Scientific Research Publishing

Journal of Biosciences and Medicines, 2022, 10, 79-89
https://www.scirp.org/journal/jbm
ISSN Online: 2327-509X
ISSN Print: 2327-5081

Comparative Study of Collagen Stimulation Using Semi-Ablative and Non-Ablative Radiofrequency

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How to cite this paper: Meyer, P.F., da Silva, R.M.V., De Morais Carreiro, E., Maia, R.R., Farias, S.L.Q., Soares, C.D., Afonso, Y.A., Pasqual, G.F., Ribeiro, S.B. and Pinheiro, R.T.A. (2022) Comparative Study of Collagen Stimulation Using Semi-Ablative and Non-Ablative Radiofrequency. *Journal of Biosciences and Medicines*, **10**, 79-89.
https://doi.org/10.4236/jbm.2022.106007

Received: April 29, 2022
Accepted: June 13, 2022
Published: June 16, 2022

Abstract

Objective: Comparison between semi-ablative and non-ablative radiofrequency for collagen stimulation. **Method:** Three volunteers with scheduled surgical procedures of abdominoplasty were selected. After the evaluation, the abdominal area was divided into three quadrants, with the right region receiving monopolar non-ablative radiofrequency (RF) application. In contrast, the left region received fractionated radiofrequency (FRF) application, using three different needle sizes (0.5, 1.5, and 3.0 mm), and the intermediate area was kept as a control. After 24 hours, surgery was performed, and the skin flaps were collected for histological and immunohistochemical analyses. **Results:** An increase in total collagen and type I collagen was observed in the treated groups. There was also an increase in the expression of COX-2, CD68 positive cells (macrophages), and lymphocyte markers (CD3, CD20, and NK-CD57). Furthermore, it was seen that only the use of 3.0-mm needles kept the channels open in the superficial tissue after 24 hours. There were no significant differences between the modalities. **Conclusion:** Non-ablative and sub-ablative radiofrequency have positive and effective results for flaccidity, demonstrating the success in collagen production. Furthermore, this study is the first to present channel opening and permanence time, which are important to optimize the action of drug delivery.

Keywords

Collagen, Flaccidity, Radiofrequency, Drug Delivery

1. Introduction

Radiofrequency (RF) therapeutic use has been studied over the years, is considered a safe practice and presents excellent results, enabling a quick recovery and a low rate of complications [1]. Radiofrequency is a non-invasive treatment that enables thermal modification in the connective tissue of the skin through dermal heating and vasodilation and lipolysis of adipose cells to allow skin rejuvenation, including the improvement of sagging, and it can also be used for body remodeling [2].

The successful transfer of RF into thermal energy depends on the size and depth of the tissue to be treated. Its application results in collagen denaturation, localized lipolysis, and the stimulation of healing cascades that promote the smoothing and firming effect of the skin as a final result. RF is well accepted because the energy produces is an electrical current rather than a light source; thus, tissue damage is minimized, and epidermal melanin remains unchanged to allow RF application to all skin types [3].

Initially, energy was used for electrocautery to generate highly focused coagulative heat, using high doses. However, one of the leading clinical concerns with RF is the ability to control temperatures and prevent damage to soft tissue elements, with the current trend being the application of non-ablative or semi-ablative levels of RF energy to achieve tissue improvement. Several RF devices are commercially available. Based on the electrode configuration that controls the dispersion of electrical current in tissue, these devices can be classified into six generations (1st generation: monopolar RF; 2nd generation: bipolar RF; 3rd generation: RF multipolar; 4th generation: Multipolar RF with pulsed electromagnetic fields; 5th generation: Fractional RF; 6th generation: Directed internal RF cannula delivery system, non-invasive real-time temperature monitoring) [1] [4].

Although there is an inclination towards using non-ablative technologies, the histological and immunohistochemical comparison of tissues submitted to the application of different types of radiofrequency was not found in the current literature. Therefore, this study aimed to compare the results of semi-ablative and non-ablative radiofrequency on collagen stimulation.

2. Materials and Methods

This experimental study was carried out with the approval of the Ethics Committee of University Potiguar (code: 2.967.282). All volunteers signed an informed consent form before the beginning of the study, and the treatment occurred from October 2020 to January 2021 in the city of Natal, Rio Grande do

Norte state, Brazil.

Three female volunteers were evaluated 35 and 45 years old with scheduled abdominoplasty and chosen in a non-probabilistic way were evaluated. Participants who presented changes in sensitivity, who were using pacemakers, automatic internal defibrillators, or who made use of metallic implants, haemophiliacs, and pregnant women would be excluded from this study.

2.1. Assessment and Treatment Procedures

After selection, the participants were informed about the procedures to be performed, and those who agreed signed the Free and Informed Consent Term, TCLE. They were then submitted to assessment, and data were collected using the assessment protocol (PAFAL) [5]. Afterward, 10 × 10 cm quadrants were delimited for treatment and control, and the right region received the application of non-ablative radiofrequency (RF) monopolar, power of 45 W, and temperature of 41˚C. In contrast, the left region received fractionated radiofrequency (FRF) application, using three different sizes of needles (0.5, 1.5 and 3.0 mm), with 30 mJ and the intermediate area was kept as a control shown in **Figure 1**.

Twenty-four hours after receiving the application of radiofrequency, the volunteers underwent abdominoplasty surgery, and the skin flaps were removed and sent for preparation of the material in blocks and histological and immunohistochemical analyses. The analyses aimed to quantify the inflammatory cells, collagen production, determination of the amount of type I collagen, and identification of the presence of acute and chronic inflammation (macrophages and lymphocytes).

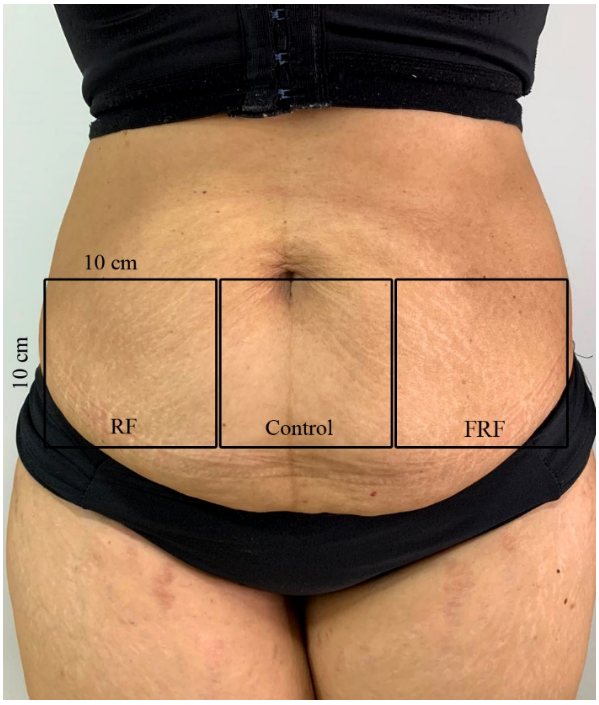


Figure 1. Demonstration of treatment quadrants.

2.2. Statistical Analysis

Histological data were analysed using GraphPad Prism (Version 8.0, GraphPad Software, San Diego, California, USA). The Mann-Whitney U test was adopted to assess the results' statistical significance (p-values < 0.05 were considered statistically significant). In addition, qualitative data were described based on the pathologist's reports (descriptive analysis of histological images and cell quantification).

3. Results

Histological analysis of inflammatory cells showed a notable increase in COX-2 expression in the groups treated with conventional radiofrequency (**Figure 2(c)**) and fractional radiofrequency (**Figure 2(d)**) when compared to the control group **Figure 2(b)**). Significant differences were observed when comparing the treated samples (RF and FRF) with the control side ($p < 0.0001$), however, these differences were not seen in the comparison between the treated groups ($p > 0.05$).

Collagen analysis was performed in total (**Figure 3**), and type I collagen was quantified (**Figure 4**). The slides showed a large amount of newformed collagen, with inflammatory cells and different degrees of fatty tissue degeneration for the treated groups. However, only the total collagen in the group treated with fractionated radiofrequency compared to the control group presented significant values, with a p value of 0.02 for the 0.5-mm needle and p-value of 0.007 for the

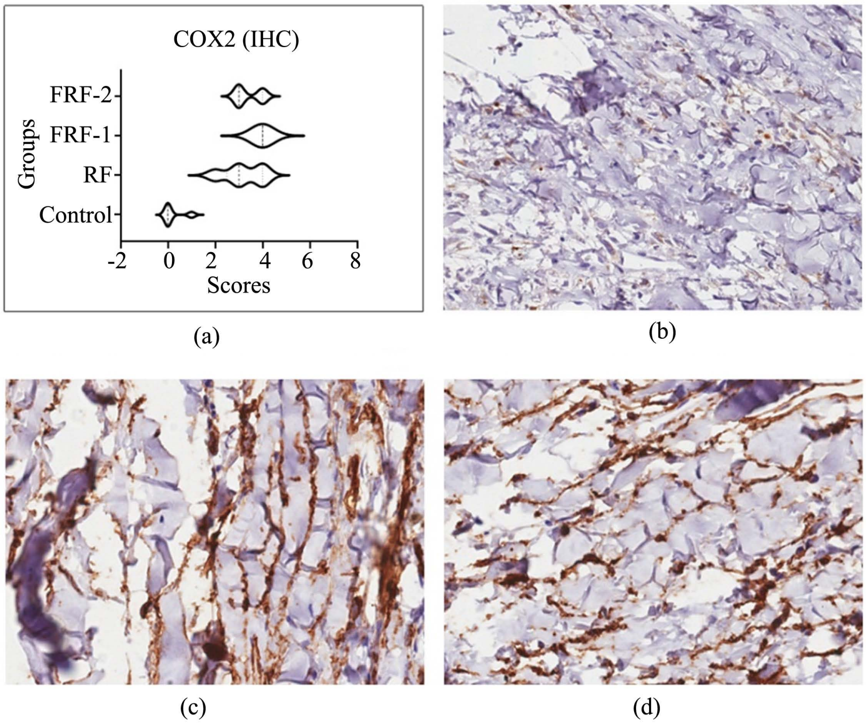


Figure 2. Analysis of COX-2 expression: (a) Scores obtained in all groups; (b) Control group; (c) Conventional RF; (d) Fractional RF.

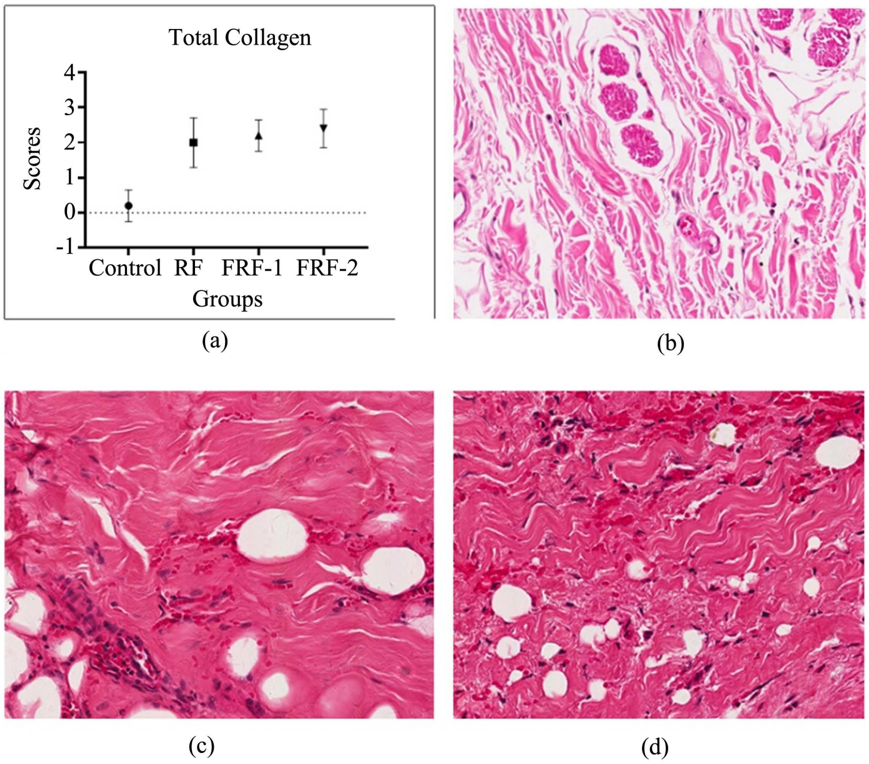


Figure 3. Analysis of total collagen expression: (a) Scores obtained in all groups; (b) Control group; (c) Conventional RF; (d) Fractional RF.

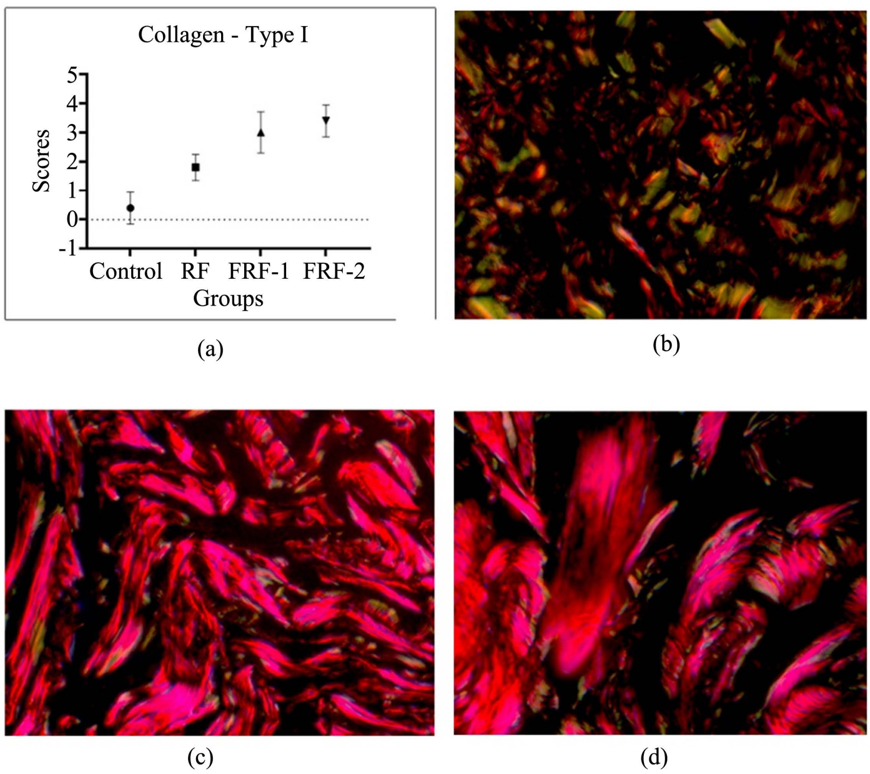


Figure 4. Analysis of type I collagen: (a) Scores obtained in all groups; (b) Control group; (c) Conventional RF; (d) Fractional RF.

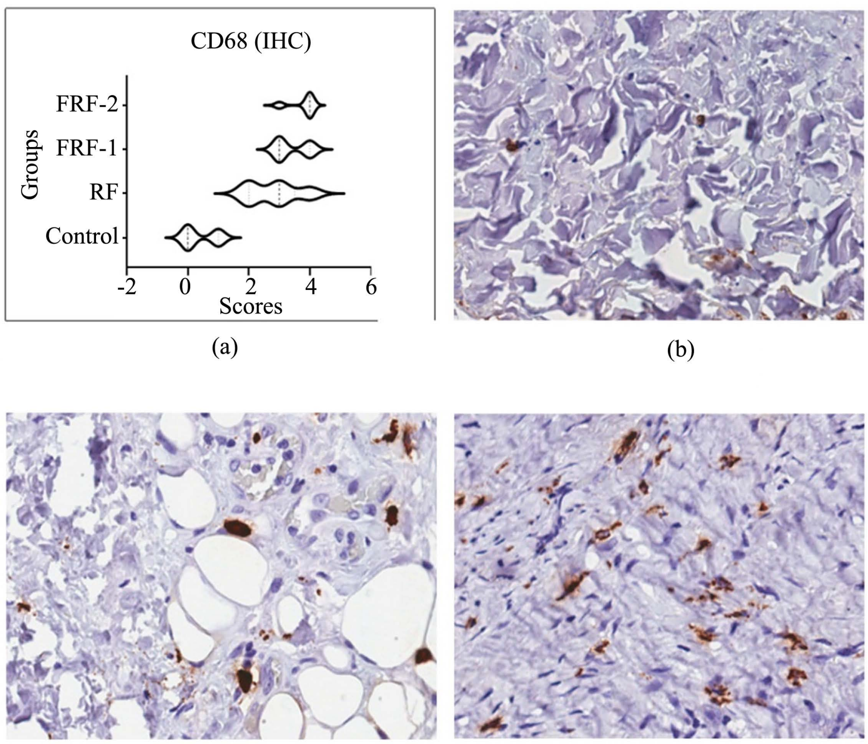
3.0-mm needle. No significant differences were observed when comparing the treated groups (p > 0.05).

There was a more significant amount of type I collagen in the treated groups compared to the control sample. However, this comparison only showed a statistically significant difference for the group treated with fractional radiofrequency, with p being 0.01 for the 0.5-mm needle and p-value of 0.002 for the 3.0-mm needle.

Figure 5 shows a notable increase in the number of CD68-positive cells (macrophages) in the groups treated with RF and FRF (both needle types) when compared to the control group (p < 0.0001).

Cells positive for lymphocyte markers (CD3, CD20, and NK-CD57) were perceived in a more significant number in the treated groups when compared to control. Significant differences were seen in most of the analyses, except when comparing the depth reach of the needles used in fractional radiofrequency, when comparing the groups treated with the control, p < 0.0001 was observed, whereas with the conventional radiofrequency versus the fractionated radiofrequency with a 0.5-mm needle, the p-value was equal to 0.03, as well as the conventional radiofrequency versus the fractionated radiofrequency with a 3.0-mm needle the p-value was equal to 0.003 (**Figure 6**).

The opening of the channels in the skin after the fractional radiofrequency application was also analysed, and the duration of this opening was determined.



(a) (b) (c) (d)

Figure 5. Analysis of CD68 expression (macrophages): (a) Scores obtained in all groups, (b) Control group; (c) Conventional RF; (d) Fractional RF.

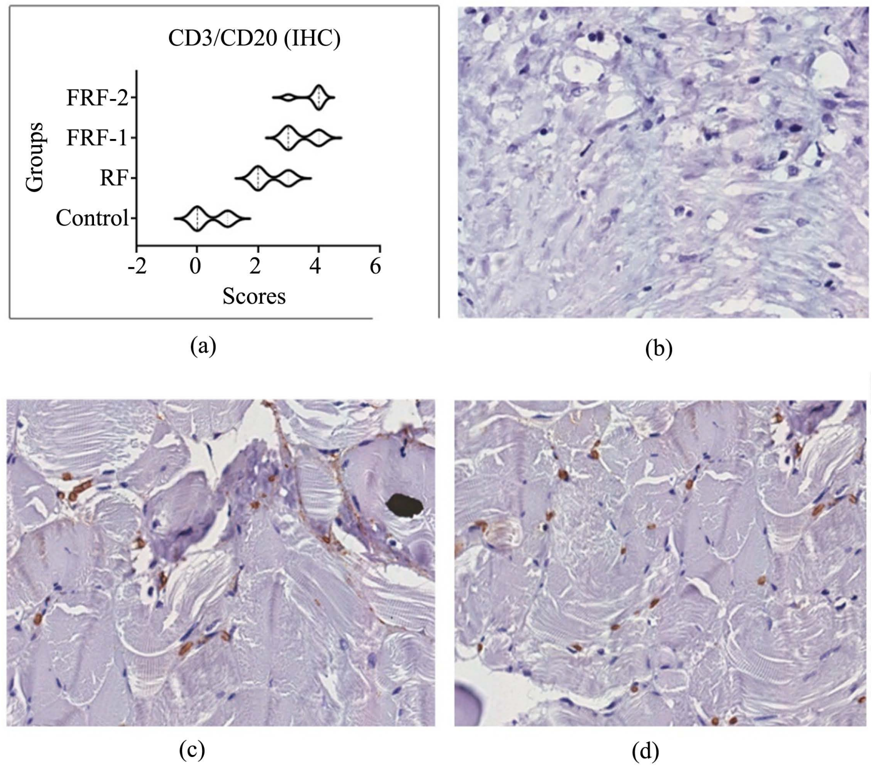


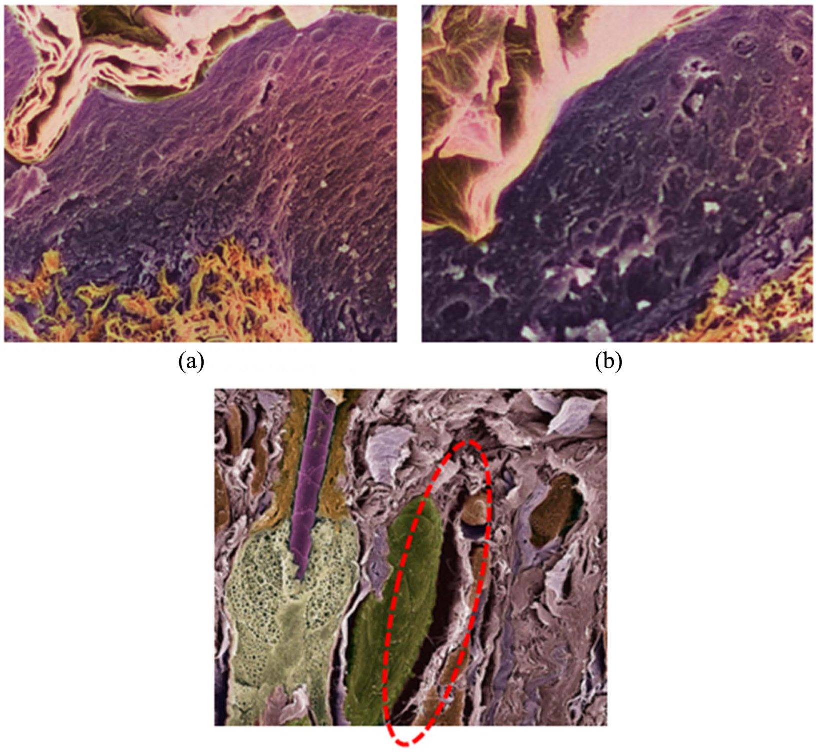
Figure 6. Analysis of lymphocyte expression (CD3, CD20, and NK-CD57): (a) Scores obtained in all groups; (b) Control group; (c) Conventional RF; (d) Fractional RF.

The results found in electron microscopy showed that the three needles of different sizes used reached a depth to the superficial dermis. With the use of 0.5- and 1.5-mm needles, after 24 hours, the skin channels were already closed. However, with the use of 3.0 mm needles, even after 24 hours, the channels remained open in the superficial tissue. Moderate to high collagen production was also observed after using these technologies, and inflammation was more consistent when using fractional radiofrequency with deeper needles (**Figure 7**).

During the period of care for the volunteers, no serious adverse effects were reported. Only hyperaemia and mild edema occurred at the sites of application of fractional radiofrequency, which were quickly resolved, without the need for interventions.

4. Discussion

Some factors are related to radiofrequency effects, mainly tissue characteristics, such as temperature and hydration, and procedure specifications, where the choice of energy and pulse duration will influence tissue response and degree of caused damage [6]. This study compared the use of conventional non-ablative radiofrequency with semi-ablative fractional radiofrequency at cellular level, demonstrating its effects on collagen, the inflammatory and immune activity of the body, and the permanence time of the channels in the superficial dermis after the application of fractional radiofrequency.



(a) (b) (c)

Figure 7. Analysis by electron microscopy of the opening of skin channels before and after the application of fractionated RF with 0.5- (a), 1.5- (b), and 3.0-mm (c) needles.

The samples analysed in this study showed an increase in the amount of newformed collagen and type I collagen after therapy application, but these values only showed a significant difference for the FRF group, using 0.5- and 3.0-mm needles, when compared to the control samples. Despite this, there was no superiority between the modalities. The synthesis of new collagen is the consequence of the initial contraction caused by radiofrequency thermal energy, which promotes dermal remodelling and skin firmness, as seen in previous studies [7].

Dayan *et al.* (2020) [8] carried out a retrospective multicentre study between 2013 and 2018 using a combination of bipolar and fractional radiofrequency for the treatment of facial aging. Two hundred and forty-seven patients initially treated with bipolar RF (internal temperature of 68˚C and external temperature of 38˚C), maintained for 1 minute after reaching the temperatures described above, were analysed. Fractional RF was then used with a 2-mm depth and 30-energy with 50% overlap, reduced in some cases. There was an improvement in the area observed with the reduction in the Baker Face/Neck classification, great satisfaction of the treated individuals, and a low rate of complications. This study presents a significant advance concerning use of these technologies, despite methodological limitations.

Immune cells secrete cytokines and inflammatory mediators, which contribute to inflammation. In particular, proinflammatory cytokines are mainly produced by activated macrophages, and they play important roles in the upregulation of inflammatory responses, such as the recruitment of additional immune cells [9]. The inflammatory process analysed in this study was significantly greater, caused by the fractional radiofrequency, and the use of larger needles, such as 3.0-mm compared to 1.5- and 0.5-mm, was related to the greater inflammatory process.

It is understood that the increase in inflammatory activity occurs as an expected response to the procedure. Therefore, the presence of a pro-inflammatory enzyme, such as COX-2, which is associated with cell proliferation and differentiation, as seen in the study by Meyer *et al.* (2017) [10], that after applying conventional RF in Wistar rats, he observed neoangiogenesis, modulation of COX-2 expression, with consequent neocollagenesis and increased rate of dermis thickness. The samples analysed in this study did not show differences in COX-2 expression between the treated groups; however, this was significant compared to the control, demonstrating a similar response in human skin.

Another study [11] identified a pro-inflammatory reaction with progressive growth from 2 days to 10 weeks after treatment, with an increase in heat shock proteins HSP72 and HSP47 and an increase in anabolic metalloproteinases MMP-13 and MMP9, leading to dermal remodelling in patients that were treated with FRF. Hantash *et al.* (2009) [12] also observed denatured collagen zones in the reticular dermis, generated at pre-selected temperatures between 60 and 80˚C, but unlike our study, there was a preservation of adipose tissue structures after the application of FRF.

Macrophages and neutrophils produce cytokines, which amplify the immune response, promote the stimulation or activation of other immune cells, such as lymphocytes, and act by directly modulating repair mechanisms through their effects on skin components [13]. Dermal tissue immune response was seen by a greater number of cells positive for lymphocyte markers (CD3, CD20, and NK-CD57) in the treated samples. There was a significantly greater difference when compared to conventional and fractionated RF using 3.0-mm needles. A notable increase in the number of CD68 positive cells (macrophages) was also observed. The macrophage is the central cell of the inflammatory phase, responsible for the injured tissue degradation and the stimulation of the influx and fibroblasts proliferation, enabling the remodelling of the region [14].

The exact influence of RF pulse energy corresponds to ablation depth, ablation width, coagulation, and necrosis. Previous studies [15] have demonstrated the microthermal zone after the application of RFF. It was seen that the ablation depth increased in proportion to the fractional RF energy. However, the zone diameter was relatively constant, regardless of the device energy.

Another relevant finding is the presence of open channels after the application of FRF, as these can favour the delivery of drugs to optimize the results. For Shin *et al.* (2014) [16], the fractional RF resulted in a superficial and broad microthermal zone of the "crater" type. This study is the first one to use electron microscopy to demonstrate the depth reached and the time to close the channels. The sizes of needles used reached a depth to the superficial dermis, and only the

3.0 mm needle remained open even after 24 hours.

The study by Park *et al.* (2016) [17] investigated the effects of FRF on transdermal drug delivery. Swine skin was exposed to fractionated RF and/or sonophoresis, followed by topical application of 5-aminolevulinic acid (ALA). It has been observed that this technology can increase substance penetration, increasing the effectiveness of these therapies. The authors emphasize the need for future studies to confirm this finding.

5. Conclusion

Conventional (non-ablative) and fractional (ablative) radiofrequency have positive and effective results for flaccidity. Both stimulated collagen type I, in addition to presenting an inflammatory process associated with the presence of important immune responses to the dermal tissue, demonstrated the RF modalities' effectiveness. Furthermore, this study presents the channels' open time and their permanence as so, not yet seen in the literature, which is something important for drug delivery action. This study also reinforces that the size of the needles has an influence in this aspect, but not in the production of collagen.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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