ORIGINAL RESEARCH

# Experimental Study of Skin Contraction Induced by Bipolar Radiofrequency

Jia Liu, PhD; Zhijie Zhao, MM; Jun Zhang, PhD; Zhibing Ma, PhD; Haonan Peng, BD; Jinlong Huang, PhD

## ABSTRACT

**Background** • Facial skin relaxation has become an important part in solving the problem of facial rejuvenation. Minimally invasive or noninvasive skin-tightening procedures have become a trend for facial rejuvenation. Bipolar radiofrequency (RF) is a new option for treating skin relaxation and is more effective than noninvasive surgery without surgical incision.
**Objective** • To explore the effect of different bipolar RF powers on the area of the original box, changes of skin and subcutaneous tissue thickness and numbers of fibroblasts in rabbits.
**Design** • The research team performed an animal study.
**Setting** • This study took place in Affiliated Hospital of Nanjing University of Chinese Medicine
**Participants** • Eighteen common-grade adult New Zealand rabbits (female, 2.5-3.0 kg).
**Methods** • Bipolar radiofrequency therapy was given to a girl rabbit on the left side of the treatment area. Standard HE and Masson staining were performed to assess the pathological changes, area of the original box and the number of fibroblasts in skin and subcutaneous tissues.
**Outcome Measures** • (1) The area of the original box, changes of skin and subcutaneous tissue thickness, and numbers of fibroblasts under different bipolar RF temperatures or under different bipolar RF powers immediately after surgery, 1 month after surgery and 3 months after surgery were observed. (2) Standard HE and Masson staining results.
**Results** • Under the condition of certain instrument power, at 36de 38d and 40nd the area of the original box shrank to different degrees immediately after surgery (16.54±0.37, 17.78±0.03, 17.19±0.01), 1 month after surgery (16.59±0.31, 17.82±0.01, 18.34±0.30) and 3 months after surgery (16.89±0.12, 18.16±0.14, 19.23±0.32) compared with that before surgery ($P < .05$). Under specific temperature conditions, at 16 W, 18 W, 20 W, and 22 W, the area of the original box shrank to different degrees immediately after surgery (16.40±0.49, 15.55±0.57, 17.54±0.12, 16.19±0.27), 1 month after surgery (16.88±0.12, 17.46±0.02, 18.05±0.35, 19.41±0.08) and 3 months after surgery (19.09±1.01, 18.30±0.69, 20.00±0.29, 21.20±0.90) compared with that before surgery ($P < .05$). When the power was fixed, the thickness of skin and subcutaneous tissue decreased immediately after surgery (6.7, 6.8, 7), 1 month after surgery (6, 6.1, 6.3) and 3 months after surgery (6.4, 6.5, 6.2) at different temperatures ($P < .05$). When the temperature was fixed, the thickness of skin and subcutaneous tissue decreased immediately after surgery (6.1, 6.08, 6.03), 1 month after surgery (6.2, 6.15, 6.13), and 3 months after surgery (6.2, 6.23, 6.03) under different powers ($P < .05$). Under the condition of certain instrument power, at 36de 38d and 40n, the number of fibroblasts increased to different degrees immediately after surgery (26.54±2.37, 30.78±3.03, 37.19±4.01), 1 month after surgery (28.59±2.31, 34.82±3.01, 40.34±4.30), and 3 months after surgery (30.89±0.12, 38.16±0.14, 42.23±0.32) compared with that before surgery, and all were statistically significant ($P < .05$). Under specific temperature conditions, at 16 W, 18 W, 20 W, and 22 W, the number of fibroblasts increased to different degrees immediately after surgery (28.29±2.49, 30.97±3.57, 38.74±3.12, 45.68±4.27), 1 month after surgery (30.88±3.12, 32.46±4.02, 41.05±0.35, 50.41±0.08), and 3 months after surgery (29.99±2.01, 33.30±2.69, 39.00±3.29, 23.20±2.90) compared with that before surgery, and all were statistically significant ($P < .05$).
**Conclusions** • Our study clarifies that bipolar RF can decrease the skin and subcutaneous tissue thickness and increase the numbers of fibroblasts at the temperature of 36°C, 38°C, and 40°C and frequency of 16-22 W, which has a therapeutical effect on skin contraction. Our study might effectively improve the skin slack of patients, and the postoperative maintenance rate is high, and will not cause obvious complications. This study may provide a theoretical direction for clinicians to tighten the skin of patients using bipolar RF. (*Altern Ther Health Med.* [E-pub ahead of print.])

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## INTRODUCTION

Facial skin relaxation has become an important part in solving the problem of facial rejuvenation.[1] As people grow older, bone aging and retreat are accompanied by the relaxation of supporting ligaments.[2] In addition, under the long-term influence of gravity, an important sign of facial aging will be manifested as the skin relaxation of the face and neck, which will affect the life and confidence of patients.[3] Traditional facelift can solve the problem of skin relaxation to a certain extent, but the trauma is larger, the recovery time is longer, intraoperative nerve damage easily, postoperative hematoma and other problems, leading to the beauty seekers of this operation is not high acceptance.[4] In addition, other medical and aesthetic items such as the round trip mechanical movement of liposuction can easily lead to postoperative complications such as hematoma and seroma, and for patients with skin laxity, postoperative skin laxity is often aggravated.[5] Therefore, it is very important for beauty seekers to find a device that can not only damage the tissue but also tighten the skin.

It has been reported that radiofrequency (RF) acts on the deep skin can stimulate collagen hyperplasia, so as to achieve the effect of skin tightening, wrinkling.[6] The efficacy analysis of a large number of clinical cases has confirmed that radiofrequency technology does have the effect of tightening the skin and improving the contour.[7] For example, Aniseh Samadi et al. have indicated that RF is efficient and safe for facial skin rejuvenation.[8] RF is a kind of electromagnetic wave with high frequency AC variation, also known as radio frequency current.[9] RF current is generated by the flow of charged particles through a closed circuit, when the RF current passes through the human tissue, the skin and subcutaneous tissue become conductive bodies, which produce resistance to the electromagnetic waves, causing high-speed oscillation and friction of water molecules in the tissue cells to produce heat, and achieving the therapeutic effect through the tissue thermal effect.[10] Through its diathermy principle, radio frequency denatures dermal collagen and melts the triple helix structure of collagen. With the cooling of the skin, collagen recombines to form a tighter and more orderly structure, and the tight binding restores the original sense of firmness to the skin.[11] According to the different electrode design, RF can be divided into single-machine RF, bipolar RF, three-stage RF, and multi-stage RF.[12] Unipolar RF only has a single magnetic head to release RF energy, and the RF wavelength of unipolar RF has a deeper effect, which can penetrate into the bottom of the skin, stimulate the fibroblast cells and secrete more new collagen to fill the gap of atrophy and loss of collagen.[13] Bipolar RF refers to the use of two-stage radio frequency technology for functional operation. There are positive and negative poles in it, and the RF energy is distributed and diffused between the electrodes. Its radiofrequency energy can act on the dermis of the skin, but the depth of action is relatively shallow, but with strong controllability.[14] The two electrodes of bipolar RF are closely arranged in the attachment of the treatment head, and the current generated only acts on a short distance between the two electrodes, and the penetration depth is only half the distance of the two electrodes.[15] Therefore, the tissue in the treatment area is lightly heated, about 2 mm, which is generally used for the treatment of weak skin.[16] A large number of clinical treatments have proved the exact skin tightening effect of monopole and bipolar RF technology.[17] There are many experimental evidences for single radiofrequency, but few histological experimental evidences for double-click radiofrequency.

This study aimed to investigate whether bipolar RF has a therapeutic effect on skin contraction in a rabbit model. This study carried out a series of experiments to observe the morphological structure changes of skins and the number of fibroblasts in the skin of rabbits, in order to explore whether bipolar RF has a therapeutic action on skin contraction. Our study indicated that bipolar RF activates the growth of fibroblasts, and then secret collagen, thereby increasing the thickness of skins and recovering skin elasticity.

## METHODS

### Materials

Eighteen common-grade adult New Zealand rabbits (female, 2.5-3.0 kg) were provided by Nanjing Ruizhi Kang Biotechnology Co., LTD. The RF instrument was provided by Shenzhen Peninsula Medical Co., LTD.

### Procedures

Eighteen rabbits were randomly numbered 1-18. Two 5 cm × 5 cm square areas on the abdomen were selected as the experimental areas and used a marker to mark.

**Anesthesia and radiofrequency operation.** The rabbits were placed on the fixed table supine, and 10% chloral hydrate 5 ml/kg was injected intraperitoneally. After the rabbits did not resist, 3% pentobarbital sodium was used for ear edge intravenous anesthesia (1 mL/kg). The onset time of anesthesia was about 5 minutes, and the anesthesia was maintained for about 50 minutes. After anesthesia was stable, the rabbits were placed in the supine position. Skin was prepared in the operation area, and 5% polyvinyl pyrrolidone (PVP) iodine (Shanghai Vidone Material Technology Co., LTD., China) was used to disinfect the operation area. Subcutaneous swelling anesthesia (0.9% sodium chloride solution 200 ml, lidocaine 200 mg, and epinephrine 1:100000) provided by Sigma-Aldrich (USA) was performed in the abdominal operation area, and about 50 ml was injected into each position. The skin was broken in the middle point below the marked area, and a 10 ml needle was used to make a tunnel under the skin. The coupler was applied to the skin surface, and the energy power (16, 18, 20 and 22 W) and temperature parameters (36, 38 and 40°C) of the radiofrequency treatment instrument (provided by Shenzhen Peninsula Medical Co., LTD., China) were adjusted. The swelling anesthesia solution was injected into the subcutaneous tissue using a water injection needle, with the injection rate was 60 mL/min, the radiofrequency electrode was inserted into the subcutaneous tissue, the receiver was placed on the skin surface, the foot was stepped, the sound of "drip" was heard from the normal operation of the machine, and the operation was carried out by the stamping method. The ablation duration was 3 min. Immediately after the treatment, ice was applied for 10 min.

**Specimen acquisition and preparation.** Immediately after operation, 1 month after operation, and 3 months after operation, 3 pieces of skin epidermis were cut with medical scissors and subcutaneous tissue (0.5 $cm^3$/ piece) were taken from different positions of the abdomen, and one piece was fixed in 10% neutral formaldehyde for routine hematoxylin-eosin (HE) staining pathological section. Another specimen was fixed in 2.5% glutaraldehyde solution and prepared for electron microscopy. The last piece was used for Masson staining. After the specimen was obtained, the skin incision was closed intermittently with No. 1 suture.

**Histological assessment**. Fixed tissues were embedded in paraffin and sectioned at 2-3 μm on the plane perpendicular to the surface of the specimen. Standard HE and Masson staining was performed to assess the pathological changes in collagen fibers of skin and subcutaneous tissues. HE staining is one of the commonly used dyeing methods in paraffin section technology. Hematoxylin dye solution is alkaline, which mainly makes chromatin in the nucleus and nucleic acid in the cytoplasm purplish blue. Eosin is an acidic dye that mainly makes the cytoplasm and extracellular matrix components red. The Masson dyeing principle is related to the size of anionic dye molecules and tissue penetration: the size of the molecule is reflected by the molecular weight, small molecular weight can easily penetrate the dense structure and low permeability of the tissue, while large molecular weight can only enter the loose structure and high permeability of the tissue. However, the molecular weight of light green or aniline blue is very large, so the muscle fiber is red after Masson staining, and the collagen fiber is green or blue, which is mainly used to distinguish the collagen fiber from the muscle fiber. For HE staining, the sections were treated with xylene (Sigma-Aldrich, USA) for 20 min and gradient alcohol (Sigma-Aldrich, USA) for 5 min for dewaxing and hydration. Then, the sections were stained with hematoxylin (Beyotime, China) for 5 min and eosin (Beyotime, China) for 30 s respectively, followed by dehydration with alcohol for 5 min and sealing with neutral mounting medium (Shanghai Maokang Biotechnology Co., LTD., China). For Masson staining, the sections were treated with xylene d for 5 min, xylene for 5 min, xylene for 5 min, anhydrous ethanol for 1 min, 95% ethanol for 1 min, 75% ethanol for 1 min, and rinsed with tap water for several seconds. Then, the sections were stained with the prepared Weigert iron hematoxylin solution for 8 minutes, followed by washing using acid ethanol differentiation solution for 15 s. Next, the sections were stained with Masson blue solution back blue for 5 minutes, and washed using distilled water. Afterwards, the sections were treated with ponceau magenta staining solution for 5 min, followed by treating phosphomolybdic acid solution for 1 min and aniline blue dye for 2 min. Sections were quickly dehydrated with 95% ethanol for 2-3 s, anhydrous ethanol for 3 times, each time for 5-10 s, xylene transparent for 3 times, each time for 1-2 minutes, and sealed by neutral gum. An optical microscope (Olympus, Japan) was used for mounting.

**Count of fibroblasts**. The Masson stained sections were observed under 400 magnification microscope, and three fields of the same size were randomly selected for each image to take photos, and then analyzed to calculate the number of fibroblasts (blue color) in each image. The average of the counts of the three fields was taken as a sample value, with 8 samples in each group, and the number of fibroblasts in each group was calculated.

### Statistical Analysis

Statistical analysis was performed using SPSS 23.0 statistical software (International Business Machines Corporation, USA). Measurement data were expressed as the mean ± standard deviation (SD), and one-way analysis of variance (ANOVA) with post-hoc test was used for comparison. $P < .05$ were considered significant.

**Figure 1.** Hematoxylin-eosin (HE) staining of rabbit skin after bipolar radiofrequency treatment

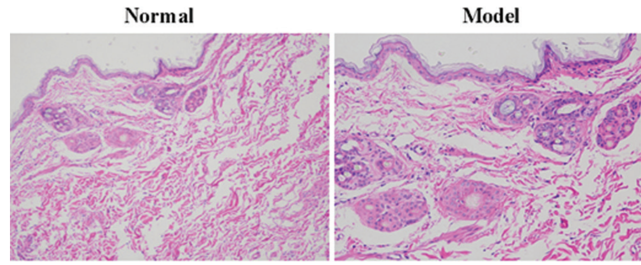



**Table 1**. The area of the original box under different bipolar RF temperatures immediately after surgery, 1 month after surgery and 3 months after surgery ($cm^2$)

| Temperature (°C) | T0 | T1 | T2 | T3 |
|---|---|---|---|---|
| 36 | 25.00±0.00 | 16.54±0.37$^a$ | 16.59±0.31$^a$ | 16.89±0.12$^a$ |
| 38 | 25.00±0.00 | 17.78±0.03$^a$ | 17.82±0.01$^a$ | 18.16±0.14$^{a,b}$ |
| 40 | 25.00±0.00 | 17.19±0.01$^a$ | 18.34±0.30$^a$ | 19.23±0.32$^{a,b,c}$ |
| F value | | 16.17 | 25.716 | 58.814 |
| *P* value | | .025 | .013 | .004 |

$^a P < .05$ compared with T0 in the group
$^b P < .05$ compared with 36 degrees between groups
$^c P < .05$ compared with 38 degrees.

## RESULTS

### HE staining of rabbit skin after bipolar radiofrequency treatment

It was observed by naked eye that, the skin was slightly red immediately after surgery at each power temperature, and there was no skin rupture or subcutaneous nodule after bipolar radiofrequency treatment, as shown in Figure 1.

Immediately after surgery, the area in the box delimited by each parameter had different degrees of contraction. Under the condition of certain instrument power, at 36°C, 38°C and 40°C, the area of the original box shrank to different degrees immediately after surgery, 1 month after surgery and 3 months after surgery compared with that before surgery ($P < .05$), as displayed in Table 1. These results suggested that different bipolar RF temperatures could reduce the area of the original box in the skin of rabbits.

Under specific temperature conditions, at 16 W, 18 W, 20 W, and 22 W, the area of the original box shrank to different degrees immediately after surgery, 1 month after surgery, and 3 months after surgery compared with that before surgery ($P < .05$), as displayed in Table 2. These results suggested that different bipolar RF powers could reduce the area of the original box in the skin of rabbits.

### Light microscopic observation

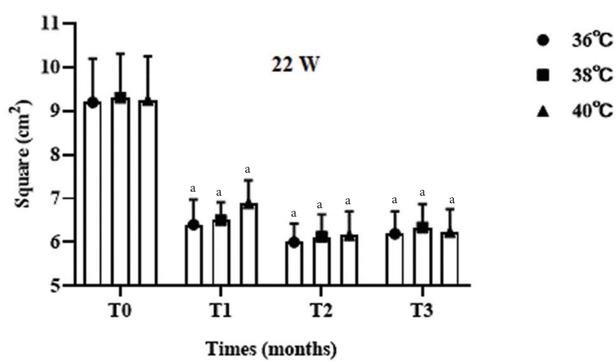
HE staining results showed that the thickness of skin and subcutaneous tissue decreased to different degrees immediately after surgery, 1 month after surgery, and 3 months after surgery compared with that before surgery. When the power was fixed, the thickness of skin and subcutaneous tissue decreased immediately after surgery, 1 month after surgery, and 3 months after surgery at different temperatures ($P < .05$), and there was no statistical significance between them ($P > .05$), as shown in Figure 2. When the temperature was fixed,

**Table 2**. The area of the original box under different bipolar RF powers immediately after surgery, 1 month after surgery and 3 months after surgery (cm$^2$)

| Power (W) | T0 | T1 | T2 | T3 |
|---|---|---|---|---|
| 16 | 25.00±0.00 | 16.40±0.49[a] | 16.88±0.12[a] | 19.09±1.01[a,b] |
| 18 | 25.00±0.00 | 15.55±0.57[a] | 17.46±0.02[a] | 18.30±0.69[a] |
| 20 | 25.00±0.00 | 17.54±0.12[a#] | 18.05±0.35[a,d] | 20.00±0.29[a,e] |
| 22 | 25.00±0.00 | 16.19±0.27[a] | 19.41±0.08[a,b,d,e] | 21.20±0.90[a,b,c,d,e,f] |
| F value | | 8.494 | 65.831 | 78.214 |
| P value | | .033 | .015 | .001 |

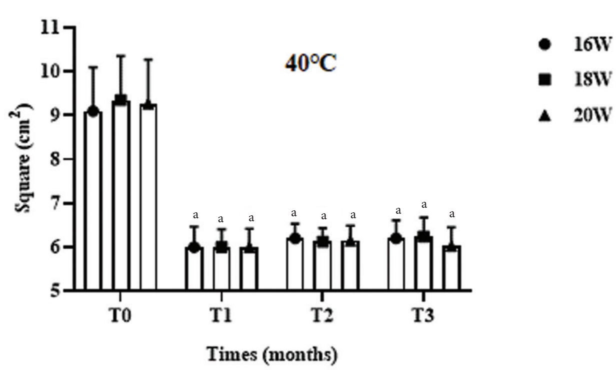
[a]*P* < .05 compared with T0 in the group
[b]*P* < .05 compared with T1
[c]*P* < .05 compared with T2
[d]*P* < .05 compared with power 16 across groups
[e]*P* < .05 compared with power 18
[f]*P* < .05 compared to power 20 within the group

**Figure 2.** Changes of skin and subcutaneous tissue thickness at different temperatures.




[a]*P* < .05 compared with T0 in the group.

**Figure 3**. Changes of skin and subcutaneous tissue thickness under different power.




[a]*P* < .05 compared with T0 in the group.

**Table 3**. Numbers of fibroblasts under different bipolar RF temperatures immediately after surgery, 1 month after surgery and 3 months after surgery

| Temperature (°C) | T0 | T1 | T2 | T3 |
|---|---|---|---|---|
| 36 | 23.00±0.00 | 26.54±2.37[a] | 28.59±2.31[a] | 30.89±0.12[a] |
| 38 | 23.00±0.00 | 30.78±3.03[ab] | 34.82±3.01[a,b] | 38.16±0.14[a,b] |
| 40 | 23.00±0.00 | 37.19±4.01[a] | 40.34±4.30[a,b] | 42.23±0.32[a,b,c] |
| F value | | 15.28 | 25.96 | 40.21 |
| *P* value | | .028 | .017 | .005 |

[a]*P* < .05 compared with T0 in the group
[b]*P* < .05 compared with 36 degrees between groups
[c]*P* < .05 compared with 38 degrees

**Figure 4.** Masson staining of rabbits' skin and subcutaneous tissues

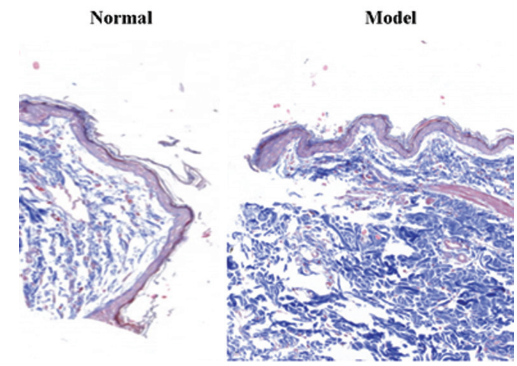



**Table 4**. Numbers of fibroblasts under different bipolar RF powers immediately after surgery, 1 month after surgery and 3 months after surgery

| Power (W) | T0 | T1 | T2 | T3 |
|---|---|---|---|---|
| 16 | 23.00±0.00 | 28.29±2.49[a] | 30.88±3.12[a] | 29.99±2.01[a] |
| 18 | 23.00±0.00 | 30.97±3.57[a] | 32.46±4.02[a] | 33.30±2.69[a,b] |
| 20 | 23.00±0.00 | 38.74±3.12[a,c,d] | 41.05±0.35[a] | 39.00±3.29[a] |
| 22 | 23.00±0.00 | 45.68±4.27[a,e] | 50.41±0.08[a,b] | 23.20±2.90 |
| F value | | 16.42 | 23.87 | 34.61 |
| *P* value | | .026 | .019 | .004 |

[a]*P* < .05 compared with T0 in the group
[b]*P* < .05 compared with T1
[c]*P* < .05 compared with power 16 across groups
[d]*P* < .05 compared with power 18
[e]*P* < .05 compared to power 20 within the group

the thickness of skin and subcutaneous tissue decreased immediately after surgery, 1 month after surgery, and 3 months after surgery under different powers (*P* < .05), and there was no statistical significance between them (*P* > .05), as shown in Figure 3. These results suggested that different bipolar RF temperatures and powers could reduce the thickness of skin and subcutaneous tissue of rabbits.

Masson staining results showed that after radiofrequency treatment, the collagen fibers of skin and subcutaneous tissue were reorganized, the original arrangement order was broken, the space between collagen was compressed, and the tissue arrangement was tighter than that before operation, so as to achieve the effect of skin tightening, as shown in Figure 4.

As revealed in Table 3, under the condition of certain instrument power, at 36 r 38  and 40nd the number of fibroblasts elevated to different degrees immediately after surgery, 1 month after surgery and 3 months after surgery compared with that before surgery, and all of them were statistically significant (*P* < .05). These results suggested that different bipolar RF temperatures could increase the number of fibroblasts in the skin of rabbits.

As revealed in Table 4, under specific temperature conditions, at 16 W, 18 W, 20 W, and 22 W, the number of fibroblasts increased to different degrees immediately after surgery, 1 month after surgery, and 3 months after surgery compared with that before surgery, and all were statistically significant (*P* < .05). These results suggested that different bipolar RF powers could increase the number of fibroblasts in the skin of rabbits.

## DISCUSSION

### Advantages of bipolar RF

Transient posttreatment erythema and edema were the most frequently reported complications for both unipolar and bipolar devices, with unipolar devices occurring more often.[18] Burns were the most common major complication with unipolar devices, while no burns were reported with bipolar devices.[19] These complications may be more common in unipolar devices, where the form of energy delivery is less controlled compared to bipolar devices.[20] The incidence of major complications with monopolar devices was 7%, including burns, scarring, ocular irritation, and erosion of the corneal epithelium.[21] The incidence of major complications with bipolar devices is 0.5%, which includes scar formation and seroma formation. The incidence of minor complications was 51% for unipolar devices and 66.7% for bipolar devices. Transient posttreatment erythema was the most frequently reported complication for unipolar (33.6%) and bipolar devices (19.5%).[22] In bipolar RF, the current flows only a short distance between the two electrodes without the need for a loop electrode. The main advantage of bipolar RF over unipolar RF is that the current distribution is easy to control. The energy provided can also act on the deep and epidermal skin.[23] The results of this study showed that bipolar RF had a good function of tightening the skin. According to previous systematic review,[24] there is objective clinical evidence that unipolar and bipolar RF devices can effectively tighten the skin, improve skin smoothness, brightness, and hydration, improve the appearance of orange peel tissue, reduce subcutaneous fat, and the incidence of major complications is relatively low, which provides evidence for supporting our finding.

### Bipolar RF reduces skin and subcutaneous tissue thickness

RF devices have been used in clinical practice for many years, but there is no standard operation guide or expert consensus. Clinicians operate by their own clinical experience, and young doctors lack guidance from theoretical data. Through a series of animal experiments, this study tried to find out the effective temperature, power and other indicators of bipolar RF to tighten the skin, while avoiding various complications, so as to better guide clinical operations. The experimental results showed that when the temperature of the instrument was set at 36°C, 38°C, and 40°C, or the power parameters were 16-22 W, bipolar RF could play a macroscopic effect on the lateral and longitudinal contraction of the skin, and there were no complications such as nodules and burns at the treatment site. The statistical results showed that the treatment effect was still statistically significant 3 months after surgery compared to the preoperative area. Consistently, a study carried out on the skin tissues of rabbits indicated that RF had tissue-tightening treatment on the dermal tissues.[25]

### Bipolar RF increases the numbers of fibroblasts

Fibroblasts are the most numerous cells in the dermal connective tissue and are the main cells that synthesize various protein molecules in the extracellular matrix.[26] When the fibroblasts are stimulated and acted upon, the fibroblasts become active and participate in the synthesis of collagen and elastin.[27] In our study, it was demonstrated that the number of fibroblasts increased when the temperature of the instrument was set at 36°C, 38°C, and 40°C, or the power parameters were 16-22 W, which indicated that bipolar RF could activate the growth of fibroblasts, and then secret collagen, thereby increasing the thickness of skins and recovering skin elasticity. This may be because that the working principle of bipolar RF is to apply radiofrequency energy to subcutaneous and dermal reticular fascia tissue through minimally invasive incision and transfer electromagnetic energy. When electromagnetic energy meets the resistance of the target tissue, it is converted into heat energy, which stimulates the growth of fibroblasts, thereby promoting contraction and regeneration of collagen and producing the effect of skin tightening.[28] In line with our results, María Luisa Hernández-Bule et al. have discovered that RF can promotes proliferation and migration in human fibroblasts.[29] More importantly, Stefania Pacini et al have proved that RF has significant biological effects on human skin fibroblasts.[30] Therefore, clinicians can refer to the use of the temperature of 36°C, 38°C, and 40°C and frequency of 16-22 W when performing skin tightening for patients with bipolar RF.

### Limitations

Due to the small sample size, short inspection time, and only morphological information, the long-term maintenance effect of bipolar RF on skin tightening is unclear. Therefore, further large-scale studies and long-term follow-up are required to address these limitations.

## CONCLUSIONS

Our study clarifies that bipolar RF can decrease the skin and subcutaneous tissue thickness and increase the numbers of fibroblasts at the temperature of 36°C, 38°C, and 40°C and frequency of 16-22 W, which has a therapeutical effect on skin contraction. Our study might effectively improve the skin slack of patients, and the postoperative maintenance rate is high, and will not cause obvious complications. This study may provide a theoretical direction for clinicians to tighten the skin of patients using bipolar RF. Our study also needs to perform large-scale studies and long-term follow-up to validate long-term effectiveness of bipolar RF for skin contraction.

## DATA AVAILABILITY STATEMENT

Original data from this study is available from the corresponding author upon reasonable request.

## AUTHORS' DISCLOSURE STATEMENT

All authors declare that they have no conflicts of interest related to the study.

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## AUTHOR CONTRIBUTIONS

Jia Liu and Zhijie Zhao contributed equally to this work.

## REFERENCES

1. Robinson H, Jarrett P, Broadbent E. The Effects of Relaxation Before or After Skin Damage on Skin Barrier Recovery: A Preliminary Study. *Psychosom Med*. 2015;77(8):844-852. doi:10.1097/PSY.0000000000000222
2. Pamplona DC, Velloso RQ, Radwanski HN. On skin expansion. *J Mech Behav Biomed Mater*. 2014;29:655-662. doi:10.1016/j.jmbbm.2013.03.023

3. Pailler-Mattei C, Laquièze L, Debret R, et al. Rheological behaviour of reconstructed skin. *J Mech Behav Biomed Mater*. 2014;37:251-263. doi:10.1016/j.jmbbm.2014.05.030
4. Cohen SR, Hewett S, Baraf P, Crowley SJ, Atlan M. Facelift With Power-Assisted Dissection: A Preliminary Report. *Aesthet Surg J*. 2021;41(6):641-651. doi:10.1093/asj/sjaa213
5. Chen Y, Niu Z, Jin R, Lei Y, Han Y. Treatment of Complications following Facial Thread-Lifting. *Plast Reconstr Surg*. 2021;148(1):159e-161e. doi:10.1097/PRS.0000000000008012
6. Kleidona IA, Karypidis D, Lowe N, Myers S, Ghanem A. Fractional radiofrequency in the treatment of skin aging: an evidence-based treatment protocol. *J Cosmet Laser Ther*. 2020;22(1):9-25. doi:10.1080/14764172.2019.1674448
7. Alessa D, Bloom JD. Microneedling Options for Skin Rejuvenation, Including Non-temperature-controlled Fractional Microneedle Radiofrequency Treatments. *Facial Plast Surg Clin North Am*. 2020;28(1):1-7. doi:10.1016/j.fsc.2019.09.001
8. Samadi A, Nasrollahi SA, Janani L, et al. Combination of Fractional Radiofrequency and Thermo-Contraction Systems for Facial Skin Rejuvenation: A Clinical and Histological Study. *Aesthet Surg J*. 2018;38(12):1341-1350. doi:10.1093/asj/sjy152
9. Gentile RD, Kinney BM, Sadick NS. Radiofrequency Technology in Face and Neck Rejuvenation. *Facial Plast Surg Clin North Am*. 2018;26(2):123-134. doi:10.1016/j.fsc.2017.12.003
10. Dayan E, Chia C, Burns AJ, Theodorou S. Adjustable Depth Fractional Radiofrequency Combined With Bipolar Radiofrequency: A Minimally Invasive Combination Treatment for Skin Laxity. *Aesthet Surg J*. 2019;39(suppl 3):S112-S119. doi:10.1093/asj/sjz055
11. Gold MH, Biron J, Wilson A. Improvement of skin texture and wrinkles using radiofrequency ultra-thin electrode technology. *J Cosmet Dermatol*. 2020;19(2):388-392. doi:10.1111/jocd.13239
12. Aydin AM, Gage K, Dhillon J, et al. Focal bipolar radiofrequency ablation for localized prostate cancer: safety and feasibility. *Int J Urol*. 2020;27(10):882-889. doi:10.1111/iju.14321
13. Fritz K, Bernardy J, Tiplica GS, Machovcova A. Efficacy of monopolar radiofrequency on skin collagen remodeling: a veterinary study. *Dermatol Ther*. 2015;28(3):122-125. doi:10.1111/dth.12195
14. Stochaj AS, Jezierska DH, Kubisz L. Comparing the Efficacy of Monopolar and Bipolar Radiofrequency Treatment on Facial Skin in Women. *J Clin Aesthet Dermatol*. 2022;15(12):22-27.
15. Cook J, DiBernardo BE, Pozner JN. Bipolar Radiofrequency as an Adjunct to Face and Body Contouring: A 745-Patient Clinical Experience. *Aesthet Surg J*. 2021;41(6):685-694. doi:10.1093/asj/sjaa417
16. Kislevitz M, Lu KB, Wamsley CE, et al. Bipolar Fractional Radiofrequency Treatment of Suprapatellar Skin Assessment Using Noninvasive Devices and Microbiopsy. *Aesthet Surg J*. 2021;41(12):NP1997-NP2008. doi:10.1093/asj/sjab210
17. Kulick MI. Commentary on: Bipolar Fractional Radiofrequency Treatment of Suprapatellar Skin Assessment Using Noninvasive Devices and Microbiopsy. *Aesthet Surg J*. 2021;41(12):NP2009-NP2010. doi:10.1093/asj/sjab220
18. Watanabe M, Osada H, Shimizu S, et al. Characterization of platelet-activating factor-induced cutaneous edema and erythema in dogs. *Am J Vet Res*. 2016;77(9):969-975. doi:10.2460/ajvr.77.9.969
19. Jeschke MG, Herndon DN. Burns in children: standard and new treatments. *Lancet*. 2014;383(9923):1168-1178. doi:10.1016/S0140-6736(13)61093-4
20. Rohrich RJ, Schultz KP, Chamata ES, Bellamy JL, Alleyne B. Minimally Invasive Approach to Skin Tightening of the Face and Body: Systematic Review of Monopolar and Bipolar Radiofrequency Devices. *Plast Reconstr Surg*. 2022;150(4):771-780. doi:10.1097/PRS.0000000000009535
21. Odell RC. Surgical complications specific to monopolar electrosurgical energy: engineering changes that have made electrosurgery safer. *J Minim Invasive Gynecol*. 2013;20(3):288-298. doi:10.1016/j.jmig.2013.01.015
22. Janssen PF, Brölmann HA, Huirne JA. Effectiveness of electrothermal bipolar vessel-sealing devices versus other electrothermal and ultrasonic devices for abdominal surgical hemostasis: a systematic review. *Surg Endosc*. 2012;26(10):2892-2901. doi:10.1007/s00464-012-2276-6
23. Fritz K, Tiplica GS, Salavastru C. [Radiofrequency : Monopolar, bipolar, multipolar and fractional]. *Dermatologie (Heidelb)*. Oct 2023;74(10):740-747. Radiofrequenz : Mono-, bi- und multipolar sowie fraktional. doi:10.1007/s00105-023-05202-w
24. Oh S, Rho NK, Byun KA, et al. Combined Treatment of Monopolar and Bipolar Radiofrequency Increases Skin Elasticity by Decreasing the Accumulation of Advanced Glycated End Products in Aged Animal Skin. *Int J Mol Sci*. Mar 10 2022;23(6)doi:10.3390/ijms23062993
25. Choi S, Cheong Y, Shin JH, et al. Short-term nanostructural effects of high radiofrequency treatment on the skin tissues of rabbits. *Lasers Med Sci*. 2012;27(5):923-933. doi:10.1007/s10103-011-1016-9
26. Correa-Gallegos D, Jiang D, Rinkevich Y. Fibroblasts as confederates of the immune system. *Immunol Rev*. 2021;302(1):147-162. doi:10.1111/imr.12972
27. Hwang SJ, Ha GH, Seo WY, Kim CK, Kim K, Lee SB. Human collagen alpha-2 type I stimulates collagen synthesis, wound healing, and elastin production in normal human dermal fibroblasts (HDFs). *BMB Rep*. 2020;53(10):539-544. doi:10.5483/BMBRep.2020.53.10.120
28. Cala Uribe LC, Perez Pachon ME, Zannin Ferrero A, et al. Effects of Bipolar Radiofrequency on Collagen Synthesis from Patients with Brachial Ptosis. *Plast Reconstr Surg Glob Open*. 2023;11(4):e4924. doi:10.1097/GOX.0000000000004924
29. Hernández-Bule ML, Toledano-Macías E, Naranjo A, de Andrés-Zamora M, Úbeda A. In vitro stimulation with radiofrequency currents promotes proliferation and migration in human keratinocytes and fibroblasts. *Electromagn Biol Med*. 2021;40(3):338-352. doi:10.1080/15368378.2021.1938113
30. Pacini S, Ruggiero M, Sardi I, Aterini S, Gulisano F, Gulisano M. Exposure to global system for mobile communication (GSM) cellular phone radiofrequency alters gene expression, proliferation, and morphology of human skin fibroblasts. *Oncol Res*. 2002;13(1):19-24. doi:10.3727/096504002108747926