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Received: 13 October 2023 | Revised: 9 February 2024 | Accepted: 28 February 2024

DOI: 10.1111/jocd.16273

JCD Journal of Cosmetic Dermatology WILEY

ORIGINAL ARTICLE

Treatment with synchronized radiofrequency and facial muscle stimulation: Histologic analysis of human skin for changes in collagen and elastin fibers

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Abstract

Background: Skin's exposure to intrinsic and extrinsic factors causes age-related changes, leading to a lower amount of dermal collagen and elastin.

Aim: This study investigated the effects of a novel facial muscle stimulation technology combined with radiofrequency (RF) heating on dermal collagen and elastin content for the treatment of facial wrinkles and skin laxity.

Methods: The active group subjects ($N=6$) received four 20-min facial treatments with simultaneous RF and facial muscle stimulation, once weekly. The control subject ($N=1$) was untreated. Skin biopsies obtained at baseline, 1-month and 3-month follow-up were evaluated histologically to determine collagen and elastin fibers content. A group of independent aestheticians evaluated facial skin appearance and wrinkle severity. Patient safety was followed.

Results: In the active group, collagen-occupied area reached $11.91 \pm 1.80 \times 10^6\,\mu m^2$ (+25.32%, $p<0.05$) and $12.35 \pm 1.44 \times 10^5\,\mu m^2$ (+30.00%, $p<0.05$) at 1-month and 3-month follow-up visits. Elastin-occupied area at 1-month and 3-month follow-up was $1.64 \pm 0.14 \times 10^5\,\mu m^2$ (+67.23%, $p<0.05$), and $1.99 \pm 0.21 \times 10^5\,\mu m^2$ (+102.80%, $p<0.05$). In the control group, there was no significant difference ($p>0.05$) in collagen and elastin fibers. Active group wrinkle scores decreased from 5 (moderate, class II) to 3 (mild, class I). All subjects, except the control, improved in appearance posttreatment. No adverse events or side effects occurred.

Conclusion: Decreased dermal collagen and elastin levels contributes to a gradual decline in skin elasticity, leading to facial wrinkles and unfirm skin. Study results showed noticeable improvement in facial appearance and increased dermal collagen and elastin content subsequent to simultaneous, noninvasive RF, and facial muscle stimulation treatments.

KEYWORDS
collagen, elastin, radiofrequency, rejuvenation

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1 | INTRODUCTION

Facial aging is a complex process that is a result of alterations in multiple layers of the face: the skin, subcutaneous fat, superficial musculoaponeurotic system (SMAS), muscle, and bone.[1,2] The facial muscles tend to lose their resting tone, especially when affecting the elevator muscles such as zygomaticus major muscles or frontalis muscles, leading to the repositioning of facial tissue. The elevator's inability to hold the overlying tissue in place inevitably results in soft tissue sagging. The skin and SMAS are affected by the loss of collagen and elastin, the principal structural and connective tissue proteins. Depletion of these proteins leads to wrinkle formation, reduced elasticity, and sagging appearance. Additionally, thinning of the SMAS layer contributes to laxity and jowling.[3,4] As such, when approaching facial aging, it is necessary to take into account all of the factors and ideally target all involved components. However, current noninvasive approaches focus solely on treating the skin or artificially providing facial volume to compensate for the repositioned tissue or inhibiting certain muscles to balance the loss of tone in others.

Yet, recently a novel approach has been introduced, a novel device using a noninvasive applicator that administers HIFES technology and synchronized radiofrequency (RF) simultaneously has been proposed to enhance the facial appearance.[5] HIFES selectively stimulates the elevator muscles by inducing supramaximal, involuntary, muscle contractions by generating strong electrical currents.[1] The electrical current depolarizes the motor neurons' membrane creating a signal for the muscle to contract. The recruited heat shock proteins and satellite cells can support muscle microprotein structure alterations.[6] Studies have shown that repeated application of HIFES induces muscle protein synthesis, which may result in an increase in muscle tissue density and overall enhancement of muscle tissue together with increasing the resting tone of carefully selected facial muscles in the forehead (frontalis muscle) and cheeks (zygomaticus major, zygomaticus minor, and risorius muscles).[7–10] The monopolar RF is an oscillating electrical current that is partially absorbed by the tissue, where it creates heat. The heating for a period of time is able to increase fibroblast activity and unwind the H-bonds to denaturate the collagen. After the heating stimuli stops, the body starts to induce normal regenerative process, which lead to the neocollagenesis and neoelastinogenesis. With repetition the collagen and elatine is rebuilded repeatedly.[11]

This study includes histological examination of facial skin tissue collected from the foreheads and cheeks of patients who underwent treatment using the non-invasive HIFES and RF applicator. The study objective was to assess changes related to the connective tissue structural proteins—collagen and elastin—and to evaluate the effectiveness of this combined treatment modality in enhancing the structural integrity of the skin (as shown in previous animal model studies)[12,13] and aesthetic facial appearance.

2 | MATERIALS AND METHODS

2.1 | Study design and subject population

This prospective, single-center, interventional, two-arm, open-label design study enrolled seven (7) female subjects interested in participating in a clinical study with a device intended for noninvasive facial rejuvenation. Six (6) subjects in the active group [39–62 (55.83 ± 8.56) years old, BMI 22.73–38.59 (27.65 ± 5.87) kg/m^2, Fitzpatrick skin types I to IV], received active treatment with simultaneous HIFES + RF to the cheeks and forehead. One (1) subject served as a control and was not treated [62 years old, BMI 36.9 kg/m^2, Fitzpatrick skin type IV].

The subjects from the Active group ($N = 6$) received four 20-min facial treatments, 1 week apart, with single-use, self-adhesive, noninvasive applicator electrodes (EMFACE device BTL Industries Ltd., Boston, MA). The whole face (i.e., forehead and cheeks simultaneously; see Figure 1 for applicator placement) was treated. Subjects

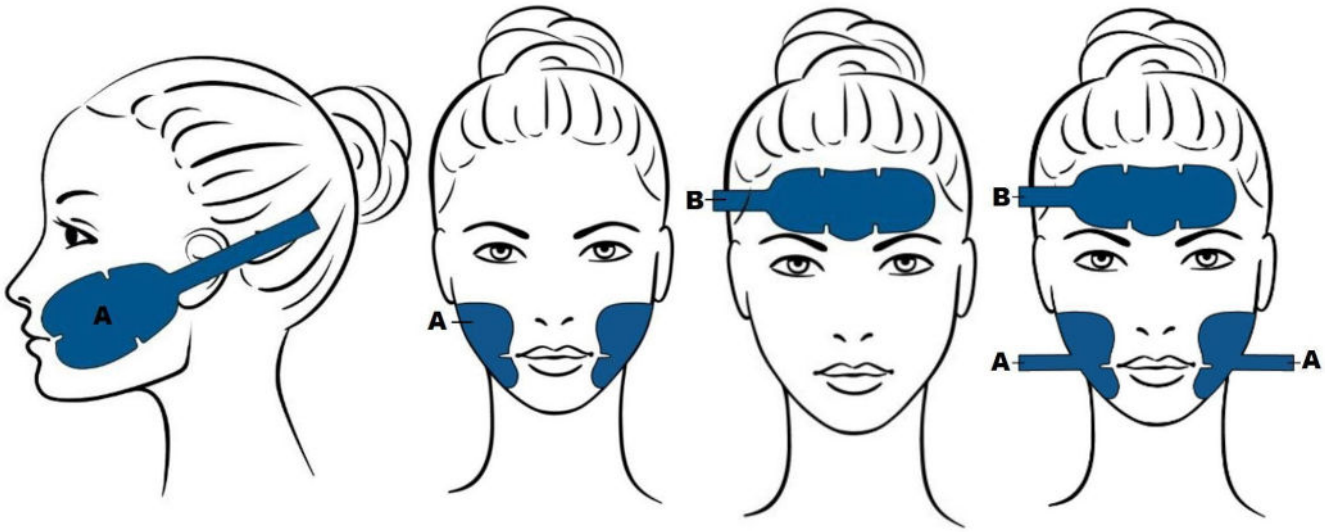

FIGURE 1 Illustration of the noninvasive, self-adhesive, hands-free device applicator placement during the therapy (A = cheek, B = forehead). The patient is treated in the supine position. The forehead and both cheeks are treated simultaneously for a 20-min duration.

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were in a supine position, anesthesia was not required. Both energy intensities (HIFES and RF) were individually set according to patients' feedback and tolerability on a scale of 0%–100%. Jewelry and prominent hair were removed from the face and the skin before the treatment.

Punch biopsies of the skin tissue from the periauricular area were performed at baseline, followed by 1-month and 3-month follow-up visits. All biopsies, 3 mm in diameter (Kruse Buster), were obtained full-thickness (approximate depth 2.0–3.0 mm) at the investigator's site. Local anesthetic was used to numb the biopsy collection area. The biopsy wounds were subsequently closed and disinfected, and the healing process was monitored throughout the study. Participants received detailed instructions for home wound care to ensure proper healing. Inclusion criteria were healthy subjects aged over 21 years, with clearly visible wrinkles on the cheeks and forehead (face) when relaxed, who were willing to abstain from any facial treatments other than the study procedure during study participation. Exclusion criteria prohibited subjects with any active psoriasis, eczema, local infection in the treatment, neurological disorders, or electroanalgesia. The study was IRB approved and registered on clinicaltrials.gov (NCT05524662).

2.2 | Histological evaluation

The obtained skin tissue samples were processed for histological examination. All samples were correctly labeled using subject ID and the description of the corresponding visit—pretreatment (baseline) and posttreatment (1-month and 3-month follow-up visits). The samples were sliced, and the sections were then stained to visualize and examine collagen and elastin under a microscope.

A clinical histologist analyzed the stained samples to evaluate the changes in the tissue, including any pathological changes to rule out potential skin damage resulting from treatment with HIFES and RF noninvasive electrode applicators.

The staining process employed conventional Masson's trichrome procedure, which enables a detailed visual assessment of collagen and the general tissue structure. Collagen fibers developed a green color. Orcein staining was used to specifically visualize elastin in the samples. The elastin fibers developed a brown-dark color. The stained samples were mounted to microscopy slides.

The slides were observed and photographed using an automated slide scanning microscope (Hitachi Axio Scan.Z1, Carl Zeiss AG, Germany; 20×/0.8NA Plan-Apochromat objective) in a bright field. Collagen and elastin quantification was performed with the Image J software based on semi-automatic segmentation in the HSB (Hue-Saturation-Brightness) color system. The appropriate threshold differentiating the collagen and elastin fibers from the background was identified in the selected regions of interest (ROI = 1800 × 1200 µm; $2.16 \times 10^6\,\mu m^2$) at a defined scale, allowing for the quantification of the area covered by collagen and elastin within the tissue sections. After selecting the collagen and elastin fibers, their densities were expressed as the occupied area (square micrometers) encompassed by the fibers in the evaluated images' in the ROI.

Statistical analysis Student's *t*-test and Friedman's one-way repeated measures ANOVA test were performed with significance level set at $\alpha = 0.05$. Post hoc Tukey HSD test was done for multiple comparisons.

This process was repeated for the seven subjects included in this two-arm study design, which consisted of one control subject and six active treatment subjects. The changes in the average areas representing elastin and collagen content before and after treatment were compared for the active group subjects and the control subject.

2.3 | Aesthetic improvement and wrinkle severity

Subjects had digital photographs of their faces taken before treatment (baseline) and at the follow-up visits. Three reviewers, independent of each other and the clinic, evaluated the facial skin appearance according to the Global Aesthetic Improvement Scale by comparing the before and after photos marked by follow-up visit.

A different trio of evaluators received the same set of photographs and evaluated the extent of skin wrinkles and lines (wrinkle severity) according to the Fitzpatrick Wrinkle Elastosis Scale (FWES). The evaluators were blinded to the follow-up stage (i.e., the photos were not labeled according to the follow-up visit and the order in which the evaluators were shown the photos was randomized). Each evaluator assigned a FWES grade per photograph.

2.4 | Safety

The occurrence of treatment or study-related adverse events and side effects was followed/monitored during the course of the trial.

3 | RESULTS

3.1 | Collagen

In the Active group, the average area occupied by collagen was $9.50 \pm 1.77 \times 10^5\,\mu m^2$ at baseline. The average collagen-occupied area increased to $11.91 \pm 1.80 \times 10^6\,\mu m^2$ ($p < 0.05$) and $12.35 \pm 1.44 \times 10^5\,\mu m^2$ ($p < 0.05$) at the 1-month and 3-month follow-up visits. Compared to baseline, in the active group, the average collagen amount increased ($p < 0.05$) at both posttreatment follow-ups. In the control group, there was no significant difference ($p > 0.05$) in collagen fibers, since the collagen content fluctuated in the range of $9.68 \pm 0.45 \times 10^5\,\mu m^2$–$9.51 \pm 0.23 \times 10^5\,\mu m^2$ throughout the study. There was a significant difference ($p < 0.05$) in the collagen-occupied sample area at both the 1-month (+25.32%) and 3-month (+30.00%) follow-ups (see Figure 2) in the treated (active) group.

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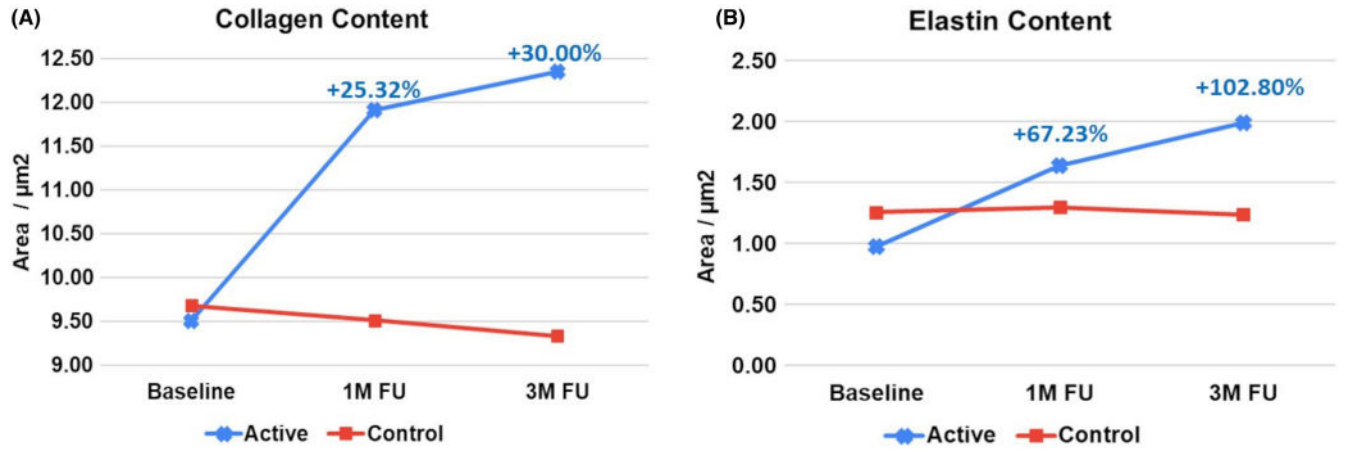

FIGURE 2 Bright-field visualization of collagen fibers by Masson's Trichome method. In the active group, the collagen fibers (A) and elastin fibers (B) increased significantly ($p < 0.05$) and occupied a greater area after 3 months follow-up compared to baseline. No significant change occurred in the Control group. (1M FU: 1-month follow-up; 3M FU: 3-month follow-up).

3.2 | Elastin

In the Active group, the mean elastin-occupied area at baseline was $0.98 \pm 0.34 \times 10^5\,\mu m^2$. At the 1-month follow-up, the average elastin amount was $1.64 \pm 0.14 \times 10^5\,\mu m^2$. At the 3-month follow-up, the area encompassed by elastin increased further to $1.99 \pm 0.21 \times 10^5\,\mu m^2$. Compared to baseline, the average elastin-occupied area increased at both follow-ups ($p < 0.05$). At the 1-month (+67.23%) and 3-month (+102.80%) follow-ups, the amount of elastin was significantly different ($p < 0.05$) comparing the active and control group (Figure 2). In the control group, there were no significant changes ($p > 0.05$) in elastin density at the follow-up points, elastin content ranged between $1.26 \pm 0.16 \times 10^5\,\mu m^2$–$1.30 \pm 0.12 \times 10^5\,\mu m^2$.

Exemplary samples of the collagen (Figure 3) and elastin (Figure 4) microscopic evaluation results of the Active group are shown below (Figure 5).

3.3 | Safety

No adverse events or side effects related to the treatment occurred. Histopathological analysis of the biopsy sample did not reveal any complications or unwanted and unexpected effects.

3.4 | GAIS & FWES results

Overall, the average score of the GAIS evaluation reported that all subjects, except the control, had an improved appearance post-treatment.

The average scores of the active group decreased by 2 grade points on the FWES and GAIS from 5 (moderate, class II wrinkles) to 3 (mild, class I fine lines). The evaluators did not find any change in the control subject's appearance.

Detailed results of the GAIS and FWES independent reviewers' evaluations can be found in Tables 1 and 2.

4 | DISCUSSION

This study demonstrates that noninvasive facial treatment with simultaneous HIFES and RF can induce collagen and elastin expression. In this study, a significant increase ($p < 0.05$) of collagen (+30%) and elastin (+103%) content was observed in dermal biopsy analysis 3 months posttreatment.

The observed increase in elastin content surpasses that of collagen by a factor of around three, marking a noteworthy finding. Upon initial observation, one might perceive the response to changes in elastin levels as deviating from the usual. However, upon examining the baseline data of the active group, it becomes apparent that the levels of elastin are significantly diminished, accounting for approximately 3.5% of the total cross sectional area. In contrast, typical physiological levels of elastin typically fall within the range of 5%–10%. Following the administration of the therapies, there was a twofold increase in elastin levels, resulting in the occupation of 7% of the slice area. This observation indicates a restoration of skin elastin levels to a state consistent with healthy physiological levels.[14–16] Additionally, as SMAS is a tissue that is intertwined with the rest of the facial structures and is made of collagen and elastin, we believe the SMAS layer was also affected and helped with the results.[17] Furthermore, the observed outcomes should not be regarded as a malfunction (e.g., flawed staining process) or an uncommon occurrence. This is supported by the findings of a study conducted by Kinney et al., which examined the effectiveness of HIFES+RF technology on an animal model. In their study, they reported a significant 110.8% increase in elastin levels, which aligns with the outcomes of our own investigation. A similar trend is observed in the case of collagen, as reported by Kinney et al., where there was a notable increase of 26.3%.[5]

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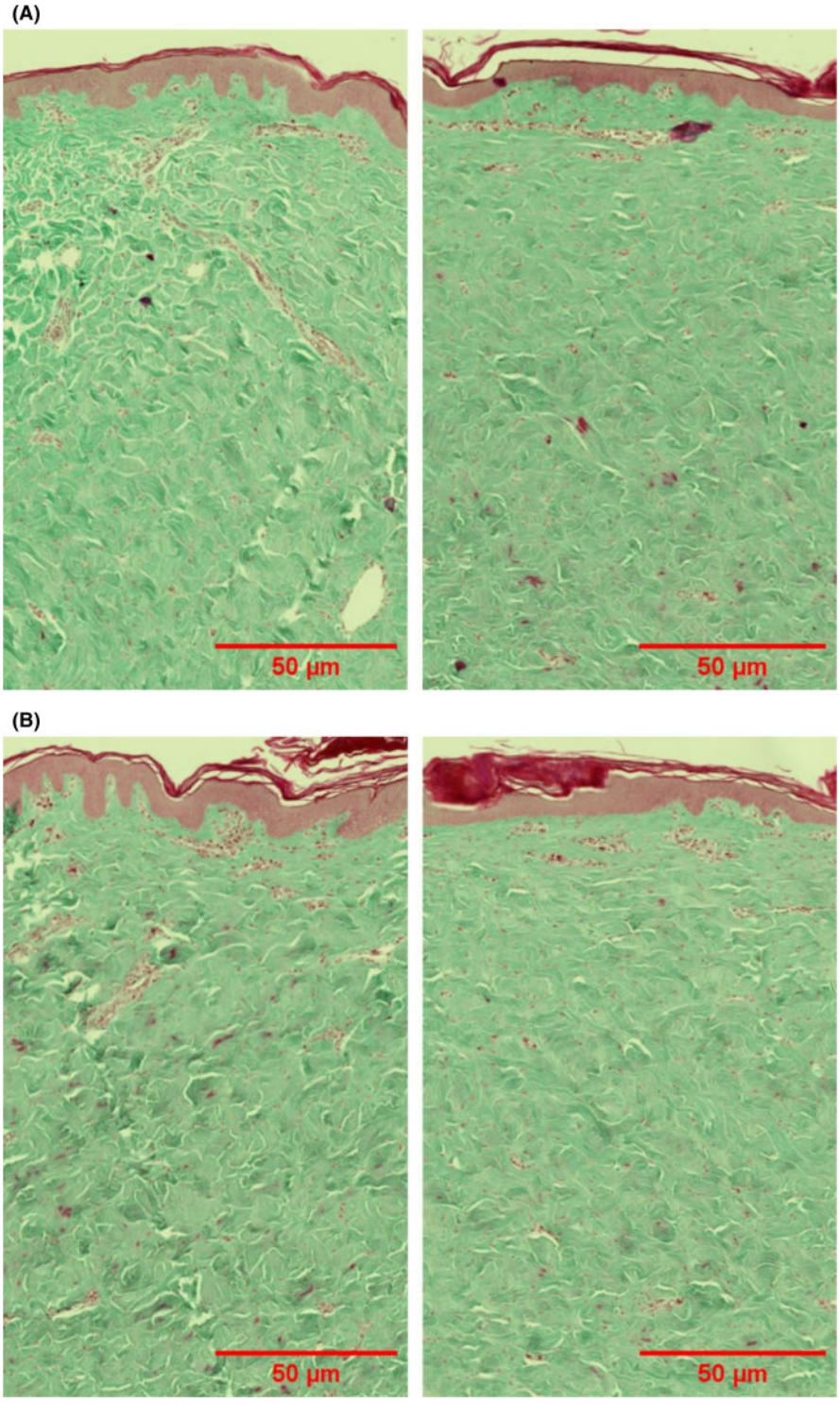

FIGURE 3 Bright-field visualization of collagen fibers stained by trichrome stain, active group samples of two patients (A, B). The collagen fibers appear in green color. At the 3-month follow-up (right), the collagen fibers are noticeably denser, occupying a greater area when compared to the baseline (left).

The substantial increase in the production of elastin and collagen could be attributed to the multimodal approach of the HIFES+RF technology. The application of RF energy has been found to elicit a thermal response in fibroblasts, whereas the HIFES technology induces muscle contractions that mechanically stimulate the skin. These processes have been shown to cause similar responses in fibroblast cells.[18–21] The findings of the study demonstrate a consistent elevation in the concentrations of collagen and elastin, which reached their highest point after a period of 3 months. This aligns with existing understanding on the time it takes for fibroblasts to react to external stimuli of this nature. However, additional research is required to examine the longevity of such an increase.

Besides histological methods, this study further incorporates FWES and GAIS evaluations (by six qualified, independent, clinical aestheticians), all objective basis. As such, the objective evaluation of patient appearance provides insight on significant increase in crucial skin components, namely elastin and collagen. The results

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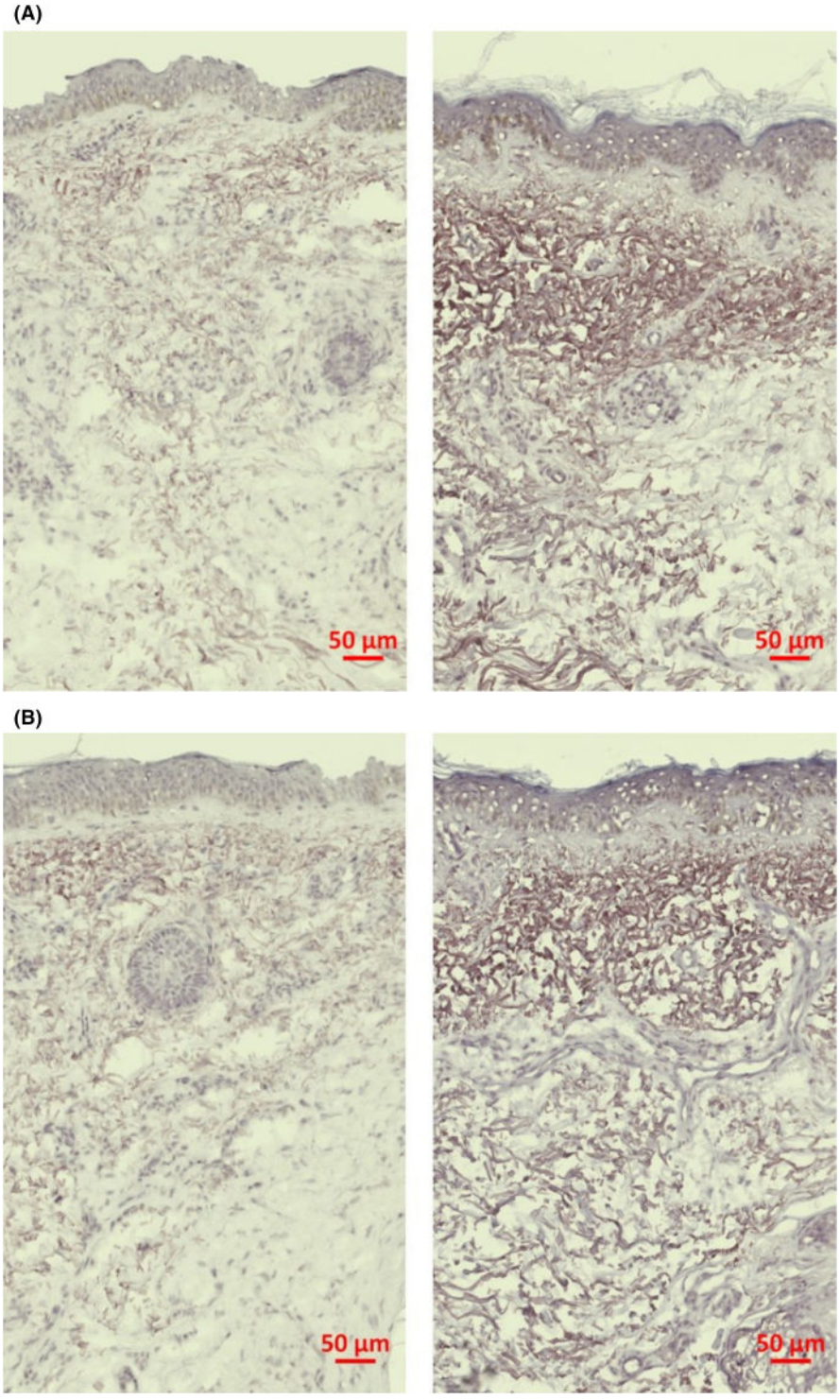

FIGURE 4 Bright-field visualization of elastin fibers by Orcein staining (active group samples of two patients, A and B). The baseline sample (left) has noticeably fewer elastin fibers, observed as dark/brown filaments, than the 3-month follow-up (right). 3-month follow-up shows elastotic fibers replaced with new, longer elastic fibers.

of both evaluation scales indicate a substantial enhancement in the active group, but the control group exhibited no change. These findings provide empirical support for the rejuvenation effect of the HIFES+RF modality.

This objective evaluation approach exhibited a favorable outcome. The findings from the Facial Wrinkle Evaluation Scale (FWES) demonstrate that patients who had scores of 6 or above, indicating moderate to severe wrinkles, experienced more substantial improvements (see Table 1). Comparable results have been observed when comparing the FWES findings to the Global Aesthetic Improvement Scale (GAIS), (Table 2). In contrast, subject number 2, who had only minor wrinkles at the baseline, did not have a major improvement according to the FWES. However, evaluators rated this subject best possible score on the GAIS. The observed discrepancy indicates that the involvement of facial muscles in the aging process holds considerable importance in the context of face rejuvenation, as outlined in the introductory section. The findings of this study may indicate that the utilization of both modalities (RF and HIFES) in combination is

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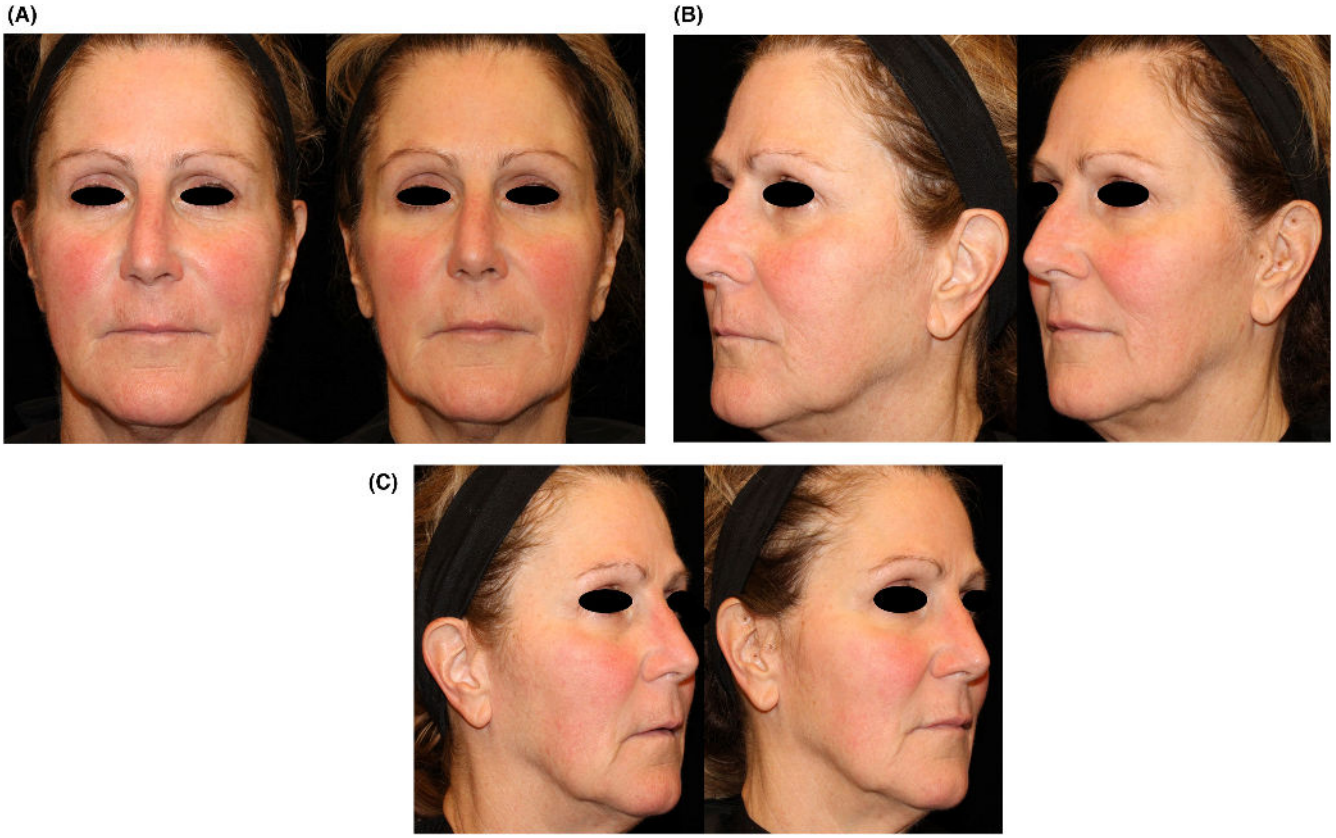

FIGURE 5 Female patient (Skin Type I, 60 years old, BMI 22.6 kg/m^2) at baseline (left) versus 3 months (right)—frontal view (A), left (B) and right (C) lateral oblique angles. The fine lines around the eyes present at baseline have reduced in severity and are less prominent, as well as lifted brows are visible after the treatments. GAIS score = 2 (i.e., much improved), FWES score decreased from 6 (moderate, class II) at baseline to 4 (moderate, class II) at 3-month follow-up.

TABLE 1 Fitzpatrick Wrinkle Elastosis Scale evaluation.

Subject ID	EV 1	EV 2	EV 3	Average	EV 1	EV 2	EV 3	Average
Subject 1	6	5	6	6	4	3	4	4
Subject 2	3	2	2	2	2	1	1	1
Subject 3	4	4	5	4	3	3	4	3
Subject 4	5	5	5	5	4	4	4	4
Subject 5	6	6	6	6	3	3	3	3
Subject 6	7	6	7	7	4	5	4	4
Subject 7[a]	6	5	6	6	6	5	6	6
	Average of active group			5	Average of active group			3

[a]Control subject.

more effective compared to therapies that solely incorporate either one of them.

Furthermore, the study device's practicality increases the comfort for both the operator and patient. The hands-free, self-adhesive applicator provides a significant enhancement in terms of safety and comfort aspects. Operator fatigue is eliminated, and the patients underwent the facial rejuvenation procedures without recovery time or impact to their daily routine. Adverse effects common with some facial rejuvenation therapies such as hyperpigmentation, dyschromia, hematoma and nerve damage did not occur in this study.[22–24]

This clinical study was limited due to the low subject count. Despite that the all-female patient set is also a limiting factor, it must be considered that real-world data have shown women as the vast majority seeking facial cosmetic and rejuvenation procedures.[25] This may be driven by an increased interest and diversity of the patients motivated to undergo treatments aimed at regenerating the body in a more natural way, as opposed to augmentative and

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TABLE 2 Global aesthetic improvement score evaluation.

Subject ID	EV 1	EV 2	EV 3	Average
Subject 1	2	2	2	2
Subject 2	3	3	2	3
Subject 3	1	1	1	1
Subject 4	1	2	2	2
Subject 5	3	3	3	3
Subject 6	3	3	2	3
Subject 7[a]	1	0	0	0

[a]Control subject.

invasive procedures, which provide a different less appealing result. Nonetheless, further in-vivo investigations of this method are warranted to expound the clinical aspects.[26]

5 | CONCLUSION

The cumulative effects of reduced collagen and elastin, lead to an overall loss of skin elasticity, resulting in skin laxity or loose skin. This is especially noticeable in the face, where laxity leads to sagging, the formation of wrinkles and fine lines, and a generally "aged" appearance. This study histologically evaluated facial skin tissue samples for collagen and elastin content pre and post-treatment with the simultaneous HIFES and RF noninvasive. Results demonstrated increased levels of dermal collagen and elastin at the 3-month follow-up.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

ETHICS STATEMENT

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to and the appropriate ethical review committee approval has been received. The study was conducted in line with Good Clinical Practice and the Decalaration of Helsinki. The study protocol and informed consent form were approved by the Advarra IRB institutional review board on the 3rd of May 2022 (reference number Pro00063141). Prior to enrolment, subjects provided voluntary written informed consent (date and signature). Subjects further provided voluntary written consent (date and signature) to have photographs of their face taken for the study records, publication and marketing purposes.

CONSENT STATEMENT

Prior to enrolment, subjects provided voluntary written informed consent (date and signature) to have photographs of their face taken for the study records and publication.

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How to cite this article: Goldberg DJ, Lal K. Treatment with synchronized radiofrequency and facial muscle stimulation: Histologic analysis of human skin for changes in collagen and elastin fibers. *J Cosmet Dermatol*. 2024;00:1-9. doi:10.1111/jocd.16273

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