의학석사 학위논문

Clinical and histological effects of fractional microneedling radiofrequency treatment on rosacea

Fractional microneedling radiofrequency 치료의 안면 주사에 대한 임상적 및 조직학적 효과 연구

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ABSTRACT

Introduction: Fractional microneedling radiofrequency (FMR) is an emerging treatment modality, but its effect on rosacea has not been studied. We sought to investigate the potential impact of FMR treatment on clinical improvement and histological changes in rosacea patients.

Methods: A 12-week, prospective, randomized, split-face clinical trial was conducted. Two sessions of FMR were performed on one side of the face with a 4-week interval between treatment sessions, and the other side of the face remained untreated. Clinical evaluation and photometric measurement of erythema and histologic analysis of skin samples were carried out.

Results: Clinical evaluation and photometric measurement revealed reduction of redness on the treated side compared with the untreated side and baseline. The erythema index decreased 13.6% and the a* value decreased 6.8% at week 12 compared with baseline. Reduced expression of markers related to inflammation, innate immunity, and angiogenesis was observed in the immunohistochemical staining of tissue obtained

after FMR treatment.

Conclusions: FMR can result in clinical and histological improvement of rosacea, and it can be used as an alternative or in combination with other treatment methods.

Keywords: Rosacea, Erythema, Fractional microneedling radiofrequency

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LIST OF ABBREVIATIONS

FMR: fractional microneedling radiofrequency

ETR: erythematotelangiectatic rosacea

IGA: Investigator’s Global Assessment scale

IHC: immunohistochemical

IL: interleukin

TLR: toll-like receptor

VEGF: vascular endothelial growth factor

TRPV: transient receptor potential transient receptor potential vanilloid

PPR: papulopustular rosacea

INTRODUCTION

Rosacea is a chronic inflammatory skin disease, characterized by persistent erythema and flushing in the central face. With prevalence up to 22%, it severely affects the appearance of the sufferers, resulting in considerable psychological stress and low quality of life [1, 2].

Although many treatment methods have been studied, there is no standard cure for rosacea. Currently, topical drugs, such as metronidazole, or oral agents, such as tetracycline antibiotics, are commonly used. With increasing concerns of antibiotics resistance, other treatment methods have been emerging. The effectiveness of other therapies, including pulsed dye laser and intense pulsed light treatments, has been also studied; however, these studies focused mainly on erythematotelangiectatic rosacea (ETR) [3, 4].

Fractional microneedling radiofrequency (FMR) is an emerging treatment modality in dermatology. FMR can deliver bipolar radiofrequency energy directly to the dermis with an array of microneedles with minimal damage of the epidermis [5]. Bipolar radiofrequency has been reported to induce neoelastogenesis and neocollagenesis and, thus, has been used in the treatment

of cutaneous wrinkles [6-8]. Recently, clinical and histological improvement of post-inflammatory erythema in acne patients by FMR treatment was reported [9]. Both acne and rosacea are categorized as sebaceous gland diseases and underlying inflammation is regarded as an important factor in the pathogenesis of these conditions. Therefore, FMR can be possible option for rosacea treatment by reducing inflammation.

The aim of this study was to investigate the impact of FMR treatment on the clinical improvement and histological changes in rosacea patients. To the best of our knowledge, this is the first study using FMR on rosacea.

MATERIALS AND METHODS

1. Study design and subjects

This study was performed as a 12-week, prospective, single-blind, randomized, and split-face clinical trial at the Department of Dermatology, Seoul National University Hospital, from January through August 2015. Subjects whose Fitzpatrick skin types were III or IV and who had rosacea were recruited. This study protocol was conducted in accordance with the Declaration of Helsinki and approved by the Institutional Review Board of Seoul National University Hospital (No. 1410-148-622). Informed consent was acquired from all subjects prior to enrollment. Subjects were not allowed to use any systemic or topical agent for rosacea treatment during the study.

2. Devices and laser treatment

After random allocation, one side of the cheek was treated with FMR (INFINI®, Lutronic, Goyang, Korea; treated side) and the other side remained untreated (untreated side). Topical anesthesia with EMLA® (AstraZeneca, Södertälje, Sweden) was applied 30 minutes before the treatment. The FMR device can

deliver radiofrequency energy to the skin with applicator tip comprising of 49 insulated microneedles and the energy level (1–20) and conduction time (10–1,000 ms) are adjustable. The treatment in this study was done at energy level 2–3 (5–7.5 W) for 50–70 ms, as our previous study [9]. FMR treatment was performed with slight overlap in two passes. Each patient received two sessions of treatment with a 4–week interval between treatments.

3. Clinical outcome assessment

Subjects visited our clinic at weeks 0 and 4 for FMR treatment and 4 and 8 weeks after the second session for clinical assessment. Digital photographs were taken at every visit using the same camera settings (EOS 600D®; Canon, Tokyo, Japan). Two independent dermatologists conducted clinical assessments with the photographs. Improvement of rosacea was assessed using Investigator' s Global Assessment scale (IGA; Table 1) and the Kappa value was 0.77 ($p < 0.001$), showing good agreement between assessors. The objective measurement of erythema was performed with two photometric devices (DermaSpectrometer®; Cortex Technology, Hadsund,

Table 1. Investigator's global assessment

Grade	Degree of improvement
0	No improvement
1	0−10%
2	11−20%
3	21−30%
4	31−40%
5	41−50%
6	51−100%

Denmark; Spectrophotometer CM−2002®; Konica Minolta, Tokyo, Japan). Patients' subjective assessments of pain were surveyed just after each treatment session. Subjective therapeutic effectiveness and satisfaction score were surveyed at weeks 4, 8, and 12. Some patients also subjectively assessed the change in their rosacea symptoms (persistent erythema, flushing, papule or pustule, telangiectasis, burning or pricking sensation, itching sensation, edema, and heat sensation) by percentage at Week 12.

4. Histopathology and immunohistochemistry

Skin biopsies were performed from one side at week 0 and from both sides of the face at week 8 in 5 patients. Tissue sections were stained with hematoxylin-eosin (H&E) and toluidine blue. The number of mast cells with granules stained purple with toluidine blue was counted. For assessing molecular changes before and after FMR treatment, tissue samples were processed for immunohistochemical (IHC) staining for interleukin(IL)-8, NF-κB, toll-like receptor (TLR)-2, LL-37, vascular endothelial growth factor (VEGF), and transient receptor potential transient receptor potential vanilloid (TRPV) 2, 3, and 4 (Abcam, Cambridge, England). The intensity of the IHC stain was measured with a semi-quantitative method using an image analysis program (Leica QWin version 3.5.1, Leica Microsystems, Wetzlar, Germany) by detecting the area of NovaRED™ (Vector Laboratories, CA, USA) staining with a color threshold.

5. Statistical analyses

We performed statistical analyses using the Statistical Package for the Social Sciences version 20 (SPSS Inc., Chicago, IL, USA). We used the Student's t test or paired t-test to

compare continuous variables obtained with photometric devices and the Mann–Whitney U test for comparing IGA and intensity of IHC staining. *P*–values < 0.05 were considered statistically significant.

RESULTS

Twenty-one patients (1 man, 20 women) completed the study. The mean age of the subjects was 42.9 ± 10.3 years. Thirteen patients had Fitzpatrick skin type III, and 8 patients had Fitzpatrick skin type IV. Fourteen patients had ETR, and 7 had papulopustular rosacea (PPR). The mean values of the erythema index were 17.8 and 17.0 for the treated and untreated sides, respectively, and those of the a* values were 10.6 and 10.4 for the treated and untreated sides at baseline, respectively, showing no significant differences between the two sides (p = 0.441 and 0.672). There was no serious adverse effect resulting in a patient discontinuing from the study.

Clinical improvement and safety

Clinical improvement of rosacea was observed in 17 (81.0 %) of 21 total patients on the FMR-treated side. The mean IGA scores were 1.05, 1.57, and 2.00 for the treated side and 0.29, 0.38, and 0.38 for the untreated side at weeks 4, 8, and 12, respectively (p = 0.016, p = 0.007, p < 0.001) (Figure 1). Among the 17 patients with improvement, the mean IGA score

was 2.47 at week 12, which indicates approximately 20 % improvement. Figure 2 represents improvement of erythema on the treated side and minimal change on the untreated side.

Photometric measurements revealed significant reduction of redness on the treated side compared with the untreated side and baseline (Figure 3). The erythema index decreased 11.9 %, 10.7 %, and 13.6 % ($p = 0.003$, $p = 0.006$, $p < 0.001$) and the a* value decreased 6 %, 5.8 %, and 6.8 % ($p < 0.001$, $p = 0.001$, $p < 0.001$) at weeks 4, 8, and 12, respectively, compared with baseline. The a* value decreased 10.5 % in the PPR patients and 4.9 % in the ETR patients, which was a statistically significant difference ($p = 0.009$).

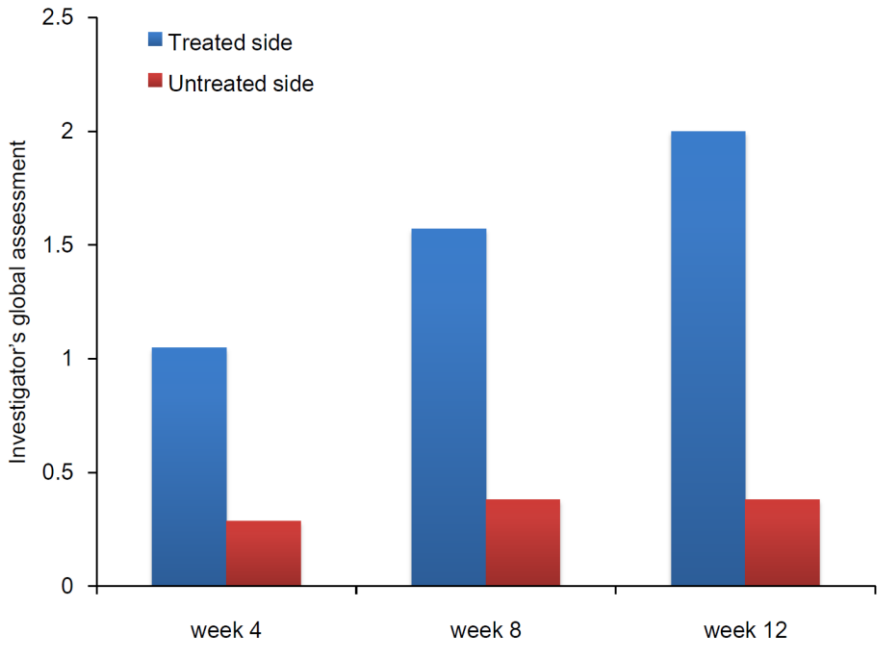


Figure 1. Changes in investigator's global assessment

Treated side showed improvement of rosacea with increasing pattern, whereas untreated side showed minimal change.

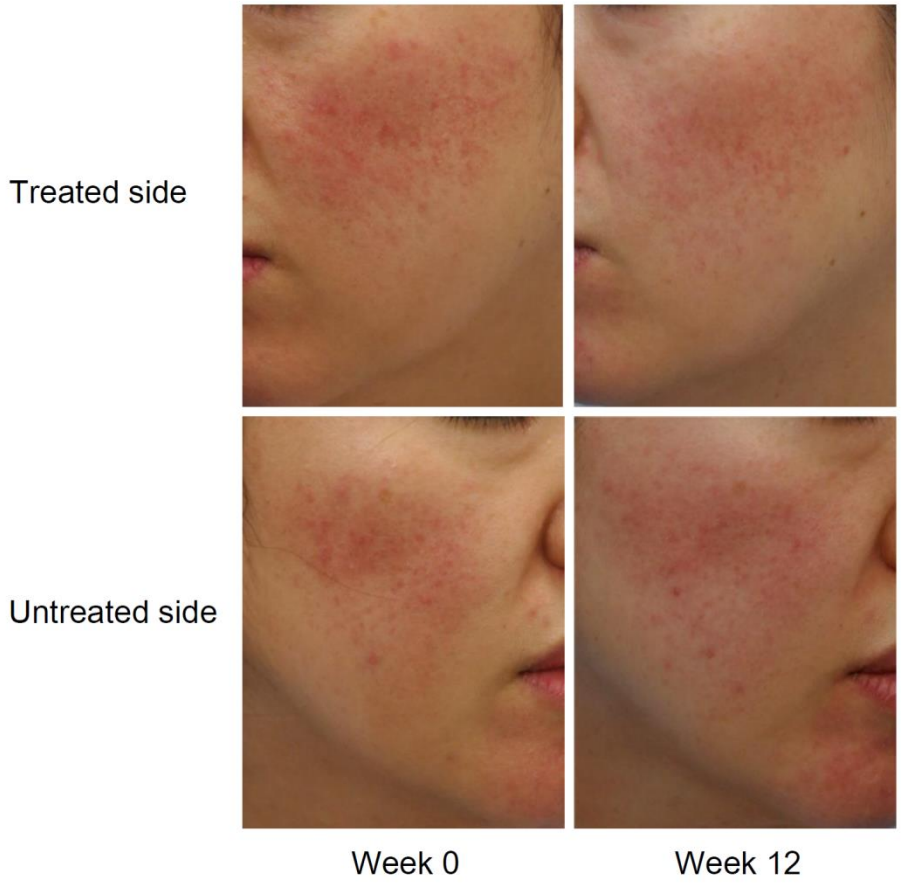
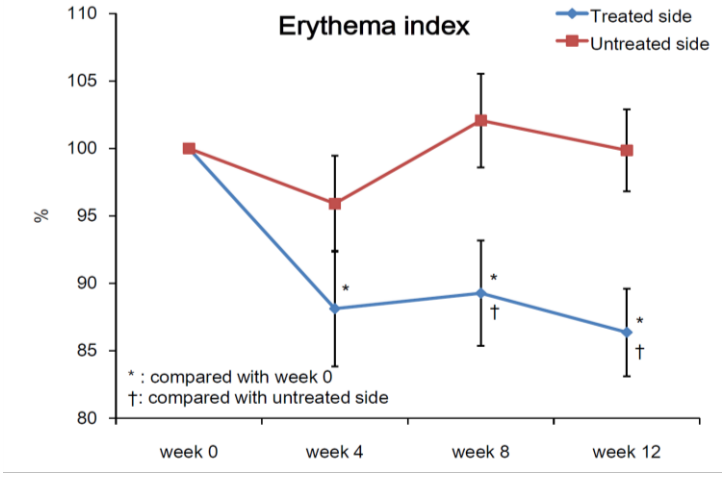


Figure 2. Clinical photographs showing improvement of rosacea.

The treated side showed decreased erythema and papules, while the lesion was widened on the untreated side.

(A)



(B)

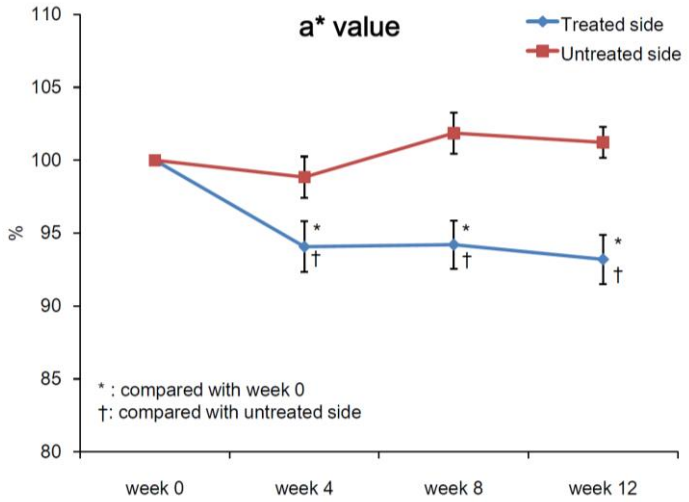


Figure 3. Photometric measurements of erythema in the treated side and untreated side.

Mean erythema index (A) and a* scale (B) decreased in the treated side, compared with baseline (week 0) and the untreated side. *: $p < 0.05$ compared with week 0; †: $p < 0.05$ compared with the untreated side.

Patient's subjective assessment

The mean values of subjective therapeutic effectiveness were 3.7 ± 1.1 at week 4, 5.3 ± 1.3 at week 8, and 5.9 ± 1.7 at week 12. Satisfaction scores were 5.1 ± 1.4 at week 4, 5.7 ± 1.0 at week 8, and 6.2 ± 1.6 at week 12. The mean pain scores were 3.1 ± 1.6 and 3.1 ± 1.4 after the first and second treatment sessions, respectively.

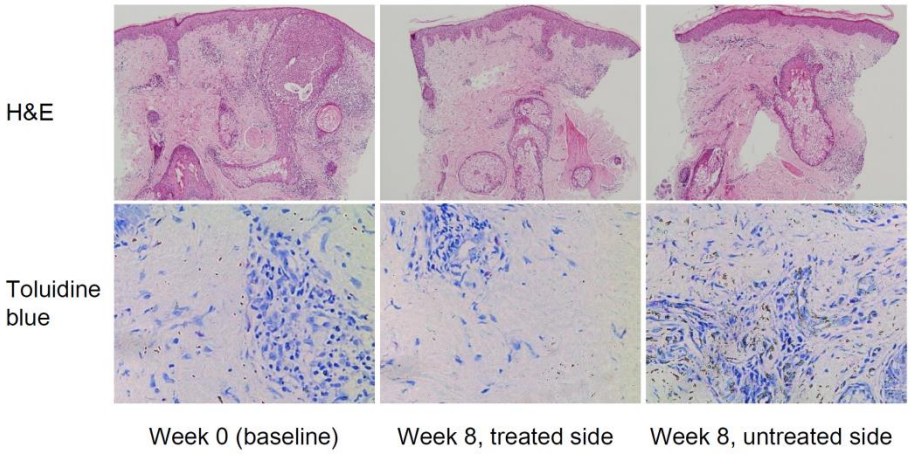
Seventeen patients (81.0%) provided assessments of their rosacea symptoms at week 12. Patient-reported improvement of symptoms were as follows: papule or pustule, 57.5 % (n = 4); itching sensation, 48.3 % (n = 3); heat sensation, 28.3 % (n = 15); persistent erythema, 28.2 % (n = 17); burning or pricking sensation, 27.9 % (n = 7); transient erythema, 25.6 % (n = 17); telangiectasis, 18.6 (n = 7); and edema, 5 % (n = 1).

Histologic findings

H&E staining of skin samples revealed reduced dermal inflammation after 2 sessions of FMR treatment (Figure 4A). The average mast cell count decreased from 10.8 at week 0 to 5.0 on the treated side at week 8 (p = 0.024). The cell count on the treated side was also significantly lower than that on the

untreated side (5.0 vs. 12.2; p = 0.036). In image analysis, an overall decrease of IHC intensity in the tissue obtained from the treated skin at week 8 was observed (Figure 4B). All the markers related to angiogenesis (VEGF), inflammation (NF-κB and IL-8), innate immunity (LL-37 and TLR2), and neuroimmunity (TRPV 2, 3, and 4) showed significantly decreased intensities for the treated side compared with baseline (week 0). Compared with the untreated side, all the markers except TLR2 and TRPV3 were reduced in the tissue from the treated side.

(A)



(B)

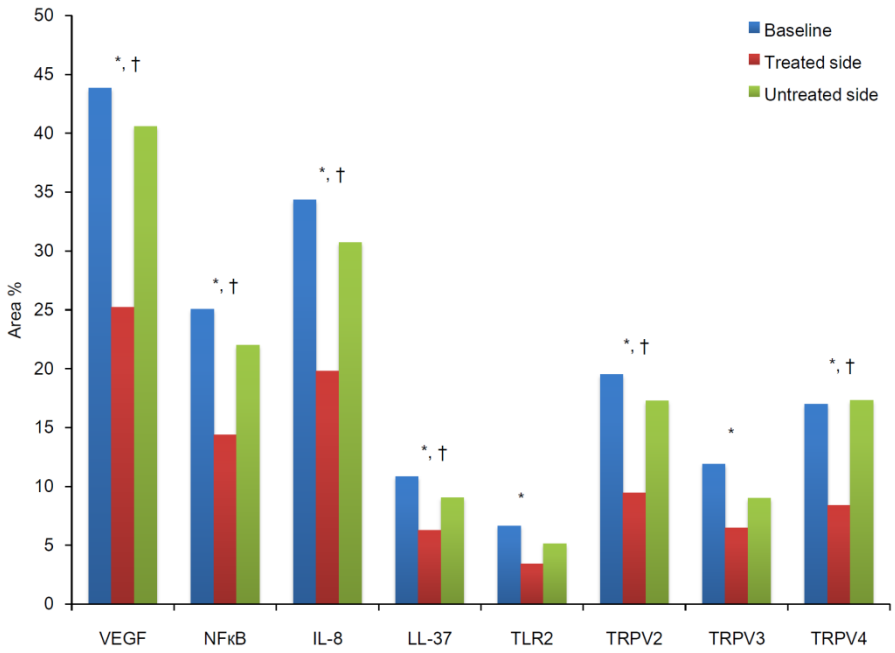


Figure 4. Histologic analysis of skin specimen.

H&E and toluidine blue staining showed decreased overall

dermal inflammation and mast cell counts (A) (H&E original magnification X100 and toluidine blue original magnification X400). Image analysis of immunohistochemistry staining showed overall decrease of markers related to angiogenesis, inflammation, innate immunity, and neuronal cation channels (B). ∗: $p < 0.05$ compared with week 0; †: $p < 0.05$ compared with the untreated side.

DISCUSSION

Although radiofrequency devices are not lasers, they have been gaining attention in dermatology. Ruiz-Esparza et al. reported a possible role of radiofrequency for the treatment of rosacea [10]; however, the treatment of rosacea with radiofrequency devices and its mechanism have rarely been reported. Our results confirmed that FMR can result in modest improvement of rosacea. The effectiveness was maintained until 2 months after the last treatment. FMR was slightly more effective in reducing erythema in patients with PPR than in those with ETR, suggesting inflammatory lesions, such as papules and pustules, could be more effectively treated with this device. This result agreed with reports showing that FMR is effective in treating inflammatory acne [11].

Subjective symptom improvements showed more dramatic differences than other assessments. All the patients who answered questions regarding symptom changes assessed that not only persistent erythema but also transient erythema (flushing) decreased. This means that FMR can alleviate the aggravation of erythema between treatment sessions without other treatments. While there was an overall increase of

measured erythema at week 8 compared with week 4 on the untreated side, the increase in measured erythema on the treated side was minimal. Reduction of other subjective symptoms including sensations of itching, heat, and burning or pricking also showed the effectiveness of FMR on rosacea symptom control.

The mechanism of FMR had been suggested as remodeling of dermal structure and decreasing sebaceous gland activity by skin needling and the thermal effect of radiofrequency energy [12, 13]. However, we previously found that FMR may have anti-inflammatory and anti-angiogenesis effects, because NF-κB, IL-8, and VEGF decreased *in vivo* after FMR treatment [9]. The expression of VEGF is up-regulated in the lesional skin of rosacea patients, and NF-κB can up-regulate IL-8 and VEGF expression through binding to the NF-κB sites of the promoter of these genes [14, 15]. Although a detailed mechanism of regulating NF-κB by FMR is unclear, the results of this study show that FMR can reduce inflammation and angiogenesis in rosacea.

Furthermore, the decrease of markers related to the innate immune system (TLR2 and LL-37) was also observed after

FMR treatment. Dysregulation of the innate immune system is thought to be important in the pathogenesis of rosacea. In rosacea patients, increased levels of TLR2 lead to higher expression of kallikrein 5, resulting in increased expression of LL-37 [16, 17]. LL-37 can induce leukocyte chemotaxis and angiogenesis [16, 18]. The candidates of triggers for TLR2 activation include Demodex mites and the Demodex-associated bacterium *Bacillus oleronius* [19, 20]. The heat produced by FMR treatment possibly killed the mites or bacteria, resulting in suppression of the overactivated innate immune system.

Mast cells are also thought to contribute to inflammation and angiogenesis in rosacea patients and are regarded as key mediators of cathelicidin LL-37-initiated skin inflammation [21, 22]. Although the exact mechanism remains elusive, we found that mast cell count decreased after FMR treatment, which could be a possible pathway to reduce inflammation and angiogenesis. The possible mechanisms of FMR against rosacea are shown in Figure 5.

Sulk et al. observed increased dermal immunostaining of TRPV 2, 3, and 4 in rosacea [23]. TRPVs are thought to be involved in inflammation, innate immunity, nociception, heat sensation,

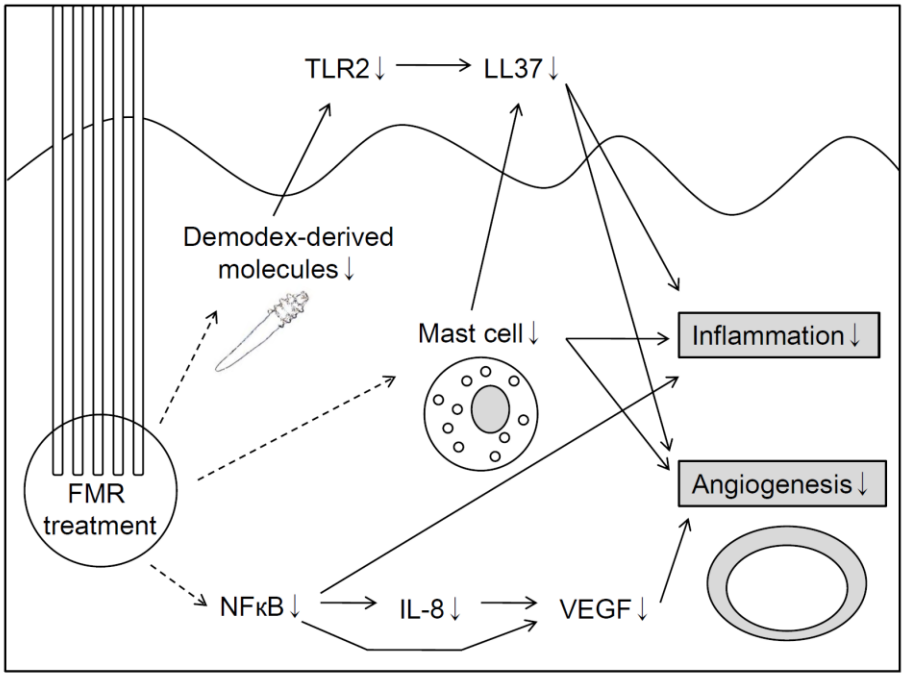


Figure 5. Possible mechanisms of fractional microneedling radiofrequency (FMR) treatment in rosacea

FMR may regulate inflammation and angiogenesis in the rosacea skin via various pathways.

and vascular regulation, and they can mediate and maintain the symptoms of rosacea [24]. In our study, reduced expression of TRPVs after FMR treatment correlated with improvements of subjective symptoms including flushing and sensations of burning or pricking and heat. However, because these markers can be expressed in immune cells, decreased expression of

TRPVs could be the result of fewer inflammatory cells in the tissue from the treated side.

The limitation of this study is that the number of patients enrolled was rather small. Also, there were fluctuations of erythema at the time of visit due to flushing the nature of rosacea. Because this study was performed during the winter through summer, there could be seasonal variations in UV and temperature, which influence aggravation or improvement of rosacea symptoms.

In conclusion, FMR results in clinical and histological improvement of rosacea. FMR can be an option for patients who fail other medical therapy, who are resistant to long-term oral therapy, or who do not want take any oral or topical medication, such as pregnant women. We also expect that FMR may have synergistic effects in combination with other treatment methods. Further study is to be focused on optimizing parameters as well as evaluating the efficacy of multiple FMR treatments and combination therapy with other medications.

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국문 초록

서론: Fractional microneedling radiofrequency (FMR)는 새로 떠오르고 있는 치료법이지만, 주사에 대한 효과에 대해서는 기존에 연구된 바 없다. 이에 본 연구에서는 주사 환자에서 FMR 치료의 임상적 및 조직학적 효과에 대해 알아보고자 한다.

방법: 12 주 동안의 전향적, 무작위배정, split-face 방식의 임상 연구를 시행하였다. 안면 한 쪽에 4 주 간격으로 2 회의 FMR 치료를 시행하였고, 다른 쪽 안면은 치료하지 않고 두었다. 임상 평가와 홍반에 대한 광학적 측정, 조직학적 분석을 시행하였다.

결과: 임상 평가와 광학적 측정 결과 치료한 쪽 안면에서 치료하지 않은 쪽 안면 및 치료 전 안면과 비교하여 모두 홍반의 감소가 나타났다. 치료 전과 비교하여 12 주 째 치료한 쪽 안면에서 erythema index 는 13.6%, a* 값은 6.8% 감소하였다. 면역조직화학 염색에서 FMR 치료 후 염증, 선천면역, 혈관생성 관련 인자들의 발현이 감소됨이 관찰되었다.

결론: FMR 치료는 임상적 및 조직학적으로 주사를 호전시킬 수 있으며, FMR 은 다른 치료법에 대한 대체방법으로, 또는 다른 치료법과 함께 사용될 수 있겠다.

주요어 : 주사, 홍반, Fractional microneedling radiofrequency

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