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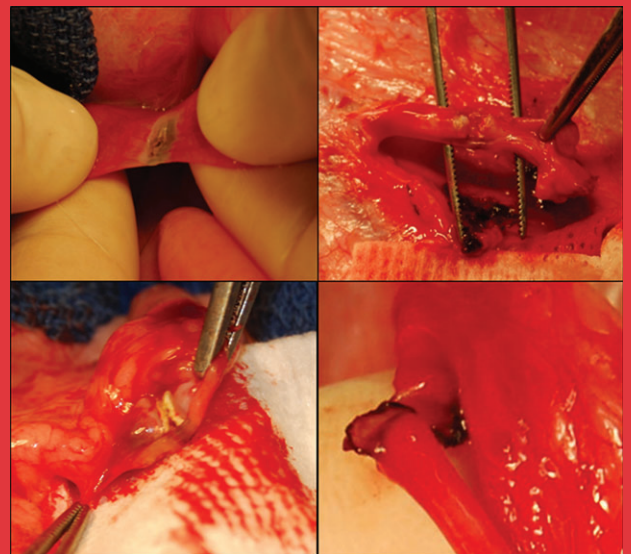


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HIGHLIGHTS IN THIS ISSUE

★ **Comparison Between Er:YAG Laser and Bipolar Radiofrequency Combined With Infrared Diode Laser for the Treatment of Acne Scars: Differential Expression of Fibrogenetic Biomolecules May Be Associated With Differences in Efficacy Between Ablative and Non-Ablative Laser Treatment**

Development of laser technology has provided effective options for acne scar treatment. However, the difference between ablative laser and non-ablative laser in detailed mechanism has not been known. Our study presents that ablative fractional Er:YAG laser is significantly more effective to acne scar treatment than non-ablative bipolar radiofrequency combined with diode laser, with tolerable adverse effects. Moreover, we investigated the expression of TGF β -associated fibrogenetic molecular changes for the suggestion of mechanism of treatment modalities.

★ **Fractional Laser-Assisted Drug Uptake: Impact of Time-Related Topical Application to Achieve Enhanced Delivery.**

Ablative fractional laser (AFXL) is known to increase uptake of topically applied agents in skin. The time frame for applying a drug after AFXL exposure remains to be established. In this study we demonstrated that the time frame to maintain enhanced drug delivery of a small, water soluble test molecule, sodium fluorescein, sustained for several hours after AFXL exposure. Results corresponded to channel morphology by optical coherence tomography and loss of skin integrity.

★ **Targeting of Sebaceous Glands to Treat Acne by Micro-insulated Needles With Radio Frequency in a Rabbit Ear Model**

Although many therapeutic options exist for acne, relapse often occurs after treatment is stopped. Some preliminary evidence suggests that selective electrothermolysis of the sebaceous glands may represent a novel therapeutic intervention. In this study, we evaluated the efficacy and safety of micro-needles radiofrequency technology in the treatment of depressed acne in a REA (Rabbit Ear Acnes) model. We demonstrated the effective destruction of sebaceous glands with minimal downtime or adverse effects.

Targeting of Sebaceous Glands to Treat Acne by Micro-Insulated Needles With Radio Frequency in a Rabbit Ear Model

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Background and Objectives: Many studies have investigated the application of micro-insulated needles with radio frequency (RF) to treat acne in humans; however, the use of a micro-insulated needle RF applicator has not yet been studied in an animal model. The purpose of this study was to evaluate the effectiveness of a micro-insulated needle RF applicator in a rabbit ear acne (REA) model.

Study Design/Materials and Methods: In this study, we investigated the effect of selectively destroying the sebaceous glands using a micro-insulated needle RF applicator on the formation of comedones induced by application of 50% oleic acid and intradermal injection of *P. acnes* in the orifices of the external auditory canals of rabbits. The effects of the micro-insulated needle RF applicator treatment were evaluated using regular digital photography in addition to 3D Primos imaging evaluation, Skin Visio Meter microscopic photography, and histologic analyses.

Results: Use of the micro-insulated needle RF applicator resulted in successful selective destruction of the sebaceous glands and attenuated TNF-alpha release in an REA model. The mechanisms by which micro-insulated needles with RF using 1MHz exerts its effects may involve inhibition of comedone formation, triggering of the wound healing process, and destruction of the sebaceous glands and papules.

Conclusion: The use of micro-insulated needles with RF applicators provides a safe and effective method for improving the appearance of symptoms in an REA model. The current *in vivo* study confirms that the micro-insulated needle RF applicator is selectively destroying the sebaceous glands. *Lasers Surg. Med.*

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Key words: acne; radiofrequency; REA model

INTRODUCTION

Radiofrequency energy (RF) is chromophore independent and depends on the electrical properties of the target

tissue, and thus is expected to have good safety profiles for all skin types [1]. Over the last decade, clinical treatment systems using RF have been proven to be safe and effective for both non-ablative skin tightening of the face and body, and for fractional RF skin resurfacing [2]. Recently, RF has revolutionized the fields of skin rejuvenation, acne scarring, and acne vulgaris [3,4,5]. Although, conventional fractional treatment has the disadvantage of possible indirect damage to the epidermis [6], a recently introduced minimally invasive fractional radiofrequency microneedle device has been used to overcome such problems by creating radiofrequency thermal zones with minimal epidermal injury [5,7].

Acne is a disease of the pilosebaceous units of the skin due to an inflammatory reaction in the follicle [8]. The basic lesion of acne is a comedo, which is an enlargement of the sebaceous follicle. Acne is graded according to the type of lesions present which can include inflammatory papules, pustules, open and closed comedones, cysts, and nodules [9]. Proliferation of the bacteria *Propionibacterium acnes* (*P. acnes*) leads to the production of inflammatory compounds resulting in neutrophil chemotaxis. *P. acnes* also secrete chemotactic factors for leukocytes which subsequently infiltrate the hair follicle, resulting in the destruction of the hair follicle wall. This bacterium is thought to contribute to inflammation through activation of the toll-like receptors TLR2 and TLR4 expressed on

Conflict of Interest Disclosures: All authors have completed and submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest and none were reported.

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keratinocytes, sebocytes, and monocytes, leading to the release of proinflammatory cytokines/chemokines such as interleukin (IL)-1 α , IL-6, and tumor necrosis factor (TNF)- α [10]. TNF- α is an important pathophysiologic factor involved in the development of acne [11,12]. Currently, the therapeutic agents available for the treatment of acne target and kill bacteria, thereby preventing inflammation or comedo production [13]. Therefore, the development of a novel device that can selectively destroy sebaceous glands without side effects would be an important advancement in the treatment of acne.

The rabbit ear assay (REA) is the most common animal model utilized to determine compound comedogenicity [14]. Agents that demonstrate marked comedogenicity in the REA also tend to produce comedones in humans. Rabbit inner ear follicles share similarities with human follicles in that they have small pili, and large adjacent sebaceous glands [15]. Therefore, we evaluated the utility of micro-insulated needles with RF applicators in the REA model. Through this method, we were able to selectively destroy the sebaceous glands and attenuate TNF- α release in the REA model.

MATERIALS AND METHODS

Animals and Experimental Design

Female New Zealand white rabbits (3 to 3.5 kg; Yonam Laboratory Animals, Cheonan, Korea) were used in animal experiments. Rabbit auricles were injected with 0.02 ml of

P. acnes (107 CFU/ml) or PBS at six points that avoided the blood vessels. Seven days after the injection, topical treatment with 50% oleic acid (Sigma, St Louis, MO) in 70% propylene glycol/30% ethanol was carried out once a day for 28 days. The animals were then divided into three experimental groups of two animals each (the center of the pinna in the concave area just external to the ear canal of rabbits, $n = 4$). AGNESTM micro-insulated needles with RF applicators (Gwoonsesang Dermatology Clinic, Seoul, Korea) were used for treatment on day 29. This device uses a monopolar handpiece and is based on the principle of facilitating discharge of pustules after treatment of acne by transferring the heat generated by the load or the contact resistance when 1 MHz RF energy is disembugued to the surface and the deep part of the skin (Fig. 1A). A 1.2 mm needle was inserted into papules and pustules of the upper layer of the rabbit ear, and a high-frequency electrical current was treatment with micro-insulated needles at a penetration depth of 1.2 mm, and a power of 2, 3, 5, 46 W, respectively. Rabbit ears were examined for clinical appearance before and after RF applicators treatment. As per the manufacturer's recommendation, treatment parameters were determined based on the anatomical location and the proximity of underlying bones.

Animals were sacrificed and analyzed 7 days after treatment. The treatment regimens varied extensively depending on the clinic involved. Needle depths ranged from 1.2 mm (C type needle) and exposure times ranged from 100 ms using a power 5 W. All procedures involving

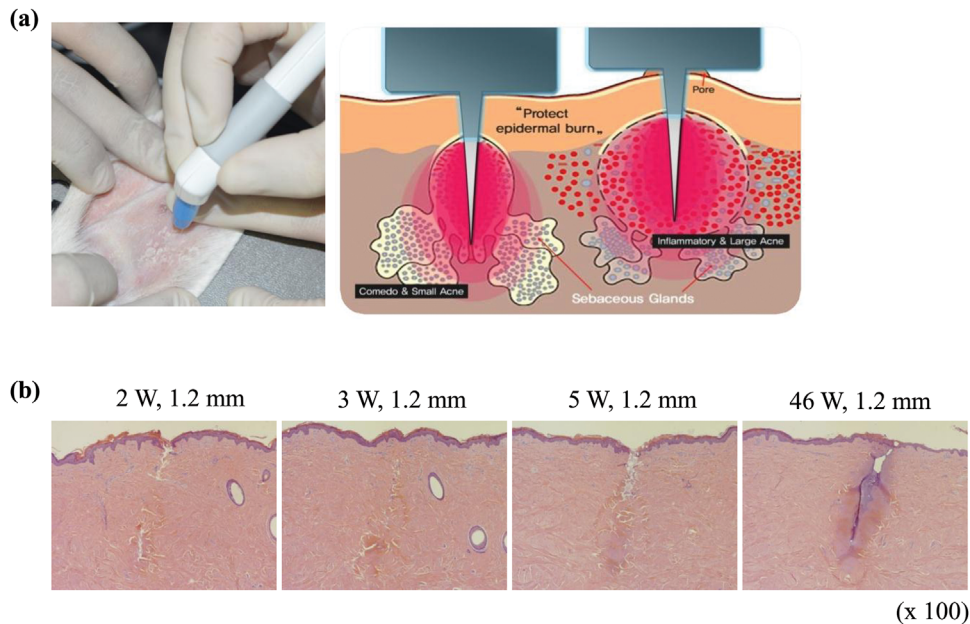


Fig. 1. Non-insulated microneedle radiofrequency applicator and technique for selectively destroying sebaceous glands in a rabbit ear acne model. (a) This device is a medical appliance designed to combine coagulation and pain relief functions using a high frequency current. (b) H&E stains of *in vivo* pig skin immediately after treatment with micro-insulated needles at a penetration depth of 1.2 mm and a power of 2, 3, 5, 46 W, respectively. The micro ablation effect is visible into the upper dermal layer. Original magnification, 100 \times .

animals were conducted in accordance with the guidelines of the Institutional Animal Care and Use Committee of Chung Ang University in Korea.

P. Acnes

P. acnes (ATCC[®] 6919) were grown on Brucella broth agar (BD, Sparks, MD) containing 5% v/v defibrinated sheep blood (Lampire Biological Laboratories, Pipersville, PA), vitamin K (5 µg/ml, Remel, Lenexa, KS), and hemin (50 µg/ml, Remel) under anaerobic conditions using GasPak (BD, Sparks, MD) at 37°C. The concentration of bacteria was adjusted to 10⁷ CFU/ml prior to inactivation at 95°C for 5 minutes. Bacteria were suspended in the appropriate amount of PBS for use in experiments.

Clinical Evaluation

Clinical symptoms were measured using images captured daily with a digital camera (Canon 3000D, Canon Inc., Tokyo, Japan). After treatment, the overall area covered with comedones relative to the overall area covered by follicular orifices was calculated by digital image analysis, performed using a phototrichogram system (Folliscope, Lead-M, Seoul, Korea). The acquisition and evaluation of the surface of human skin to evaluate effects on skin roughness were performed using optical *in vivo* measurements with a three-dimensional (3-D) micro-topography imaging system (PRIMOS, GFM, Teltow, Germany) [16].

Biopsy Specimens and Histologic Measurements

Paired 8mm-punch biopsy specimens were obtained from each side of the facial skin at baseline and at the end of treatment. Post-treatment biopsy specimens were taken near the previous biopsy site. Tissue samples were fixed in 10% buffered formalin and embedded in paraffin. Tissues were fixed with 4% PFA, embedded in paraffin, and sliced into 5 micrometer thick sections. Sections were then transferred to probe-on-plus slides (Fisher Scientific, Pittsburgh, PA). Standard hematoxylin-eosin (H&E) and immunofluorescence staining, including TNF-α staining, were performed. For immunofluorescence, sections were blocked at room temperature for 2 hours in PBS containing 0.2% Triton X-100 and normal horse serum. Sections were stained using mouse monoclonal antibodies against TNF-α (1:100, sc-12744, Santa Cruz Biotechnology, Santa Cruz, CA) and incubated at 4°C overnight. Following incubation, sections were washed three times for 5 minutes with 0.2% Triton X-100 in PBS. Sections were then incubated at room temperature with FITC-conjugated anti-Armenian hamster secondary antibodies (1:200, sc-2446, Santa Cruz Biotechnology) for 30 minutes. Sections were then counterstained with DAPI for 5 minutes.

Statistical Analyses

Statistical comparisons between the treated and untreated groups were performed using a two-tailed student *t*-test for paired samples. The results are expressed as mean ± standard deviation from at least three

independent experiments, and *P*-values of **P* < 0.05, ***P* < 0.01, and ****P* < 0.001 were considered statistically significant.

RESULTS

Determination of Penetration Depth and Morphologic Features of the Injection Site Using Micro-Insulated Needles With RF Applicators

The micro-insulated needle with RF method (MRF) includes an electrode array configured to ablate tissue during insertion of the needle and form a coagulation zone at the edges of the needle (1.2 mm). In our evaluation of the depth and thermal lesion changes on pig back skin, H&E images taken using a microscope showed regions of coagulative thermal lesions surrounded by undamaged epidermal tissue (Fig. 1B).

To induce comedone formation, each rabbit ear received 50 µl of *P. acnes* followed by a subcutaneous quad injection in the dermis. After the injections, 50% oleic acid was applied to the orifices of the external auditory canals of the rabbit ears daily for 4 weeks. The animals were divided into three experimental arms with eight animals each. The normal group served as the untreated controls. The control group received a single intradermal injection of a 50 µl *P. acnes* suspension in the ear. The MRF group began treatment with micro-insulated needles with RF applicators after 4 weeks. All groups were sacrificed and analyzed 7 days after treatment.

Histologic Analysis and Non-Invasive Objective Skin Measurement Using 3D Skin Imaging

External auditory canals of the rabbit ears were examined for changes in skin morphology 4 weeks post-treatment. The reduction in papules and pustules of the upper layer of the ear was confirmed by examination of photographs using the phototrichogram system (Fig. 2A). Rabbit ears in the normal group showed no physical signs of acne, whereas rabbit ears in the group treated with *P. acnes* and 50% oleic acid (control group) showed the four typical acne symptoms: (i) hyperkeratinization of the follicle, leading to a microcomedo that eventually enlarges into a comedo; (ii) increased sebum production by sebaceous glands, in which androgens have an important role; (iii) colonization of the follicle by the anaerobe *P. acnes*; and (iv) an inflammatory reaction in the ears.

As shown in Figure 2B, H&E stained sections were used to evaluate the presence of sebaceous glands in the dermis. Examination of the MRF treated-group showed destruction of the sebaceous glands, as evidenced by the magnified view of a damaged sebaceous gland in Figure 2B. Moreover, the epidermis was mostly preserved whereas the dermis was damaged and displayed a decrease in inflammation status.

The PRIMOS imaging system, used to generate 3D virtual models of the skin surface at 0, 3, 7 days, allowed us to compare the skin surface micro topography in an objective and quantifiable manner. Images using this system to evaluate the rabbit ear acne model treated with

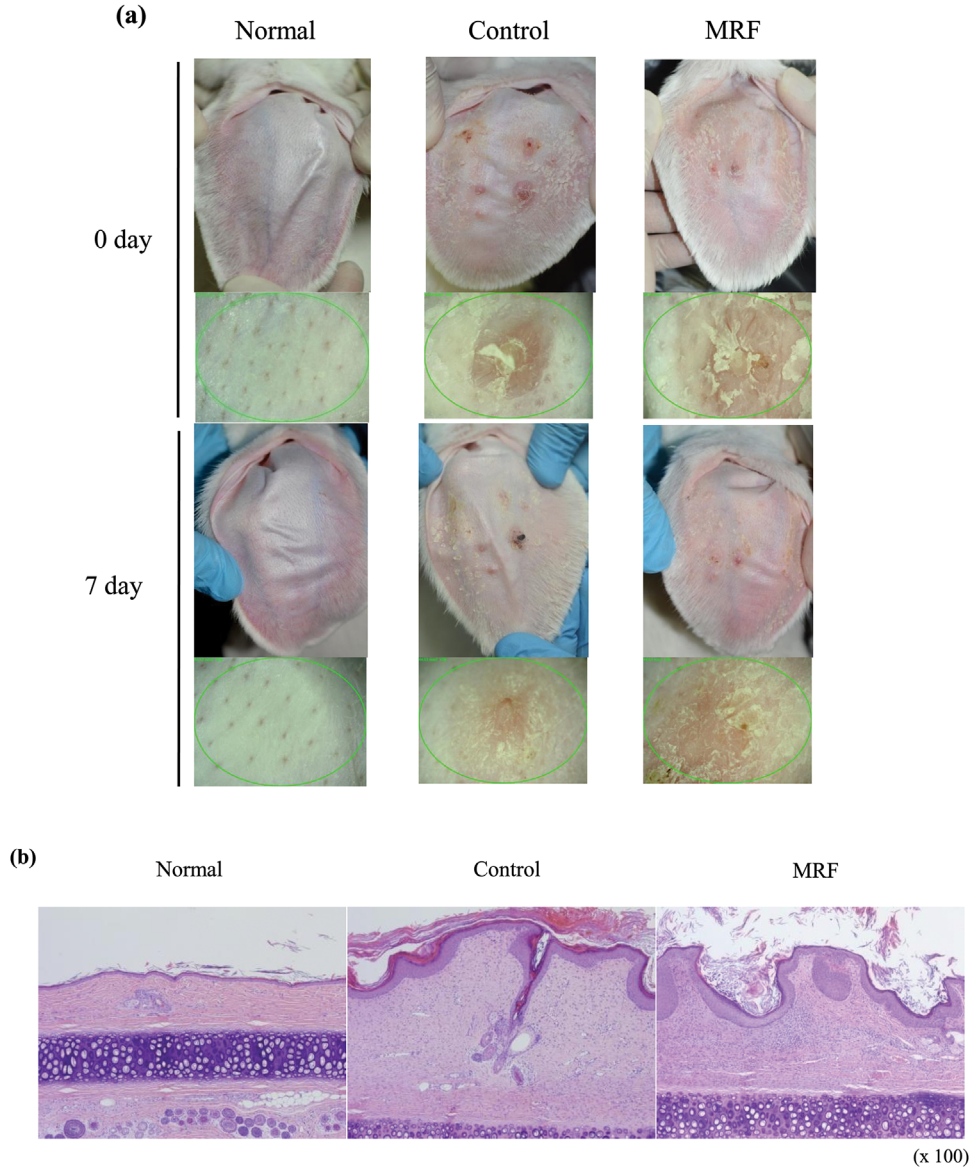


Fig. 2. Histologic analysis showing *in vivo* changes in the rabbit ear skin surface. (a) Treatment with micro-insulated needles at a penetration depth of 1.2 mm and a power of 5 W. Photographs taken with a folliscope (30 \times) of the treated areas 7 days after cooling device application showed clear surface changes. (b) H&E staining shows the effect of non-insulated microneedle radiofrequency on sebaceous ducts in the rabbit ear acne model. Original magnification, 100 \times . MRF, micro-insulated needle RF.

micro-insulated needles with RF are shown in Figure 3A. These images were used to calculate the roughness parameters of arithmetic average height and volume. Both parameters increased during the procedure and decreased at the completion of the procedure, resulting in significant differences ($P > 0.01$) (Fig. 3B). Image analysis revealed that the volume of papules decreased 67.1% and the height of papules decreased 64.7% in the MRF group compared to the control group. Overall, the morphological and histologic findings showed that the use of micro-insulated needles with RF applicators

resulted in a decrease in the physical signs of acne and effective destruction of sebaceous glands.

Destruction of Rabbit Ear Skin Sebaceous Glands and Decreased Tnf- α Synthesis Caused by Micro-Insulated Needles With Radio Frequency

To investigate inflammatory signaling and evaluate the mechanism underlying the release of TNF- α in the control and MRF groups, we performed immunofluorescence microscopy on ear skin specimens using antibodies specific

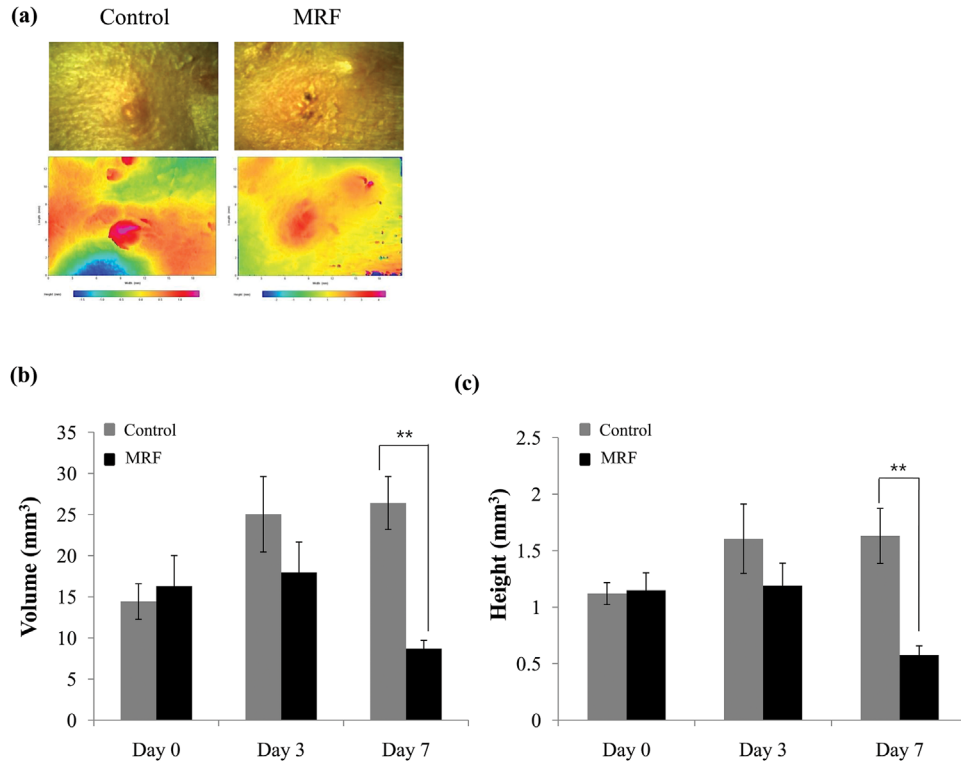


Fig. 3. 3D *in vivo* optical skin imaging for topographical quantitative assessment of non-insulated microneedle radiofrequency. (a) Using the optical 3D skin measurement system PRIMOS premium, roughness measurements can be determined on a precise area prior to and at different time points after treatment with non-insulated microneedle radiofrequency. (b) and (c) Color-coded surface topography images of an animal with acne scarring before treatment on day 0–7 days after the end of treatment. Treatment with micro-insulated needles at a penetration depth of 1.2 mm and a power of 5 W. A reduction in the measured volume and height from a given area is shown. This can also be visualized as a reduction in green color in this area translating to a reduction in papules. Values shown are mean \pm S.D ($n=4$ /ear canal of rabbit; ** $P < 0.01$ vs. control group). MRF, micro-insulated needle RF.

to TNF- α . Immunofluorescence revealed strong depositions of TNF- α around the sebaceous gland areas. Immunostaining studies showed that the MRF group had markedly decreased TNF- α expression levels compared with the control group (Fig. 4). Furthermore, the dermis and the associated sebaceous glands were completely destroyed. These results suggest that MRF alters certain functions that block an increase in lipid synthesis. Additionally, the results confirm that our electrothermolysis method using sufficient electrical heat resulted in a permanent reduction of sebum excretion through the precise destruction of hyperactive sebaceous glands without causing skin surface damage.

DISCUSSION

Acne vulgaris is a chronic dermatologic condition that can affect patients both physically and psychologically due to the presence of lesions on the face [17]. This has a negative effect on quality of life and can cause psychological difficulty such as social avoidance [18]. In the search for potential alternative candidate modalities for the treatment of acne, a micro-needle radiofrequency device

that allowed effective destruction of sebaceous glands was investigated as a promising candidate using *in vivo* animal studies.

There are many types of acne treatment. Acne patients routinely receive years of topical or systemic therapies. Common treatment options include topical anti-inflammatories, topical and oral antibiotics, hormonal agonists and antagonists, and topical and oral retinoids [19].

Although minimally invasive, the treatment of acne with fractional lasers is accompanied by significant downtime and pain.

Recently, various light-based therapies are under development for the treatment of acne. A reduction in acne lesion count upon exposure to blue [20], red [21], violet [22], and ultra-violet light [23] has been reported. Blue light is thought to reduce acne symptoms due to the absorption of endogenous porphyrins produced by *P. acnes* that results in a phototoxic effect on *P. acnes* [24]. However, these treatment options must be used over long periods of time and are associated with multiple side effects. Additionally, because these therapies do not target

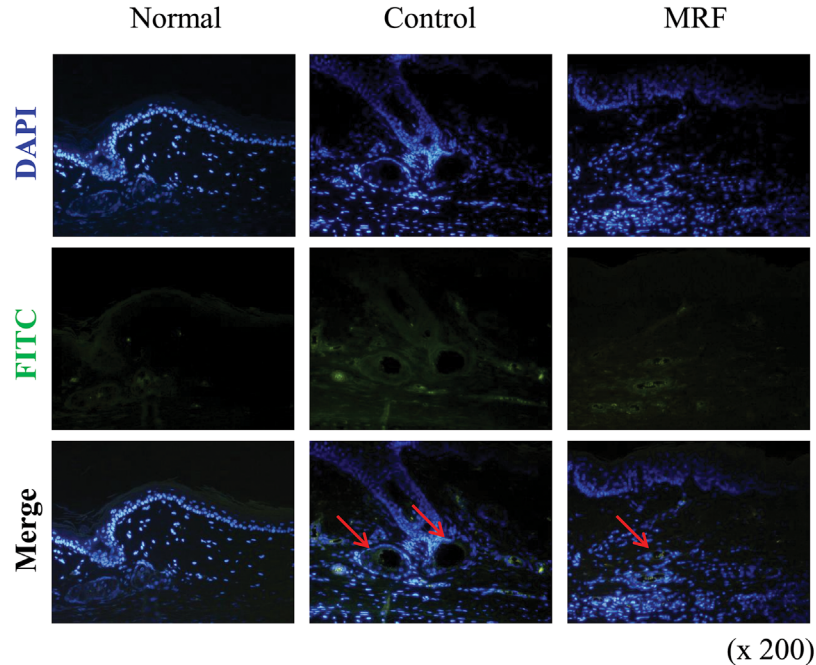


Fig. 4. Representative sections of rabbit ear skin tissue stained for TNF- α expression. Dorsal skin biopsies were taken after 7 days and immunofluorescence staining for TNF- α (green) was performed. Nuclei were counterstained with DAPI (blue), $n = 3$ ears per group. Original magnification, 200 \times . MRF, Micro-insulated needle RF.

sebaceous glands, long-term remission has not yet been demonstrated and may not be possible due to repopulation by *P. acnes*.

Our data show comparable or better clinical results with high efficiency and less downtime compared to other treatment methods. In particular, the morphological and histologic findings demonstrated that treatment with micro-insulated needles with RF applicators resulted in a decrease in the physical signs of acne, and effective destruction of sebaceous glands.

Sebaceous glands are involved in the genesis of acne and serve as the platform for important immunological phenomena, including innate immunity and the synthesis of antimicrobial peptides [25]. Our study revealed that micro-insulated needles with RF applicators can specifically target pilosebaceous glands, leading to an improvement in acne. In addition, we confirmed a reduction in the levels of inflammatory mediators, such as TNF- α , and presented clinical histological evidence of a reduction in inflammation after treatment with micro-insulated needles with RF.

The presented results evaluated the efficacy and safety of micro-needles radiofrequency technology in the treatment of depressed acne in a REA model. The treatment was minimally invasive and caused minimal discomfort and downtime. This study also demonstrated the effective destruction of sebaceous glands with minimal downtime or adverse effects. However, further human clinical trials are needed to clarify the optimal regimen for acne treatment.

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