**Purpose:** Oro-facial fibrosis in systemic sclerosis (SSc) causes significant impairment in mouth function and patient’s quality of life. Lipotransfer is a reconstructive technique that may be used to treat facial fibrosis. This study aimed to prospectively assess the use of lipotransfer to reverse orofacial fibrosis and restore facial volume in patients with SSc. Adipose derived stem cells (ADSCs) within the lipotransfer may mediate the anti-fibrotic effect of the surgical treatment but the precise mechanism by which ADSCs reverse fibrosis is unknown. To understand the mechanism by which lipotransfer acts, an *in vitro* co-culture model of ADSCs with human dermal fibroblasts derived from patients with SSc was performed.

**Methods:** Prospective analysis of patients undergoing lipotransfer for oro-facial scleroderma was performed between 2011-2018. Eighty-eight female patients were included (average age 58 years) in the study with diffuse and limited SSc. Improvement in aesthetic and functional outcome was assessed at a minimum of 12 months (range 12-84 months) following surgery by the patient and surgeon. Clinical evaluation included assessment of mouth function using the validated Mouth Handicap in Systemic Sclerosis Scale (MHISS) and pre and postoperative volumetric assessment using 3D-surface imaging systems. The effect of lipotransfer on the psychological health of the patients was assessed using the Derriford Appearance Scale (DAS24), Short Form Health Survey (SF-36), Hospital Anxiety and Depression Scale (HADS) and Visual Analogue Scale (VAS). Following isolation of adipose stem cells (SSc-ADSCs) and dermal fibroblasts (SSc-HDFs) from 6 female patients within the cohort, *in vitro* co-culture assays were performed for 14 days. The SSc-HDF proliferation using DNA content and toxicity with LDH assay was analysed. Furthermore, invasion and migration using wound scratch assays of SSc-HDF co-cultured with SSc-ADSCs were analysed over 14 days. At 7 and 14 days a fibrosis pathway specific qPCR array was performed of SSc-HDFs gene expression in monoculture and co-culture. Furthermore, the SSc-HDF secretion of fibrotic protein’s TGF-β1 and CTGF at day 7 and 14 was assessed using enzyme-linked immunosorbent assay (ELISA).

**Results:** All patients reported aesthetic and functional improvement in their orofacial fibrosis following lipotransfer without any complications. The patient demonstrated significant improvement in postoperative MHISS scores compared to preoperative scores, demonstrating enhanced mouth function (p < 0.05). Post-operative psychological health questionnaires scores were significantly improved following lipotransfer compared to pre-operative scores (p < 0.05). Volume analysis of the orofacial region confirmed the long-term lipotransfer restoration at 12-months for all patients, with significant retention in the cheek regions compared to all other facial regions (p < 0.05). Proliferation, migration and invasive capacity of SSc-HDFs was significantly enhanced in co-culture with SSc-ADSCs than monocultures (p < 0.05). A down regulation of fibrotic genes (PDGF, MMP8, SMAD3, Raf, MEK, Erk) and secretion of CTGF and TGF-β1 proteins by SSc-HDFs was observed over 14 days in co-culture with SSc-ADSCs (p < 0.05).

**Conclusions:** Lipotransfer effectively reverses orofacial fibrosis in SSc and may mediate this through MEK-Erk pathways. Further understanding into the mechanism by which lipotransfer reverses fibrosis will improve the efficacy of such treatment for SSc skin fibrosis.

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**Fat Grafting Rescues Radiation-induced Groin Contracture And Results In Diminished Numbers Of Profibrotic Prrx1-positive Dermal Fibroblasts**

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**Purpose:** Contracture is a long-term complication of radiotherapy (RT) that results from pathologic fibrosis of soft tissue in the radiation field. Fat grafting can regenerate fibrotic soft tissue by decreasing collagen density and reorganizing collagen fiber networks. Fibroblasts are the predominant cell involved in extracellular matrix (ECM) synthesis, and dermal fibroblasts possess distinct embryonic origins. We recently identified a profibrotic fibroblast in the mouse ventral dermis marked by embryonic expression of paired related homeobox 1 (Prrx1). Here we sought to investigate how fat grafting alters Prrx1-positive subpopulation distribution as well as functional contractures.

**Methods:** Adult Prrx-1Cre;R26eYFP reporter mice (n=9) underwent whole body lethal irradiation with 9 Gy for hematopoietic depletion. Mice were immediately reconstituted with 2 million nucleated bone-derived cells from donor NSG (NOD.CB17-Prkdcscid/J) mice via retro-orbital sinus injection. The success of reconstitution and
immunodepletion was assessed by fluorescence-activated cell sorting (FACS) analysis of peripheral blood. After 4 weeks, 30 Gy was delivered to the right hindlimb in five fractionated doses to generate limb contracture. The irradiated, contracted limb was then grafted with 200 μl fresh human lipoaspirate and limb extension was measured over the subsequent 8 weeks, at which point skin was harvested for assessment of fibroblast subtypes for FACS and immunofluorescence. A group of mice with radiation-induced groin contracture did not undergo fat grafting and served as the control group.

Results: FACS analysis indicated successful immunodepletion and engraftment by 3 weeks post bone marrow transplantation. At one month following groin irradiation, mice had developed significant right hind limb contracture with significantly reduced limb extension (****p≤0.0001). Histologically this was paralleled by thickening of the dermis, and substantial expansion of the fibrogenic Prrx-1-positive fibroblast subpopulation. While human fat graft volume retention was reduced over 8 weeks following implantation, this was associated with significantly improved in limb extension. The skin overlying the grafted fat showed reduced collagen density, as indicated by trichrome staining, as well as a reduction in the fibrogenic Prrx-1-positive fibroblast subpopulation by immunofluorescence imaging, as compared to the control mice.

Conclusion: Here we show that fat grafting improves the extensibility of irradiated and contracted hind limbs and reverses radiation-induced skin fibrosis by both reducing the collagen content and by altering the composition of dermal fibroblast subpopulations. Specifically, fat grafting results in a depletion of the Prrx-1-positive fibroblast subpopulation. Further elucidating how this profibrotic fibroblast subpopulation is involved in ventral surface soft tissue fibrosis will facilitate development of novel strategies to treat/prevent debilitating side-effect of radiotherapy.

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Novel Lineage Tracing System To Identify Site-specific Ectopic Skeletal Stem Cells

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Purpose: Traumatic heterotopic ossification (HO) is the formation of bone outside of the healthy skeletal system after traumatic injury causing decreased range of motion and discomfort in affected patients. Previous research has identified potential progenitor cells of HO, however, this is still contended. Better understanding of the cells contributing to HO will allow for targeted treatment to reduce HO in high-risk patients. It was recently discovered that cells expressing Hoxa11, an embryonic patterning transcription factor expressed exclusively in the zeugopod, is expressed by mesenchymal stem cells (MSCs) into adulthood and all skeletal lineages are marked downstream of these progenitors using a CreERT2 lineage tracing system. Currently, most lineage tracing systems label MSCs throughout the body, making it difficult to determine if genetic deletion is due to systemic or local effects. We hypothesize, using a mouse model of HO that Hoxa11+ MSCs will differentiate into site-specific HO-progenitor cells, and eventual form HO.

Methods: Hoxa11-CreERT2;ROSA-TdTomato mice were treated with tamoxifen at 6 weeks of age, chased for one week, then injured with an established and reproducible HO-forming Achilles’ tenotomy with concurrent 30% total body surface area back burn. Injured and uninjured hind limb samples were harvested one week, three weeks, and nine weeks post-injury, sectioned for immunofluorescent histology, and imaged by confocal microscopy.

Results: Examining the uninjured hindlimb of the Hoxa11-CreERT2;ROSA-TdTomato mouse, tdTomato-marked Hoxa11 lineage-positive cells were found throughout the zeugopod within tendon, enthesis, and bone. These cells were PDGFRα+ (not shown). Three weeks after injury, Hoxa11 lineage-positive cells are found at regions of heterotopic ossification in the burn/tenotomy model, specifically at the cut end of the tendon and calcaneus. After injury, Hoxa11 lineage+/PDGFRα+ cells express chondrocyte marker, SOX9, and pSMAD3, a downstream TGFβ1 signaling pathway transcription factor central to HO formation. Three weeks after injury, condensing chondrocytes within the HO anlagen are Hoxa11 lineage-positive and are colocalized with early bone marker, RUNX2. At nine weeks post-injury, SP7+ osteoblast and RUNX2+ pre-osteoblasts are marked with tdTomato. Hoxa11 cells were also able to