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Efficacy of adipose stromal cells-enriched high-density fat graft combined with BTX-A for Raynaud's phenomenon: a prospective cohort study

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Abstract

Background Conventional treatments for Raynaud's phenomenon (RP) often show limited effectiveness due to their inability to address both vascular and inflammatory aspects. This study evaluates the combination of high-density fat grafting (HDFG) with botulinum toxin A (BTX-A) for treating RP.

Methods Eleven patients with 20 affected hands diagnosed with RP were recruited and randomly assigned to receive either HDFG combined with BTX-A (intervention group, $n=11$) or HDFG alone (control group, $n=9$). Efficacy was assessed using Visual Analog Scale (VAS) pain scores and McCabe Cold Sensitivity Scores, along with finger ulcer healing time and infrared thermal imaging to evaluate blood perfusion improvements.

Results The HDFG-BTX group showed significant improvements in hand symptoms. VAS pain scores decreased from a pre-treatment mean of 5.33 to 0.84 post-treatment (mean reduction of 4.49, $p=0.018$), indicating effective pain relief. McCabe scores improved from 272.73 to 75.00 (mean reduction of 197.73, $p=0.001$), demonstrating reduced cold sensitivity. Ulcer healing time was shorter in the HDFG-BTX group (14.25 days) compared to HDFG alone (25.6 days, $p<0.001$), highlighting faster recovery. Infrared imaging indicated significant enhancements in blood perfusion.

Conclusion HDFG combined with BTX-A is a reliable and beneficial intervention for RP, leading to high patient satisfaction.

Key message

• Combining high-density fat grafting with botulinum toxin A significantly improves symptoms in Raynaud's phenomenon.
• This novel therapy enhances pain relief, blood flow, and ulcer healing, demonstrating strong patient satisfaction.

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Keywords Raynaud's phenomenon, Fat grafting, Botulinum toxins type A, Adipose-derived stromal cells

Introduction

Raynaud's phenomenon (RP) is characterized by recurrent spasms in peripheral blood vessels, causing significant cold sensation and a distinctive "three-phase color" change in the affected fingers [1]. Predominantly affecting young and middle-aged women, RP has an annual incidence approaching 5%, significantly impacting patients' quality of life due to pain, ulceration, and severe ischemia, which can lead to bone exposure and amputation [2–4]. Current treatments aim to prevent and reduce attacks through lifestyle modifications and pharmacological interventions, such as calcium channel blockers and endothelin receptor blockers [5, 6]. However, these treatments often have limited efficacy and can result in severe adverse reactions like ulcers and gangrene [7]. Surgical treatments for RP are invasive, costly, and often have high recurrence rates, thus are mainly used for severe cases, particularly with complications like Sjögren's syndrome, digital ulcers, or necrosis [8]. These challenges highlight the urgent need for more effective and less invasive treatment options.

Autologous fat grafting (AFG) has gained traction in plastic surgery for its dual role as a filler and its ability to promote angiogenesis and wound healing [9–11]. AFG has shown promise for RP, despite low stromal cell concentrations in non-centrifuged fat [12, 13]. Research indicates that stromal cells play a significant role in pain relief and ulcer healing in RP [14, 15]. High-density fat grafting (HDFG) enhances AFG by incorporating a higher concentration of stromal cells, potentially leading to better therapeutic outcomes [16]. Botulinum toxin type A (BTX-A) is popular for its ability to inhibit neurotransmitter release, performing chemical denervation [17]. BTX-A-assisted fat transplantation improves fat graft retention by reducing muscle movement, enhancing angiogenesis [18, 19]. Clinical studies have shown BTX-A's feasibility in treating RP, though some side effects have been noted [20–22].

Preliminary studies indicate that HDFG enhances vascularization and modulates inflammation in ischemic tissues, while BTX-A improves fat graft survival and has muscle-relaxing effects [23]. Both have anti-inflammatory properties via different mechanisms. The proposed combination of HDFG's regenerative and immunomodulatory effects with BTX-A's benefits may synergistically enhance outcomes. This study hypothesizes that combining these therapies will provide superior pain relief, improved blood flow, and faster ulcer healing in RP patients compared to individual treatments.

Methods

Patient selection and study design

This prospective cohort study aimed to evaluate the efficacy of HDFG combined with BTX-A injection in patients with RP. Prior to inclusion in this study, all patients had undergone standard treatments, which included phosphodiesterase 5 (PDE5) inhibitors and other conventional therapies, under the supervision of rheumatologists. These patients were selected for the experimental procedure only after it was determined that they had not responded to these standard treatments, making them suitable candidates for the new intervention. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013) and was approved by the Clinical Research Ethics Committee of the Affiliated Hospital of Zunyi Medical University (No.20210202). All patients gave written informed consent, and the authors were allowed to use their case details and photographs for scientific purposes. Clinical trial registration number: Chinese Clinical Trial Registry (https://www.chictr.org.cn/) ChiCTR2200057580.

Eleven patients (5 men and 6 women) were enrolled between April 2022 and May 2023. Inclusion criteria contained (1) all patients showed typical symptomatic RP with symptoms lasting for at least 3 months; (2) the age of the patients was 40 to 80 years and the BMI was 18.5 to 23.9 kg/m^2. Exclusion criteria included (1) patients who had whole finger necrosis requiring amputation; (2) patients with a history of pharmacological or surgical interventions in the past 1 month; (3) patients with severe cardiopulmonary involvement who could not tolerate the operation. Among the 11 patients included, one had primary RP, and ten had secondary RP (four associated with scleroderma, one with mixed connective tissue disease, and five secondary to other underlying diseases). Five patients had ulcers in both hands, four had ulcers in the right hand, and two had no ulcers.

Patients were prospectively followed after enrollment. The intervention group received HDFG combined with BTX-A ($n = 11$), while the control group received HDFG alone ($n = 9$). Randomization was done using a computer-generated table. Each hand received 35 mL of high-density fat graft and 100 units of BTX-A (Fig. 1). Data was collected from 20 hands in 11 patients via a questionnaire survey conducted by independent plastic surgeons. Two patients had one hand treated, and nine had both hands treated, with no repeated interventions. Analyses treated each hand as independent. Reductions in Visual Analog Scale (VAS) and McCabe cold sensitivity scores pre- and post-operation showed no significant difference between one-hand and two-hand treatments (VAS: $p = 0.327$, Z =

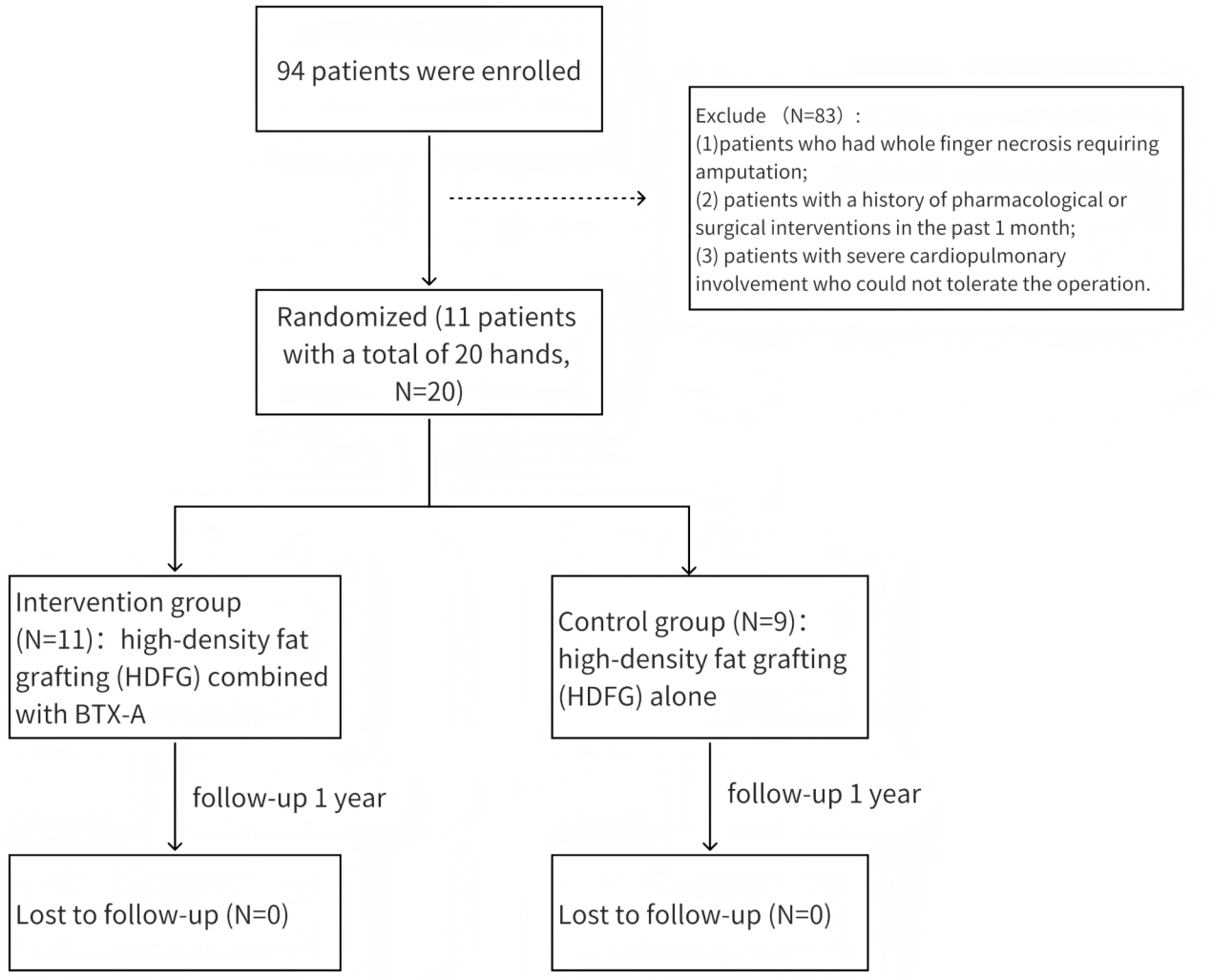

Fig. 1 Flow chart of this prospective cohort study

-1.063; McCabe: $p = 1.000$, $Z = 0.000$), indicating similar effectiveness regardless of the number of hands treated. Patients were assessed at baseline and followed for one year to monitor outcomes and adverse effects.

Preparation of high-density fat and postoperative management

High-density fat was prepared using the Coleman technique and centrifugation, as previously described, with the patient under anesthesia [24]. Briefly, adipose tissue was harvested from the lower abdomen after infiltration with 1000 mL of Ringer's solution containing 15 mL of 5% xylocaine and adrenaline 1/100,000. The harvested fat tissue was centrifuged at 1200 g for 3 min, and high-density fat was obtained by introducing a glass float labeled (DGA/FLOAT, British Ray-Ran Testing Equipment Co., Ltd., UK) with a density of 0.935 g/ml during centrifugation. High-density fat was defined as > 0.935 g/ml (Fig. 2a).

A stab incision was made using an 18-gauge needle, and then fat grafting was performed using 1-mL syringes connected with a blunt 1.2-mm diameter cannula. High density fat was diffusely distributed and injected into several planes (palmar, dorsal, and metacarpal spaces): 4 mL along the superficial palmar arch, 2 mL in the volar webspaces, 3 mL in the first webspace, 10 mL in the dorsum of the hand, 2 mL in each dorsal webspace, and 3 mL in the snuffbox (Supplementary Video 1). Simultaneously, 100 units of BTX-A mixed with 5 mL of normal saline were injected [22, 25]. The neurovascular bundles at the base of all five fingers were injected with equal doses of BTX-A (Fig. 2b). At the end of the liposuction, the incisions were sutured with 5 – 0 nylon thread, and sterile gauze was applied. Subsequently, the patient was instructed to wear compression garments regularly to

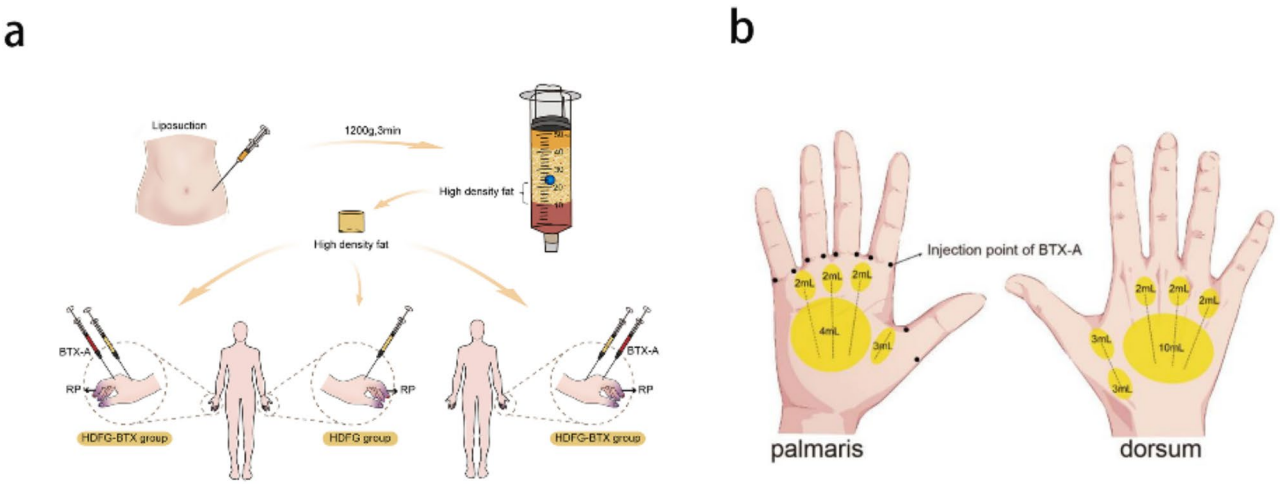

Fig. 2 (**a**) Schematic illustration of the preparation of high density fat and the combination therapy of HDFG with botulinum toxin A injection (BTX) for RP (**b**) HDFG combined with BTX-A hand injection

reduce discomfort and swelling. The affected hand was sterilely covered with gauze and bandaged, with reduced activity within one week. We adopted the approach of fat grafting along with injecting BTX-A based on existing literature that demonstrates the effectiveness of this strategy in improving fat retention and patient outcomes. Studies indicate that BTX-A enhances fat graft retention through mechanisms such as promoting angiogenesis and reducing muscle movement [19, 26–28]. This combined effect can lead to better long-term results in managing the symptoms of autoimmune hand diseases.

Isolation, culture, and flow cytometry assay of adipose-derived stem cells (ADSCs)

Ten milliliters of high-density adipose tissue were digested with type I collagenase (0.75 mg/mL, Solarbio, Beijing, China) in phosphate-buffered saline at 37℃. Subsequently, the mixtures were filtered, centrifuged, and resuspended in Dulbecco's modified Eagle's medium (Gibco, Thermo Fisher Scientific Inc) containing 10% fetal bovine serum (Gibco). The stromal vascular fraction cells were cultured at 37℃ in 5% CO_2 in DMEM supplemented with 10% FBS and 1% penicillin-streptomycin (Gibco). ADSCs were subcultured at 80% confluence, and passage-3 cells (1×10^6) were incubated with fluorescence-conjugated antibodies (CD105-PE, CD90-FITC, CD73-APC, CD45-PE, CD19-PE, CD34-PE, CD11b-PE, HLA-DR-PE) (1 mg/mL; Abcam, Cambridge, UK) and analyzed by a flow cytometer (BD FACSAria™ III system; BD Pharmingen).

Outcome assessment

The McCabe cold sensitivity score was employed to assess finger sensitivity to cold before and after treatment, according to previous reports [29]. The VAS pain score was utilized to gauge the extent of finger pain relief before and after treatment. Infrared thermal imaging was used to observe changes in finger temperature before and after the treatment. The percentage of blood perfusion units in the hands was then calculated using Image J software (Version 1.54j). In addition, the healing time of finger ulcers was recorded.

Statistical analysis

Data are reported as means with standard deviations ($M \pm SD$) for continuous variables and as frequencies with percentages (n, %) for categorical variables. Statistical analysis was conducted using SPSS Statistics for Windows, version 26.0 (IBM Corp., Armonk, NY). For within-group comparisons of non-normally distributed data, the Wilcoxon signed-rank test assessed pre-treatment versus post-treatment values. Between-group comparisons used the Mann-Whitney U test for changes between treatment groups. To evaluate the impact of individual and combined factors on post-treatment outcomes, multiple linear regression analysis included variables such as treatment group, age, gender, ulcer presence, and number of pre-operative ulcers. Additionally, one-way ANCOVA adjusted for potential confounding covariates to verify multiple linear regression results, with differences between pre- and post-operative outcomes as dependent variables and treatment group as the independent variable. The repeated measures ANOVA test evaluated changes over time (pre-operation, 7 days, 14 days, 1 month, 3 months, and 12 months) in VAS pain scores and McCabe Cold Sensitivity Scores within each group and assessed the interaction between treatment and time.

Results

Demographics data of patients

The average age of patients in the intervention and control groups was 57.82 ± 11.29 years and 60.56 ± 10.56 years, respectively, with no significant difference between the two groups ($p = 0.541$). The average BMI of all patients was 22.5 kg/m^2, ranging from 18.5 to 23.9, with no significant differences observed between the groups ($p > 0.05$). Regarding RP types, one patient (9.1%) had primary RP, while ten patients (90.90%) had secondary RP: four with scleroderma (36.4%), one with mixed connective tissue disease (9.10%), and five with other diseases (45.50%). Ulcer distribution among patients was as follows: five had ulcers in both hands (45.5%), four had ulcers only in the right hand (36.4%), and two had no ulcers (18.20%). The average duration of ulcers was 17.33 ± 21.25 weeks. Post-surgery, slight bruising and swelling were observed in the first week, but no severe discomfort or complications occurred during the follow-up period. Additional characteristics of the patient groups are detailed in Table 1.

VAS pain score

In the HDFG-BTX group, preoperative scores ranged from 2.2 to 9 (average 5.33 ± 2.04), decreasing postoperatively to 0.2 to 1.4 (average 0.84 ± 0.82). This resulted in an average reduction of 4.49 ± 1.41 (Wilcoxon signed-rank test, Z = -2.67, $p = 0.008$), an 84.2% pain reduction ($p = 0.018$). The HDFG group showed preoperative scores from 2 to 7 (average 4.27 ± 1.53), dropping to 0.4 to 3.0 postoperatively (average 1.57 ± 0.92). This was a reduction of 2.70 ± 1.41, representing a 63.2% pain decrease (Z = -2.94, $p = 0.003$). The greater pain reduction in the HDFG-BTX group was statistically significant compared to the HDFG group (Mann-Whitney U test, Z = -2.36, $p = 0.018$). These findings are detailed in Table S1 and shown in Fig. 3a-b.

A repeated measures ANOVA assessed treatment effects over time at five points (7 days, 14 days, 1 month, 3 months, 12 months). There was a significant main effect of time (F = 67.898, $p < 0.001$) and a significant interaction between time and treatment (F = 5.964, $p < 0.001$). Post-hoc Bonferroni analyses highlighted significant differences in VAS pain score reductions at specific times within each group. At 3 and 12 months, the VAS score reduction in the HDFG-BTX group was significantly greater than in the HDFG group ($p = 0.025$ and $p = 0.011$, respectively) (Fig. 4a-c).

McCabe cold sensitivity scores

After the treatment, all patients reported significant relief in the symptom of finger sensitivity to cold. In the HDFG-BTX group, the mean McCabe cold sensitivity score prior to treatment was 272.73 ± 75.38. Following treatment, this mean score decreased significantly to 75.00 ± 48.73. This change reflects a substantial mean reduction of 197.73 ± 49.31, equating to a 72.5% decrease in cold sensitivity (Z = -2.70, $p = 0.003$). Similarly, the average McCabe cold sensitivity score in the HDFG group was 258.33 ± 51.54 before treatment. After treatment, this average decreased to 136.11 ± 50.17, indicating a significant reduction in cold sensitivity. The average decrease of 122.22 ± 51.54 corresponds to a 47.3% reduction in sensitivity (Z = -2.95, $p = 0.007$) (Fig. 3c). Comparatively, the reduction in McCabe cold sensitivity scores was greater in the HDFG-BTX group than in the HDFG group (Fig. 3d), with this difference being statistically significant (Z = -3.31, $p = 0.001$). This significant difference highlights the superior efficacy of the combined HDFG and BTX-A treatment in alleviating cold sensitivity symptoms in patients with RP, as evidenced by a greater reduction in McCabe cold sensitivity scores compared to HDFG alone.

To further evaluate treatment effects over time, a repeated measures ANOVA was conducted to analyze the reduction in McCabe cold sensitivity scores across five time points (7 days, 14 days, 1 month, 3 months, 12

Table 1 Demographics data of patients

Patient	Gender	Age (years)	Diagnosis	Ulcers (pre-operation)	Duration of Ulcers (week)	Prior Intervention
1	M	61	SRP	None	None	None
2	F	43	Scleroderma	R	52	Botox, sympathectomy
3	M	54	SRP	BH	9	calcium channel inhibitors
4	M	63	Scleroderma	BH	56	Botox, sympathectomy
5	F	46	MCTD	R	13	calcium channel inhibitors
6	F	54	Scleroderma	R	1	None
7	F	48	Scleroderma	R	8	Botox
8	F	78	SRP	BH	1	None
9	M	50	PRP	BH	4	None
10	M	72	SRP	BH	12	calcium channel inhibitors
11	F	67	SRP	None	None	None

Note: PRP, primary Raynaud's Phenomenon; SRP, secondary Raynaud's Phenomenon; MCTD, mixed connective tissue disorder; R, right hand; BH, both hands. None indicates that the patient did not receive the specific treatment listed, but it does not imply that the patient was treatment-naive. All patients had previously received standard treatments but did not achieve satisfactory results before being considered for the experimental procedure

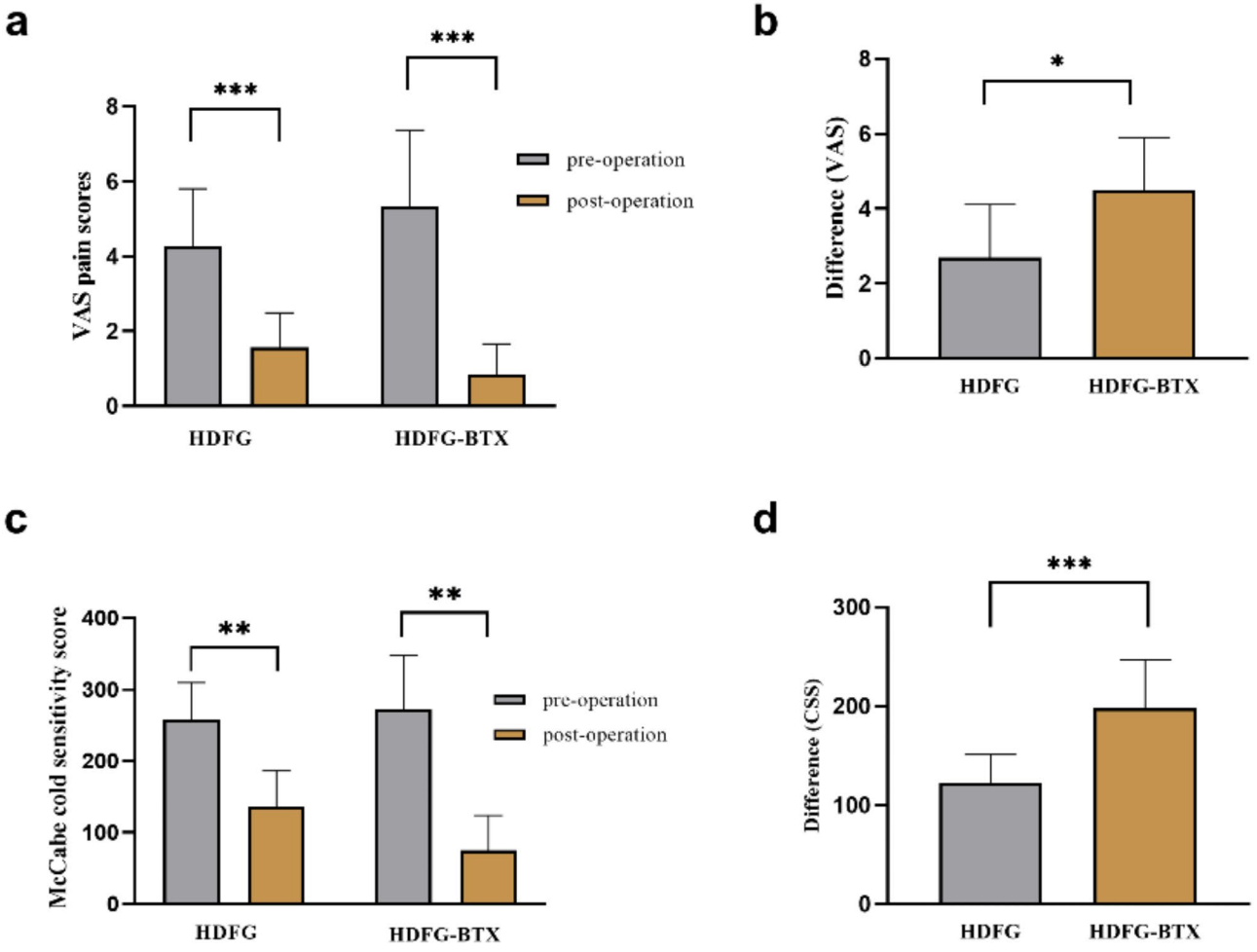

Fig. 3 (**a**) and (**c**) Comparison of VAS pain scores and McCabe cold sensitivity scores for HDFG-BTX and HDFG treatments; (**b**) and (**d**) Reduction in VAS pain scores and McCabe cold sensitivity scores between two groups. Note: *** means $p < 0.001$, ** means $p < 0.01$* means $p < 0.05$

months). The analysis demonstrated a significant main effect of time ($F = 131.78$, $p < 0.001$) and a significant interaction between time and treatment ($F = 12.50$, $p < 0.001$). Post-hoc analyses with the Bonferroni method revealed notable differences in McCabe cold sensitivity scores reduction at specific time points within each group. Notably, at 3 months and 12 months, the reduction in scores for the HDFG-BTX group was significantly greater than that for the HDFG group ($p = 0.001$ for both groups) as shown in Fig. 4e-f.

Fingertip ulcer healing time

A total of 14 out of 20 hands (70%) had ulcers before treatment, and among them, 13 hands healed after treatment. One patient underwent distal finger amputation 2 weeks after the operation due to pre-existing fingertip necrosis. All ulcers were completely healed within one month after the operation and did not recur during the 1-year follow-up period. Figure 5a presents representative images depicting ulcer healing before and after treatment. Follow-up results revealed that hand ulcers in the HDFG-BTX group exhibited shorter healing times compared with the HDFG group (Fig. 5b). The ulcer healing time was 14.25 ± 2.49 days for the HDFG-BTX group and 25.6 ± 4.34 days for the HDFG group. This difference was statistically significant (Z = -2.93, $p = 0.002$) as determined by the Mann-Whitney U test, suggesting that HDFG-BTX was more effective than HDFG in promoting ulcer healing.

Blood supply of the hands

Interestingly, following treatment with HDFG-BTX, significant improvements were reported not only in pain relief and sensitivity to cold but also in hand skin color, soft tissue texture, elasticity (Supplementary Video 2). Figure 5c illustrates representative images of the hand's appearance before and after treatment. Infrared thermal imaging was utilized to assess blood flow in the hand, revealing a significant improvement in blood supply after treatment with HDFG-BTX. Figure 5d presents a

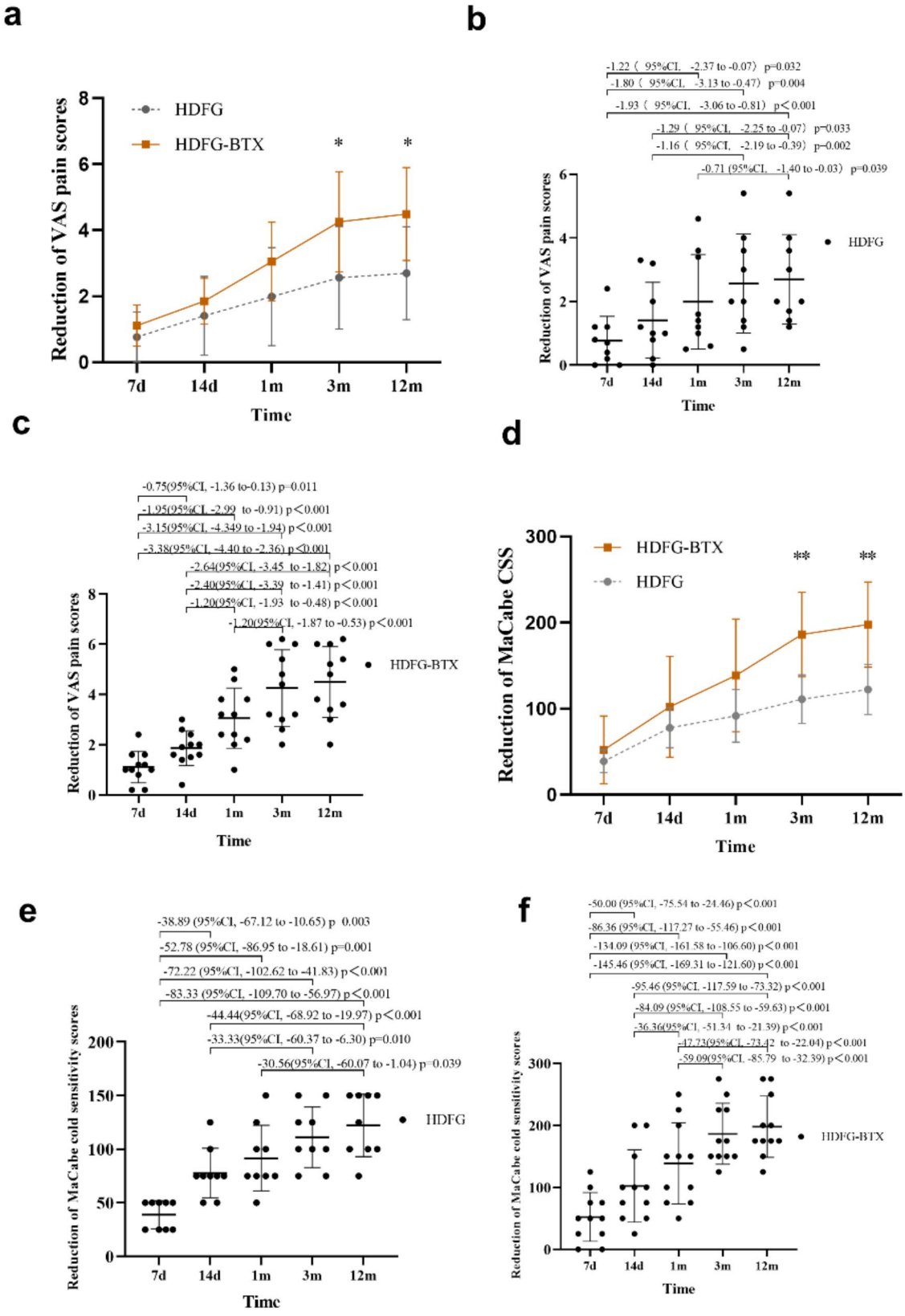

Fig. 4 (**a**) Reduction in VAS Pain Scores over time for HDFG and HDFG-BTX groups; Post-hoc comparisons of VAS pain score reductions in the (**b**) HDFG group and (**c**) HDFG-BTX group; (**d**) Reduction in McCabe cold sensitivity scores over time for HDFG and HDFG-BTX groups; Post-hoc comparisons of McCabe cold sensitivity scores reductions in the (**e**) HDFG group and (**f**) HDFG-BTX group. Note: * means $p < 0.05$, ** means $p < 0.01$

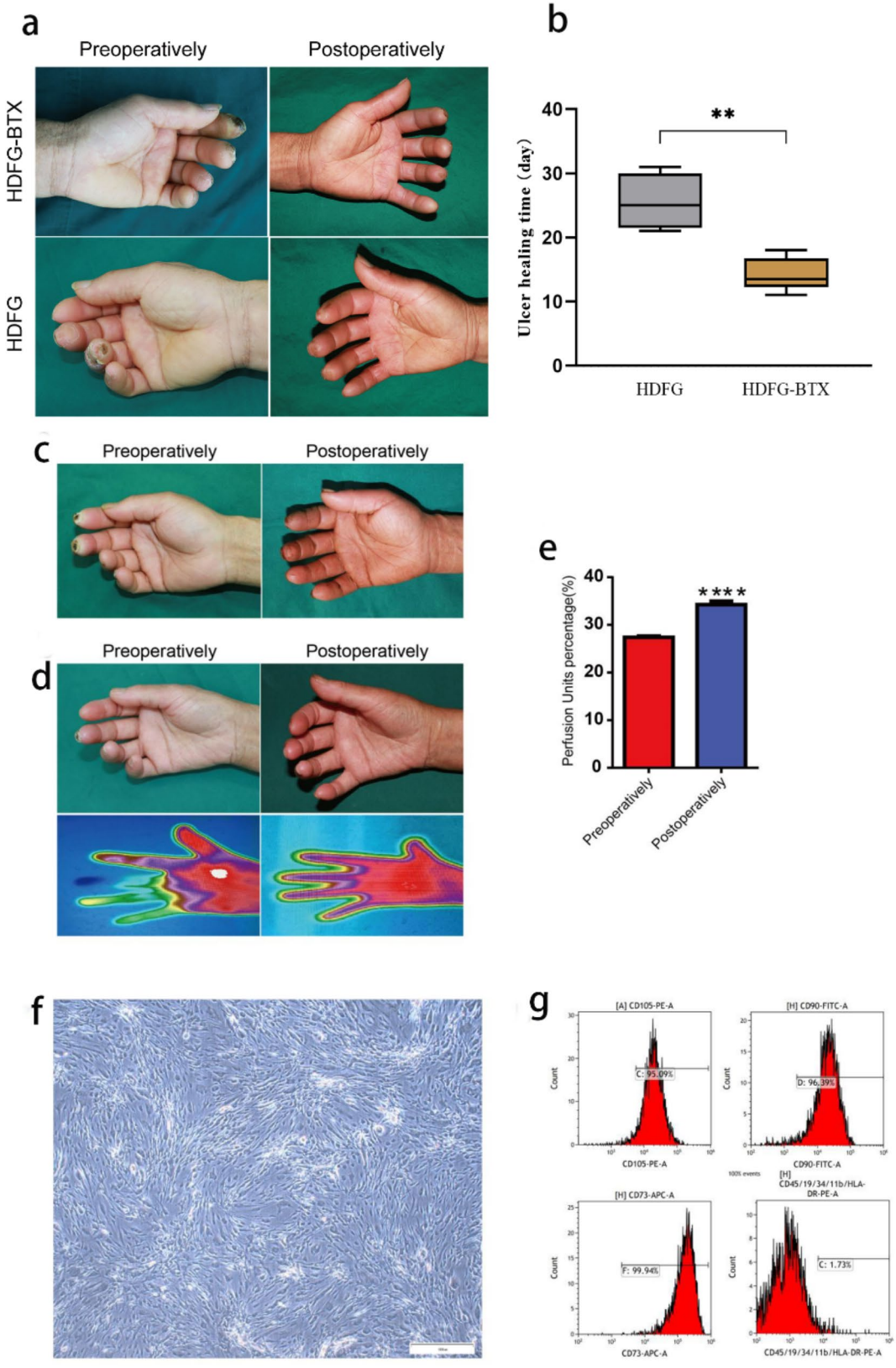

Fig. 5 (See legend on next page.)

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Fig. 5 (**a**) Representative images depicting ulcer healing before and after treatment; (**b**) Ulcer healing time comparison between HDFG and HDFG-BTX groups; Hand images and the results of infrared thermal imaging: (**c**) The representative images of the appearance of the hand before and after treatment one year. The fingertip ulcers on the index and middle fingers healed 16 days after surgery and had not recurred after 1 year of follow-up; (**d**) A representative image of the hand's blood supply before and after treatment. There was evident insufficiency of blood supply before surgery, particularly in the fingers with ulcers. One year post-surgery, the blood supply to the fingers was essentially restored; (**e**) There were significant differences in the percentage of blood perfusion units in the hands of HDFG-BTX group before and after treatment; Identification of Adipose-derived stromal cells: (**f**) Adipose-derived stromal cells from patients with RP (ADSCs-RP) subcutaneous adipose tissue displayed mesenchymal stem cell characteristics. Phase-contrast micrograph of the ADSCs-RP demonstrated fibroblast morphology. Magnification: 40×; (**g**) Flow cytometric analysis of ADSCs-RP

representative image of the hand's blood supply before and after treatment. Notably, evident insufficiency of blood supply was observed before surgery, particularly in the fingers with ulcers. However, one year post-surgery, the blood supply to the fingers was essentially restored. Significant differences in the percentage of blood perfusion units in the hands of the HDFG-BTX group before and after treatment are shown in Fig. 5e.

Flow cytometry analysis of ADSCs-RP

We measured the number of SVF cells in high-density fat tissue. The number of isolated SVF cells per milliliter of fat tissue was $3.87 \pm 1.63 \times 10^5$, ranging from 1.67 to 6.82×10^5 cells. Flow cytometry analysis demonstrated that the surface markers of ADSCs were highly positive in ADSCs-RP, including CD105 (PE 95.09%), CD90 (FITC 96.39%), CD73 (APC 99.94%), while surface markers, such as CD45/CD19/CD34/CD11b/HLA-DR were negative (PE 1.73%) (Fig. 5f-g). The results suggested that typical stromal stem cells, which have a powerful therapeutic effect, can still be found in autologous high-density fat from patients with RP.

Multiple linear regression and one-way ANCOVA analysis for evaluation of treatment

To assess the impact of individual and combined factors on post-treatment outcomes, multiple linear regression analysis was conducted. The variables included in the model were treatment group, age, gender, and ulcer presence and its number pre-operation. The regression model revealed that the treatment group was a significant predictor of the reduction in VAS pain scores ($R^2 = 0.49$, $p<0.01$) and McCabe cold sensitivity scores ($R^2 = 0.63$, $p<0.001$), with the HDFG-BTX group showing significantly better outcomes compared with the HDFG group. Specifically, the HDFG-BTX treatment resulted in greater reductions in pain and cold sensitivity scores, indicating a superior therapeutic effect. In contrast, age, gender, ulcer presence and number of ulcers were not significant predictors in the model, suggesting that these factors did not significantly influence the post-treatment outcomes within the context of this study. These findings reinforce the effectiveness of the HDFG-BTX combination therapy in enhancing clinical outcomes for patients with Raynaud's phenomenon, positioning it as a potentially superior treatment modality.

To further validate these results, we conducted a one-way ANCOVA to control for potential confounding variables. This analysis confirmed that the treatment group was the sole significant factor affecting difference in post-treatment outcomes, with a significant effect observed ($p = 0.006$, $F = 10.244$). This underscores the enhanced efficacy of HDFG-BTX therapy in reducing VAS pain and McCabe cold sensitivity scores compared with HDFG alone. Importantly, the adjustment for covariates did not alter the significance of the treatment group's impact, reinforcing the robustness of the observed therapeutic benefits.

Discussion

Traditional treatments for RP often have side effects and may not always provide satisfactory outcomes [30, 31]. This study explored a new therapeutic strategy by integrating HDFG with BTX-A injection for RP treatment. The HDFG-BTX group showed a significantly greater reduction in VAS pain scores (4.49 ± 1.41 vs. 2.70 ± 1.41; $p = 0.018$) and McCabe cold sensitivity scores (197.73 ± 49.31 vs. 122.2 ± 29.17; $p = 0.001$) compared with the HDFG group. Ulcer healing time was shorter in the HDFG-BTX group (14.25 ± 2.49 days vs. 25.6 ± 4.34 days; $p < 0.001$). Infrared thermal imaging demonstrated significant improvements in blood flow post-treatment. Multiple linear regression analysis confirmed the HDFG-BTX group as a significant predictor of reduced VAS pain scores ($R^2 = 0.49$, $p < 0.01$) and McCabe cold sensitivity scores ($R^2 = 0.63$, $p < 0.001$), while age, gender, ulcer presence and number of ulcers were not significant predictors.

Our findings align with studies demonstrating the benefits of AFG in treating various conditions. Abergel et al. [32] reported that AFG improves hand rejuvenation, skin texture, and elasticity, consistent with our observed enhancement in skin quality and blood perfusion. Another study [12] showed AFG effectively treated RP in patients unresponsive to medical management, similar to our results with HDFG and BTX-A. AFG also effectively treated skin complications and digital ulcers in RP patients with systemic sclerosis [33]. However, conflicting results exist, such as a case where fat injection did not prevent necrosis in a patient with 10-digit ischemia due to scleroderma [34].

The discrepancy in results highlights the need for further exploration of fat grafting mechanisms. Fat grafting transfers a ADSCs from donor tissue to the recipient area, promoting vascularization and anti-inflammatory responses mainly through paracrine signaling [35, 36]. However, low ADSC concentration in conventional fat may limit its effectiveness in treating RP. Enhancing ADSC concentration can be achieved through centrifugation, which increases adipose tissue density and functional cell content [37–39]. High-density fat, richer in SVF, enhances the graft's regenerative potential. Our study used high-density fat, maintaining SVF content and stem cell properties, consistent with previous findings [24]. ADSCs secrete growth factors like VEGF and FGF, crucial for angiogenesis and blood flow enhancement, and anti-inflammatory cytokines such as IL-10 and TGF-β, which reduce inflammation and support tissue repair. These ADSC properties explain the superior outcomes of high-density fat grafting in terms of pain relief, improved blood flow, and accelerated ulcer healing in Raynaud's phenomenon patients.

BTX-A has proven effective in treating various conditions such as strabismus, hyperhidrosis, neuropathic pain, finger muscle spasticity, facial wrinkles, facial rejuvenation, and hair restoration, while also improving the symptoms of RP [40]. However, there is limited clarity on how BTX-A can improve the vasospasm and pain symptoms of RP. The current study categorizes this into three aspects. First, the mechanism by which BTX-A improved RP may be related to the blockage of the release of neurotransmitter such as norepinephrine by cleaving SNAP-25 in sympathetic neuron [41]. This suggests that BTX-A could inhibit vasoconstriction and increase blood perfusion by blocking the cholinergic system. Second, BTX-A is able to inhibit the release of calcitonin gene-related peptide and substance P, thus improving neuropathic pain symptoms [42]. Third, BTX-A may promote vascular regeneration by regulating angiogenesis regulator genes, thereby improving the blood supply to the fingers of Raynaud patients [43].

The combination of HDFG with BTX-A proves superior to HDFG alone in the treatment of RP, suggesting a potential synergistic effect between the two. It has been reported that the retention rate of AFG can be improved by combining with botulinum toxin injection, and the mechanism involves the augmentation of angiogenesis and adipogenesis [23, 44]. We hypothesize two causes for this synergistic effect. First, both HDFG and BTX-A can be utilized in treating RP. Second, BTX-A injection enhances the retention rate of fat grafting, thereby improving the therapeutic effect of HDFG. Nevertheless, further studies are required to explore the underlying mechanisms.

This study is the first to report using HDFG combined with BTX-A injection for treating RP. Patient feedback was highly positive, with 90.9% (10 out of 11) recommending the treatment. This high satisfaction suggests the therapy is well-received, and its single-session approach enhances convenience. However, limitations include a small sample size and a short follow-up period, which may not fully capture long-term efficacy and safety. Future research should involve multicenter trials with larger samples and longer follow-ups. Additionally, the study assumed interventions on each hand were independent, with no significant outcome differences between one-hand and two-hand treatments (VAS: $p = 0.327$; McCabe: $p = 1.000$). However, the small number of one-hand cases ($n = 2$) limits the robustness of this finding. Further investigation is needed to confirm these preliminary observations and explore the impact of treating one or both hands.

Conclusion

In summary, we introduced a novel therapeutic method to treat RP-HDFG combined with BTX-A injection. This method not only significantly improved the clinical symptoms of RP but also promoted the rejuvenation of the skin of the hand. The mechanism may be related to the immune regulation and angiogenic effect of ADSCs and the anti-vasospasm effect of the botulinum toxin, which have a synergistic effect. Overall, this method seems to be a safe and beneficial intervention for RP. Future research should focus on conducting randomized controlled trials (RCTs) to further evaluate the mechanisms underlying the combined therapy. Such studies would provide more definitive evidence regarding the efficacy and safety of this innovative treatment, as well as insights into its effects and potential applications in broader clinical contexts.

Abbreviations

ADSCs	Adipose-derived stem cells
AFG	Autologous fat grafting
BTX-A	Botulinum toxin A
HDFG	High-density fat grafting
PDE5	Phosphodiesterase 5
RP	Raynaud's phenomenon
VAS	Visual Analog Scale

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13075-025-03533-8.

Supplementary Material 1: Supplementary Table S1. Detailed results of patients

Supplementary Material 2: Supplementary Video 1. High density fat were diffusely distributed and injected into several planes

Supplementary Material 3: Supplementary Video 2. After treatment with HDFG-BTX, the skin color, soft tissue texture, and elasticity of the hand were also significantly improved

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Author contributions
Conception and design: YZ, PM; Analysis and interpretation: CD, MW, BW, TZ, SX; Data collection: CD, XL, MW; Writing the article: CD, XL; Critical revision of the article: MW, BW, TZ, SX, PM, YZ; Statistical analysis: CD, YZ; Obtained funding: CDOverall responsibility: YZ. All authors reviewed the manuscript.

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Data availability
No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
The study was conducted in compliance with all relevant national regulations and institutional policies and was approved by the Clinical Research Ethics Committee of the Affiliated Hospital of Zunyi Medical University (No.20210202). Furthermore, informed consent forms were signed by all participants to ensure their voluntary participation in the study.

Consent for publication
Written informed consent for publication of their clinical details and/or clinical images was obtained from the patient.

Competing interests
The authors declare no competing interests.

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