

Use of Lipotransfer in Scleroderma

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Abstract

Lipotransfer for soft tissue filling is a well-established plastic and aesthetic surgical technique. Elective fat grafting is considered a safe and well-tolerated procedure. Coleman and others have reported that fat grafting may have tissue regenerative properties and not only serve as a soft tissue filler. There have been reports from our group and others that it may improve fibrosis secondary to many different pathological aetiologies including scleroderma, burn injury, lichen sclerosis, graft vs host disease, and radiation. The mechanism of action remains unclear but has been postulated that it is adipose derived stem cells (ADSCs) related. Lipoaspirate has been characterised and shown to contain several cell populations including fibroblasts, endothelial cells, and ADSCs. The ADSCs have shown to secrete angiogenic, immunodulatory, and antiapoptotic factors as well as proliferate and differentiate into different cell types similarly to other stem cell sources. We have shown that ADSCs are functionally different in scleroderma patients but despite this lipotransfer produces a significant reversal in the effects of fibrosis in these patients. The advantage of lipoaspirate containing a valuable source of regenerative properties, ease of access, isolation, and processing may serve a significant future role in the treatment of fibrotic conditions.

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Systemic sclerosis (SSc) is a multisystem disease characterised by cutaneous and internal organ fibrosis.¹ Typically the disease affects the skin tissue but also the lungs, heart, or digestive tract. A wide range of cells play a role in the formation of fibrosis in scleroderma, though the secretion of mediators or with interactions with other cells.² Fibroblasts play a pivotal role through the secretion of extracellular matrix formation. The dermis becomes acellular and intensively packed with dense collagen and extracellular matrix proteins with the loss of the microvasculature.² Besides excessive collagen build up in tissues, vascular abnormalities in SSc cause peripheral vascular disease including Raynaud's phenomenon.³ Clinical manifestations in SSc are highly variable but generally SSc has a very poor prognosis due to complications such as pulmonary fibrosis.³

Facial involvement associated with oral complications and aesthetic changes causes severe impairment to the patient's self-image.^{1,3,4} Although not life threatening, the resulting limited facial expression and disfigurement is very difficult to hide, leading to a poor quality of life.^{1,3,4}

Current therapeutic interventions offer minimal improvement in facial appearance and thus these patients pose a difficult clinical challenge for aesthetic and reconstructive surgeons to treat.^{1,3,4}

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Lipotransfer for soft tissue filling is a well-established plastic and aesthetic surgical technique.¹⁻⁴ Elective fat grafting is considered a safe and well-tolerated procedure.³ In 2006, Coleman et al postulated that fat grafting may have tissue regenerative properties and not only serve as a soft tissue filler.³ Lipoaspirate has shown to be beneficial for wound healing and localized forms of scleroderma including “en coup de sabre” scleroderma.²⁻⁵

Furthermore, lipoaspirate has been characterized and shown to contain several cell populations including fibroblasts, endothelial cells, and a stem cell population, termed adipose derived stem cells.⁶⁻¹⁰ This adult stem cell population has generated interest due to the ease of isolation of and high abundance compared to traditional sources of adult stem cells such as bone marrow aspirate.⁸⁻¹¹

The ADSCs have shown to secrete angiogenic, immunomodulatory, and antiapoptotic factors as well as proliferate and differentiate into different cell types similarly to other stem cell sources.⁶⁻¹⁰ Whether these cells are the effector mechanism in the beneficial effects of fat transfer is proposed but not definitively proven.

Hence with the advantage of lipoaspirate containing a valuable source of regenerative stem cells, ease of isolation, and little processing time, lipotransfer has been investigated as a novel therapeutic approach for the treatment of scleroderma.

Lipotransfer Therapy for Scleroderma

The senior author has treated over 90 patients with SSc oro-facial fibrosis with lipotransfer and found both improvement in facial appearance and facial function using a standard technique.¹⁰ Mouth related disability is assessed by the Mouth Handicap in Systemic Sclerosis (MHSS scale), which is a specifically designed validated tool for patients with SSc.¹⁰

There have been reports of using lipotransfer for the treatment of SSc.¹¹⁻¹⁴ Del Papa et al treated 20 patients with perioral thickening due to diffuse SSc with autologous fat.¹¹ The lipoaspirate was centrifuged at $700 \times g$ for 3 minutes. The middle layer was then used for injection using a blunt cannula into 8 sites around the mouth. Each perioral area received 2 mL of lipoaspirate. After 3 months of treatment, both the interincisional distance and oral perimeter were increased significantly ($P < 0.001$). Furthermore, skin neovascularization was increased as shown by lip videocapillaroscopy and microvessel density scoring of the perioral skin biopsy sections. Similarly, Sautereua et al treated 14 patients with SSc with lipoaspirate.⁵ The lipoaspirate was purified using a PureGraft system (Puregraft LLC, Solana Beach, CA). At 6 months, improvement in mouth opening, facial pain, and MHSS scores were observed.⁵

Due to the regenerative properties of ADSCs with the fat transfer, it has been postulated whether direct injection of ADSCs may offer increased benefits compared to lipotransfer alone. Onesti et al has compared the effects of lipotransfer and enriched ADSCs in 10 patients with systemic sclerosis.¹⁵ In the enriched ADSCs group, lipoaspirate from the abdomen was harvested and then processed for ADSC isolation.¹⁵ The ADSCs were expanded and 8×10^5 cells/mL were then injected into patients 3 weeks post the harvesting procedure, by means of a hyaluronic acid gel. At one-year follow-up, both procedures provided significant results but neither technique offered superior results.¹⁵

A recent trial using the Cytori system (Cytori Therapeutics, Inc., San Diego, CA) has commenced after a promising report of an open label trial in hand fibrosis secondary to scleroderma.^{16,17} Whether it is superior to standard fat transfer or other processing techniques in terms of clinical efficacy and cost effectiveness will only be shown by prospective, randomized, and blinded clinical trial.¹⁷

Lipotransfer and Other Fibrotic Conditions

The use of lipotransfer for reversing fibrosis is currently being explored in other fibrotic conditions.¹⁸⁻²⁰ Lipotransfer for scar revision as an alternative to traditional scar revision has been reported. Gentile et al demonstrated improved contour restoration in 63% after 1 year using lipotransfer compared to only 39% of the control group.¹⁹ Kingler et al further demonstrated that lipotransfer improved scar elasticity and patient satisfaction in 20 patients at 6 years follow up.²⁰ Lipotransfer has shown to be promising for scars of different origins including burn, trauma scars, postsurgery, and ulcers.²⁰

Radiation induced fibrosis is a common late complication causing tissue scarring, induration, and contracture that has been shown to be improved by lipotransfer by our group and others.²¹⁻²⁴ Preclinical studies have further demonstrated promising results for the treatment of radiotherapy-induced fibrosis with ADSCs. Luan et al demonstrated that supplementation of fat grafts with ADSCs improved fat volume retention and vascularity in a radiation induced soft tissue injury in a rodent model.²⁵ Forcheron et al demonstrated that ADSCs improved the wound healing after cutaneous radiotherapy induced fibrosis in a minipig model.²⁶ A number of clinical reports have also demonstrated that lipotransfer can improve radiation-induced fibrosis. Rigotti et al showed improved symptom and visible scores after lipotransfer in twenty patients with an average follow up of 31 months.²⁴ Phulpin et al also showed that in 11 patients with head and neck radiation induced fibrosis, lipotransfer provided both aesthetic and functional improvement.²⁷

Mechanisms of Action of Lipotransfer in Fibrotic Conditions

An increasing number of studies are emerging to investigate MSCs from patients with SSC.³ It has been demonstrated that bone marrow stem cells from SSc patients and healthy controls were similar in their phenotype including their differentiation, immunosuppressive, and haematopoietic potential.²⁸ However, other studies have shown differences in osteogenic and adipogenic differentiation potential.²⁹ We have shown that ADSCs from SSc patients and healthy matched controls had a similar surface marker phenotype differentiation potential but differed in their migration and invasion potential.³⁰ Scuderi et al also reported no alterations in phenotype, differentiation, or population potential.³¹

Current exploration of the therapeutic benefits of lipotransfer on reversing fibrosis has been centered around investigating the effect of the ADSC on the fibrotic pathway.³ Several mechanisms have been proposed by which ADSCs may reverse the fibrotic pathway.³ Three main areas are currently being investigated as potential mechanisms by which lipotransfer reverses fibrosis including modulation of: (1) transforming growth factor beta-1 (TGF β 1); (2) angiogenesis; and (3) immune modulation and response.

The most studied pathway is the transforming growth factor beta-1 (TGF β 1)/Smad pathway. TGF- β 1 plays a major role in the pathogenesis of fibrosis, being secreted by numerous cell types including fibroblasts, immune cells, and platelets.² TGF- β 1 is a potent inducer of collagen synthesis and dysfunction can lead to fibrosis.² Chen et al examined the use of ADSCs in a bleomycin fibrotic C57BL/6 murine model.³² Compared with the control group, the ADSC treatment group had reduced skin thickness and total content of hydroxyproline.³² The ADSC group also showed lower levels of TGF- β 1 and higher levels of vascular endothelial growth factor (VEGF) compared to the control group.³² Furthermore, Sun et al illustrated that the suppression of TGF- β 1 may have contributed to the alleviation of irradiation skeletal muscle.³³ Jiang et al further demonstrated that TGF- β 1 suppression after ADSC treatment through intravenous injection could suppress radiation induced lung injury.³⁴ However, the exact mechanism by which TGF- β 1 is downregulated by the ADSCs is unknown.^{33,34}

Increasing evidence has shown lipotransfer may decrease collagen deposition and dermal thickening and enhance angiogenesis in fibrotic conditions.³⁴ The ADSCs have been postulated to contribute to vessel formation through the secretion of angiogenic growth factors including insulin growth factor, VEGF, and platelet-derived growth factor (PDGF).³⁴ Furthermore, the ADSCs may differentiate into endothelial cells and contribute directly to the formation of a vascular structure.³⁵ Serratrice et al

demonstrated that in a scleroderma nude mouse model that lipotransfer reversed the skin fibrosis and showed vascular improvement. However, with few reports, further evidence is required to evaluate how lipotransfer may induce vascularization of surrounding tissue.³⁶

ADSCs may modulate immune responses, inflammation, and improve wound healing at the graft site.³⁷⁻⁴⁰ Dou et al demonstrated in 2016 that ADSCs in a bleomycin-induced model of scleroderma alleviated inflammation of the lungs and fibrosis of the skin using an immune regulating function.⁴⁰ Dermal thickness and collagen deposition was decreased after the ADSCs therapy. T helper 17 (Th17) and regulatory T cell (Treg cell) were detected using flow cytometry. The levels of cytokines in the lung tissues and in the serum were detected by real time fluorescence quantification.⁴¹ Levels of cytokines IL-17, IL-6, tumor necrosis factor- α (TNF- α) mRNA in the lung tissue decreased of ADSC therapy as well as IL-6 in the serum. The expression of Th17 and Treg was also modulated after ADSC therapy.⁴⁰ Alternatively ADSCs may exert an antioxidant effect by protecting against hypoxia by the secretion of growth factors such as hepatocyte growth factor (HGF).⁴²

Overall cellular mechanisms and mediators involved in the treatment of fibrotic tissues with lipotransfer remain poorly understood. The ability to model the fat grafting environment will aid the underpinning by which lipotransfer reverses fibrosis.⁴¹ Ranjagam et al has recently demonstrated that 3-dimensional cell masses of ADSCs on a maltose binding protein-basic fibroblast growth factor substrate may mimic the secretion of TGF- β 1 responsible for tissue fibrosis.⁴³

DISCUSSION

The reversal of fibrotic symptoms with lipotransfer has been observed in the treatment of facial scleroderma. Within the studies identified there have been differences in harvest site, purification method, and implantation procedure. To fully appreciate the effect of lipotransfer on reversal of fibrosis for SSc randomised controlled studies need to be performed, which control for harvesting procedures, purification, and implantation techniques. The harvest site and cannula needs to be controlled to obtain predictable results. Further controlled studies need to determine whether purification with controlled systems, decantation, or centrifugation improve the lipoaspirate quality and efficacy. If the fat is chemically digested to enrich ADSC content it is considered an advanced therapy medical product according to the European and United States regulatory bodies the production procedure needs to be according to Good Manufacturing Practices (GMPs).⁴⁴ The ideal number of ADSCs and the ADSC fat ratio to be delivered at the

fibrotic area to deliver reproducible and effective effects is still unclear. Furthermore, whether the ADSCs need to be delivered within fat or another carrier system must also be determined. The implementation of relevant control measures also need to be adopted to ensure patient safety and reproducible treatment effects.⁴⁴ Some practitioners have suggested intravenous administration of ADSCs as a therapeutic approach with reported benefits in fibrotic and inflammatory conditions. If an intravascular approach is to be attempted there needs to be baseline phase 1 and 2 trials to determine both safety and efficacy.

The Food and Drug Administration (FDA) have issued guidance on what is allowable.⁴⁵ The FDA will allow a tissue product such as fat that is “minimally manipulated.” Minimal manipulation may include washing and sizing for homologous use. Chemical digestion to produce ADSCs is considered “more than minimal manipulation.” A tissue product that is more than minimally manipulated is considered an advanced cell therapy and is subject to similar regulations to a biologic or drug therapy requiring prior FDA approval before use in patients. Unregulated practice opens the profession to significant reputational risk and potential patient harm.

CONCLUSION

Lipotransfer for the treatment of facial scleroderma has been shown to restore facial lipoatrophy and improve mouth function. Enhancement of harvesting techniques and implantation protocols which result in stem cell enrichment may improve the clinical benefit in facial fibrosis but these need to be demonstrated in a controlled clinical trial setting. Significant risks exist to this very promising treatment for fibrotic conditions by application of this technology in unregulated and potentially clinically unsafe applications.

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