Clinical, Cosmetic and Investigational Dermatology Dovepress

Open Access Full Text Article ORIGINAL RESEARCH

Effects of Cell-Free Fat Extract and Platelet-Rich Fibrin on Scar Maturation in an Experimental Rabbit Ear Wound Model

Zhenni Wei[1,2,*], Mingliang Zhang[3,*], Minjian Chen[1], Yong Song[1], Yi Wang[1]

[1]Fujian Maternity and Child Health Hospital, Affiliated Hospital of Fujian Medical University, Fuzhou, Fujian, People's Republic of China; [2]Fujian Obstetrics and Gynecology Hospital, Fuzhou, Fujian, People's Republic of China; [3]Fuzhou First General Hospital Affiliated with Fujian Medical University, Fuzhou, People's Republic of China

*These authors contributed equally to this work

Correspondence: Minjian Chen, Fujian Maternity and Child Health Hospital, Affiliated Hospital of Fujian Medical University, Fuzhou, Fujian, People's Republic of China, Email 1367120447@qq.com

Background: Multiple methods have been used to treat hypertrophic scarring; however, an optimal treatment method remains to be established. We aimed to research and compare the effects of cell-free fat extract (CEFFE) and platelet-rich fibrin (PRF) on hypertrophic scar formation based on histomorphological analysis in this study.

Methods: Twelve rabbits were divided into four groups randomly. (CEFFE+PRF group, n=3; CEFFE group, n=3, PRF group, n=3 and Control group, n=3). After the ear hypertrophic scar model were established, the two ears of each rabbit in the four groups were injected with CEFFE 0.05 mL/cm^2 + PRF 0.05 mL/cm^2, CEFFE 0.1 mL/cm^2, PRF 0.1 mL/cm^2, and saline 0.1 mL/cm^2, respectively. The scar elevation index and histological analysis using hematoxylin-eosin and Masson staining were evaluated after injection on day 40.

Results: The CEFFE+PRF group was significantly more effective in the prevention of pathological scar formation than the CEFFE-only, PRF-only, and control groups in terms of capillary count, collagen organization, fibroblast count, and scar elevation index ($p<0.05$).

Discussion: CEFFE combined with PRF was the most effective treatment for the prevention of hypertrophic scar formation in our study.

Keywords: effects of cell-free fat extract, platelet-rich fibrin, hypertrophic scar, rabbit

Introduction

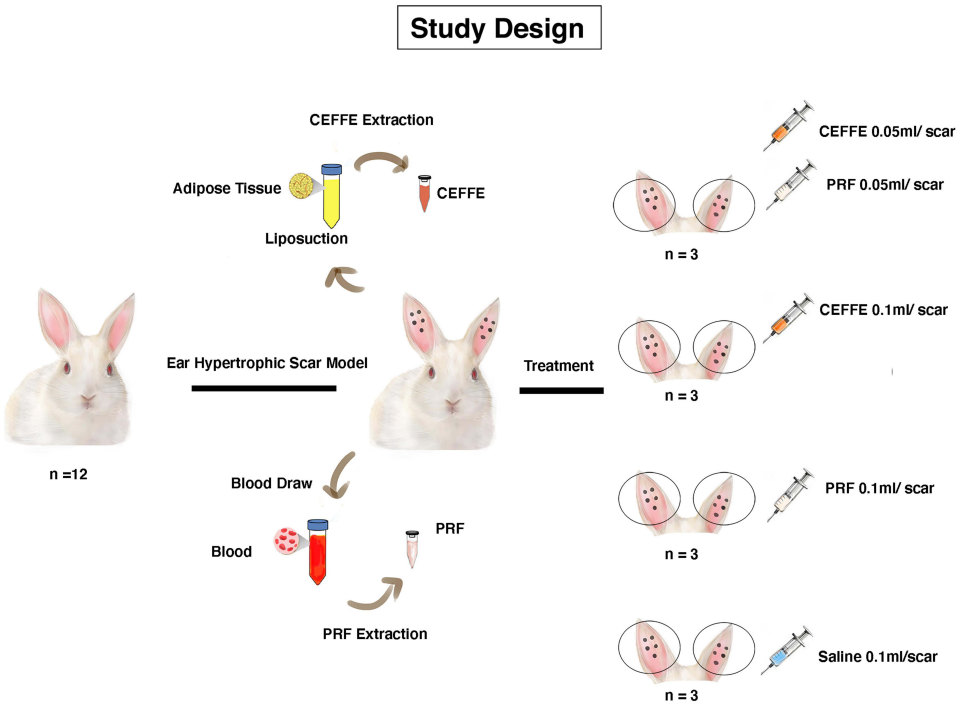
Hypertrophic scars are pathological scars with excessive proliferation of fibroblasts, fibrosis, chronic inflammation, and excessive deposition of fibroblast-derived extracellular matrix proteins.[1] Hypertrophic scars have become a major skin complication that seriously affect the physical and mental health of patients with burns, trauma, and surgery in recent years.[2,3]

There are various methods to treat scars, such as surgical excision,[4] pressure treatment,[5] silicone gel dressings,[6] 5-fluorouracil,[7] laser therapy,[8] radiotherapy,[9] local injection of corticosteroids,[10] and local injection of botulinum toxin.[11] However, the treatment of hypertrophic scars is still in the research stage and has great prospects.[12,13] The use of autologous fat and its components is one of the most promising treatments.[14] Autologous fat transplantation demonstrated significant improvement of scar appearance, skin characteristics, and pain, improving itch and restoration of volume and three-dimensional contour.[15–17]

In recent years, an emerging technology for mechanically extracting cell-free cellular components and lipid remnants from human adipose tissue has entered the field of fat transplantation.[18,19] Cell-free fat extract (CEFFE)[19] has been shown to contain a huge number of cytokines and growth factors, including vascular endothelial growth factor (VEGF),

Received: 24 October 2024
Accepted: 12 December 2024
Published: 17 December 2024

Graphical Abstract



transforming growth factor-β1 (TGF-β1), insulin-like growth factor 1, hepatocyte growth factor (HGF), platelet-derived growth factor (PDGF), basic fibroblast growth factor (bFGF), brain-derived neurotrophic factor, and glial-derived neurotrophic factor. Cai et al[20] demonstrated that the combination of adipose tissue extract and fractional carbon dioxide laser treatment can enhance the adipogenesis and decrease the collagen deposition in hypertrophic scars. Similar to CEFFE, platelet-rich fibrin (PRF)[21–23] is rich in various growth factors, including VEGF, bFGF, PDGF, epithelial growth factor, and cytokines. PRF with three-dimensional structure promotes growth factor bonding and platelet capture, which strengthens the gradual and long-lasting release of growth factors and cytokines. Moreover, Xiong et al[24] demonstrated that platelet-rich fibrin could improve the outcomes of fat grafting in rabbit models.

Accordingly, we assumed that the combination of CEFFE and PRF could enhance adipogenesis and decrease collagen deposition in hypertrophic scars. Thus, in our study, we compared the effects of PRF and CEFFE on the prevention of hypertrophic scar formation by histological evaluation in a rabbit ear wound model.

Methods

Twelve New Zealand white rabbits (weight: approximately 2.5–3.5 kg, male, 6–8 months) were used in our study. All rabbits were purchased at the Experimental Animal Center of Fujian Medical University. This study was approved by the ethics committee of our hospital and the Experimental Animal Center of Fujian Medical University (IACUC-FMCHH -2024-002). All rabbits were housed in a well-ventilated holding room with a 12 h light–dark cycle at an ambient temperature of 23 ± 2 °C and 70% humidity, with free access to water and food. All procedures were conducted in accordance with the "Guiding Principles in the Care and Use of Animals" (China).

Ear Hypertrophic Scar Model

The ear hair of the rabbits was shaved one day before. All rabbits were anesthetized by inhalation of 2%–2.5% isoflurane. The rabbit ear hypertrophic scar model has been defined previously.[25,26] Five-millimeter circular defects were fabricated

on the ventral surfaces of both ears. The dermis, epidermis, and perichondria within the defective area were removed. Wounds were cleaned daily until red, hard hypertrophic scars protruding from the skin surface had formed.

CEFFE Preparation

CEFFE was prepared as described previously.[19] Fat tissue was harvested from both inguinal regions of the rabbits by using suction. The harvested adipose tissue was rinsed with physiological saline to remove red blood cells, then the tissue was centrifuged at 1200 × *g* for 3 min. The lower fluid layers and upper oily were removed, then the middle fat layer was collected and mechanically emulsified. The emulsified fat was subsequently frozen (−80 °C) and thawed (37 °C). The fat was centrifuged (2000 × *g* for 5 min) and separated into four layers after one cycle of the freeze and thaw process. The third layer containing CEFFE was harvested and frozen (−80 °C). The extract was harvested by sterilizing and removing cell debris using a 0.22 μm filter eventually.[19]

PRF Preparation

PRF was prepared as previously described.[24] 10 mL of blood was drawn from the rabbits and centrifuged immediately (12 min at 1000 × *g*). After centrifugation and naturally polymerized fibrinogen, the blood was separated into three-layer. The middle layer containing a PRF clot was harvested and placed in a 4 °C refrigerator.[24]

Grouping and Intervention

After establishing the rabbit ear hypertrophic scar mode, twelve New Zealand white rabbits (total of 120 rabbit ear wounds) were randomly divided into four groups as follows:

- CEFFE+PRF Group: CEFFE 0.05 mL/cm^2 + PRF 0.05 mL/cm^2 (30 rabbit ear wounds)
- CEFFE Group: CEFFE 0.1 mL/cm^2 (30 rabbit ear wounds)
- PRF Group: PRF 0.1 mL/cm^2 (30 rabbit ear wounds)
- Control Group: Saline 0.1 mL/cm^2 (30 rabbit ear wounds)

All rabbits were anesthetized by inhalation of 2%–2.5% isoflurane. CEFFE and PRF were prepared as described before. Injection was conducted at 1mm lateral to the wound margin subcutaneously.

Scar Elevation Index

The scar elevation index (SEI) was used to evaluate scar and wound healing as previously described.[20,27] The vertical distance from the highest point of scar tissue (a) and the surrounding normal skin surface (b) to the ear cartilage surface were measured using Image J software (SEI = a/b, a, the vertical distance from the highest point of scar tissue to the ear cartilage surface; b, the vertical distance from the surrounding normal skin surface to the ear cartilage surface). SEI was evaluated before and 40 days after injection.

Tissue Harvesting and Histopathological Analysis

On day 40 after injection, standardized digital photographs of all scars were taken, and the rabbits were euthanized with an overdose of anesthetic; the complete thickness of the epidermis and dermis were harvested, including at least 5 mm of normal skin at the edge of the scar, for histopathological analysis. The samples were fixed in 10% formaldehyde solution and embedded in paraffin blocks. Specimens were subsequently sectioned serially (4 μm thickness) along the longitudinal axis. All sections were examined under a microscope (Olympus, Tokyo, Japan). The sections were stained with hematoxylin and eosin (HE) and Masson's trichrome staining. One experienced pathologist obtained the results. HE staining was performed 40 days after the treatment to examine the histological morphology. Fibroblasts were counted at 400× magnification in three randomized fields. The number of capillaries formed was counted at 40× magnification. Collagen structure was assessed with a scale from 0 to 3 ("3" being the least organized collagen structure and "0" being normal organized collagen structure).[28]

Statistical Analysis

IBM SPSS Statistics 21.0 were used for statistical analyses. To compare both groups, the values indicating normal distribution were expressed as mean ± SD, and for independent samples, a *t*-test was used. Comparisons of continuous variables were studied using the one-way analysis of variance test. Values indicating non-normal distributions were expressed as [P50 (P25, P75)]. Values indicating non-normal distributions or heterogeneity of variance were analyzed using the Mann–Whitney *U*-test and Kruskal–Wallis test. A p-value <0.05 was deemed statistically significant.

Results

Hyperplastic scars with hard textures and red bulges were observed pre-injection, and the hyperplastic area did not extend beyond the edges of the original wound in all rabbit wounds. No infections or ulcer formation were observed during the intervention. The color of the scars gradually faded and those treated with CEFFE or PRF gradually attenuated, softened, or narrowed over time (Figure 1). The control group demonstrated thicker epidermis and dermis, excessive deposition of the extracellular matrix and irregular collagen fibers. The combination treatment group (CEFFE +PRF) showed the least collagen deposition, thinner epidermis and dermis, more regular and orderly collagen arrangement, followed by CEFFE-only and PRF-only groups (Figures 2 and 3).

The results demonstrated that, on day 40 after injection, the scarred epidermis and dermis in the saline group showed an abundant infiltration of inflammatory cells. The CEFFE+PRF, CEFFE-only, and PRF-only groups showed the

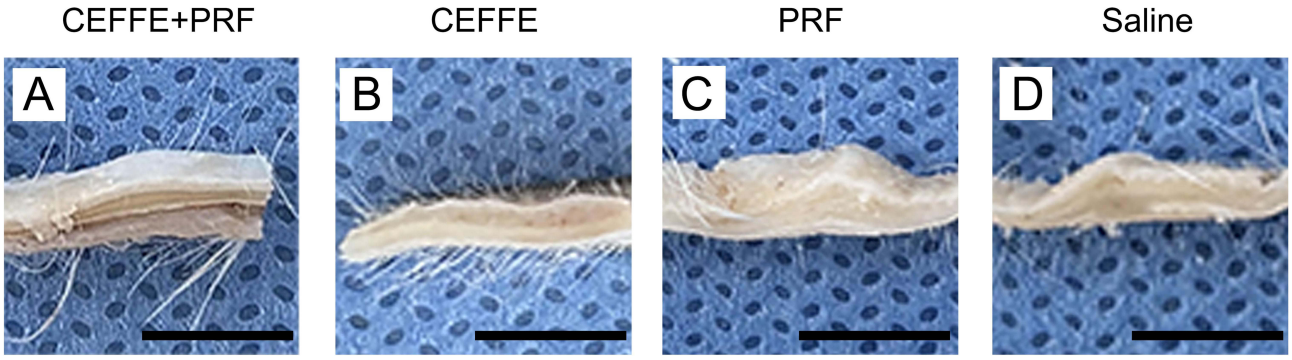


Figure 1 Appearance of scars in each group 40 days after injection. (**A**) CEFFE+PRF Group, scale bar = 5mm. (**B**) CEFFE-only Group, scale bar = 5mm. (**C**) PRF-only Group, scale bar = 5mm. (**D**) Control Group, scale bar = 5mm.

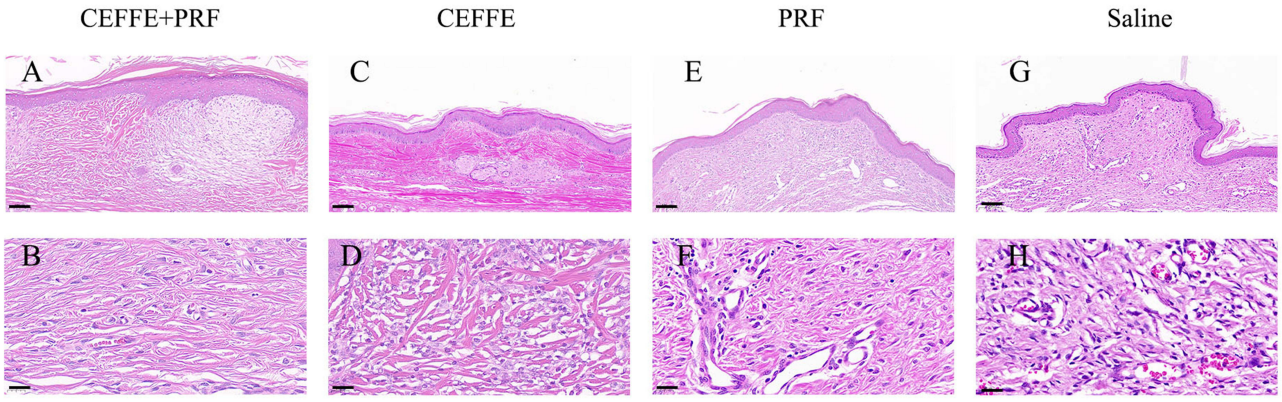


Figure 2 Sample images by HE staining in the scar tissues. HE staining showed that the infiltration of inflammatory cells decreased after treatment, especially in the combination group, but almost no improvement in the control group. (**A**) CEFFE+PRF Group: regular collagen fibers (x100). scale bar = 100 μm. (**B**) CEFFE+PRF Group: (x400). scale bar = 20 μm. (**C**) CEFFE-only Group: (x100). scale bar = 100 μm. (**D**) CEFFE-only Group: (x400). scale bar = 20 μm. (**E**) PRF-only Group: (x100). scale bar = 100 μm. (**F**) PRF-only Group: (x400). scale bar = 20 μm. (**G**) Control Group: excessive deposition of the extracellular matrix and irregular collagen fibers. (x100). scale bar = 100 μm. (**H**) Control Group: Fibroblast cells and capillary formations, accompanied by a substantial infiltration of inflammatory cells. (x400). scale bar = 20 μm.

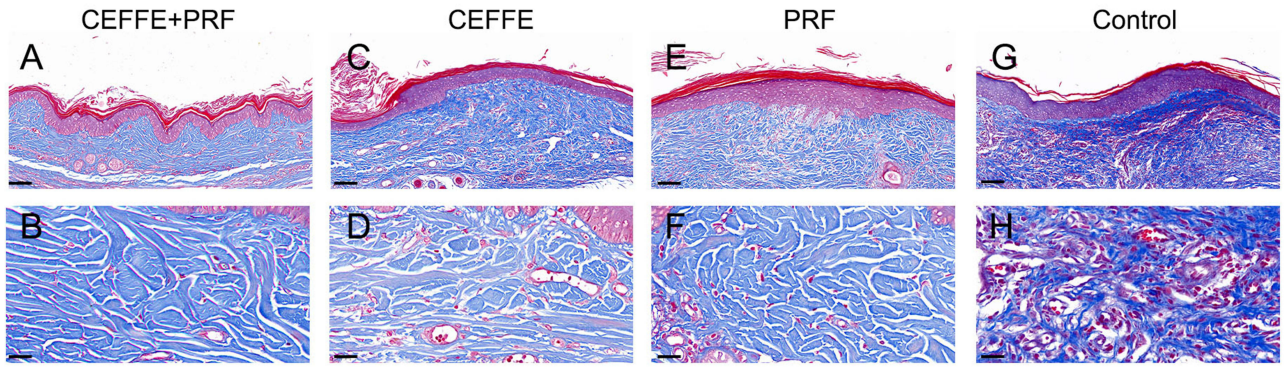


Figure 3 Sample images by Masson's trichrome staining in the scar tissues. (**A**) CEFFE+PRF Group: the arrangement of collagen fibers in the combination group was the loosest and regular (x100). scale bar = 100 μm. (**B**) CEFFE+PRF Group (x400). scale bar = 20 μm. (**C**) CEFFE-only Group: (x100). scale bar = 100 μm. (**D**) CEFFE-only Group: (x400). scale bar = 20 μm. (**E**) PRF-only Group: (x100). scale bar = 100 μm. (**F**) PRF-only Group: (x400). scale bar = 20 μm. (**G**) Control Group: the collagen fibers were disordered and dense. (x100). scale bar = 100 μm. (**H**) Control Group: (x400). scale bar = 20 μm.

reduction of the infiltration of inflammatory cells on the contrary. Adipocytes were observed in the scar dermis of the CEFFE+PRF and CEFFE-only groups.

The mean SEI scores were 2.20 ±0.05, 2.19 ±0.09, 2.20 ±0.08, and 2.21 ±0.13 in the CEFFE+PRF, CEFFE-only, PRF-only, and control groups pre-injection, respectively (p >0.05). Mean SEI scores were 1.75 ±0.03, 1.93 ±0.05, 1.95 ±0.03, and 2.17 ±0.04 in the CEFFE+PRF, CEFFE-only, PRF-only, and control groups post-injection, respectively (p <0.05). As for SEI scores, the CEFFE+PRF, CEFFE-only, PRF-only groups demonstrated statistically significant differences pre- and post-injection (p <0.05). There was also a significant difference in SEI scores between the CEFFE +PRF and CEFFE-only group post-injection (p <0.05) (Figure 4A).

The mean epithelial thicknesses were 0.08 ±0.008, 0.09 ±0.012, 0.11 ±0.021, and 0.12 ±0.017 mm in the CEFFE +PRF, CEFFE-only, PRF-only, and control groups, respectively. There was significant difference among the CEFFE +PRF, CEFFE-only, PRF-only, and control groups in mean epithelial thickness post-injection (p <0.05). There was no significant difference between PRF-only and control groups in mean epithelial thickness post-injection (p >0.05) (Figure 4B).

The mean numbers of fibroblasts in the CEFFE+PRF, CEFFE-only, PRF-only, and control groups were 322 ±16.1, 601 ±17.3, 650 ±22.4, and 750.8 ±20.7, respectively. The difference among CEFFE+PRF, CEFFE-only, PRF-only, and control groups were statistically significant (p <0.05) (Figure 4C).

The mean numbers of capillaries in the CEFFE+PRF, CEFFE-only, PRF-only, and control groups were [4.5 (4.0. 5.0)], [7.5 (7.0, 8.0)], [8.0 (8.0, 9.0)], and [10.0 (9.0, 10.0)], respectively. There was significant difference among CEFFE +PRF, CEFFE-only, PRF-only, and control groups in the mean numbers of capillaries (p <0.05). There was no significant difference between CEFFE-only and PRF-only group in the mean numbers of capillaries (p >0.05) (Figure 4D).

The mean collagen organization scores of the CEFFE+PRF, CEFFE-only, PRF-only, and control groups were [2 (1,2)], [2 (2,3)], [3 (2,3)], and [3 (3,3)], respectively. There was significant difference among CEFFE+PRF, CEFFE-only, PRF-only, and control groups in the mean collagen organization scores (p <0.05). There was no significant difference between CEFFE-only and PRF-only group in the mean collagen organization scores (p >0.05) (Figure 4E).

Discussion

Hypertrophic scarring is a clinical challenge, characterized by a disorganized structure and abnormal biomechanical properties.[29] Various methods have been used to treat abnormal pathological scars. However, there is no specific treatment standard.[30] With the development of regenerative medicine, autologous fat and its components have played an important role in tissue regeneration and remodeling, providing new ideas for the treatment of scars.[14–17,31]

Clinical observations by Gentile[32] have shown that fat transplantation can soften and thin scars, thereby improving scar texture. Sultan et al[33] found through animal experiments that after fat transplantation, the levels of pro-angiogenic factors such as VEGF and stromal cell derived factor-1 in the recipient area increased, while the levels of pro-fibrotic

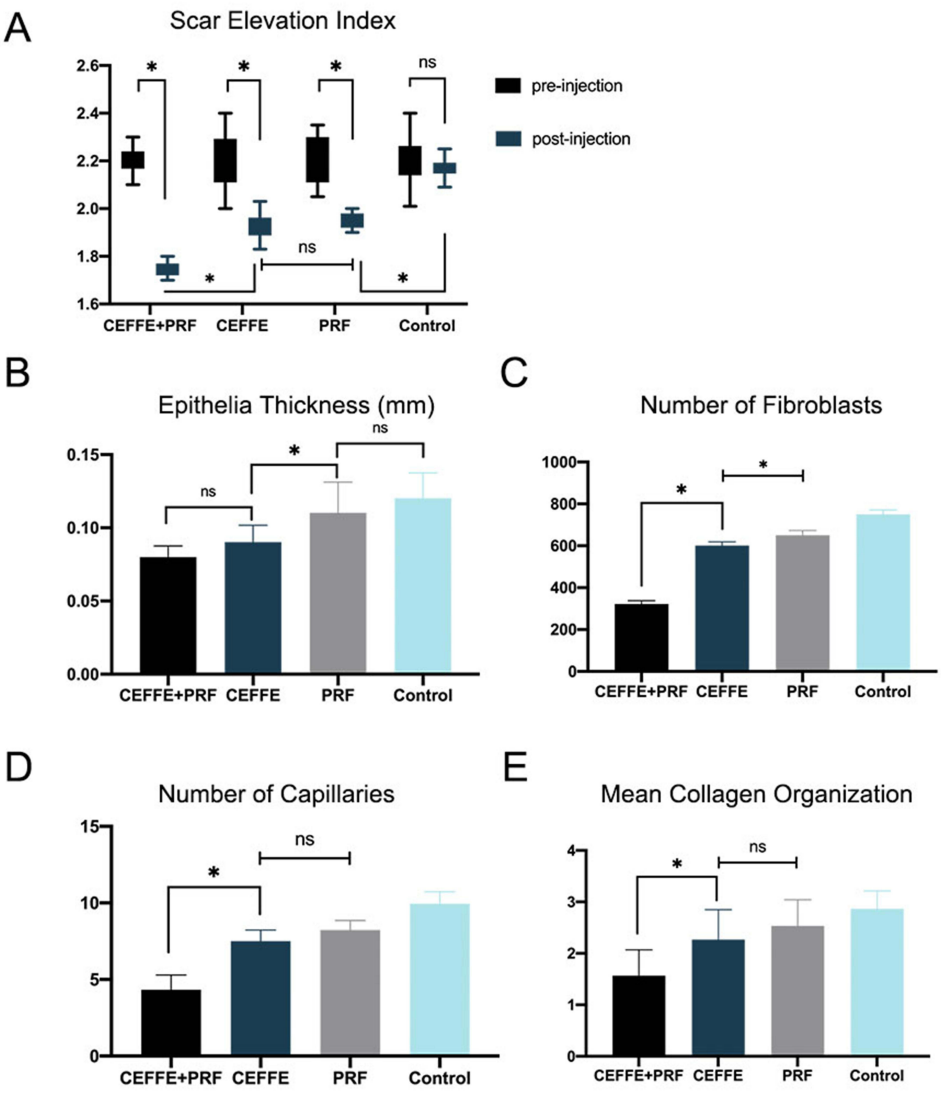


Figure 4 Summary of histopathological results. (**A**) Mean value of SEI score pre-injection and post-injection in each group. SEI index decreased after treatment, especially in the CEFFE+PRF group (SEI is defined as ratio of the vertical distance from the highest point of scar tissue to the ear cartilage surface and the vertical distance from the surrounding normal skin surface to the ear cartilage surface. (**B**) Mean value of epithelial thickness for each group in millimeters. (**C**) Mean number of fibroblasts in each group. (**D**) Mean number of capillary formations in each group. (**E**) Mean collagen organization score for each group. (ns, no significant difference. *p < 0.05.).

cytokines such as TGF-β1 decreased, thereby improving the appearance and texture of scars. These findings demonstrated the significant potential of fat transplantation in scar treatment. In recent years, cell therapies have been extended to the field of fat transplantation, including nano-fat[34] and stromal vascular fraction gel[35,36] transplantation, which mainly exert their effects through active adipose-derived stromal cells (ADSCs).

Currently, the mechanism by which ADSCs regulate fibrosis response in scar is still inconclusive. Yang et al[37] found through animal experiments that ADSCs exert regenerative effects through paracrine secretion and direct differentiation, with paracrine function being the main one. The paracrine products of ADSCs include various cytokines, such as VEGF, HGF, and so on, which can regulate cell apoptosis, angiogenic activity, and inflammatory response.[38] Zhang et al[39] injected rabbit ADSCs and their culture medium into the hypertrophic scars of rabbit ear wounds and found that the scar proliferation index decreased after 35 days of treatment through skin ultrasound monitoring. Moreover, the arrangement of dermal collagen was regular, and the expression of alpha smooth muscle actin and type I collagen decreased by histologically, indicating that ADSCs inhibited scar proliferation through paracrine inhibition. Uysal et al[40] found through animal experiments that injecting ADSCs locally around scars can downregulate the expression of pro fibrotic

markers α-SMA, TG-β 1, and type I collagen genes, upregulate the expression of anti-fibrotic fibroblast growth factor and VEGF genes, reduce the deposition of type I and III collagen and elastin fibers, improve collagen fiber arrangement, and prevent scar formation. Moreover, ADSCs can upregulate the expression of TGF-β 3, adjust the ratio of TGF-β1/TGF-β3, and enhance anti fibrotic effects.[41] In summary, ADSCs can regulate extracellular matrix deposition degradation, improve extracellular matrix structure, and inhibit scar hyperplasia. However, currently, the application of human ADSCs in scars remains challenging. First, ADSCs require in vitro enzymatic digestion, isolation, amplification, and culture, and cell safety still needs to be studied. Secondly, ADSCs have unlimited proliferation and multidirectional differentiation potential, and there may be a risk of teratogenicity or tumorigenesis after implantation into the human body. CEFFE, which is rich in cytokines and growth factors, can be harvested without in vitro culture, may regard as a preferred substitute for ADSCs treatment.[42,43]

Recently, CEFFE, which was purified from nano-fat by removing cellular components and lipid remnants, has gained an increasing amount of interest among researchers.[19] CEFFE comprises various growth factors that are similar to those secreted by ADSCs.[19] Several studies have investigated the effects of cell-free fat extracts on adipogenesis.[44,45] Lu et al[46] showed that fat extracts create a beneficial microenvironment for adipose tissue formation in the adipose tissue engineering compartment model. Furthermore, Cai et al[20] showed that adipose tissue extract had a remarkable improvement in scar appearance using a rabbit ear hypertrophic scar model, as evidenced by a reduction in the scar elevation index, more regular collagen fibers, a decrease in the Vancouver scar scale score and expression of α-Smooth muscle actin, and an increase in the expression of adipogenic markers PPARγ and C/EBPα.

Notably, adipocytes were found in the scar dermis of the CEFFE-only groups and combination groups in our study. It seemed the number of adipocytes in the scar can improve the scar appearance. The results are similar to those reported by Cai et al.[20] Therefore, we hypothesized that adipogenesis is one of the mechanisms involved in the treatment of hypertrophic scars. In our study, the CEFFE+PRF group obtained the lowest SEI score (1.75 ±0.03), indicating a less prominent superficial scar by injecting CEFFE combined with PRF. Besides, the CEFFE+PRF group showed less epithelial thickness (0.08 ±0.008), less fibroblasts cells (322 ±16.1), less capillaries [4.5 (4.0. 5.0)], and more regular collagen fibers [2 (1,2)] than other groups, suggesting that CEFFE combined with PRF may have a synergistic effect, which substantially improve the structure and appearance of scar tissue (Figure 4). This is possibly due to the specific three-dimensional structure of PRF. Unlike platelet-rich plasma, a first-generation platelet concentrate, PRF does not contain anticoagulants, which enables natural and progressive polymerization during centrifugation. This structure tremendously facilitates the incorporation of platelets, cytokines and circulating growth factors into the fibrin mesh. Therefore, PRF markedly prolongs the release time of cytokines and growth factors, which could have long lasting effects on adipogenesis by more gradually releasing these growth factors.[24]

Furthermore, Masson's trichrome staining revealed that the combination group showed more orderly and regular collagen arrangement, and the least collagen deposition in the CEFFE+PRF group (Figure 3). The mean collagen organization scores of the CEFFE+PRF group [2 (1,2)] is lower than the CEFFE-only [2 (2,3)], PRF-only [3 (2,3)], and control groups [3 (3,3)] ($p<0.05$), indicating more normal organized collagen structure (Figure 4E). These results suggest that the combination CEFFE and PRF treatment promotes collagen remodeling in scars.

In conclusion, the combination of CEFFE and PRF effectively improved the outcome of hypertrophic scars in our study. Possible mechanism in regulating scar formation may be the induction of adipogenesis and extracellular matrix remodeling. Further studies to explicate the potential molecular pathways are warranted.

Conclusion

CEFFE combined with PRF was the most effective treatment for the prevention of hypertrophic scar formation in our study. This study provides basic research data and a new adipogenic acellular therapy for the clinical management of CEFFE and PRF in the treatment of hypertrophic scars.

Declaration of Generative AI in Scientific Writing

No AI was used.

Research Ethics

This study was approved by the ethics committee of our hospital and the Experimental Animal Center of Fujian Medical University (IACUC-FMCHH-2024-002).

Funding

This work was supported by Startup Fund for scientific research, Fujian Medical University (Grant number:2021QH1184).

Disclosure

Zhenni Wei and Mingliang Zhang should be considered co–first authors. The author reports no conflicts of interest in this work.

References

1. Atiyeh BS. Nonsurgical management of hypertrophic scars: evidence-based therapies, standard practices, and emerging methods. *Aesthetic Plast Surg*. 2007;31:468–492. doi:10.1007/s00266-006-0253-y
2. Liu C, Tang L, Hou C, Zhang J, Li J. Intralesional axitinib injection mitigates hypertrophic scar by inhibiting angiogenesis pathway: a preliminary study in a rabbit ear model. *Clin Cosmet Invest Dermatol*. 2023;16:3023–3034. doi:10.2147/CCID.S430852
3. Lee HJ, Jang YJ. Recent understandings of biology, prophylaxis and treatment strategies for hypertrophic scars and keloids. *Int J Mol Sci*. 2018;19:1.
4. Ogawa R. The most current algorithms for the treatment and prevention of hypertrophic scars and keloids: a 2020 update of the algorithms published 10 years ago. *Plast Reconstr Surg*. 2022;149:79e–94e. doi:10.1097/PRS.0000000000008667
5. Anzarut A, Olson J, Singh P, et al. The effectiveness of pressure garment therapy for the prevention of abnormal scarring after burn injury: a meta-analysis. *J Plast Reconstr Aesthet Surg*. 2009;62:77–84. doi:10.1016/j.bjps.2007.10.052
6. O'Brien L, Jones DJ. Silicone gel sheeting for preventing and treating hypertrophic and keloid scars. *Cochrane Database Syst Rev*. 2013;2013:CD003826. doi:10.1002/14651858.CD003826.pub3
7. Beibei Y, Yating D, Yifeng S, et al. Bilayer dissolving microneedle array containing 5-fluorouracil and triamcinolone with biphasic release profile for hypertrophic scar therapy [J]. *Bioact Mater*. 2021;6:2400–2411. doi:10.1016/j.bioactmat.2021.01.014
8. Lecl_ere FM, Mordon SR. Twenty-five years of active laser prevention of scars: what have we learned? *J Cosmet Laser Ther*. 2010;12:227–234. doi:10.3109/14764172.2010.514923
9. Moon SJ, Byun IH, Chang JS, Song SY, Lee WJ, Lee DW. A prospective comparative study of radiotherapy effect upon scar quality. *J Plast Reconstr Aesthet Surg*. 2021;74:1801–1806. doi:10.1016/j.bjps.2020.11.044
10. Jalali M, Bayat A. Current use of steroids in management of abnormal raised skin scars. *Surgeon*. 2007;5:175–180. doi:10.1016/S1479-666X(07)80045-X
11. Naeini FF, Najafian J, Ahmadpour K. Bleomycin tattooing as a promising therapeutic modality in large keloids and hypertrophic scars. *Dermatol Surg*. 2006;32:1023–1030. doi:10.1111/j.1524-4725.2006.32225.x
12. Gauglitz GG, Korting HC, Pavicic T, Ruzicka T, Jeschke MG. Hypertrophic scarring and keloids: pathomechanisms and current and emerging treatment strategies. *Mol Med*. 2010;17:113–25.
13. Khansa I, Harrison B, Janis JE. Evidence-based scar management: how to improve results with technique and technology. *Plast Reconstr Surg*. 2016;138:165S–178S. doi:10.1097/PRS.0000000000002647
14. Al Qurashi AA, Siddiqi AK, Alghamdi AA, et al. Effectiveness of autologous fat transfer in the treatment of scar-related conditions: a systematic review and meta-analysis. *Aesthetic Plast Surg*. 2022;46:2564–2572. doi:10.1007/s00266-022-02869-9
15. Negenborn VL, Groen JW, Smit JM, Niessen FB, Mullender MG. The use of autologous fat grafting for treatment of scar tissue and scar-related conditions: a systematic review. *Plast Reconstr Surg*. 2016;137:31e–43e. doi:10.1097/PRS.0000000000001850
16. Xu X, Lai L, Zhang X, et al. Autologous chyle fat grafting for the treatment of hypertrophic scars and scar-related conditions. *Stem Cell Res Ther*. 2018;9:64. doi:10.1186/s13287-018-0782-8
17. Hoerst K, van den Broek L, Sachse C, et al. Regenerative potential of adipocytes in hypertrophic scars is mediated by myofibroblast reprogramming. *J Mol Med*. 2019;97:761–775. doi:10.1007/s00109-019-01772-2
18. Nie M, Tian Y, Xiao Y, Lei S, Wu D. Enhancing high-quality fat survival: a novel strategy using cell-free fat extract. *FASEB J*. 2024;38:e23733. doi:10.1096/fj.202400523RRRR
19. Yu Z, Cai Y, Deng M, et al. Fat extract promotes angiogenesis in a murine model of limb ischemia: a novel cell-free therapeutic strategy. *Stem Cell Res Ther*. 2018;9:294. doi:10.1186/s13287-018-1014-y
20. Cai Y, Tian J, Li J, et al. A novel combined technology for treating hypertrophic scars: adipose tissue extract combined with fractional CO2 laser. *Front Physiol*. 2023;14:1284312. doi:10.3389/fphys.2023.1284312
21. Dohan DM, Choukroun J, Diss A, et al. Platelet-rich fibrin (PRF): a second-generation platelet concentrate. Part I: technological concepts and evolution. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod*. 2006;101:e37–44. doi:10.1016/j.tripleo.2005.07.008
22. Dohan DM, Choukroun J, Diss A, et al. Platelet-rich fibrin (PRF): a second-generation platelet concentrate. Part II: platelet-related biologic features. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod*. 2006;101:e45–50. doi:10.1016/j.tripleo.2005.07.009
23. Jiménez-Aristizabal RF, López C, Álvarez ME, et al. Long-term cytokine and growth factor release from equine platelet-rich fibrin clots obtained with two different centrifugation protocols. *Cytokine*. 2017;97:149–155. doi:10.1016/j.cyto.2017.06.011
24. Xiong S, Qiu L, Su Y, Zheng H, Yi C. Platelet-rich plasma and platelet-rich fibrin enhance the outcomes of fat grafting: a comparative study. *Plast Reconstr Surg*. 2019;143:1201e–1212e. doi:10.1097/PRS.0000000000005624

25. Saulis AS, Mogford JH, Mustoe TA. Effect of mederma on hypertrophic scarring in the rabbit ear model. *Plastic Reconstr Surg*. 2002;110:177–183. doi:10.1097/00006534-200207000-00029
26. Wang HJ, Gao WC, Ma SL. Effect of Abnormal Savda Munziq on hypertrophic scar formation in a rabbit ear model. *Chin J Integr Med*. 2015;21:537–541. doi:10.1007/s11655-014-1622-4
27. Uzun H, Bitik O, Hekimoğlu R, Atilla P, Kaykçoğlu AU. Angiotensin-converting enzyme inhibitor enalapril reduces formation of hypertrophic scars in a rabbit ear wounding model. *Plast Reconstr Surg*. 2013;132:361e–371e. doi:10.1097/PRS.0b013e31829acf0a
28. Kurt M, Akoz Saydam F, Bozkurt M, Serin M, Caglar A. The effects of valsartan on scar maturation in an experimental rabbit ear wound model. *J Plast Surg Hand Surg*. 2020;54:382–387. doi:10.1080/2000656X.2020.1814312
29. Kim S, Choi TH, Liu W, Ogawa R, Suh JS, Mustoe TA. Update on scar management: guidelines for treating Asian patients. *Plast Reconstr Surg*. 2013;132:1580–1589. doi:10.1097/PRS.0b013e3182a8070c
30. Celeste C, Finnerty, Marc G, et al. Hypertrophic scarring: the greatest unmet challenge after burn injury. *Lancet*. 2016;388:1427–36.
31. Li Y, Zhang J, Shi J, et al. Exosomes derived from human adipose mesenchymal stem cells attenuate hypertrophic scar fibrosis by miR-192-5p/IL-17RA/Smad axis. *Stem Cell Res Ther*. 2021;12:221. doi:10.1186/s13287-021-02290-0
32. Gentile P, De angelis B, Pasin M, et al. Adipose-derived stromal vascular fraction cells and platelet-rich plasma: basic and clinical evaluation for cell-based therapies in patients with scars on the face. *J Craniofac Surg*. 2014;25:267–272. doi:10.1097/01.scs.0000436746.21031.ba
33. Sultan SM, Stern CS, Allen RJ, et al. Human fat grafting alleviates radiation skin damage in a murine model. *Plast Reconstr Surg*. 2011;128:363–372. doi:10.1097/PRS.0b013e31821e6e90
34. Tonnard P, Verpaele A, Peeters G, Hamdi M, Cornelissen M, Declercq H. Nanofat grafting: basic research and clinical applications. *Plast Reconstr Surg*. 2013;132:1017–1026. doi:10.1097/PRS.0b013e31829fe1b0
35. Wang J, Liao Y, Xia J, et al. Mechanical micronization of lipoaspirates for the treatment of hypertrophic scars. *Stem Cell Res Ther*. 2019;10:42. doi:10.1186/s13287-019-1140-1
36. Xiao S, Qi J, Li J, et al. Mechanical micronization of lipoaspirates combined with fractional CO2 laser for the treatment of hypertrophic scars. *Plast Reconstr Surg*. 2023;151:549–559. doi:10.1097/PRS.0000000000009915
37. Yang D, Wang W, Li L, et al. The relative contribution of paracine effect versus direct differentiation on adipose-derived stem cell transplantation mediated cardiac repair. *PLoS One*. 2013;8:e59020. doi:10.1371/journal.pone.0059020
38. Mirotsou M, Jayawardena TM, Schmeckpeper J, Gnecchi M, Dzau VJ. Paracrine mechanisms of stem cell reparative and regenerative actions in the heart. *J Mol Cell Cardiol*. 2011;50:280–289. doi:10.1016/j.yjmcc.2010.08.005
39. Zhang Q, Liu LN, Yong Q, Deng JC, Cao WG. Intralesional injection of adipose-derived stem cells reduces hypertrophic scarring in a rabbit ear model. *Stem Cell Res Ther*. 2015;6:145. doi:10.1186/s13287-015-0133-y
40. Uysal CA, Tobita M, Hyakusoku H, Mizuno H. The effect of bone-marrow-derived stem cells and adipose-derived stem cells on wound contraction and epithelization. *Adv Wound Care*. 2014;3:405–413. doi:10.1089/wound.2014.0539
41. Mattei A, Magalon J, Bertrand B, et al. Autologous adipose-derived stromal vascular fraction and scarred vocal folds: first clinical case report. *Stem Cell Res Ther*. 2018;9:202. doi:10.1186/s13287-018-0842-0
42. Czerwiec K, Zawrzykraj M, Deptuła M, et al. Adipose-derived mesenchymal stromal cells in basic research and clinical applications. *Int J Mol Sci*. 2023;24:3888. doi:10.3390/ijms24043888
43. Al-Ghadban S, Artiles M, Bunnell BA. adipose stem cells in regenerative medicine: looking forward. *Front Bioeng Biotechnol*. 2022;9:837464. doi:10.3389/fbioe.2021.837464
44. Sarkanen JR, Kaila V, Mannerström B, et al. Human adipose tissue extract induces angiogenesis and adipogenesis in vitro. *Tissue Eng Part A*. 2012;18:17–25. doi:10.1089/ten.tea.2010.0712
45. He Y, Xia J, Chen H, Wang L, Deng C, Lu F. Human adipose liquid extract induces angiogenesis and adipogenesis: a novel cell-free therapeutic agent. *Stem Cell Res Ther*. 2019;10:252. doi:10.1186/s13287-019-1356-0
46. Lu Z, Yuan Y, Gao J, Lu F. Adipose tissue extract promotes adipose tissue regeneration in an adipose tissue engineering chamber model. *Cell Tissue Res*. 2016;364:289–298. doi:10.1007/s00441-015-2322-5