

# Epidermal and Dermal Regeneration Through Sequential Monopolar and Bipolar Radiofrequency: Evidence From an Aging Mouse Model

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## Abstract

**Background:** It is well established that the aging process triggers various physiological changes, which may manifest as loss of hydration and the degradation of collagen and elastin fibers, ultimately leading to lines, wrinkles, and skin laxity. To restore the skin's youthful appearance, both noninvasive and minimally invasive procedures have been developed.

**Objectives:** The authors of this study aim to evaluate the safety and efficacy of noninvasive monopolar vs sequential monopolar–bipolar radiofrequency (RF) irradiation from a single tip on skin quality in an aged mouse model.

**Methods:** The experiment involved male mice aged 4 and 12 months. The Density RF device (Jeisys Global, Seoul, South Korea) used for irradiation employs both monopolar (Classic-Tip) and monopolar–bipolar (High-Tip) RF, operating at a frequency of 6.78 MHz. The device features an automatic impedance matching system that measures impedance in real time and determines the corresponding compensation value. RF irradiation was performed after applying coupling fluid to the dorsal skin of mice (4 cm<sup>2</sup>) using an energy density of 63.4 J/cm<sup>2</sup> per shot under anesthesia. RF was applied on Day 0; subsequently, the mice were sacrificed on Days 1 and 28 for sample harvesting and histological study.

**Results:** The RF-irradiated group has shown a significant increase in skin hydration and elasticity. The aging control group showed a 1.7-fold reduction in epidermal thickness compared with the young control group. However, in comparison with the aging control group, after 28 days, the RF irradiation group showed a substantially thicker epidermis, up to 1.5-fold thicker (monopolar group: 15.797 ± 1.093 μm, monopolar–bipolar group: 15.600 ± 0.963 μm). The monopolar group showed a 1.8-fold ( $P < .05$ ) increase in collagen density; however, the monopolar–bipolar group showed a 2.4-fold ( $P < .05$ ) increase. Notably, the monopolar–bipolar group demonstrated a substantial increase in collagen density compared with the controls. Elastin fiber expression significantly increased in both monopolar and monopolar–bipolar groups compared with the controls. However, the highest expression of elastin fibers was observed in the monopolar–bipolar group. Further, polymerase chain reaction analysis confirmed a significant increase in gene markers, such as Blimp1, Fsp1, and Dlk1, suggesting that RF irradiation promotes fibroblast differentiation.

**Conclusions:** The sequentially monopolar–bipolar RF irradiation (Density High-Tip) system significantly increases epidermal thickness, collagen, and elastin fiber production and promotes fibroblast cell differentiation compared with the monopolar RF alone.

## Level of Evidence: 4 (Therapeutic)

From antiquity, both men and women invested their time and energy in the pursuit of a “fountain of youth” to live longer and maintain a youthful appearance. They tried various modalities to preserve their youthfulness. As the largest organ, the skin, exposed to the external environment, shows early signs and symptoms of aging. Skin aging is generally divided into 2 types: extrinsic and intrinsic.<sup>1–3</sup> Extrinsic aging

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is induced by external factors and lifestyle, most notably environmental ultraviolet exposure, which causes pigmentation, coarse wrinkles, reduced skin elasticity, and loss of hydration. Intrinsic aging is a genetically regulated process characterized by the formation of fine wrinkles, a decrease in the extracellular matrix (ECM), and a reduction in skin thickness.<sup>4-7</sup>

Furthermore, from a biological perspective, oxidative stress can cause DNA damage and nonspecific cross-linking of collagen fibers, resulting in skin aging.<sup>8,9</sup> In aesthetic medicine, the development of noninvasive technologies, particularly lasers and radiofrequency (RF), has emerged as a significant advancement.<sup>10,11</sup> Specifically, RF devices operate at a frequency of 6.78 MHz to deliver electromagnetic waves that induce regulated heat within the dermal layers. The induced heat penetrates the skin depending on its condition; this selective dermal heating drives collagen remodeling and supports tissue regeneration. In conclusion, RF treatment has been widely utilized to improve skin sagging and facial laxity.<sup>12</sup>

RF energy is delivered in various ways, depending on the number of electrodes, and is generally categorized into monopolar, bipolar, unipolar, and multipolar frequencies. In monopolar systems, energy is generated from a single electrode that flows toward a grounding pad. This system enables deep thermal penetration, reaching fibrous septae, deep dermis, and subcutaneous tissues.<sup>12,13</sup> Bipolar energy is delivered between 2 electrodes, resulting in more localized and superficial heating within the epidermis and upper dermis. Unipolar RF uses a single electrode to generate heat by oscillating water molecules and does not require a grounding pad.<sup>14</sup> Lastly, a multipolar system generates thermal heating through 3 or more electrodes.<sup>15</sup> It enables uniform and controlled heat distribution in the treatment area.<sup>16</sup> Based on thermal theory, the depth of energy penetration is calculated by the distance between the electrodes.

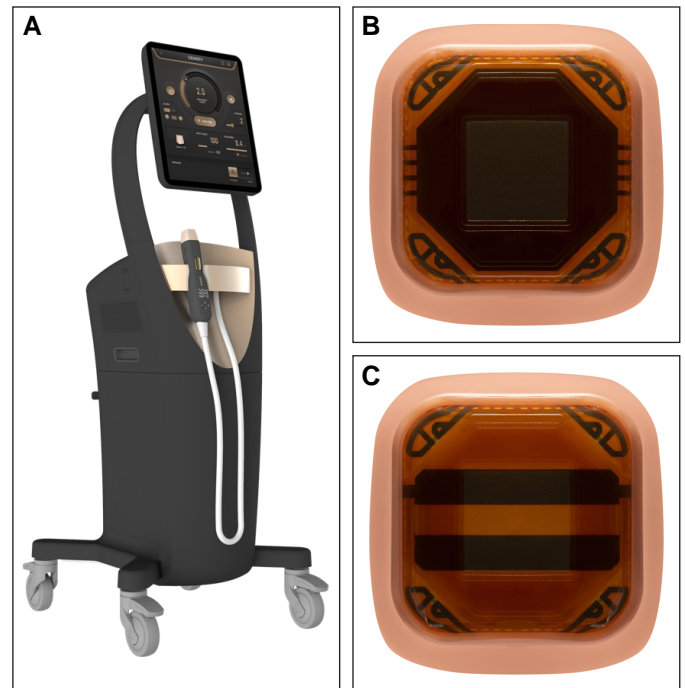
Evidence suggests that sequential irradiation using monopolar and bipolar (MONO-BI) RF with microneedling significantly increased the density of collagen and elastin fibers compared with the MONO-MONO, BI-MONO, and BI-BI modes.<sup>17</sup> These studies were conducted at a frequency of 1 and 2 MHz. Therefore, we decided to study the most effective irradiation mode using a noninvasive monopolar (Classic-Tip) and a combined monopolar–bipolar (High-Tip) RF system, employing sequential pulse treatments in an aged mouse model operating at 6.78 MHz.

## METHODS

### Animals and Radiofrequency Irradiation

Six-week-old male and female C57BL/6N mice were purchased from Orient Bio (Seoul, Korea). Mice were housed in a 12 h light/dark cycle at  $24 \pm 2^\circ\text{C}$  under 30% to 70% humidity at the Gachon University animal facility. After the acclimation period, the mice were bred, and their offspring were raised until 12 months of age. We also bought 4-month-old male mice, which underwent a 1-week acclimation period following their purchase. Subsequently, the animals were randomly assigned to 1 of 6 groups (6 groups  $\times$  3 animals per group), either the young, aging control, or intervention group ( $n = 18$ ).<sup>18</sup>

Gachon University adheres to standard laboratory animal facility practices that align with international guidelines, ensuring the safety of personnel and the welfare of animals. The Center of Animal Care approved all animal experiments and Use Ethical Board of Gachon



**Figure 1.** (A) Density radiofrequency device. (B) Density monopolar tip (Classic-Tip). (C) Density monopolar–bipolar (High-Tip).

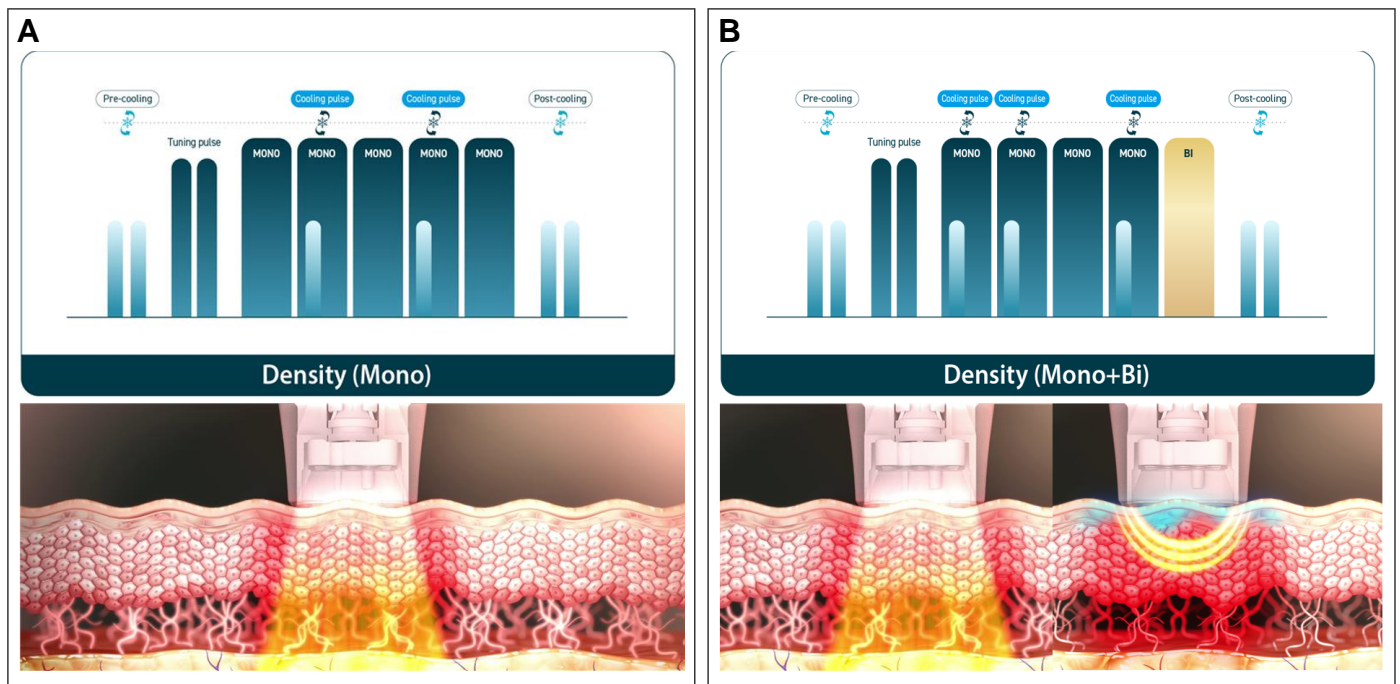
University (approval number LCDI-2021-0066), and conducted under the Institutional Animal Care and Use Committee.

### Radiofrequency System

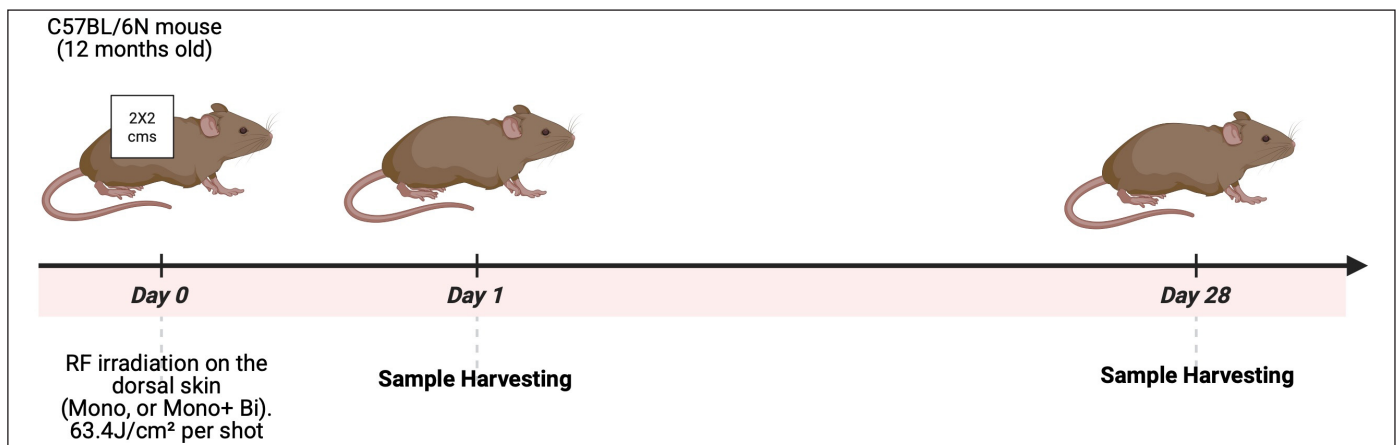
DENSITY (Jeisys Medical Inc, Seoul, South Korea) was used to irradiate the RF. The device operates at a power output of 400 W and a frequency of 6.78 MHz. The system incorporates an impedance-based, real-time energy feedback mechanism that adjusts output power dynamically to maintain an optimal thermal effect. The tips are of 2 types: the first type is a monopolar tip referred to as Classic-Tip (4 cm<sup>2</sup>), which delivers monopolar RF energy through a square-shaped electrode, whereas the second type is a combination of MONO-BI in a single tip referred to as High-Tip, which sequentially emits both MONO-BI RF energy. High-Tip features a square-shaped monopolar electrode, 2 bipolar electrodes, and an area of 4 cm<sup>2</sup> (Figure 1A-C). Density RF system incorporates a cryogenic gas-based contact cooling system that delivers powerful, rapid cooling before, during, and after RF irradiation.

The sequential delivery of MONO-BI energy occurs within a single shot. Each shot consists of 5 pulses: 4 monopolar pulses followed by 1 bipolar pulse, allowing for the continuous delivery of both energy types in sequence (Figure 2A, B). Each pulse lasts 200 ms, with a 50 ms pause between pulses. Additionally, using the same fluence, thermal penetration was assessed for both tips. Thermal imaging shows that the Classic-Tip (monopolar) heats to a depth of 3 mm, whereas the High-Tip (monopolar–bipolar) reaches a depth of 2.5 mm.

On Day 0, RF irradiation was performed after applying sufficient coupling fluid to the dorsal skin of the mice (4 cm<sup>2</sup>) using an energy density of 63.4 J/cm<sup>2</sup> per shot, under anesthesia. All measurements were conducted on Days 0, 1, and 28. Before irradiation and



**Figure 2.** (A) Classic-Tip (monopolar radiofrequency [RF]) irradiation pattern. (B) High-Tip (monopolar–bipolar RF) irradiation pattern.



**Figure 3.** Mouse model to evaluate skin quality postradiofrequency irradiation.

measurement, the hair on the treatment site was removed. All the animals were euthanized for sample acquisition (Figure 3).

## Evaluation of Skin Hydration and Elasticity

Skin hydration and elasticity were measured using a skin diagnostic device (Aram Huvis, API-100) before RF irradiation and tissue sampling. A total of 5 measurements were taken per treatment site. The 3 values closest to the median were selected to calculate the average.

## Sample Preparation

For histological and molecular analyses of skin properties, tissue samples were collected on Days 1 and 28 after RF irradiation.

Animals were randomly selected for the sample acquisition. The tissues were homogenized using the RNAiso Plus reagent (Takara, Shiga, Japan), according to the manufacturer's instructions. The extracted RNA was quantified using the Nanodrop 2000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA).

## Histological Analysis

The collected tissues from each group were fixed in 4% paraformaldehyde (Sigma-Aldrich, St Louis, MO) and rinsed for 30 min to prepare for embedding. The tissues were embedded in paraffin and sectioned at 7  $\mu$ m using a Leica microtome (Wetzlar, Germany). The slides were stained with hematoxylin and eosin (H&E), Verhoeff–Van Gieson

(VVG), Herovici's trichrome, and Masson's trichrome (MT) to evaluate skin architecture and density of elastin and collagen.

## Immunohistochemistry

Immunohistochemical (IHC) staining was performed to assess the expression of collagen 1A1 (COL1A1) and collagen 3A1 (COL3A1). The sections were processed according to standard protocols. In brief, sectioned slides were deparaffinized in xylene and serially rinsed with ethanol and distilled water. Then, endogenous peroxidase activity was blocked with 0.3% hydrogen peroxide, and nonspecific binding was reduced by preincubation with normal goat serum. Next, sections were incubated with primary antibodies anti-col1a1 (Santa Cruz, sc-293182, 1:50), anti-col3a1 (bioss, bs-0549R, 1:100) at 4°C overnight. Signal detection was developed using an HRP/DAB detection kit (Sigma-Aldrich) with hematoxylin counterstaining, where applicable. Finally, immunostained slides were examined using an optical microscope (Olympus Optical Co, Tokyo, Japan), and data were analyzed using ImageJ software (NIH, Bethesda, MD).

## Quantitative Analysis of Histological Staining Images

Tissue sections were scanned and magnified using an automated slide scanner (Motic Scan Infinity 100; Motic, Hong Kong). H&E staining was performed to assess epidermal skin thickness in young and aged mice. Measurements were obtained from magnified images, and pixel values were converted to micrometers using ImageJ software (NIH).

## Quantitative Real-Time Polymerase Chain Reaction

The quantified RNA was synthesized into cDNA, and quantitative real-time polymerase chain reaction (PCR) was performed using a QuantStudio 3 real-time PCR system (Thermo Fisher Scientific) with SYBR Green reagent (Takara) mixed with the synthesized cDNA.

## Statistical Analysis

All experimental data were expressed as mean  $\pm$  standard deviation. Group comparisons were analyzed using the Kruskal–Wallis test and post hoc analysis with the Mann–Whitney *U* test. All statistical analyses were conducted using SPSS software v.26 (IBM Corporation; Armonk, NY).

## RESULTS

RF irradiation increases skin hydration and epidermal thickness. To evaluate the effect of RF irradiation on skin quality, skin hydration and elasticity were measured in both MONO and MONO-BI groups. Four-month-old mice were used as a young control group. The aging control group consisted of 12-month-old mice, which, without any RF irradiation, exhibited significantly reduced skin hydration and elasticity. Compared with the young control group, there was a nearly 4.3-fold decrease in hydration and elasticity, indicating an age-related loss of skin hydration and elasticity.

On Day 1, there was no significant difference in skin hydration and elasticity between the RF-irradiated group (MONO or MONO-BI) and the control group. By Day 28, both the MONO and MONO-BI groups exhibited a significant increase in skin hydration and elasticity compared with the aging control. However, no significant change was observed between the MONO and MONO-BI groups (Figure 4A, B).

Epidermal thickness was measured using H&E staining. The epidermal thickness in the young and aging control groups was measured at  $17.183 \pm 1.063$  and  $10.324 \pm 0.843$   $\mu\text{m}$ , respectively. The aging control group showed a 1.7-fold reduction in epidermal thickness compared with the young control group. However, 28 days after RF irradiation, the epidermis became substantially thicker, up to 1.5-fold (28 days MONO group  $15.797 \pm 1.093$   $\mu\text{m}$ , MONO-BI group  $15.600 \pm 0.963$   $\mu\text{m}$ ). There was no statistically significant difference between the MONO and MONO-BI groups. RF irradiations promoted the elevation of skin barrier components through epidermal differentiation (Figure 4C, D).

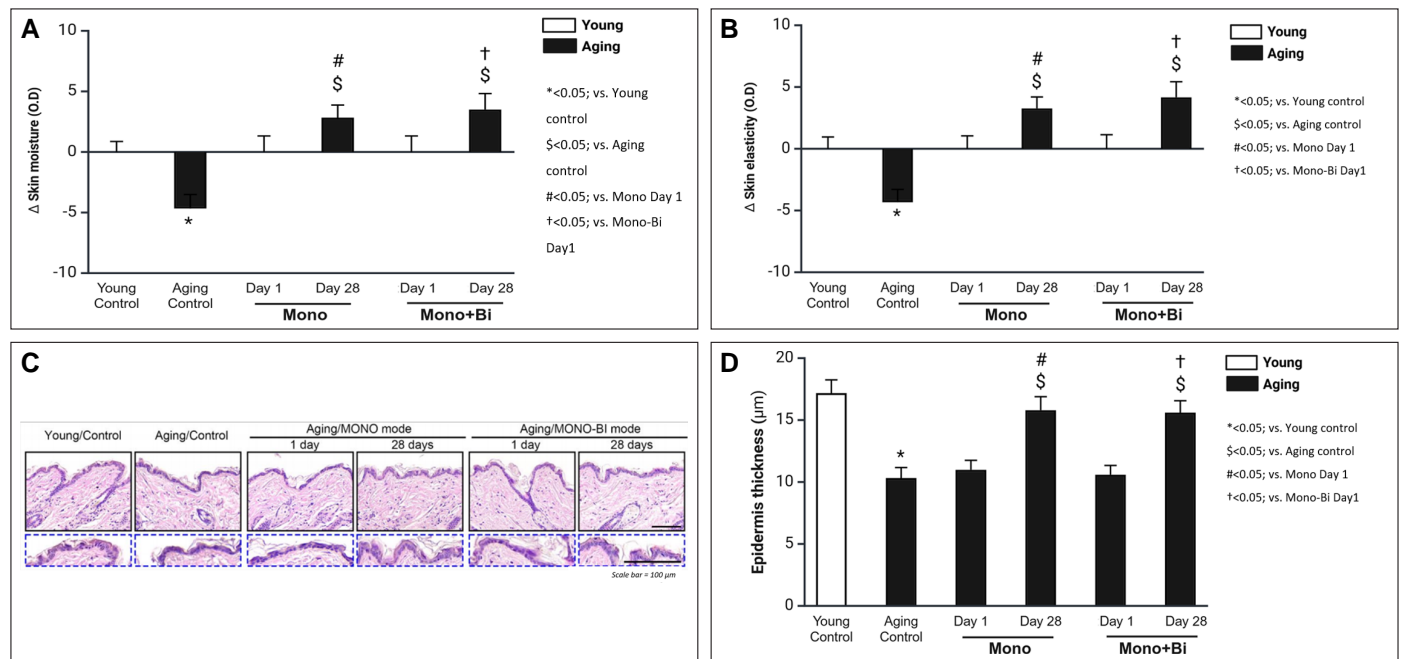
RF irradiation enhances the density of collagen and elastin fibers and stimulates the synthesis of newly formed collagen. MT staining revealed lower collagen expression in the aging control group compared with the young control group. The amount of collagen fibers was measured in the RF-irradiated groups compared with the aged control group. There was a significant increase in collagen with MONO, 1.8-fold ( $P < .05$ ), and MONO-BI, 2.4-fold ( $P < .05$ ). Notably, the MONO-BI group exhibited a substantial increase in collagen density compared with the controls (Figure 5A, B).

Furthermore, VVG staining demonstrated a lower density of elastin fibers in the aging control group compared with the young control group. In the RF-irradiated groups, elastin fiber expression significantly increased in both MONO and MONO-BI groups compared with controls. Moreover, the highest expression of elastin fibers was observed in the MONO-BI group (Figure 5C, D).

Additionally, to confirm the synthesis of newly formed collagen fiber, Herovici's collagen staining was performed; newly synthesized collagen is stained blue, whereas mature collagen is stained red. In the young control group, newly formed collagen was significantly more prominent. In contrast, the aging control group predominantly showed mature collagen. However, following RF irradiation, both the MONO and MONO-BI groups significantly promoted the synthesis of newly formed collagen fibers. A slight increase in mature collagen density is likely because of the natural aging process. These results indicate that RF irradiation accelerates the synthesis of both collagen and elastin fibers (Figure 5E-G).

RF irradiation upregulates the expression of genes involved in collagen synthesis. To investigate whether RF irradiation affects the expression of key factors related to collagen and elastin production, we performed quantitative PCR analysis of the related genes *col1a1*, *col3a1*, elastin (*ELN*), and elastin-binding protein (*EBP*). Type I collagen, encoded by the  $\alpha 1$  (COL1A1) gene, is a major structural component of skin connective tissue and is crucial in maintaining dermal strength and integrity.<sup>19</sup> Type III collagen  $\alpha 1$  (COL3A1), synthesized as a precursor molecule (preprocollagen), is involved in tissue remodeling and wound healing.<sup>20</sup> The expression levels of *col1a1* and *col3a1* were significantly lower in aged mice compared with the young control group. RF irradiation significantly increased expression of both genes in the MONO and MONO-BI groups (Figure 6A, B).

Elastin (ELN) and EBP are essential for forming elastic fibers, with EBP supporting the regulation and synthesis of elastin-related genes.<sup>21-27</sup>



**Figure 4.** (A) Skin hydration improved because of MONO or MONO-BI RF irradiation. (B) Skin elasticity improved because of MONO or MONO-BI RF irradiation. (C) Histology demonstrates improvement in skin quality because of MONO or MONO-BI RF irradiation. (D) Epidermal thickness increased because of MONO or MONO-BI RF irradiation. MONO-BI, monopolar and bipolar; RF, radiofrequency.

RF irradiation significantly upregulated the expression of genes in both irradiation modes (Figure 6C, D).

To confirm the more specific changes, we conducted IHC staining. In the aging control group, staining for COL1A1 and COL3A1 showed low-intensity levels. However, the RF-irradiated groups exhibited significantly increased staining intensity for both genes with MONO and MONO-BI. Notably, the MONO-BI mode showed the highest intensity with both COL1A1 and COL3A1 (Figure 6E-H).

RF irradiation affects the dermal differentiation of fibroblasts; therefore, we conducted quantitative PCR analysis to investigate whether dermal fibroblast differentiation is affected. Dermal fibroblasts have 2 distinct subpopulations—papillary and reticular dermis fibroblasts—each exhibiting unique properties.<sup>28-30</sup> Several relevant marker genes, including *Blimp1* and *Fsp1*, were selected for study, as they are associated with papillary dermis fibroblasts. B lymphocyte-induced maturation protein 1 (*Blimp1*) marks papillary dermal fibroblast precursors, whereas ferroptosis suppressor protein 1 (*Fsp1*) is associated with papillary fibroblast identity.<sup>31-33</sup> The RF-irradiated groups demonstrated a significant increase in the expression of both genes compared with the aging control group (Figure 7A, B).

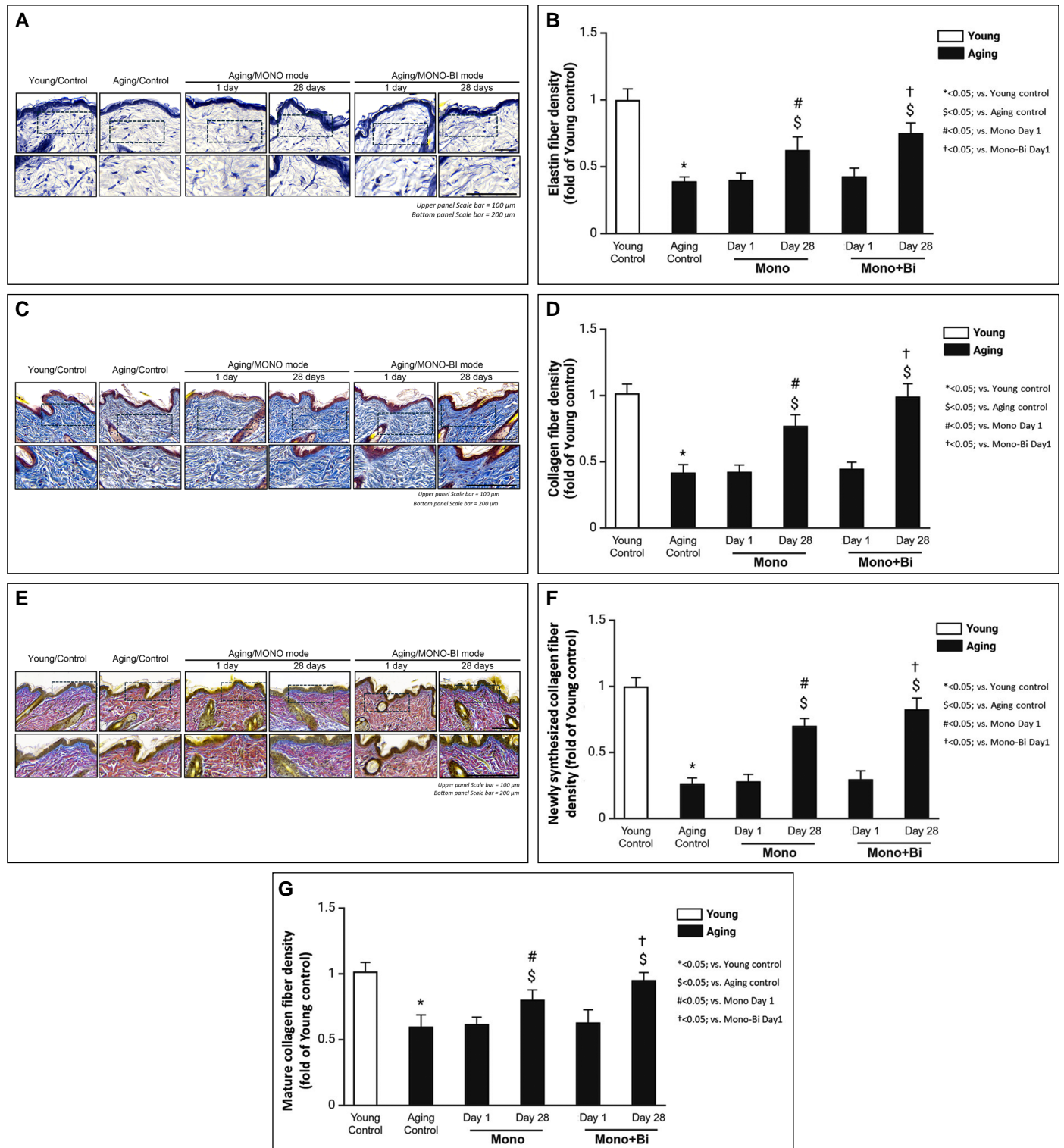
Additional marker genes were examined for the reticular dermis. Delta-like homolog 1 (*Dlk1*) serves as a marker for reticular and hypodermal fibroblasts and leucine-rich repeats. Immunoglobulin-like domain 1 (*Lrig1*) is recognized as a marker for dermal fibroblast precursor.<sup>34-37</sup> The expression of both *Dlk1* and *Lrig1* was significantly increased in RF-irradiated groups compared with the aging control groups. However, there is no statistically significant difference in expression level between the MONO and MONO-BI groups (Figure 7C, D). These findings suggest that RF irradiation affects the differentiation of dermal fibroblasts. Moreover, it may stimulate epidermal stem cell activity, enhancing the dermal-epidermal junction.

## DISCUSSION

There has been a growing interest in improving treatment outcomes by combining different energy-based devices in aesthetic practices. RF combined with intense pulsed light or CO<sub>2</sub> laser has been widely introduced. These combination techniques often synergize, even doubling or tripling clinical advantage. However, they may also increase the risk of unexpected adverse events, particularly when thermal effects are poorly controlled.<sup>38,39</sup>

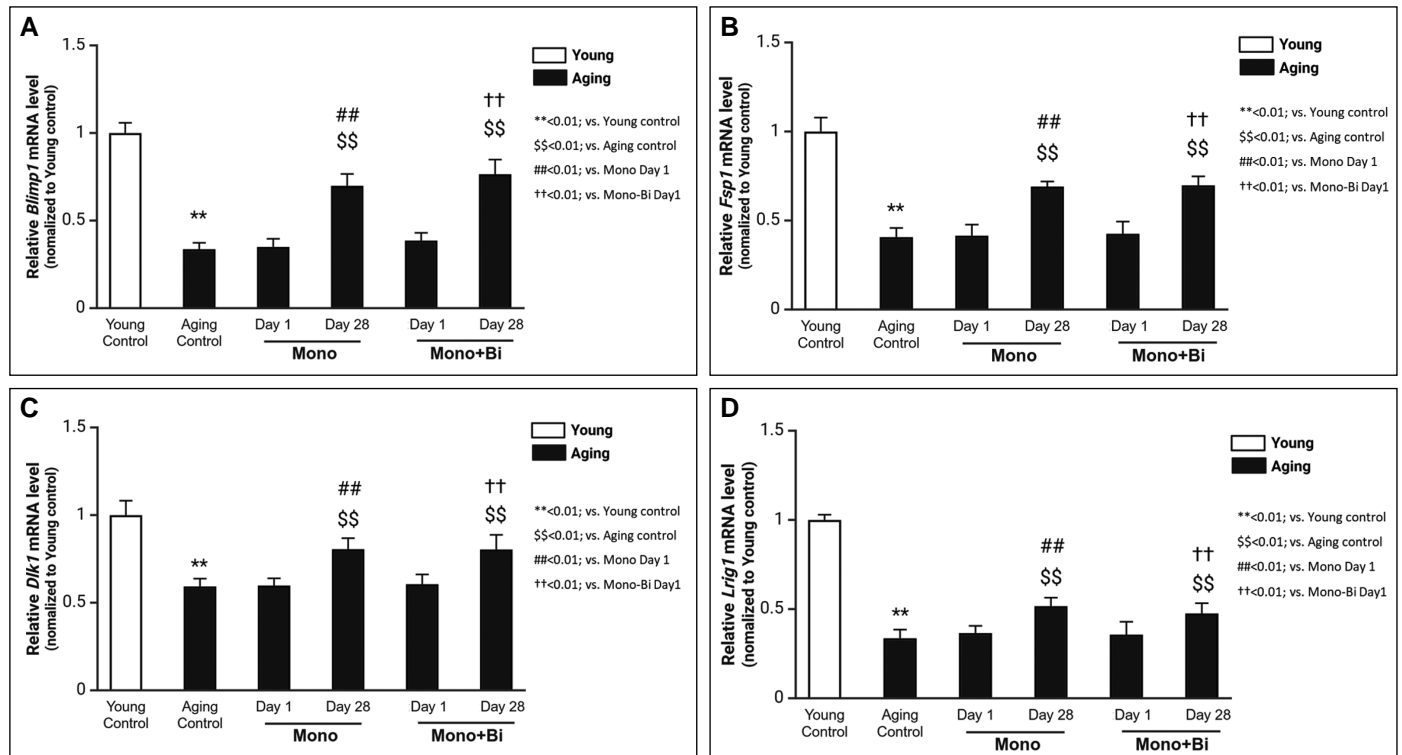
Therefore, with the newer generation of RF devices, which are more efficient, the goal should be the development of protocols and techniques focusing on both safety and efficacy to optimize patient outcomes. As mentioned, monopolar RF induces volumetric dermal heating into the mid-dermis, whereas bipolar RF induces superficial dermal heating. Monopolar RF contributes to the regenerative density of collagen and elastin fibers, with a visible result for 4 to 6 months.<sup>40</sup> Interestingly, combining MONO-BI RF energy in a single tip, such as the Density High-Tip, has the advantage of delivering more concentrated thermal energy to the papillary dermis, thereby enhancing fibroblast activation. Just after the procedure, a visible tissue lift and thickening can be observed because of partial collagen denaturation, contraction of fibrils, and thermal manipulation of fibrous septae. Such an immediate, visible outcome can be beneficial to patients seeking faster results.<sup>41,42</sup> Furthermore, subsequent tissue tightening is attributed to the wound healing process, which facilitates collagen synthesis, angiogenesis, and elastin reorganization.<sup>43</sup>

However, to our knowledge, no preclinical study has evaluated the effectiveness of a multipulse system combining sequential MONO-BI RF irradiation at 6.78 MHz with a power output of 400 W. It is essential to note the difference in skin architecture between humans and mouse models, specifically the thinner epidermal layer and a loosely



**Figure 5.** (A, B) Masson's trichrome staining showing an increase in dermal collagen composition in both MONO and MONO-BI RF irradiated groups. (C, D) Verhoeff–Van Gieson staining demonstrated a significant increase in elastin fiber expression in both MONO and MONO-BI groups. (E–G) Herovici's staining shows newly synthesized collagen in the young control groups and mature collagen in the aging control groups. Both the MONO and MONO-BI groups significantly promoted the synthesis of newly formed collagen fibers. MONO-BI, monopolar and bipolar; RF, radiofrequency.





**Figure 7.** Radiofrequency treatment upregulates mRNA expression of fibroblast precursor markers in aged mice. (A) *Blimp1*. (B) *Fsp1*. (C) *Dlk1*. (D) *Lrig1*.

Based on the findings, the authors demonstrate the effect of a combined monopolar–bipolar RF irradiation from a single tip. The results show an unexpected increase in the expression of genes related to collagen and elastin synthesis. Delivering thermal energy to the deep dermis and superficial epidermis is expected to achieve a dual effect on skin rejuvenation. The dual effect was demonstrated by upregulated expression of *Lrig1*, *Blimp1*, *Dlk1*, and *Fsp1*, which are representative papillary and reticular dermal fibroblast markers. These observations are consistent with the IHC staining results, which confirmed gene expression at the protein level. Notably, COL1A1 and COL3A1 expression were markedly increased in MONO-BI dual mode. These observations are in line with previous reports. Our findings are consistent with previous studies reporting increased dermal thickness and fibroblast activation following thermal-based RF treatment.

The present study lacks an analysis of the signaling pathways involved in the mechanism underlying the increased density of collagen and elastin fibers. Recent studies have reported that RF-induced thermal stimulation may activate fibroblast precursors and promote ECM synthesis through heat shock protein-mediated pathways. Another limitation is that the study was conducted using a mouse model. Nevertheless, it provides novel insights by directly comparing monopolar and sequential monopolar–bipolar RF irradiation systems using a multipulse mode in aged animal models.

Lastly, our research suggests that RF devices, particularly those using a MONO-BI sequential pulse such as Density High-Tip, are an innovative and effective tool for noninvasive skin rejuvenation with minimal discomfort. Further study will be necessary to elucidate the underlying mechanisms of collagen and elastin regeneration, and progress toward clinical trials will be made to demonstrate these findings.

## CONCLUSIONS

An RF device with sequential MONO-BI functions on a single tip (High-Tip; Density) showed notable increases in epidermal thickness, collagen, and elastin fiber production, and it encouraged fibroblast differentiation in a mouse model experiment compared with monopolar RF alone. More clinical studies are necessary to confirm these results in humans.

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## Disclosures

Dr Kumar is a consultant for Jeisys Medical Inc (Seoul, Korea) and receives a fee, whereas Ms Baek is an employee who receives a salary.

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