaluate the differences in the AEH, MEH, PS score, and VAS score from the baseline. McNemar's or exact McNemar's test was used to determine the percentage of patients who exhibited

improvement in the GLS score. $P < 0.05$ was considered to indicate statistical significance. Paired-sample *t*-test or Wilcoxon signed-rank test was utilized to evaluate the differences in collagen expression levels between MFU-treated areas and the control area.

Ethics declaration

The study protocol was approved by the Institutional Review Board (2024-0521) of Asan Medical Center and performed in compliance with the principles of the Declaration of Helsinki. This trial is registered with NCT06457607. The rat experiments were approved by the Animal Institutional Review Board of Asan Life Science Research Center and conducted in accordance with the institutional guidelines.

RESULTS

1.1. Baseline patient characteristics

A total of 38 patients (32 women and 6 men) were enrolled in this study. No dropout was recorded. The mean age of the patients was 41.68 ± 3.08 years and the median age was 41.50 (range, 39 – 43) years. All the patients had a GLS score of 2 or higher. (Table 1) A total of 33 patients were treated with a combination of 2.0-mm and 3.0-mm transducers, whereas 5 patients were treated with a combination of 3.0-mm and 4.5-mm transducers.

1.2. Average eyebrow heights and maximal eyebrow heights

The mean baseline AEH was 27.78 ± 2.04 mm. At week 4, the mean AEH was 29.76 ± 2.14 mm, indicating an increase of 1.99 ± 0.58 mm from the baseline. The mean AEH gradually decreased throughout the follow-up visits: 29.59 ± 1.92 mm, 29.36 ± 2.02 mm, and 29.34 ± 0.48 mm at weeks 8, 12, and 16, respectively. All the mean differences in the AEH were statistically significant ($P < 0.0001$).

The mean baseline MEH was 31.67 ± 2.58 mm. At week 4, the mean MEH was 33.88 ± 2.63 mm, indicating an increase of 2.21 ± 0.73 mm from the baseline. The mean MEH was 33.67 ± 2.39 mm at week 8, 33.41 ± 2.59 mm at week 12, and 33.35 ± 2.53 mm at week 16. Although the mean MEH gradually decreased from week 4 to 16, the difference in the MEH between weeks 16 and baseline was still statistically significant ($P < 0.0001$). (Table 2, Figures 3 and 4)

1.3. Patient satisfaction scores

The mean PS scores were 6.29 ± 0.52, 6.05 ± 0.52, 5.84 ± 0.55, and 5.45 ± 0.50 at weeks 4, 8, 12, and 16, respectively. Compared with the baseline PS score, which was 4, indicating neutrality, all the PS scores were statistically significant ($P < 0.0001$) All the PS scores measured at the follow-up periods were 5 or higher. (Table 3).

1.4 Glabellar line scale scores

At week 4, 16 patients exhibited mild glabellar wrinkles with a GLS score of 1, 16 had moderate glabellar wrinkles with a GLS score of 2, and 6 had severe glabellar wrinkles with a GLS score of 3. At week 8, the number of people with mild glabellar wrinkles decreased to 9, 19 patients had moderate glabellar wrinkles, and 10 showed severe wrinkles. At week 12, only one patient showed mild glabellar wrinkles, whereas all the other patients had moderate or severe wrinkles. At week 16, no patient met the criteria of a GLS score of 1. (Table 4, Figures 5 and 6)

The percentage of patients who showed an improvement in GLS score of 1 point or more was 71.05% at week 4, 47.37% at week 8, 21.05% at week 12, and 5.26% at week 16. The percentage of patients with an improvement of 1 point or more was statistically significant at week 4 ($P = 0.0169$) and week 16 ($P = 0.0023$), but it was not significant at week 8 ($P = 0.6776$) or week 12 ($P = 0.776$).

The percentage of people who achieved an improvement in GLS score of 2 points or more was 15.79% at week 4, 10.53% at week 8, 2.63% at week 12, and 0.00% at week 16. While the percentage of patients with an improvement in GLS score of 2 points or more was statistically significant at week 8 ($P = 0.0192$) and week 12 ($P = 0.0005$), it was not significant at week 4 ($P = 0.0784$) or week 16.

1.5 Visual analogue scale scores and adverse events

The mean VAS score was 6.35 ± 10.55 measured 30 min after the treatment. All the patients reported a VAS score of 0 (no pain) at the follow-up visits. (Table 5) In terms of safety, no patient developed severe adverse events whereas one patient experienced transient facial palsy in the periorbital area. The patient reported difficulty closing the right eye on day 5 after the MFU treatment. After treatment with prednisolone 30 mg for 7 days and 20 mg for 4 days, the patient's symptoms completely resolved on day 16. (Figure 7)

2.1. Histological analyses of the dorsal skin of MFU-treated rats

On H&E staining, there was no significant difference in the epidermis between the MFU-treated areas and the control areas. However, the dermis of MFU-treated areas appeared more eosinophilic compared to that of the control areas. (Figure 8a–d) The dermis of MFU-treated

areas was more bluish on Masson's Trichrome staining, indicative with the altered collagen density as observed on H&E staining. (Figure 8e–h) Collagen IV staining was more conspicuous at the basal membrane of the tissues treated with MFU than those of control samples. (Figure 8i–n)

2.2. Quantification of the expression levels of collagen

The quantities of collagen in the dermis and collagen IV of the basement membrane were measured from the tissues stained with Masson's Trichrome or collagen IV, respectively. Each expression level of MFU-treated tissues was normalized against the mean value measured in control tissues to reflect relative changes. (Table 6, Figure 9a-b)

The terms of dermal collagen expression levels, the ratios of step2/ mCTRL (mean value of control tissues) were 1.094 ± 0.005 in group A, 1.123 ± 0.026 in group B, 1.193 ± 0.027 in group C, and 1.021 ± 0.184 in group D. Additionally, the ratios of step 3/ mCTRL were 1.177 ± 0.024 in group B, and 1.139 ± 0.043 in group C. (Figure 9a)

In group D, the ratios of collagen IV expression levels (step 1/ mCTRL, step 2/ mCTRL, step 3/mCTRL) were 1.166 ± 0.006, 1.213 ± 0.102, 1.227 ± 0.094 in group D. These ratios were all higher than 1, indicating that collagen IV expression levels were elevated in the MFU-treated areas compared to control areas. (Figure 9b)

DISCUSSION

This prospective, single-arm, rater-blinded study demonstrated that a single treatment with MFU elevated eyebrows. The AEH and MEH increased, with maximal differences from the baseline of 1.99 and 2.21 mm, respectively, at week 4. While the increases in the AEH and MEH from the baseline remained statistically significant even at week 16, which were 1.57 and 1.63 mm, respectively, the differences from the baseline gradually decreased from week 4 to 16.

There are some published data on eyebrow height, which reported the range of average eyebrow elevation from 0.47 to 1.7 mm.[8, 9, 16, 17] This result variability could be attributed to the use of different MFU devices. In our study, the mean change of the AEH at week 12, which was 1.58 mm, was comparable to those at the 90-day follow-up in other studies. In a study conducted on 30 Asian patients, the AEH and MEH at week 12 were even higher than those at week 4.[17] Contrarily, in our study, the AEH and MEH exhibited the greatest differences at week 4 and gradually declined. Such a decline is consistent with the finding of another study that reported the highest eyebrow height at 1-month follow-up, which could be attributed to the decreasing facial volume.[8]

As for the PS score, all the patients reported scores of 5 or more. The mean PS score was the highest at week 4 and slightly decreased throughout the follow-up visits, but the difference from the baseline was statistically significant. The trend of the PS score was similar to those of the AEH and MEH. Although PS is subjective and the result may be potentially biased as this study was an open-label trial, the objective outcomes of the AEH and MEH support the efficacy of the MFU treatment.

We also evaluated the GLS scores of the patients as an exploratory endpoint to whether MFU treatment can alleviate wrinkles at the frown state. A high percentage (71.05%) of patients showed improvement in the GLS score at week 4. At week 16, the percentage was 5.26%, with statistical significance. However, the percentage of patients with a GLS score of 1 or higher was not significant at weeks 8 and 12. Furthermore, no patient achieved a GLS score of 2 or higher at week 16. The initial alleviation of wrinkles at the frown state could be due to the increase in the upper facial volume and skin tightening.[8, 19] Although our result

indicated a relatively short-term effect on glabellar wrinkles, our study demonstrated that MFU can also alleviate wrinkles at a contracted state aside from a relaxed state.

In our study, the procedure was performed without local anesthesia, which enabled the patients to experience minimal pain but increased the potential need for more shots. The pain in our study was tolerable, as the mean VAS score evaluated 30 min after the treatment was 6.35 out of 100, and no residual pain was reported throughout the follow-up visits. The main difference between this study and similar previous ones is that we evaluated the VAS score 30 min after the treatment, not during or immediately after the treatment.[8, 16, 17] This was done to evaluate the perioperative pain in the treatment session, as the energy of each treatment was set at the level that each patient could tolerate.

No serious adverse events resulting in any drop were reported. However, one of our patients reported difficulty closing the right eye on day 5 after treatment. Several literatures documented nerve-related complications such as numbness, paresthesia, and dysesthesia after MFU treatment.[6, 7, 13, 20-22] Sabet-Peyman et al. reported that one patient had transient brow ptosis, which lasted for 2 months, as a complication of MFU and demonstrated that the atypical, more superficial branches of the facial nerves may be susceptible to treatment.[23] Marr et al. reported a case of a 62-year-old woman who developed neuropraxia and brow ptosis of the right eye after a single treatment and completely recovered after 49 days.[24] Although previous studies showed that MFU can cause nerve paralysis, our patient did not develop brow ptosis which is a sign of a damaged temporal branch of the facial nerve.[24, 25] Thus, the partial periorbital paralysis experienced by our patient may not be a result of nerve dysfunction but rather caused by tissue edema, or is independent of the treatment.

Previous studies have reported that MFU treatment induces increased dermal collagen and thickening of the dermis in human skin, especially in the reticular dermis.[6, 26] A study conducted on photo-aged mouse skin showed that MFU treatment increased collagen distribution and improved collagen array by inducing higher levels of TGF-β and MMP3.[27] In our study, the tissue samples stained with H&E and Masson's trichrome also suggested denser collagen in the treated dorsal skins. When quantified, the average area fractions of dermal collagen were higher in step 2-treated areas than in control areas in all groups. Particularly in group C, where rats were treated twice with a two-week interval and then

euthanized 2 weeks after the last treatment, the collagen levels were higher even when treated with the power of step 1. This suggests that MFU may induce more neocollagenesis if performed frequently. However, in group D, the ratios of dermal collagen expression levels were not lower than 1.00 when treated with the power of step 1 or step3. Regarding the difference between group C and group D being the timing of euthanasia, this implies that the changes of dermal collagen induced by MFU treatment are temporary.

We also measured the expression levels of collagen IV. Collagen IV is a main component of lamina densa, forming a network with laminin with interconnections by nidogen and perlecan.[28] It is reported that aged skin demonstrates lesser collagen IV in the basement membrane.[28-30] A study of hydrogen peroxide-induced senescent fibroblasts reported that TGF-β1 treatment significantly increased collagen IV genes in the fibroblasts.[30] There is limited data on whether MFU could stimulate the expression of collagen IV. A case report demonstrated that weekly skin-resurfacing treatments with a cosmetic preparation containing copper–glycyl-L-histidyl-L-lysine (GHK), oligo-hyaluronic acid, rhodiolar extract, tranexamic acid, and β-glucan for 12 weeks slightly increased collagen IV production without statistical significance.[31] In our study, the collagen IV expression levels were consistently higher in MFU-treated areas than the control area in group D. This result may indicate that collagen IV in the basement membrane tends to increase more slowly than dermal collagen. Moreover, this increase of collagen IV may be related to TGF-β signaling, which is also associated with the increase in dermal collagen.[27, 30] However, further studies on molecular pathways related to the increase of collagen IV are required.

This study has several limitations. First, the choice of which transducers to use in the treatment depended on the physician's clinical impression of the patient's skin thickness, not visualization by ultrasound; the treatment depth may have been too shallow or too deep to target the superficial musculoaponeurotic system.[32] Also, it was difficult to ensure proper contact of the transducer to the skin with MFU without visualization.[33] Furthermore, the study population mainly consisted of middle-aged Asian women, making it difficult to generalize the results to other races or age groups. However, this population may reflect the real clinical setting in Asia, where middle-aged women are the primary age group seeking MFU treatment.

CONCLUSION

Our study demonstrated that the new MFU device is an effective and safe treatment for temporary eyebrow lifting and glabellar wrinkle reduction. We also showed that MFU may induce a mild increase of dermal collagen and collagen IV of the basement membrane in *in vivo* experiments with hairless rats. These findings suggest that MFU is a promising non-invasive treatment option in skin rejuvenation, regarding its potential to enhance collagen levels in skin structures.

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국문요약

제목: 미세 집속 초음파의 일시적인 눈썹 리프팅과 미간 주름 개선 효과에 대한 연구

연구배경: 미세 집속 초음파는 피부 탄력 개선을 위해 널리 사용되는 비침습적 치료 장비이다. 다른 미세 집속 초음파 장비들이 이완된 피부를 개선시키는데 허가를 받았기에, 해당 연구는 새로운 미세 집속 초음파의 눈썹 리프팅 효과를 연구하였다. 또한 미세 집속 초음파가 찡그린 상태에서의 주름을 완화시키는 효과가 있는지에 대한 연구가 적기 때문에, 해당 연구에서는 미세 집속 초음파 기기가 미간 주름을 경감시키는 효과가 있는지 평가하였다. 추가적으로, 본 연구에서는 미세 집속 초음파에 의한 조직학적 피부 콜라겐의 변화를 밝히고자 하였다.

재료 및 방법: 해당 임상 시험은 전향적, 단일기관, 단일군, 공개 임상 연구였다. 각 환자는 이마와 양쪽 눈 주변에 한 차례의 미세 집속 초음파 치료를 받았다. 효과는 4주, 8주, 12주 및 16주에 평균 눈썹 높이와 최대 눈썹 높이, 환자 만족도 점수, 미간 주름 점수를 계산하여 측정하였다. 또한 미세 집속 초음파 치료에 의한 콜라겐 변화를 분석하기 위해 hairless rat을 이용한 동물 실험을 시행하였다.

결과: 임상 시험에는 총 38명의 환자가 포함되었다. 평균 눈썹 높이는 4주차에 평균 1.99 mm 상승하였으며, 16주차에도 평균의 차이가 1.57 mm로 유지되었다. 최대 눈썹 높이의 평균은 4주차에 2.21 mm, 16주차에 1.63 mm 증가되었다. 일부 환자들의 미간 주름은 일시적으로 완화되었다. Hairless rat의 등의 피부 조직에서의 조직학적 검사 결과 진피 콜라겐과 기저막의 제 4형 콜라겐은 약한 증가를 보였다.

결론: 해당 연구는 미세 집속 초음파가 기저막 및 진피 콜라겐에 영향을 미쳐 효과적으로 일시적인 눈썹 리프팅과 미간 주름 완화 효과를 보임을 시사한다.

FIGURES AND FIGURE LEGENDS

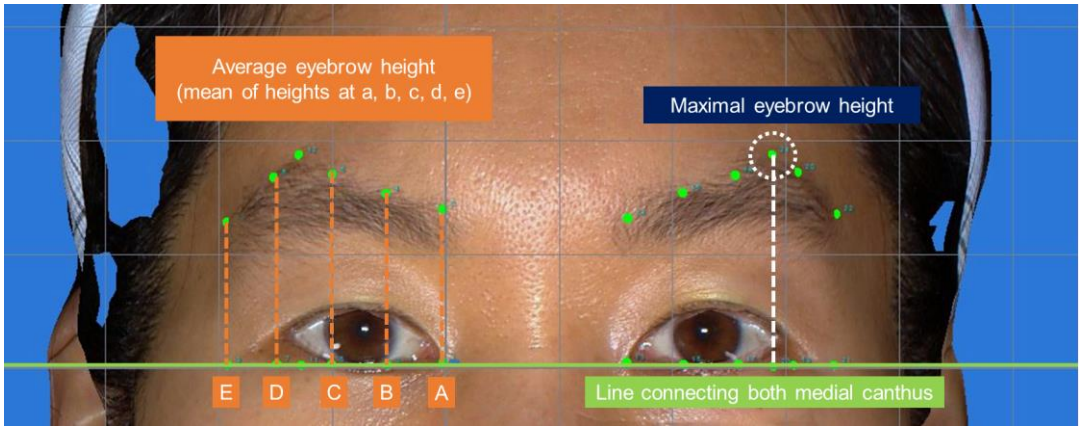
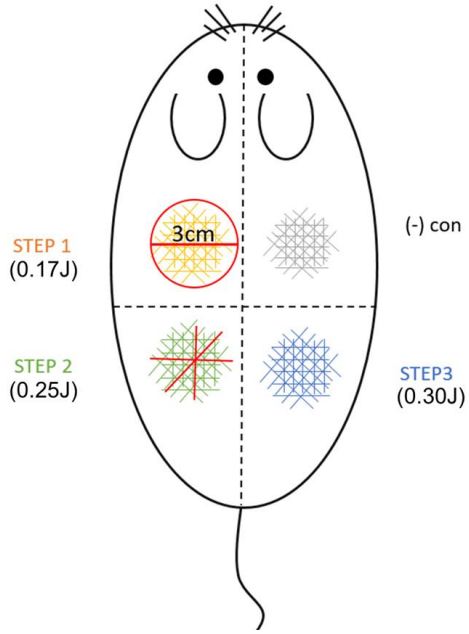


Figure 1. Eyebrow height measurement using the 3D Vectra H1-270 camera

(A)



(B)

Group	Explanation	N
A	Sacrificed 2 weeks after 1 MFU treatment	2
B	Sacrificed 4 weeks after 1 MFU treatment	3
C	Sacrificed 2 weeks after 2 MFU treatment	3
D	Sacrificed 4 weeks after 2 MFU treatment	3

(C)

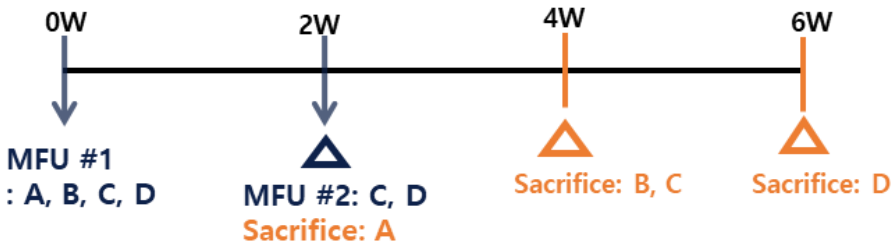


Figure 2. Experiment protocol (A) The dorsal skin of each rat was divided into four quadrants and each quadrant except the upper right quadrant was treated with an MFU device with different powers (B, C) A total of 11 rats were divided into 4 groups and treated with MFU or sacrificed in accordance with the illustrated schedule

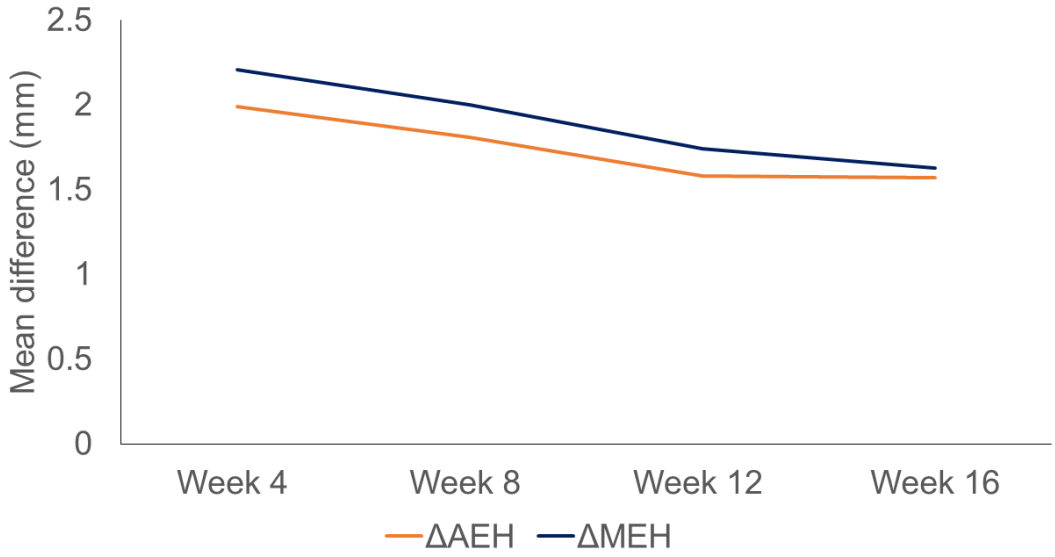


Figure 3. The mean difference of the average eyebrow height and maximal eyebrow height compared with the baseline

Abbreviation: AEH, average eyebrow height; MEH, maximal eyebrow height

(A) (B)

(C) (D)

(E)

Figure 4. Clinical improvement of eyebrow heights after the treatment from (A) baseline, (B) week 4, (C) week 8, (D) week 12, and (E) week 16

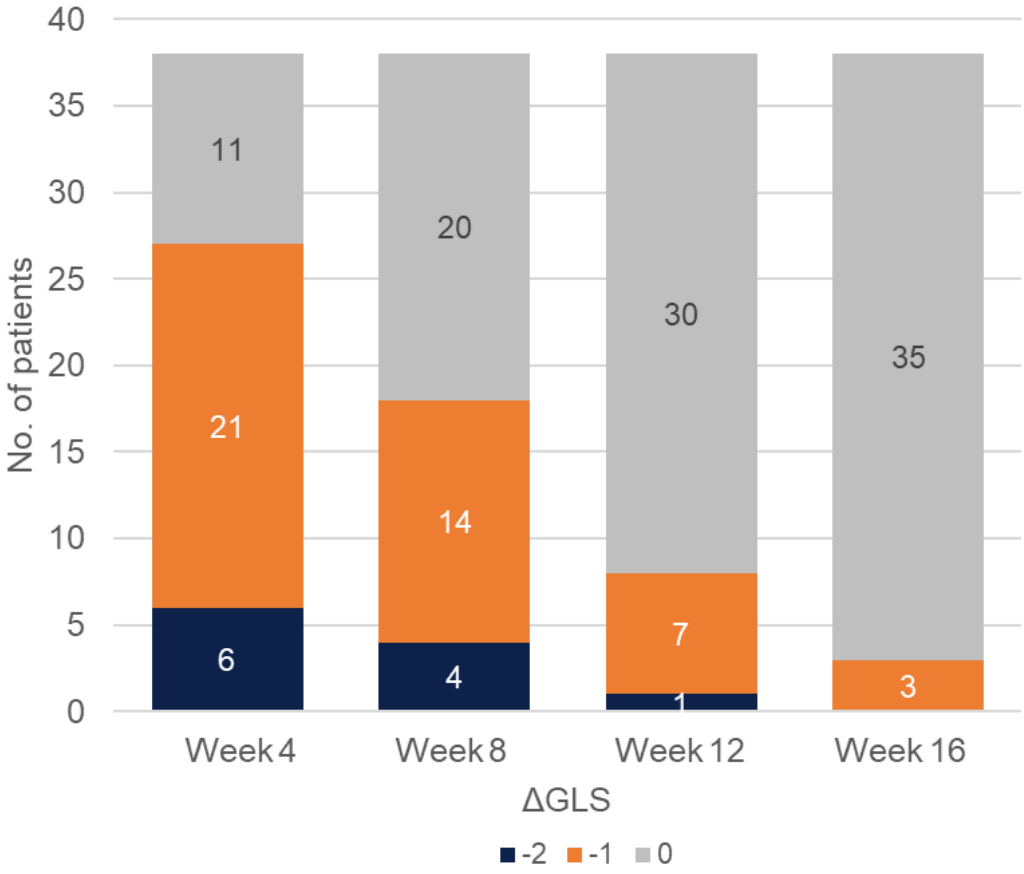
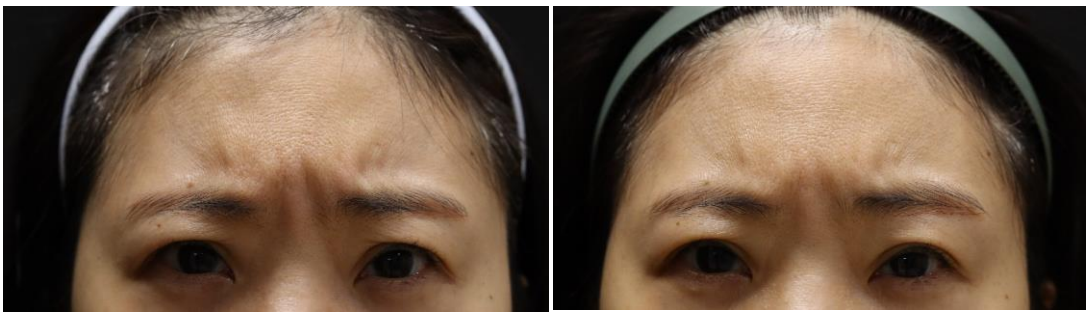


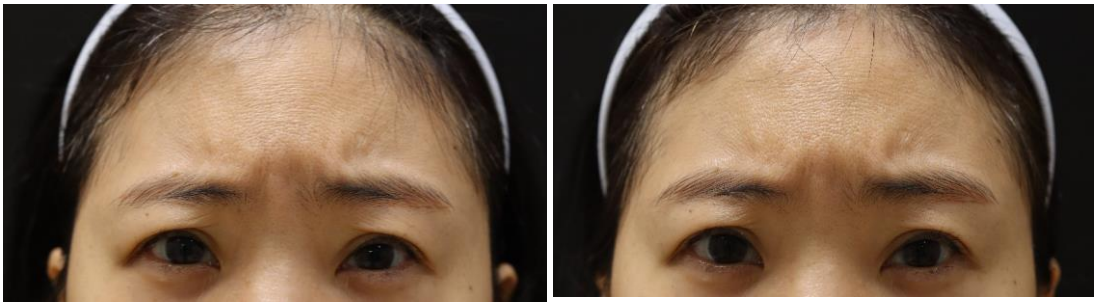
Figure 5. Patients showing improvement in GLS score

Abbreviation: GLS, glabellar line scale

(A) (B)



(C) (D)



(E)

Figure 6. Clinical improvement of glabellar wrinkles at the frowned state after treatment from (A) baseline, (B) week 4, (C) week 8, (D) week 12, and (E) week 16

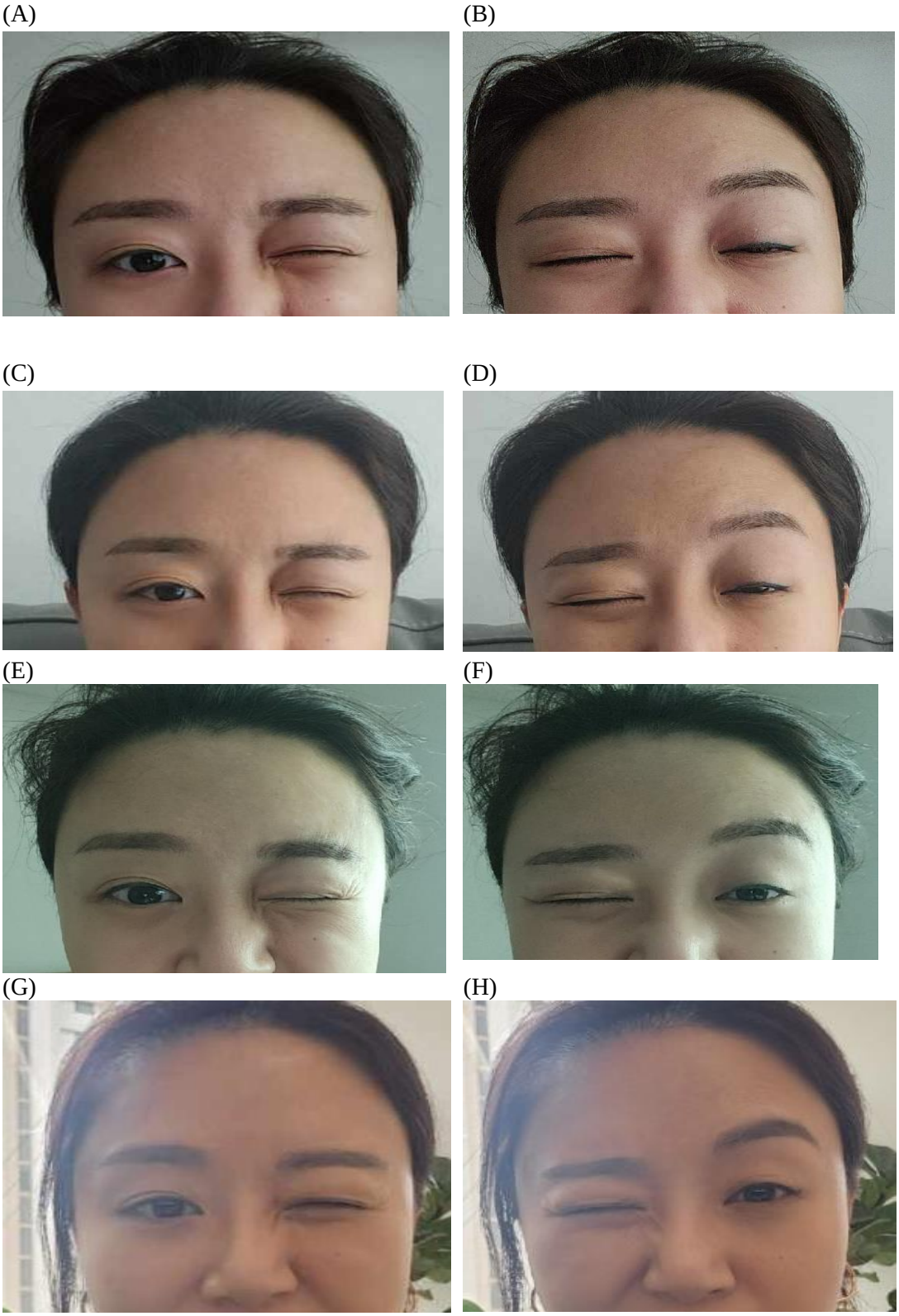


Figure 7. Transitory partial facial paralysis (A, B) 5 days after the treatment, (C, D) 8 days after the treatment, (E, F) 12 days after the treatment, and (G, H) 16 days after the treatment

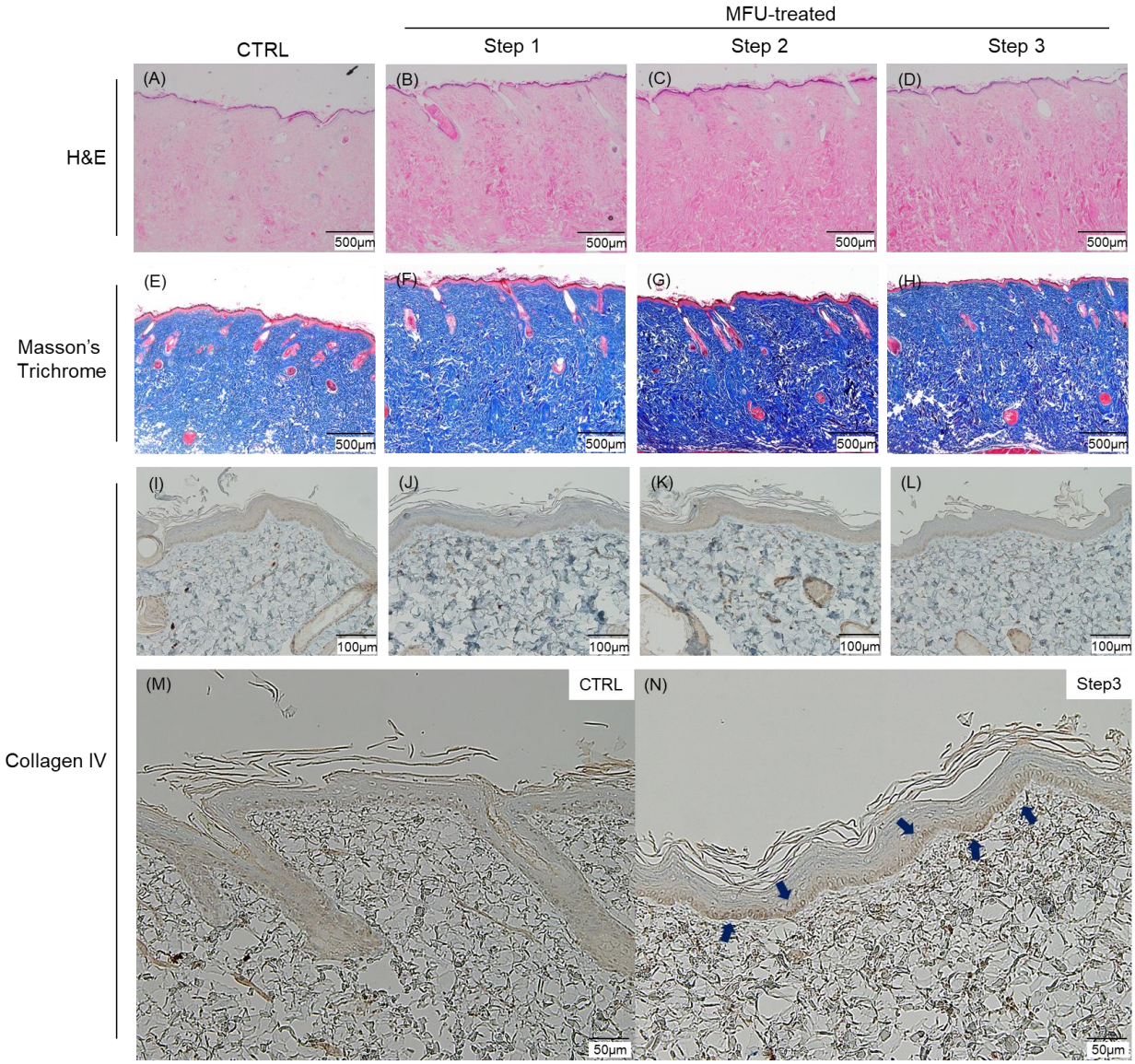
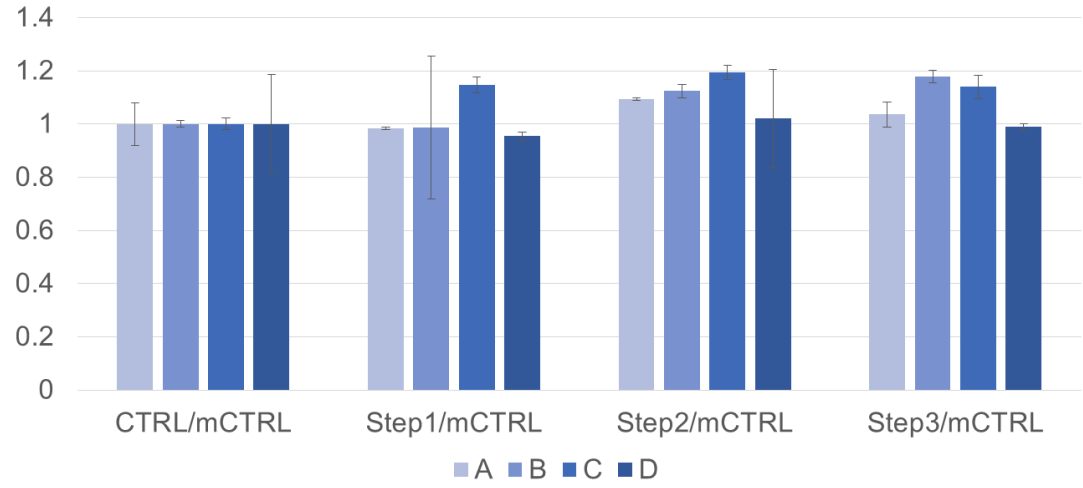


Figure 8. Histologic analysis shows the dense staining on the dermis of MFU-treated tissues (A – D) Hematoxylin and eosin (E – H) Masson's Trichrome (I – N) Immunohistochemistry of collagen IV

(A)



(B)

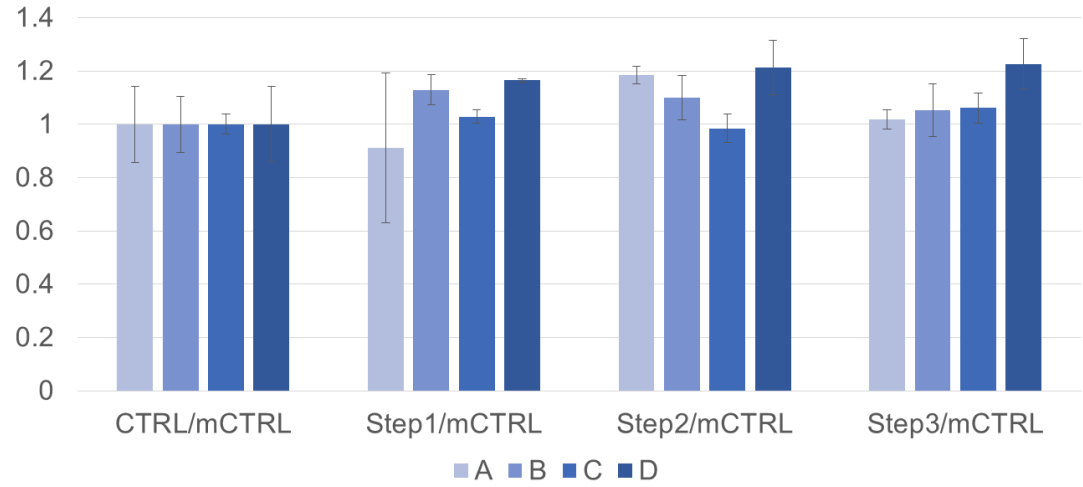


Figure 9. The expression levels of collagen measured using Image J software (A) Dermal collagen expression levels from Masson's Trichrome stained tissues (B) Collagen IV expression levels in the dermo-epidermal junction from Collagen IV-stained tissues

TABLES AND TABLE LEGENDS

Table 1. Baseline patient characteristics

Characteristic	Total (n = 38)
Age, years – Mean ± SD	41.68 ± 3.08
Sex – no. (%)	
Male	6 (15.79)
Female	32 (84.21)
Eyebrow height, mm – Mean ± SD	
Average eyebrow height	27.78 ± 2.04
Maximal eyebrow height	31.67 ± 3.58
GLS score – no. (%)	
2 (Moderate)	15 (39.47)
3 (Severe)	23 (60.53)

Abbreviation: GLS, glabellar line scale

Table 2. Average eyebrow height and maximal eyebrow height

	Average eyebrow height			Maximal eyebrow height		
	Mean ± SD (mm)	**Mean difference (mm)**	***P*- value**	**Mean ± SD (mm)**	**Mean difference (mm)**	***P*- value**
Baseline	27.78 ± 2.04	–	< 0.0001	31.67 ± 3.58	–	<0.0001
Week 4	29.76 ± 2.14	1.99 ± 0.58	< 0.0001	33.88 ± 2.63	2.21 ± 0.73	<0.0001
Week 8	29.59 ± 1.92	1.81 ± 0.64	< 0.0001	33.67 ± 2.39	2.00 ± 0.92	<0.0001
Week 12	29.36 ± 2.02	1.58 ± 0.58	< 0.0001	33.41 ± 2.59	1.74 ± 0.83	<0.0001
Week 16	29.34 ± 1.95	1.57 ± 0.48	< 0.0001	33.35 ± 2.53	1.63 ± 0.63	<0.0001

Abbreviation: SD, standard deviation

Table 3. Patient satisfaction score

	No. of patients (n = 38)				
	4	**5**	**6**	**7**	**Mean ± SD (*P*- value)**
Baseline	38	0	0	0	4.00 ± 0.00
Week 4	0	1	25	12	6.29 ± 0.52 ($P < 0.0001$)*
Week 8	0	4	28	6	6.05 ± 0.52 ($P < 0.0001$)*
Week 12	0	9	26	3	5.84 ± 0.55 ($P < 0.0001$)*
Week 16	0	21	17	0	5.45 ± 0.50 ($P < 0.0001$)*

Patient satisfaction score: 0 = very dissatisfied, 2 = dissatisfied, 3 = somewhat dissatisfied, 4 = neutral, 5 = somewhat satisfied, 6 = satisfied, 7 = very satisfied.

* *P*-value with statistically significant difference compared with the baseline.

Table 4. Glabellar line scale score

	Patients with the corresponding GLS score (n = 38)			**Patients with improvement of 1 point or more – n (%)**	**Patients with improvement of 2 points or more – n (%)**
	1	**2**	**3**		
Baseline		15	23		
Week 4	16	16	6	27 (71.05) (*P* = 0.017)*	6 (15.79) (*P* = 0.078)
Week 8	9	19	10	18 (47.37) (*P* = 0.678)	4 (10.53) (*P* = 0.019)*
Week 12	1	22	15	8 (21.05) (*P* = 0.210)	1 (2.63) (*P* = 0.001)*
Week 16	0	17	21	2 (5.26) (*P* = 0.002)*	0 (0.00)

* *P*-value with statistically significant difference compared with the baseline.

Table 5. Visual analogue scale

	Visual analogue scale (0–100)	**P- value**
Baseline (30 minutes after the treatment)	6.35 ± 10.55	
Week 4	0.00 ± 0.00	<0.0001*
Week 8	0.00 ± 0.00	<0.0001*
Week 12	0.00 ± 0.00	<0.0001*
Week 16	0.00 ± 0.00	<0.0001*

* *P*-value with statistically significant difference compared with the baseline.

Table 6. The ratios of expression levels of collagen measured from the tissues compared to the control

	Group	CTRL/ mCTRL	Step 1/ mCTRL	Step 2/ mCTRL	Step 3/ mCTRL
Dermal collagen	**A (n = 2)**	1.000 ± 0.080	0.982 ± 0.005 (*P* = 1.000)	1.094 ± 0.005 (*P* = 0.100)	1.036 ± 0.047 (*P* = 0.400)
	B (n = 3)	1.000 ± 0.013	0.987 ± 0.269 (*P* = 0.700)	1.123 ± 0.026 (*P* = 0.100)	1.177 ± 0.024 (*P* = 0.100)
	C (n = 3)	1.000 ± 0.022	1.146 ± 0.030 (*P* = 0.100)	1.193 ± 0.027 (*P* = 0.100)	1.139 ± 0.043 (*P* = 0.100)
	D (n = 3)	1.000 ± 0.185	0.955 ± 0.016 (*P* = 0.700)	1.021 ± 0.184 (*P* = 0.700)	0.990 ± 0.012 (*P* = 0.700)
Collagen IV	**A (n = 2)**	1.000 ± 0.142	0.912 ± 0.281 (*P* = 0.629)	1.186 ± 0.033 (*P* = 0.100)	1.017 ± 0.036 (*P* = 1.000)
	B (n = 3)	1.000 ± 0.104	1.130 ± 0.057 (*P* = 0.114)	1.101 ± 0.083 (*P* = 0.200)	1.054 ± 0.100 (*P* = 0.886)
	C (n = 3)	1.000 ± 0.038	1.029 ± 0.025 (*P* = 0.143)	0.985 ± 0.053 (*P* = 1.000)	1.061 ± 0.056 (*P* = 0.229)
	D (n = 3)	1.000 ± 0.141	1.166 ± 0.006 (*P* = 0.100)	1.213 ± 0.102 (*P* = 0.114)	1.227 ± 0.094 (*P* = 0.200)

Abbreviation: mCTRL, mean value of the control group

Values are given as mean ± standard deviation.