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Clinical, Histologic, and Transcriptomic Evaluation of Sequential Fat Grafting for Morphea
A Nonrandomized Controlled Trial

Juzi Liu, MD; Jing Wang, MD, PhD; Qian Zhang, MD; Feng Lu, MD, PhD; Junrong Cai, MD

IMPORTANCE Morphea is a rare disease of unknown etiology without satisfactory treatment for skin sclerosis and soft tissue atrophy.

OBJECTIVE To provide clinical, histologic, and transcriptome evidence of the antisclerotic and regenerative effects of sequential fat grafting with fresh fat and cryopreserved stromal vascular fraction gel (SVF gel) for morphea.

DESIGN, SETTING, AND PARTICIPANTS This single-center, nonrandomized controlled trial was conducted between January 2022 and March 2023 in the Department of Plastic and Reconstructive Surgery of Nanfang Hospital, Southern Medical University and included adult participants with early-onset or late-onset morphea who presented with varying degrees of skin sclerosis and soft tissue defect.

INTERVENTIONS Group 1 received sequential grafting of fresh fat and cryopreserved SVF gel (at 1 and 2 months postoperation). Group 2 received single autologous fat grafting. All patients were included in a 12-month follow-up.

MAIN OUTCOME AND MEASURES The primary outcome included changes in the modified Localized Scleroderma Skin Severity Index (mLoSSI) and Localized Scleroderma Skin Damage Index (LoSDI) scores as evaluated by 2 independent blinded dermatologists. The histologic and transcriptome changes of morphea skin lesions were also evaluated.

RESULTS Of 44 patients (median [IQR] age, 26 [23-33] years; 36 women [81.8%]) enrolled, 24 (54.5%) were assigned to group 1 and 20 (45.5%) to group 2. No serious adverse events were noted. The mean (SD) mLoSSI scores at 12 months showed a 1.6 (1.50) decrease in group 1 and 0.9 (1.46) in group 2 (P = .13), whereas the mean (SD) LoSDI scores at 12 months showed a 4.3 (1.34) decrease in group 1 and 2.1 (1.07) in group 2 (P < .001), indicating that group 1 had more significant improvement in morphea skin damage but not disease activity compared with group 2. Histologic analysis showed improved skin regeneration and reduced skin sclerosis in group 1, whereas skin biopsy specimens of group 2 patients did not show significant change. Transcriptome analysis of skin biopsy specimens from group 1 patients suggested that tumor necrosis factor α signaling via NFκB might contribute to the immunosuppressive and antifibrotic effect of sequential fat grafting. A total of 15 hub genes were captured, among which many associated with morphea pathogenesis were downregulated and validated by immunohistochemistry, such as *EDN1*, *PAI-1*, and *CTGF*.

CONCLUSIONS AND RELEVANCE The results of this nonrandomized trial suggest that sequential fat grafting with fresh fat and cryopreserved SVF gel was safe and its therapeutic effect was superior to that of single autologous fat grafting with improved mLoSSI and LoSDI scores. Histological and transcriptomic changes further support the effectiveness after treatment.

TRIAL REGISTRATION Chinese Clinical Trial Registry identifier: ChiCTR2200058003

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Multimedia

Supplemental content

Author Affiliations: Department of Plastic and Reconstructive Surgery, Nanfang Hospital, Southern Medical University, Guangzhou, China (Liu, Zhang, Lu, Cai); Department of Plastic Surgery, The Second Affiliated Hospital, Zhejiang University School of Medicine, Hangzhou, China (Wang).

Corresponding Authors: Junrong Cai, MD (drjunrongcai@outlook.com), and Feng Lu, MD, PhD (doctorlufeng@hotmail.com), Department of Plastic and Cosmetic Surgery, Nanfang Hospital, Southern Medical University, 1838 Guangzhou North Rd, Guangzhou, Guangdong 510515, China.

Morphea, or localized scleroderma, refers to a group of rare autoimmune connective tissue diseases with an incidence of 0.4 to 2.7 per 100 000 people.[1,2] Morphea is characterized by an early lymphocytic predominate infiltrate and swollen endothelial cells that are followed by collagen deposition, skin sclerosis, and eventual atrophy of the affected area, resulting in varying degrees of disfigurement that are negatively associated with the quality of life and mental health of patients.[3-6] Current treatment regimens mainly include topical immunomodulators, topical steroids, and phototherapy.[7-9] Several studies have supported the use of methotrexate with corticosteroids in managing severe morphea types.[10-12] However, these agents do not have regenerative properties and are not associated with improved cutaneous sclerosis and soft tissue atrophy of morphea. Therefore, identifying alternative treatment options for morphea is necessary.

Autologous fat grafting, a relatively low-risk and low-morbidity procedure, has been used to repair various soft-tissue defects. Several clinical studies have reported that autologous fat grafting is able to correct volume loss in patients with morphea to improve facial disfigurement.[13-16] Due to severe soft tissue depression and skin hardening, patients with scleroderma often require multiple fat grafting procedures. Cryopreserved fat grafting could be convenient and beneficial for multiple injections. Although cryopreservation of fat tissue has been studied extensively, the results remain controversial. Our previous study showed that cryopreserved stromal vascular fraction gel (SVF gel), an adipose-derived product that concentrates stromal vascular fraction cells, maintained tissue integrity and cell viability and was associated with a better long-term retention rate than that of cryopreserved fat.[17] Clinical application of cryopreserved SVF gel for aesthetic is associated with safe and effective outcomes.[18,19] However, whether it could exert a positive association with improving skin sclerosis remains unclear.

This nonrandomized controlled trial was conducted to determine whether sequential grafting with fresh fat and cryopreserved SVF gel is better than a single procedure of autologous fat grafting for treating morphea. Meanwhile, evaluation of the transcriptome in lesional skin before and after sequential fat grafting was performed to identify the mechanisms associated with its therapeutic effect.

Key Points

Question How safe and effective is sequential grafting with fresh fat and cryopreserved stromal vascular fraction gel (SVF gel) in treating morphea, and is it better than a single treatment of autologous fat grafting?

Findings In this nonrandomized trial of 44 patients with morphea, sequential fat grafting with fresh fat and cryopreserved SVF gel was safe and effective and associated with improved aesthetic contouring and alleviated skin sclerosis. The therapeutic results were significantly better than single autologous fat grafting.

Meaning The findings of this study suggest that sequential fat grafting with fresh fat and cryopreserved SVF gel has therapeutic potential for treating morphea.

Methods

Study Design and Participants

With approval from the Nanfang Hospital institutional review board, 44 patients with lesions clinically and histologically diagnosed as morphea at the Department of Cosmetic and Reconstructive Surgery at Nanfang Hospital were recruited. The sample size for this study was determined based on practical, and not statistical, considerations. Informed written consent was obtained from all patients before entering the study. We followed the Transparent Reporting of Evaluations With Nonrandomized Designs (TREND) reporting guideline.

Participants were enrolled between January 2022 and March 2023. Demographic data were collected, including date and year of birth, age, sex, and race and ethnicity. Race and ethnicity data were self-identified by participants and provided as free text without predefined categories. The major inclusion criteria for patients entering this study included a clinically and histologically confirmed diagnosis of morphea; age of 18 to 59 years; body mass index (calculated as weight in kilograms divided by height in meters squared) of 17 or greater (for adequate fat harvesting); and adequate organ function. Major exclusion criteria included newly used immunosuppressive drugs within 3 months; treatment with topical steroids or immunomodulators within 2 weeks in the surgical area; treatment with prednisolone greater than 10 mg/d; body mass index less than 16; or pregnant and nursing individuals.

The trial flow diagram is presented in eFigure 1 in Supplement 1. Patients were nonrandomly assigned to 2 treatment groups according to the time of enrollment. Group 1 patients underwent sequential fat grafting with cryopreserved SVF gel injected at the points of 1 and 2 months after the primary surgery. Group 2 patients only received single fat grafting. Methodological details of the surgical technique, SVF gel preparation, cryopreservation, and recovery are in eMethods 1 and 2 and in Supplement 1 and the **Video**. Participants' surgical fees were waived. All surgical procedures were conducted by the same plastic surgeon at Nanfang Hospital. The amount of each injection depended on the needs of each patient (eTable 1 in Supplement 1). Patients were followed up in months 1, 2, and 12 after the first surgery.

For safety assessment, vital signs and state of consciousness were monitored during the whole surgical procedure. At each visit, new adverse events (AEs) were assessed and recorded by 1 reviewer. Study participants were assessed for treatment-emergent AEs, including (1) pain, prolonged edema, skin irregularities, and nodules around the transplant area; (2) skin unevenness, paresthesia, scarring, and dyspigmentation around the liposuction area; and (3) serious AEs, including skin necrosis, infection, and embolization around the liposuction and transplant area.

Therapeutic Assessment Tool

For the primary outcome, changes in the total score of the modified Localized Scleroderma Skin Severity Index (mLoSSI) and Localized Scleroderma Skin Damage Index (LoSDI) were assessed by 2 independent masked dermatologists. Other outcome measures, including each parameter of mLoSSI and LoSDI scores, images (VISIA; Canfield) of patients at each visit, and histologic and transcriptomic changes, were also used to demonstrate skin lesion changes.[20]

Skin Biopsy Specimen Collection

The preoperative biopsy specimens were collected immediately before surgery. The postoperative biopsy specimens were collected 12 months after the first surgery. Preoperative biopsy specimens were obtained in the facial lesion, and the postoperative biopsy specimens were obtained 1 cm next to the preoperative ones within the skin lesion (ensuring that the previous scar was not included). Tissues were stored in either RNAlater (Qiagen) or liquid nitrogen and kept at -80 °C for RNA purification and in formalin for histologic analysis.

Histological Analysis of Skin Biopsy Specimens

The histopathological features of available biopsy specimens before and 12 months after surgery were reviewed by 2 masked dermatopathologists and tabulated. Light microscopic examination of tissue sections, prepared via hematoxylin-eosin and Masson trichrome staining, was performed for each available case using a semiquantitative, 4-grade scale (0 = normal, 1 = slight, 2 = moderate, and 3 = severe). In addition, immunohistochemical (IHC) staining with CD31 (for endothelial cells) and CD3 (for lymphocytes) was performed to evaluate the level of neovascularization and immune infiltration. The number of CD31 and CD3 were counted using ImageJ software (version 1.53; National Institutes of Health). Transcriptome results were further validated by IHC staining (eMethods 3 in Supplement 1).

Transcriptome Analysis of Skin Biopsy Specimens

Methodological details of RNA extraction, gene expression profiling, and transcriptome analyses are described in eMethods 4 in Supplement 1. Briefly, genes with a fold change of 1.5 or greater and adjusted *P* value of less than .05 found by DESeq2 were assigned as differentially expressed genes (DEGs).

Statistical Analysis

For all outcome measures (the total score and each parameter of mLoSSI and LoSDI, semiquantitative histological analysis), scores were self-compared and compared between the treatment groups at 12 months. Statistical significance was set at $P < .05$. For mLoSSI and LoSDI scores, descriptive statistics were computed at all points. Baseline characteristics and treatment characteristics were analyzed using descriptive statistics. Statistical analysis was performed using the SPSS, version 19.0 (IBM). All available data were included in the therapeutic analyses. No data were excluded or imputed. Data were analyzed from September 2022 to June 2023.

Results

Characteristics of the Study Population

A total of 44 patients (median [IQR] age, 26 [23-33] years) with morphea were recruited between January 2022 and March 2023, of whom 36 were women (81.8%) and 24 (54.5%) received sequential fat grafting. All patients completed clinical analyses. Eleven patients (25.0%) were lost to follow-up (7 in group 1 and 4 in group 2), leaving 33 patients for the histological analysis. No protocol deviation occurred. Demographic characteristics were well balanced in groups 1 and 2 (**Table**[21]). All patients were Asian individuals and had linear subtype (38 of 44 [86.4%]) or circumscribed subtype (6 of 44 [13.6%]), with a median age at disease onset of 16 years (IQR, 11-21 years) and disease duration of 10 years (IQR, 6-14 years). Thirty of 44 patients (68.2%) had received systemic or topical medication in the past, and 2 of 44 patients (4.5%) were still taking medication during the perioperative period. eTable 1 in Supplement 1 summarizes the morphea classification, distribution of cutaneous lesions, biopsy sites, and the amount of fat grafting per patient.

Clinical Analysis

All 44 patients exhibited facial depression before treatment and showed improvements in facial appearance, with volume loss correction and improved symmetry after treatment (representative images in eFigures 2 and 3 in Supplement 1). The images of group 1 patients showed evaluated angiogenesis and less dyspigmentation (representative images in **Figure 1**). The mean (SD) mLoSSI scores at 12 months showed a 1.6 (1.50) decrease in group 1 and 0.9 (1.46) in group 2 ($P = .13$), suggesting that group 1 did not exhibit superiority in improving morphea activity compared with group 2. However, for parameters of mLoSSI, skin thickness was significantly decreased in group 1, whereas a rebound was seen in group 2 (**Figure 2**). For LoSDI, the mean (SD) LoSDI scores at 12 months showed a 4.3 (1.34) decrease in group 1 and 2.1 (1.07) in group 2 ($P < .001$). The 3 parameters of LoSDI all decreased 12 months after therapy in group 1. In group 2, dermal atrophy, subcutaneous atrophy, and total LoSDI scores showed a gradual rebound with time (Figure 2). Compared with group 2, group 1 showed a more significant decrease in skin thickness, dermal atrophy, subcutaneous atrophy, and total LoSDI score , suggesting that group 1 experienced greater improvement in morphea skin damage compared with group 2 (eTable 2 in Supplement 1).

Adverse Events

For both groups, recorded treatment-emergent adverse events (AEs) included pain (1 of 24 [4%] vs 0 of 20 [0%] at month 1) and skin irregularities (6 of 24 [25%] vs 4 of 20 [20%] at month 1, 3 of 24 [12%] vs 1 of 20 [5%] at month 2) within the transplant area and paresthesia (5 of 24 [21%] vs 3 of 20 [15%] at month 1, 3 of 24 [12%] vs 3 of 20 [15%] at month 2) and dyspigmentation (4 of 24 [17%] vs 2 of 20 [10%] at month 1 and 2) within the liposuction area (eTable 3 in Supplement 1). No treatment-emergent AEs were recorded at 12 months. There were no serious AEs, including infection, skin necrosis, and embo-

Table. Demographic and Clinical Characteristics

Variable	No. (%)		
	Group 1 (n = 24)	Group 2 (n = 20)	Total (N = 44)
Sex			
Male	4 (17)	4 (20)	8 (18)
Female	20 (83)	16 (80)	36 (82)
Age, median (IQR), y	26 (23-30)	28 (23-35)	26 (23-33)
Asian race and ethnicity	24 (100)	20 (100)	44 (100)
Age at onset, median (IQR), y	17 (13-21)	16 (10-21)	16 (11-21)
Disease duration, median (IQR), y	10 (8-14)	11 (6-16)	10 (6-14)
Morphea subtype[a]			
Parry Romberg syndrome	9 (37)	11 (55)	20 (45)
ECDS	10 (42)	8 (40)	18 (41)
Circumscribed morphea	5 (21)	1 (5)	6 (14)
Previous medication			
None	8 (33)	2 (10)	10 (23)
Tacrolimus	3 (12)	2 (10)	5 (11)
Corticosteroids	3 (12)	4 (20)	7 (16)
Topical[b]	7 (29)	7 (35)	14 (32)
TCM	14 (58)	12 (60)	26 (59)
UV therapy	3 (12)	4 (20)	7 (16)
Surgery	2 (8)	2 (10)	4 (9)
Medication duration, median (IQR), y	4 (2-7)	3 (2-4)	3 (2-5)
Duration of drug withdrawal, median (IQR), y	3 (1-6)	4 (2-8)	4 (2-6)
Amount of transplanted fat, median (IQR), mL			
Fresh fat	20 (11-40)	25 (15-35)	20 (15-40)
First injection of SVF gel	10 (6-20)	NA	NA
Second injection of SVF gel	5 (5-10)	NA	NA
Total	35 (25-68)	NA	30 (16-54)

Abbreviations: ECDS, en coup de sabre; NA, not applicable; SVF, stromal vascular fraction; TCM, traditional Chinese medicine.

[a] Morphea classification according to Laxer and Zulian.[21]

[b] Topical medication included mucopolysaccharide polysulfate cream and asiaticoside cream.

lization, during the whole treatment and observation period in both groups.

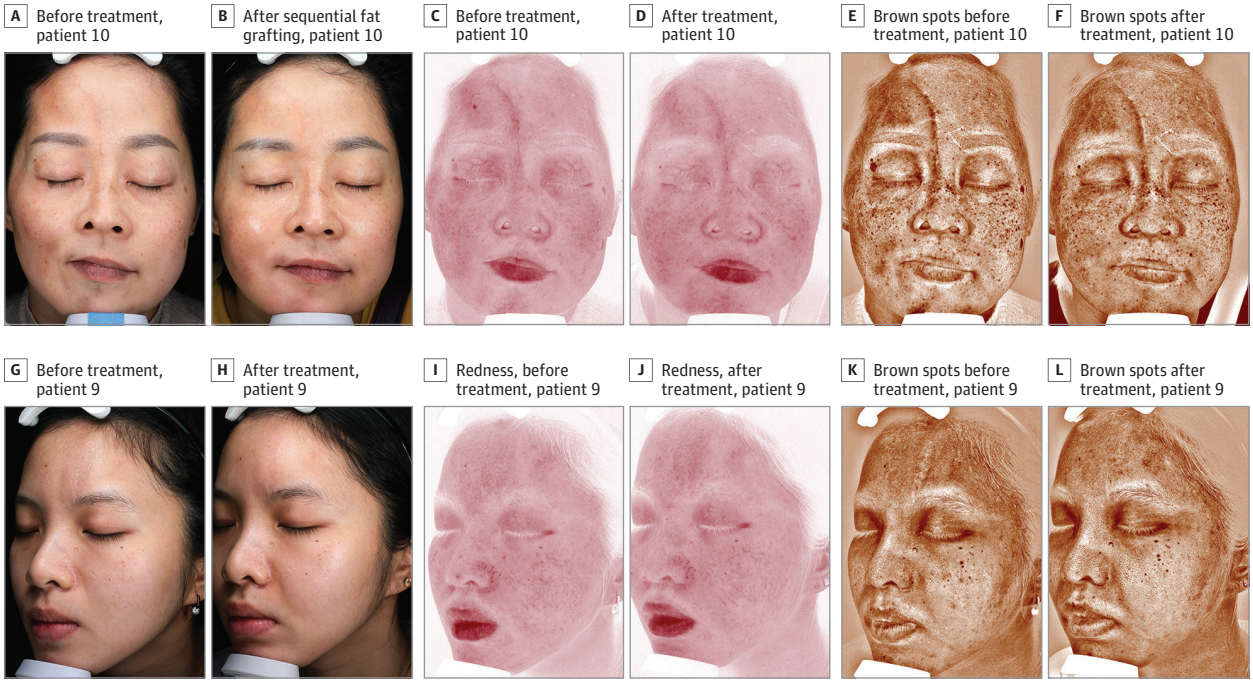
Histological Analysis

The skin of patients with morphea exhibited atrophy of adnexal and adipose tissue and dermal sclerosis (**Figure 3**). Histologic analysis demonstrated that sequential fat grafting showed better results via being associated with significantly improved skin restructure and reduced skin sclerosis. In group 1 patients, scattered clusters of adipose tissue (black asterisks) and increasing adnexal (black arrowheads) were observed in the dermis (Figure 3). Masson staining showed collagen loosening of compaction in the dermis (Figure 3). IHC staining of perilipin further confirmed the presence and viability of the adipocytes (Figure 3). IHC staining of CD31 showed a significant increase, while CD3 showed a significant decrease after sequential fat grafting treatment (eFigure 4 and eTable 4 in Supplement 1). In Group 2, only mildly reduced dermal sclerosis was seen after autologous fat grafting without significant recovery of the annexes and dermal adipose tissue (eFigure 5B in Supplement 1) and significant differences in dermal adipose tissue content (eFigure 5F in Supplement 1).

Transcriptomic Analysis

In this trial, we sought to understand the mechanism by which sequential fat grafting is associated with reduced skin sclerosis and promoted skin regeneration. Among 8 patients whose skin tissue was extracted, only 3 patients in group 1 with a diagnosis of Parry Romberg syndrome met the quality and quantity requirements for microarray analysis of the entire genome (eTable 5 in Supplement 1). We identified 247 DEGs (fold change ≥1.5; $P < .05$), among which 104 genes were upregulated and 143 genes downregulated (eFigure 6A in Supplement 1). The gene expression pattern of the lesional skin after sequential fat grafting diverged from those before treatment (eFigure 6B in Supplement 1). The top 100 dysregulated genes are listed in eTable 6 in Supplement 1. Functional enrichment analysis showed that the upmodulated DEGs were involved in adipogenesis and epidermis development, and the downmodulated DEGs were mainly involved in inflammation, hypoxia, and extracellular matrix (ECM) development (eFigure 7A in Supplement 1). Gene set enrichment analysis of all genes showed 23 significantly altered Hallmark gene sets, among which we found many that had well-established roles in the pathogenesis of morphea were down-modulated (eFigures 7 and 8 in Supplement 1). The most significantly altered tumor necrosis factor (TNF) α signaling via nuclear factor (NF) κB (NES = −2.48; $P < .001$) referred to a set of 200 genes regulated by NFκB in response to TNF, among which we found 22 genes significantly downregulated (eFigure 7D in Supplement 1). To see if proteins coded by these genes played a central role in the therapeutic effect of sequential fat grafting, we

Figure 1. Representative Images After Sequential Fat Grafting in Group 1



Patient 10 had morphea lesion on her right hemiface before stromal vascular fraction (SVF) gel treatment (A) and showed great improvement on facial symmetry 12 months after sequential fat grafting (B). C and D, Image (VISIA; Canfield) showed the redness of the skin lesion was slightly increased 6 months after sequential fat grafting (D, after 12 months of treatment). E and F, Image showed decreased brown spots 12 months after treatment. Patient 9 had a localized scleroderma lesion on her left forehead before treatment (G) and showed a normal appearance 12 months after treatment (H). I and J, Image showed the redness of the skin lesion was greatly increased 12 months after treatment. K and L, Image showed more even distribution of the brown spots 12 months after treatment, suggesting improvement in hyperpigmentation (dark spots) and hypopigmentation (white spots).

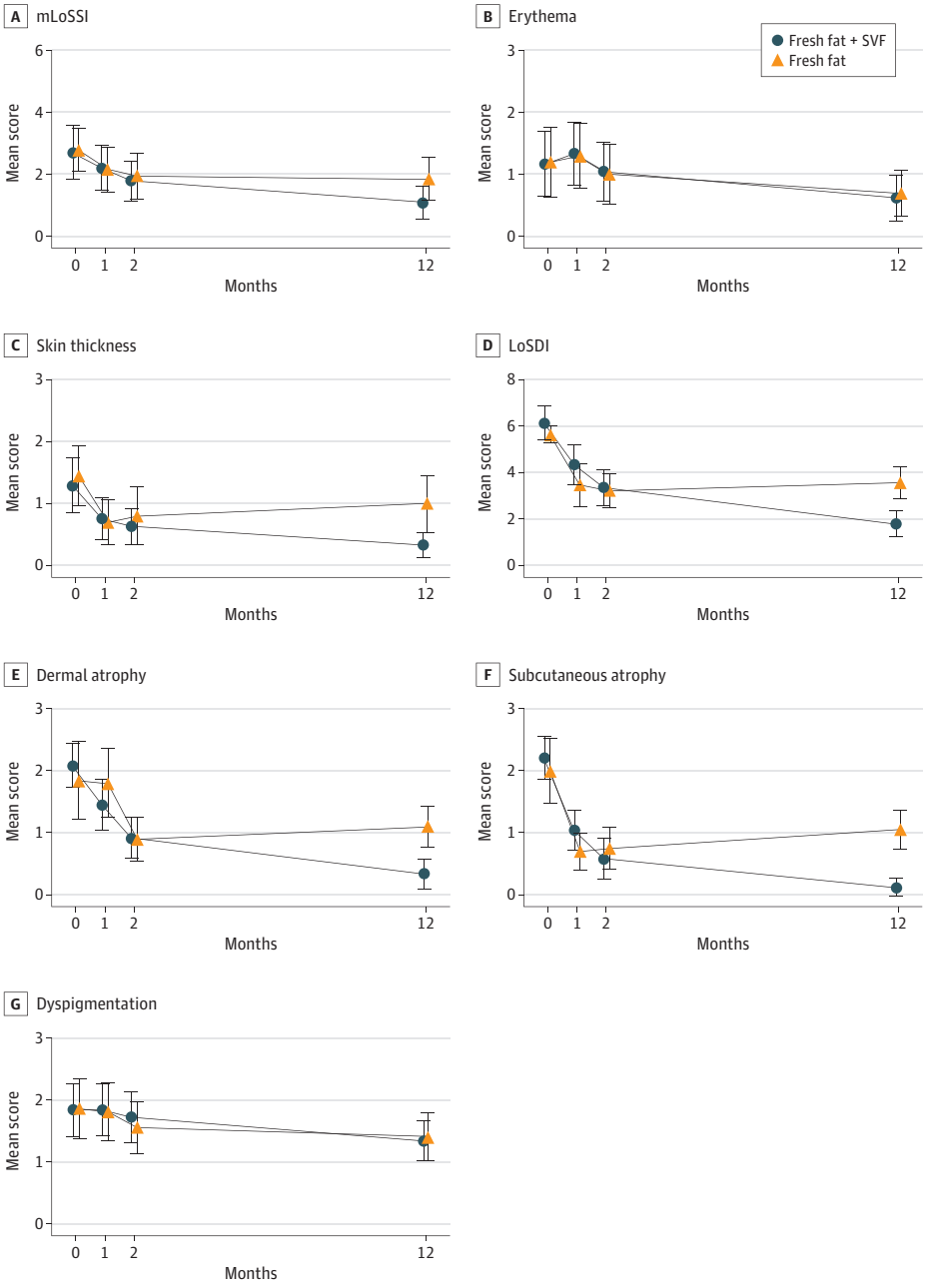
used cytoHubba to capture essential proteins from the protein-protein interaction network (eTable 7 in Supplement 1). Their intersections revealed 15 potential hub genes, 4 of which (*PAI-1*, *EDN1*, *NFKBIA*, and *MYC*) were also included in TNFα signaling via NFκB (eFigure 7D in Supplement 1). The reduced expression of *PAI-1*, *EDN1*, and *NFKBIA* was confirmed by IHC, while changes in skin tissue expression of MYC were not significant (eFigure 9 in Supplement 1). We also found several sclerosis-mediating genes among the downregulated hub genes, such as *CTGF* (eFigure 9A in Supplement 1), *CXCR4* (eFigure 9F in Supplement 1), *IGFBP3*, and *THBS1*.[22-28] To further understand how the cellular immunology of morphea is modulated by sequential fat grafting, we performed an immune cell infiltrate analysis and cell types enrichment analysis. Both results suggested reduced $CD4^+$ memory T cells after treatment (eFigure 10 in Supplement 1).

Discussion

The findings of this nonrandomized trial suggested that multiple grafting with fresh fat and cryopreserved SVF gel was safe and beneficial for patients with morphea. Multiple injections of SVF gel were able to alleviate pathological skin sclerosis and help improve the volume-filling effect. Patients receiving cryopreserved SVF gel did not experience any serious undesired complications. Meanwhile, no significant increase in the incidence of treatment-emergent AEs was noticed compared with traditional fat grafting (eTable 3 in Supplement 1). Treatment-emergent AEs in both groups were recorded during follow-up visits in the first 2 months and had recovered at 12 months. There were no serious AEs, including infection, skin necrosis, and embolization, during the entire treatment course. Thus, this treatment strategy was not associated with increased surgical risks and may be considered to be a safe treatment procedure. The mean mLoSSI and LoSDI scores at 12 months were significantly decreased after sequential grafting, suggesting that skin sclerosis was successfully improved after treatment. Histological and transcriptomic changes showed that sequential grafting could be associated with lower dermal inflammation, increased vascularization, and remodulated ECM.

It has been reported that SVF cells, especially adipose-derived mesenchymal stromal cells, could exert an antisclerotic association with morphea.[29-31] SVF gel only requires the mechanical process to eliminate most mature adipocytes and concentrate SVF cells within the adipose tissue, getting rid of using the collagenase and retaining the ECM scaffold.[32-34] SVF gel can be injected through a 30-G needle, which enables precise intradermal delivery, as traditional lipoaspirates cannot. Intradermal injection of SVF gel could directly reconstruct the dermal fat layer in the skin lesion (eFigures 3E and F in Supplement1). For scleroderma-induced soft tissue defects or even

Figure 2. Patterns in Components of Morphea Disease Activity and Damage

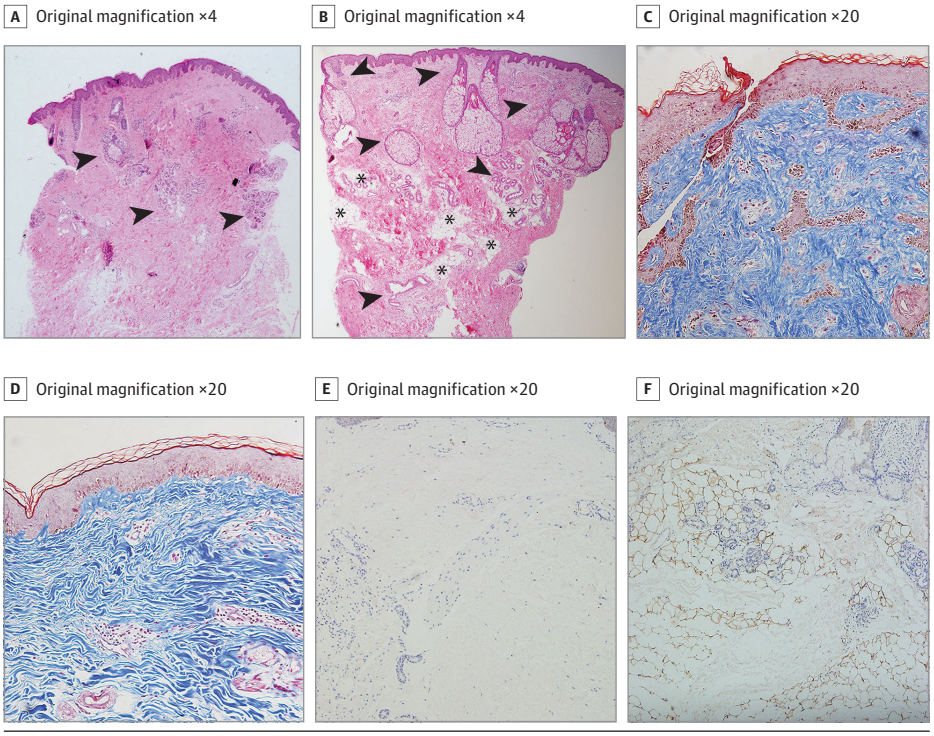


Mean (95% CI [represented by bars]) scores were from before treatment and 1, 2, and 12 months after the initial surgery. LoSDI indicates Localized Scleroderma Skin Damage Index; mLoSSI, modified Localized Scleroderma Skin Severity Index; SVF, stromal vascular fraction.

hemifacial atrophy, sclerosis of the skin increases its tension, making it difficult for surgeons to fill the defect with a single transplant and requiring multiple fillings. Our previous study suggested that SVF gel is a better alternative for cryopreservation than traditional fat since most fragile adipocytes were removed after the mechanical process.[17] Thus, we chose the SVF gel for cryopreserved use, which facilitates reinjection for future use. Multiple fillings of fat tissue not only associated with improved the texture of the skin and reduced stiffness, but also easier expansion of the skin easier for volumization.

Several immunoinflammatory mechanisms are recognized in the pathogenesis of morphea. After sequential fat grafting, $CD3^+$ lymphocytes were significantly decreased. T lymphocyte infiltration is a common histologic feature of morphea and is believed to be associated with disease progression.[35,36] It has been reported that lymphocytes can regulate the ECM and endothelial cell function to improve tumor vasculature.[37,38] After sequential fat grafting, the TNFα signaling via NFκB was the most significant down-modulated gene set. We found 3 genes (*PAI-1*, *EDN1*, and *NFKBIA*) from this gene set recognized as hub genes were reduced after sequential fat grafting (eFigure 9B-D in Supplement 1). Recognized as a potent vasoconstrictor, *EDN1* plays an indispensable role in vascular damage and matrix protein production by fibroblasts, contributing to one of the earli-

Figure 3. Representative Histological Changes Before and 12 Months After Sequential Fat Grafting in Group 1



Preoperative biopsy specimens were obtained in the facial lesion and the postoperative biopsy specimens were obtained 1 cm next to the preoperative ones within the skin lesion to ensure that scar tissue was not included. A, Preoperation biopsy specimen showing dermal sclerosis, well-defined adipose, and annex atrophy (black arrowheads). B, Postoperation biopsy specimen showing reduced dermal sclerosis with the recovery of the annexes (black arrowheads) and dermal adipose tissue (black asterisks). C, Masson trichrome stain before operation. D, Postoperation biopsy specimen showing collagen loosening of compaction in the dermis. E, Immunohistochemistry staining of perilipin, showing severe atrophy of dermal adipose tissue before operation. F, Postoperation biopsy specimen showing reproduction of dermal adipose tissue within the skin lesion.

est pathologic features of morphea.[39-43] *PAI-1* is encoded by SERPINE1, overexpression of which has been found in fibroblasts of keloids[44] and bleomycin-induced murine scleroderma.[45] Given these findings combined with (1) the proinflammatory role and serum elevation of TNFα in morphea,[46-48] (2) the presence of $CD3^+/IL22^+$ T cells in morphea skin that capacitates dermal fibroblast responses to TNF,[49] and (3) that NFκB is a known mediator between inflammation and sclerosis in scleroderma fibroblasts,[50-53] we hypothesized that TNFα signaling via NFκB could be an important mediator of the immunosuppressive effect of sequential fat grafting to improve pathological skin sclerosis of morphea.

We also found that the fat grafting procedure was associated with mild erythema in patients who had no erythema before surgery or exacerbated erythema in patients with the condition, which is supported by the images (Figure 1) and IHC staining of CD31 (eFigure 4A-B in Supplement 1). Prior studies have noticed the improvement in angiogenesis after SVF gel treatment, an effect thought to be mediated partially through providing a rich source of cells and growth factors in a paracrine fashion.[54,55] In accordance, the Hallmark_hypoxia gene set was significantly down-modulated after treatment (eFigure 7A-B in Supplement1). Thus, we believe that sequential fat grafting may contribute to improving skin vascularity in morphea skin with a downregulated hypoxia-related pathway.

After dermal injection of SVF gel into the morphea skin lesion, we found that some adipocytes presented within the dermis layer. These adipocytes might be derived from the transplanted SVF gel or in situ adipocyte regeneration stimulated by the treatment. The Hallmark_adipogenesis gene set was significantly upregulated after sequential fat grafting (NES = 1.34; $P < .001$). Moreover, among the top upregulated pathways, 3 were associated with lipid metabolism (eFigure 7A in Supplement 1). The upregulated DEGs included *IGFL2*, *LCN2*, *SPNS2*, and *SLC27A6*, which suggested increased fatty acid synthesis and enhanced fatty acid transport in the sclerotic skin. Thus, we speculated that restoring adipocyte function in the dermal and subcutaneous layer may also contribute to antisclerotic effects.

Signs of skin regeneration were observed in morphea lesions after sequential fat grafting; epidermal atrophy improved as the number of epidermal layers increased, and there was an increase in the size and number of atrophic appendages. Meanwhile, we identified multiple upregulated DEGs involved in the regulation of hair cycle and epidermis development (eFigure 7A in Supplement 1), including *SPRR4*, *KRT28*, and *PADI3*. We can only assume that the regenerative effect of sequential fat grafting is closely associated with the enriched stem cells within SVF gel. How these transplanted cells may interact with their adjacent microenvironment to regenerate normal skin structure requires further investigation.

Sequential grafting with fresh fat and cryopreserved SVF gel is a beneficial treatment that is not only associated with improved subcutaneous atrophy, but also dermal lesions, which encourages closer cooperation between dermatologists and plastic surgeons for a more comprehensive treatment plan of morphea. Moreover, this study revealed a key role of subcutaneous fat in regulating the disease progress of morphea. However, further studies are required to fully address the association between subcutaneous adipose tissue and skin sclerosis.

Limitations

This study was limited by the nonrandomization, homogeneity of race, and the number of paired skin samples for histologic and transcriptome analyses. RNA sequence analysis was also limited by the absence of group 2 skin samples.

Conclusions

This nonrandomized trial suggested that sequential fat grafting was helpful in improving morphea skin sclerosis in which an immunosuppressive effect that was at least partially associated with TNFα signaling via NFκB appears to contribute. The study also provided clinical and histologic support for the potential of sequential fat grafting to be used clinically to ameliorate morphea as a better alternative to single autologous fat grafting. Further investigation of the detailed mechanisms for sequential fat grafting to improve skin sclerosis is warranted. By investigating the adipose secretome, or inducing subcutaneous adipogenesis, we look forward to the development of new therapeutic options for morphea.

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Concept and design: All authors.
Acquisition, analysis, or interpretation of data: Liu, Wang, Zhang.
Drafting of the manuscript: Liu, Zhang.
Critical review of the manuscript for important intellectual content: All authors.
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Supervision: Wang, Lu, Cai.
Other: Wang.

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