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Fat Grafting and Adipose Stem Cells for Facial Systemic Sclerosis: a Systematic Review of the literature

Authors:

Aurora Almadori (MBBS, MSc, MSc, PhD)[1,2], Sze Ching Fung (BSc)[3], Christopher P Denton (FRCP, FMedSci)[4], Peter EM Butler (FRCS Plast)[1,2]

Affiliations:

1. Centre for Nanotechnology and Regenerative Medicine, Division of Surgery and Interventional Science, University College of London
2. Department of Plastic Surgery, Royal Free Hospital NHS London Trust
3. Department of Structural and Molecular Biology, University College London
4. Centre for Rheumatology, UCL Division of Medicine, University College London, London, UK
5. Centre for Rheumatology and Connective Tissue Diseases, Royal Free Hospital, London, UK

Corresponding author:

Miss Aurora Almadori,
aurora.almadori.14@ucl.ac.uk ; aurora.almadori@gmail.com

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Facial Systemic Sclerosis treatment with Fat Grafting and Adipose-derived Stem Cells: a Systematic Review of the literature

ABSTRACT

Background: The oro-facial modification occurring in systemic sclerosis are detrimental for patients, but the therapeutic options are limited.

Objectives: This systematic review aimed to perform an up-to-date appraisal of the literature focusing on fat grafting and other adipose stem cell-based therapies for the treatment of facial systemic sclerosis, determining its efficacy and safety, and investigating the current practice for treatment optimization.

Methods: The review was prospectively registered in PROSPERO (CRD42021286268) and followed the PRISMA principles. Multiple databases were searched and only original studies were included.

Results: Over the 12 studies matching the inclusion criteria, 174 patients were treated. Of them, 87.3% (n = 152) were considered to have improved. The complications, graded with the Clavien-Dindo grading system, were grade 1 (no treatment required) or 2 (antibiotic required).

Patients received an average of 2.5 ± 3.68, median 1.35 (range 1-14), lipotransfer procedures. Overall, an amount of 14.60 ± 6.24 ml was injected in the overall facial area, median 16 (range 27-3) ml. The average interval between procedures was 5.30 ± 2.04 months, with a median of 6 (range 3-6.91) months. At the time of inclusion, patients were diagnosed with scleroderma disease on an average 14.7 ± 7.35 years.

Conclusions: Fat grafting for facial systemic sclerosis is effective and safe. The definitive durability of the effect is still unclear, and the optimal number of treatments must be determined to define a precise evidence-based protocol. The body of evidence is highly fragmented, with disagreement in the surgical technique used and outcome assessment, making results from different studies often not comparable. The level of evidence is overall low or very low, and the risk of bias of published studies is overall medium to high. RCTs are urgently needed.

INTRODUCTION

Systemic sclerosis (SSc) is classified into two subgroups: limited cutaneous SSc (lcSSc) when the skin fibrosis involves to the face and extremities, and diffuse cutaneous SSc (dcSSc) when the skin fibrosis extends on the trunk and proximal portions of the limbs. In both the disease subsets, there is excessive extra-cellular matrix deposition and reduced remodeling, leading to fibrosis and loss of connective tissue [1,2]. Irrespectively of the disease subset, the signs of the disease are predominantly evident on the face, with a significant impact on the facial appearance and function [1,2].

Typical orofacial features include: subcutaneous tissue loss, fibrotic skin tightly adhered to the underlying planes, reduced facial expression, nasal alar resorption, perioral wrinkles, narrowing of the oral line with decreased mouth opening (microstomia), thinned lips (microcheilia), and dry mouth (xerostomia). These alterations can have a detrimental impact on patients' psychological well-being and overall quality of life.

The oro-facial alterations associated with SSc are the most challenging aspect to correct, and the therapeutic options to address the oro-facial fibrosis associated with SSc are limited. Fat grafting and adipose-derived stem cells (ASCs) represent a minimally invasive surgical technique largely used in plastic surgery to increase sub-cutaneous volumes and ameliorate the skin fibrosis and scarring in multiple conditions, including scleroderma [3].

The objective of this systematic review was to perform an up-to-date appraisal of the available data in the literature focusing on fat grafting and other adipose stem cell-based therapies for the treatment of facial systemic sclerosis to determine its efficacy and safety, and to investigate the current practice for treatment optimization.

METHODS

The systematic review was prospectively registered in the PROSPERO database (ref CRD42021286268) and followed the principles of the Preferred Reporting Items for Systematic Review and Meta-Analyses Protocols (PRISMA-P) statement (www.prisma-statement.org) [4].

Search strategy

With the support of an academic librarian, a literature search was conducted on Embase, Medline, Web of Science, Scopus, Google Scholar, SciELO, The Cochrane Library, and

ClinicalTrials.gov until May 2023. Free keywords and MeSH headings related to scleroderma and adipose stem cell-based therapies were combined with Boolean operators ‘‘and/or’’: ‘‘scleroderma’’, ‘‘systemic sclerosis’’, ‘‘SSc’’, ‘‘diffuse cutaneous’’, ‘‘limited cutaneous’’, ‘‘dcSSc’’, ‘‘lcSSc’’, ‘‘lipofilling’’, ‘‘fat grafting’’, ‘‘fat transfer’’, “lipotransfer”, ‘‘fat injection’’, ‘‘stromal vascular fraction’’, ‘‘SVF’’, ‘‘adipose derived stem cells’’, ‘‘ASCs’’.
Examples of full Boolean search strategies for EMBASE and MEDLINE are illustrated in Supplement Tables 1 and 2, respectively. The references of the included studies were also reviewed for any relevant publications that might not have been captured in the electronic search.

Study selection

After excluding duplicates, all identified articles were screened by reading the titles and abstracts. Selected studies were downloaded and included in this review after full-text reading. Table 1 presents the inclusion and exclusion criteria for this study. Only original studies were included, like RCTs, case-control studies, cohort studies, case series, and case reports. All surgical techniques used to process the adipose tissue were considered. Reports without original data such as reviews, discussions, viewpoints, editorials, conference papers, and letters to the editors were not included. The search was not limited by language, and when required, Google Translator (Google, California, USA) was adopted.

Data extraction and analysis

The full text of the selected papers was read, and their references were checked to retrieve potential papers missed via electronic search. Data from the included studies were recorded and categorized in a Microsoft Excel spreadsheet (Microsoft, Washington, USA) as follows: Study details: first author, year of publication, country; Participant demographics: age, gender, disease subset, disease duration, BMI; Information regarding the fat grafting procedure: number of treatments, amount injected per session (ml), donor site, recipient site, recipient site preparation (if applicable), fat harvesting method, fat processing method, fat injection method, length of follow-up, and loss to follow-up; Efficacy: percentage and number of patients with reported improvement; Complications: description of reported complications, percentage and number, and grade according to the Clavien-Dindo classification [5]; Outcome measures: qualitative/quantitative outcome assessment,

physician-based outcome measure (validated/non-validated), and patient-based outcome measure (validated/non-validated).

Study outcomes

The primary outcomes of this study were: to determine the efficacy of treatment, defined as the improvement of signs and symptoms, functionality, and patients' quality of life, as reported with qualitative/quantitative means, by both physicians and patients; to determine the safety of the treatment, defined as the incidence of intra-/postoperative complications such as infection, oil cysts, nodules, necrosis, and others.

Secondary outcomes were: 1. to determine the optimal treatment modality: when to start the treatment with fat grafting, if multiple procedures are required, the ideal number of treatments (defined as the number of procedures performed until a satisfactory outcome is achieved), and the ideal interval between one treatment and another; 2. to determine the optimal surgical technique: if a method of harvesting, processing, and grafting the adipose tissue is more effective, the ideal amount to be injected in each session, if recipient site preparation improves the outcome, and to determine if lipotransfer used in conjunction with other techniques presents an outcome optimization; 3. to investigate the methodology used to assess the outcome: qualitative/quantitative outcome assessment, physician-based/patient-based outcome measure (questionnaire, scales); validated /non-validated tools.

Statistical analysis

The results were summarized in a systematic qualitative synthesis and presented as text and tables. Results were assessed using descriptive statistics, such as mean (SD) or median (range), with a 95% confidence interval.

RESULTS

Search results

The database search led to the identification of 659 papers (Figure 1). After removing duplicates, a total of 458 abstracts were selected. The reference management software (EndNote) automatically identified 123 conference papers that were excluded. Thus, 335 records were screened for eligibility.

Study selection

Two authors independently screened the articles (AA, SCF). Disagreements were addressed by assessment from a senior author (PEB). The screening process took place in two phases: title reading and abstract reading. In total, 165 records that did not meet the inclusion criteria were excluded. The reasons for exclusion were: lack of fat transfer or adipose stem cell-based therapy to treat scleroderma (n = 143), letter or comment (n = 12), laboratory-based studies without clinical data (n = 6), language different to the ones mentioned in the inclusion criteria (n = 4).

For the remaining 170 records, download was attempted; of these, six were not retrievable. Therefore, 164 articles were downloaded and further screened by full-text reading. Of these, 152 were excluded because did not meet the inclusion criteria. The reasons for exclusion were as follows: use of dermal fat graft *en bloc* instead of processing the lipoaspirate (n = 12), narrative review without original results (n = 15), lack of adipose stem cell-based therapy to treat scleroderma (n = 108), treatment of localized scleroderma (n = 10), and treatment of systemic sclerosis of body areas different from the face, i.e. hands (n = 7). Therefore, 12 studies were included in this systematic review (Figure 1).

Data analysis

The data extracted from the included studies are listed in Tables 2-5 [1,6-16]. The studies were published between 2005 and 2021. The studies were conducted in the following countries: Italy (n = 4), UK (n = 2), USA (n = 2), France (n = 2), Israel (n = 1), and Iran (n = 1). The study design was mainly prospective (n = 7), followed by retrospective (n = 2), case report (n = 2), and case series (n = 1). The level of evidence ranged between 3 and 5, according to the Oxford Center for Evidence-Based Medicine [17], with the majority of studies presenting an LOE of 4 (n = 7), followed by level 3 (n = 2) and level 5 (n = 3).

Demographics:

A total of 174 patients were treated. Of these, 163 were female (93.7%) and 11 males (6.3%). The mean age was 48.89 ± 10.69 years. BMI was reported in only two studies, with an average value of 21.2 ± 2.26. Of the 12 studies, 4 did not specify the type of SSc (28 patients); from the 8 studies specifying the disease subset, 75 patients were affected by

dcSSc and 71 by lcSSc. The disease duration prior to treatment was 14.7 ± 7.35 years. Follow up was on average 8.73 ± 4.96 months, median 9.1 (range 2.25 – 12.41) months.

Primary outcome:

Efficacy: Of the 174 treated patients, 87.3% were considered to have improved (n = 152) (Table 3). Due to the high heterogeneity of the methodologies included to assess the outcome, the results were not comparable except for mouth function, which was assessed using the Mouth Handicap in Systemic Sclerosis (MHISS) scores in six studies, even if only in five actual pre- and postoperative values were provided. Overall, the average MHISS score reported in five studies was 30.89 ±5.74 pre-operatively and 22.1 ±6.75 postoperatively, with an overall improvement of 8.79 ± 3.93 points ($p < 0.001$).

Safety: Out of 12 studies, 6 reported on the complications occurring after surgery. The most frequently reported complications were bruising, pain, and swelling of the donor site (4 studies), followed by bruising (2 studies), edema (1 study), and infection of the recipient site (1 study). Only 3 studies reported the number of patients presenting complication: in one study, infection occurred and required antibiotic administration in 1 patient out of 62 (1.6%) [1]; in two studies, bruising of the donor site was reported in 10 out of 16 (62.5%) [9]; and in 1 out of 7 (14.2%) [10], respectively.

The grading of the reported complications was performed according to the Clavien-Dindo grading system [5], and was considered ‘grade 1’ (no treatment required, resolved spontaneously) in all the reported complications except one that was ‘grade 2’ because antibiotics were required (1 study, 1 patient, 0.57% of overall cases included in the review).

Secondary outcomes:

Treatment modality: Patients received one fat graft in seven studies, while in five studies two or more treatments were performed until satisfactory results were achieved. Overall, patients were offered an average of 2.5 ± 3.68, median 1.35 (range 1-14) fat grafting procedures. The average interval between procedures was on 5.30 ± 2.04 months, with a median of 6 (range 3-6.91) months. At the time of inclusion, patients were diagnosed with scleroderma disease on an average 14.7 ± 7.35 years.

Surgical technique: The most frequent donor site was the abdominal area (9 studies), followed by the trochanteric area (4 studies), thighs (3 studies), flanks (2 studies), inner knee (1 study), and buttock (1 study). In most studies (n = 6), fat was harvested from multiple

body areas, rather than just one site (n = 5), whereas in one study the donor site was not reported.

Donor site tumescent infiltration was performed in five studies and the anesthetics used were: 150 ml of Klein solution (1 study); modified Klein solution with 50 ml saline, 0.5 ml 1:1.000 adrenalin and 10 ml 2% mepivacaine (1 study); modified Klein solution with 100 ml of saline, 20 ml of mepivacaine 2%, 20 ml of ropivacaine 7.5, 1 ml of epinephrine, and 5 ml of sodium bicarbonate solution 1 mEq/ml (1 study); 500 ml of tumescent solution with normal saline, 25 ml lidocaine 2%, 0.5 ml epinephrine 1:1000 (1 study); 1 liter of sodium chloride 0.9%, 20 ml of lidocaine 2%, and 1 ml of epinephrine 1:200,000 (1 study); 0.5% lidocaine, adrenaline (1:100.000), and 0.8% bicarbonate (1 study). One study adopted the dry technique with no infiltration performed, and six studies did not disclose whether donor-site infiltration was performed.

The lipoaspirate was harvested with a blunt 3 mm cannula (4 studies) connected to 10 ml (3 studies) or 60 ml (1 study) syringes; with a 14-gauge cannula connected to a 10 ml syringe (2 studies); and a 10-gauge cannula connected to a 10 ml syringe (1 study); 5 studies did not specify the diameter of the harvesting cannula.

The lipoaspirate was processed by: centrifugation at 3,000 rpm for 3 mins (4 studies), 2,700 rpm for 5 min (1 study), 2,547 rpm for 3 min (1 study), decanted by gravity for 10 min (2 studies), or 15 minutes (1 study), and washed in a closed 50 mL device (2 studies). One study did not report on the processing method.

The processed lipoaspirate was injected in the facial area with the following tools: 2 mm blunt cannula connected to 1 ml syringe (2 studies); 0.5–0.7 mm cannula (1 study); 15-gauge infiltration cannula (1 study); 17-gauge cannula (1 study); 18-gauge cannula connected to 1 ml syringe (1 study); 19-gauge cannula connected to 2.5 ml syringe (1 study); 21-gauge 0.8 mm cannula (2 studies). In 3 studies, the type of cannula used for injection was not reported.

Overall, an amount of 14.6 ± 6.2 ml was injected in the overall facial area, median 16 (range 27-3) ml. Only in 4 studies details of the aesthetic units injected were provided, as follow: 1 study treated the nose injecting 1.2 ± 0.3 ml; 2 studies treated the cheeks with an average of 3.9 ± 1.8 ml; 1 study treated the chin with 1.9 ± 0.8 ml; 1 study the marionette lines with 2.9 ± 0.7 ml; 1 study the nasolabial fold with 0.9 ± 0.3 ml ; 4 studies the oral area with an average 10 ± 7.63 ml injected, median 8.86 (range 4-16) ml.

Of the 12 studies, nine involved the injection of adipose tissue alone. One study reported the injection of lab-expanded ASCs at a dose of 8×10^5 suspended in 4 ml of hyaluronic acid [12]. In one study, 10-12 cm^3 of PRP obtained from peripheral blood was injected into the perioral area approximately 10 min before fat injection [13]. In another study, an average of 6.26 ml of PRP was mixed with an average of 19.25 ml of lipoaspirate (microfat) to obtain a final mix, which was injected sub-dermally and intradermally in different facial aesthetic units [15].

The operative time was reported in only one study with a range of 60-90 minutes per operation, while 11 studies did not report the operative time.

Two studies described additional surgical procedures performed before or in combination with fat grafting. These include phenol peel in one case report [14] and a more comprehensive surgical reconstruction in another case report, involving full-thickness skin graft and free buccal mucosal graft to the lower lip, free abdominal mucosal graft with V-Y mucosal advancement flap to lower lip, facial suspension to chin and lower lip, tensor fascia lata graft to chin, mental silicone implant, Z-plasty to lip, V-Y advancement flap to lip, and placement of a mental implant [7].

Outcome assessment:

Qualitative/quantitative objective assessment: Standard 2D photography was adopted in 5 studies; pre- and postoperative photographs were then subjectively graded by physicians using non-validated tools. In one study, 3D imaging was used (3dMD Torso System), and volumetric analysis was performed using a designated software (Vultus) to objectively quantify the volumetric retention rate of the implanted fat and its survival over time [1].

A direct measurement of mouth opening utilizing a digital caliper was performed measure in 8 studies. These included heterogeneous measurements, such as the maximum interincisal distance (8 studies), distance between the angles of the mouth (2 studies), mouth perimeter (1 study), lip thickness (1 study), and other methods not specified (1 study).

Skin assessment was performed by evaluating different parameters: skin hardness using a handheld digital durometer in one study; skin elasticity with cutometry (Cutometer Dual MPA 580, Courage & Khazaka Electronic GmbH, Cologne, Germany) in two studies; skin

elasticity with a non-specified skin suction elastometer in one study; and skin fibrosis (collagen pattern and content) using a Reviscometer in one study.

In two studies, punch biopsies were performed; one consisted of lip biopsy assessing keratosis and fibrosis, while in the other, the samples were harvested in the lip commissure to assess dermo-epidermic junction flattening and microvascular density by counting the absolute number of CD31+ and CD34+ vessels per high-power field.

Vascularization was assessed with videocapillaroscopy of the lower lip using a computerized system called Videocap 200-DS (Medigroup) in one study; and with videodermatoscopy of the upper left lip to assess capillary density and vasal ectasia in one study.

Mouth dryness, or xerostomia, was assessed in one study using the sugar test, which consisted of measuring the time required to melt sugar without crunching it.

Physician-based assessment: This included mainly non-validated grading of clinical 2d photographs (3 studies): one as none/mild/moderate/severe; one as worsening/no improvement/some improvement/much improvement; and another with a VAS from 1 (no improvement) to 10 (maximum possible improvement).

The Modified Rodnan Skin Score (mRSS) was also used to evaluate skin thickness in two studies. In seven studies, a physician-based assessment was mentioned but not further specified.

Patient-based assessment: Mouth function assessment was included in seven studies: in one study with a non-validated VAS for mouth opening (0-100), and in six studies it was measured with the validated Mouth Handicap in Systemic Sclerosis score. Of these, only six studies reported the pre- and post-operative MHISS score. Overall, the average MHISS score reported in five studies was 30.89 ±5.74 pre-operatively, and 22.1 ±6.75 postoperatively, with an overall improvement of 8.79 ± 3.93 points ($p < 0.001$).

The majority of included studies (8 out of 12) assessed the patient satisfaction, even though none of the included tools had been previously validated: one study used a 3-point scale on degree of improvement (0: unsatisfied, 1: somewhat satisfied, and 2: very satisfied); in three studies a 4-point scale on degree of satisfaction was used (unsatisfied, mildly/moderately satisfied, rather satisfied, and very satisfied); one study adopted a 10-point scale with “1” being the lowest and “10” being the highest level of satisfaction; while in three studies details were not provided.

Pain was considered an outcome assessment in two studies; in one study, it was measured with a VAS and short-form McGill Pain Questionnaire (SF-MPQ), while in the other with a VAS (0–100) on pain induced by the palpation of the masseter and temporal muscles and another VAS (0–100) on facial pain.
In two studies, the perception of disability was quantified using the validated Health Assessment Questionnaire-HAQ.
Other quality of life measures included (in one study) were the DAS 24 (satisfaction with appearance), HADS (anxiety and depression), BFNES (preoccupation with other people's judgement), and VAS (0-10) for perceived noticeability of disfigurement.
Mouth dryness was investigated in only one study using the validated Xerostomia Inventory Questionnaire and VAS (0–100) for sicca syndrome.

DISCUSSION

This systematic review of the literature on the use of therapeutic approaches involving ASCs-based therapies showed high potential of these techniques for facial SSc. Considering the overall high effectiveness reported of 87.3% (152/174 patients), fat grafting ca be considered a valuable minimally invasive treatment to ameliorate the effects of facial scleroderma. The improvement was particularly evident in the mouth function ($p < 0.001$ across 5 studies).
The effectiveness reported was also supported by an overall contained complication rate, which was limited mainly to general post-surgical events common to all surgical procedures, such as bruising, edema, pain. Intervention (non-surgical) was required in only 0.57% of cases (1 patient out of 174) for a reported infection, and it was solved with antibiotic administration. Unfortunately, the majority of the studies reviewed did not disclose the percentage or actual number of complications; therefore, an accurate calculation of the complication rate was challenging.

With this surgical technique, multiple facial aesthetic units can be targeted both to enhance the soft tissue bulk and ameliorate fibrosis, improving mouth function and overall skin quality. Among the included studies, the amount of injected fat varied significantly in the oral area: on average the mouth injection was 10 ml with a SD of 7.63 ml, ranging between 4 and 16

ml. Conversely, the amount injected in the other facial aesthetic units was more consistent among different studies: nose 1.2 ml ± 0.3; cheeks 3.9 ± 1.8 ml; chin 1.9 ± 0.8 ml; marionette lines 2.9 ± 0.7 ml; and nasolabial fold 0.9 ± 0.3 ml.

Overall, the combined volumetric and anti-fibrotic effects exerted on the different aesthetic units of the face contributed to the improvement of mouth function and positively affected the overall quality of life of SSc patients. Across the included studies, this positive effect was attributed mainly to the ASCs; however, ASCs-mediated anti-fibrotic effects are not clearly understood yet. Only in one study ASCs were extracted, characterized, and implemented in clinical use; yet, but no statistically significant was noted between the ASCs group and the fat grafting group [12]. Further molecular studies are required to understand its mechanism of action.

Multiple considerations can be drawn from the results of this systematic review. The first regards the surgical technique adopted, which should be standardized as much as possible because a change in any of the passages of harvesting, processing, or injecting adipose tissue can alter the final by-product, adding variables, and making the surgical outcome less predictable. Among the included studies, we found that only three studies adopted the same technique, which was the pure lipostructure technique described by Coleman. The majority of the studies adopted either modification of the technique (i.e., blunt cannula connected to a 60 ml syringe instead of 10 ml) or a different method (i.e., microfat grafting technique or washing).

Another aspect regards the outcome assessment. There is a high variability in the methodology implemented to assess the outcome in studies published so far, making the results not comparable. A consensus on a core outcome set (COS) to assess SSc should be reached, and researchers/doctors carrying out future studies should be encouraged to adopt the same methodology to allow future comparisons and meta-analyses. Volumetric augmentation is one of the main indications for this technique; yet, only one study assessed this aspect using a validated 3D imaging system. 3-D imaging should be implemented as an essential component in future studies to objectively assess the retention rate over time, given that the durability of the effect remains the main unanswered question. Another

important aspect is fibrosis improvement, which was assessed only in three studies using different qualitative/quantitative methods (i.e., cutometer or histological features). The use of such methodologies should be validated in SSc patients and potentially implemented in future studies. Finally, as microstomia improvement represents one of the main endpoints achievable with this technique, standardization of mouth opening measurement is required. Out of 12, 9 studies included direct measurement of mouth opening performed with a digital caliper. However, this methods does not produce valuable results because it does not consider the soft tissue distention that is improved with soft tissue fat grafting [18]; therefore, alternative and more effective methods should be used in future studies.

Drawing conclusions from the studies published to date to inform clinical practice is still premature for a number of reasons. First, the body of evidence is highly fragmented, with disagreement in the surgical technique used and outcome assessment, making results from different studies often not comparable; the level of evidence is overall low or very low, with lack of RCTs; and the risk of bias of published studies is overall medium to high.

RCTs are urgently needed to rule out a potential placebo effect, which is usually high in cohort studies using mainly PROMs to assess the outcome.

CONCLUSIONS

Multiple studies included in this systematic review have reported innovative and effective interventions to correct form and function in facial SSc. However, studies published thus far present limitations, like small study power, heterogeneous outcome assessment (often implementing non-validated tools), and short-term follow-up.

The definitive durability of the effect is still unclear, and the optimal number of treatments must be determined to define a precise evidence-based protocol.

RCTs are required to confirm these results, and molecular studies are encouraged to clarify the mechanism of action.

REFERENCES

[1] Almadori A, Griffin M, Ryan CM, Hunt DF, Hansen E, Kumar R, Abraham DJ, Denton CP, Butler PEM. Stem cell enriched lipotransfer reverses the effects of fibrosis in systemic sclerosis. PLoS One. 2019 Jul 17;14(7):e0218068. doi: 10.1371/journal.pone.0218068. PMID: 31314805; PMCID: PMC6636710.

[2] Ho YY, Lagares D, Tager AM, Kapoor M. Fibrosis—a lethal component of systemic sclerosis. Nat Rev Rheumatol. 2014;10:390–402. pmid:24752182

[3] Almadori A, Butler PE. Scarring and Skin Fibrosis Reversal with Regenerative Surgery and Stem Cell Therapy. Cells. 2024 Mar 3;13(5):443. doi: 10.3390/cells13050443. PMID: 38474408; PMCID: PMC10930731.

[4] Page M J, McKenzie J E, Bossuyt P M, Boutron I, Hoffmann T C, Mulrow C D et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews BMJ 2021; 372 :n71 doi:10.1136/bmj.n71

[5] Clavien PA, Barkun J, de Oliveira ML, Vauthey JN, Dindo D, Schulick RD, de Santiba.es E, Pekolj J, Slankamenac K, Bassi C, Graf R, Vonlanthen R, Padbury R, Cameron JL, Makuuchi M. The Clavien-Dindo classification of surgical complications: five-year experience. Ann Surg. 2009 Aug;250(2):187-96.

[6] Strong AL, Adidharma W, Brown OH, Cederna PS. Fat Grafting Subjectively Improves Facial Skin Elasticity and Hand Function of Scleroderma Patients. Plast Reconstr Surg Glob Open. 2021;9(1):e3373. Published 2021 Jan 25. doi:10.1097/GOX.0000000000003373

[7] Jeon FHK, Griffin M, Varghese J, Butler PEM. Oro-facial fibrosis in systemic sclerosis: a reconstructive journey. BMJ Case Rep. 2020 Oct 10;13(10):e236663. doi: 10.1136/bcr-2020-236663. PMID: 33040038; PMCID: PMC7552801.

[8] Pignatti M, Spinella A, Cocchiara E, Boscaini G, Lusetti IL, Citriniti G, Lumetti F, Setti G, Dominici M, Salvarani C, De Santis G, Giuggioli D. Autologous Fat Grafting for the Oral and Digital Complications of Systemic Sclerosis: Results of a Prospective Study. Aesthetic Plast Surg. 2020 Oct;44(5):1820-1832. doi: 10.1007/s00266-020-01848-2. Epub 2020 Jul 6. PMID: 32632623.

[9] Gheisari M, Ahmadzadeh A, Nobari N, Iranmanesh B, Mozafari N. Autologous Fat Grafting in the Treatment of Facial Scleroderma. Dermatol Res Pract. 2018 Aug 1;2018:6568016. doi: 10.1155/2018/6568016. PMID: 30154838; PMCID: PMC6093005.

[10] Blezien O, D'Andrea F, Nicoletti GF, Ferraro GA. Effects of Fat Grafting Containing Stem Cells in Microstomia and Microcheilia Derived from Systemic Sclerosis. Aesthetic Plast Surg. 2017 Aug;41(4):839-844. doi: 10.1007/s00266-017-0904-1. Epub 2017 Jun 8. PMID: 28597066.

[11] Del Papa N, Caviggioli F, Sambataro D, Zaccara E, Vinci V, Di Luca G, Parafioriti A, Armiraglio E, Maglione W, Polosa R, Klinger F, Klinger M. Autologous fat grafting in the treatment of fibrotic perioral changes in patients with systemic sclerosis. Cell Transplant. 2015;24(1):63-72. doi: 10.3727/096368914X674062. PMID: 25606975.

[12] Onesti MG, Fioramonti P, Carella S, Fino P, Marchese C, Scuderi N. Improvement of Mouth Functional Disability in Systemic Sclerosis Patients over One Year in a Trial of Fat Transplantation versus Adipose-Derived Stromal Cells. Stem Cells Int. 2016;2016:2416192. doi: 10.1155/2016/2416192. Epub 2016 Jan 5. PMID: 26880939; PMCID: PMC4736419.

[13] Virzi. F, Bianca P, Giammona A, Apuzzo T, Di Franco S, Mangiapane LR, Colorito ML, Catalano D, Scavo E, Nicotra A, Benfante A, Pistone G, Caputo V, Dieli F, Pirrello R, Stassi G. Combined platelet-rich plasma and lipofilling treatment provides great improvement in facial skin-induced lesion regeneration for scleroderma patients. Stem Cell Res Ther. 2017 Oct 23;8(1):236. doi: 10.1186/s13287-017-0690-3. PMID: 29058626; PMCID: PMC5651639.

[14] Ramon Y, Fodor A, Ullmann Y. Deep phenol peeling and fat injection: treatment option for perioral wrinkles in a scleroderma patient. Dermatol Surg. 2005 Jul;31(7 Pt 1):777-9. PMID: 16029706.

[15] Philandrianos C, Magalon J, Daumas A, et al. Combined PRP and microfat graft for facial disability in systemic sclerosis. Journal of Scleroderma and Related Disorders. 2017;2(3):e7-e11. doi:10.5301/jsrd.5000261

[16] Sautereau N, Daumas A, Truillet R, Jouve E, Magalon J, Veran J, Casanova D, Frances Y, Magalon G, Granel B. Efficacy of Autologous Microfat Graft on Facial Handicap in Systemic Sclerosis Patients. Plast Reconstr Surg Glob Open. 2016 Mar 22;4(3):e660. doi: 10.1097/GOX.0000000000000621. PMID: 27257590; PMCID: PMC4874304.

[17] Jeremy Howick, Iain Chalmers, Paul Glasziou, Trish Greenhalgh, Carl Heneghan, Alessandro Liberati, Ivan Moschetti, Bob Phillips, and Hazel Thornton. "The 2011 Oxford CEBM Evidence Levels of Evidence (Introductory Document)". Oxford Centre for Evidence-Based Medicine

[18] Griffin MF, Jeon FHK, Chew BJW, Bakir A, Denton CP, Butler PEM. Comparison of non-invasive methodologies to assess mouth opening following lipotransfer techniques to

reverse oral fibrosis. Clin Exp Rheumatol. 2020 May-Jun;38 Suppl 125(3):184. Epub 2020 Aug 27. PMID: 32865174.

Table 1. Inclusion and Exclusion Criteria

INCLUSION CRITERIA	EXCLUSION CRITERIA
Original studies involving humans where ASCs-BT used to treat a form of facial systemic sclerosis	Non original studies (review, comment, letter, note, conference paper)
Clinical trials, Prospective, Retrospective, Comparative studies, Case series, Case reports	Abstract not available / Inaccessible in full text
Animal studies assessing the mechanism of action using human derived ASCs-BT	Animal studies assessing the mechanism of action using non-human derived ASCs-BT
Publication in English, Spanish, French, Italian, German, Portuguese or translatable	Localised scleroderma and systemic sclerosis of other areas than the face

Table 2. Demographics information from papers Included in this Review

Author, Year	Country	Age Mean (Sd) or Median (Range)	Gender	BMI	Other Previous Surgery Performed	Time from diagnosis *(years)*
Almadori, 2019 [1]	UK	56 (±11.59)	61 F, 1 M	NR	NR	15 (±8.81)
Strong, 2021 [6]	US	48.7 (± 17.6)	F	NR	NR	19 (± 12.11)
Jeon, 2020 [7]	UK	42 year old	F	NR	Full-thickness skin graft to lower lip, free buccal mucosal graft to lower lip, free abdominal mucosal graft with V-Y mucosal advancement flap to lower lip, facial suspension with Mitek to chin and lower lip, tensor fascia lata graft to chin, mental silicon implant, Z-plasty to lip, V-Y advancement flap to lip, replacement of mental implant.	NR
Pignatti, 2020 [8]	Italy	55.9 (± 9.25)	19 F, 6 M	NR	NR	15.39 (± 60.5)
Gheisari, 2018 [9]	Iran	39 (± 8.32)	F	NR	NR	7 (±1.79)
Blezien 2017 [10]	US (New York)	46.28 (± 6.37)	F	NR	NR	10 (± 4.2)
Papa, 2015 [11]	Italy	35 (± 15)	F	NR	NR	11 (± 10)
Onesti, 2015 [12]	Italy	33 (± 8.46)	8 F, 2 M	NR	NR	9 (± 5.45)
Virzi, 2017 [13]	Italy	41 to 63	3 F, 2 M	NR	NR	9 (± 7.09)
Ramon, 2005 [14]	Israel	64	F	NR	Deep phenol peel	20
Philandrianos, 2017 [15]	France	58 and 64	F	19.2 and 22.9	NR	NR
Sauterau, 2016 [16]	France	53.8 (±9.6)	F	22.6 (±2.4)	NR	9.4 (± 6.7)

Abbreviations:
NR *Not reported*
F *Female*
M *Male*

Table 3. Study details

Author, Year	Sample Size	No. of Treatment	Interval Between Treatments (months)	Length of F-Up (months)	Loss to F-Up (% and number)	Patients Improved (% and number)	Complication(s) and %	Complication(s) Grade	Qualitative / Quantitative Outcome Assessment	Physician-Based Outcome Measure(s)	Patient-Based Outcome Measure(s)
Almadori, 2019 [1]	62	2,96	NR	12,41	NR	100%	Infection recepient site wound, treated and solved with antibiotic 1 patient (1.61%)	2	a) 3dmd imaging (3Dmd and Vultus software); b) 2D standard photography; c) in-vitro analysis	2D photograph evaluation	a) MHISS (mouth function); b) quality of life: DAS 24, HADS, BFNES,VAS
Strong, 2021 [6]	10	1,7	NR	6,2	NR	100%	Pain, bruising, swelling at donor site and minor bruising at the recipient site	1	Photographs	N/A	Qualitative interviews
Jeon, 2020 [7]	1	14	6,91	2,25	0	100%	NR	n/a	Mouth openning	N/A	Not reported
Pignatti, 2020 [8]	25	2,41	6	6	NR	88.8%	Perioral ecchymosis	1	a) Mouth opening (interincisal) b) Photographs, c) Sialometry	Skin sclerosis (modified Rodnan Skin Score)	a) Pain with VAS and SF-MPQ), b) perception of disability HAQ and MHISS
Gheisari, 2018 [9]	16	1	NA	3	0	80%	Bruising at zone of harvest 10 patients (62.5%)	1	a) Mouth opening capacity b) Skin biophysical properties (Reviscometer to measure changes in the collagen pattern and content). c) Photographs	a) Skin sclerosis (Rodnan skin score) b) 2D photograph evaluation	a) mouth function with MHISS b) Global patients' satisfaction
Blezien 2017 [10]	7	1	NA	12	0	NR	Graft area oedema; Harvesting site ecchymosis;	1	a) labial biopsy samples; b) photographs; c) mouth opening	N/A	MHISS

							Post-operative pain persistent for more than 3 days				
Papa, 2015 [11]	20	1	NA	3	0	NR	NR	n/a	a) Skin biopsies b) Skin hardness with durometer c) Videocapillaroscopy	N/A	Non validated semiquantitative score on satisfaction with procedure (1 to 5)
Onesti, 2015 [12]	10	2	3	12	N/A	100%	NR	n/a	Mouth opening	a) VAS 1-10 for improvement	a) MHISS b) Non validated semiquantitative score on satisfaction with procedure (1 to 3)
Virzi, 2017 [13]	6	1	NA	6	0	100%	NR	n/a	a) Mouth opening b) cutaneous elasticity (elastometer) c) Vascularization (videodermatoscope)	Non validated clinical observation	Non validated semiquantitative score on satisfaction with procedure (1 to 10)
Ramon, 2005 [14]	1	1	NA	18	0	100%	None	n/a	none	Non validated clinical observation	Non validated score on satisfaction with procedure
Philandrianos, 2017 [15]	2	1	NA	12	0	100%	0 None	na	Mouth opening	(Non validated clinical observation	a) MHISS b) Non validated score on satisfaction with procedure

Sauterau, 2016 [16]	14	1	NA	12	0	91.6%	Bruising 8 patients (57.1%) Pain 4 patients (28.5%) Perioral sensitive manifestation 1 patient (7.14%)	1	a) Mouth opening b) skin elasticity (Cutometer) c) xerostomia (sugar test)	2D photograph evaluation	a) Mouth with MHISS and VAS for mouth opening (0-100); b) Xerostomia with xerostomia Inventory questionnaire and VAS (0–100) for sicca syndrome c) Pain induced by the palpation of masseters and temporal muscles (VAS 0–100) and facial pain (VAS 0–100) d) Non validated semiquantitative score on satisfaction with procedure (1 to 4) e) Global disability: HAQ adapted to SSc

Table 4. Details of the surgical procedure

Author, Year	Amount Injected	Donor Site	Recipient Site	Comparator Groups	Fat Harvesting Method	Fat Processing Method	Fat Injection Method	Product Used	Operative Time
Almadori, 2019 [1]	10,2	Abdomen or thighs	face (nose; cheeks; chin; nasolabial folds; upper lip; lower lip)	a) lcSSc versus dcSSc; b) 1-2 treatments versus 3+ treatments; c) immunosuppressed versus not immunosuppressed	Coleman technique - 15 cm x 3 mm disposable cannula connected to a 10cc Luer Lock syringe	Centrifugation at 3000rpm for 3 mins	1ml Luer-Lock syringes connected to 9 cm by 2mm blunt disposable cannulae	Fat	Not reported
Strong, 2021 [6]	19,2	Abdomen or thighs	Face (lips, nasolabial folds, malar regions, marionette lines)	Not reported	3.0mm liposuction cannula connected 60 ml syringe	Centrifugation at 3000 rpm for 3 minutes	3ml syringe. 1) face: small incisions were made with 11-blade along lateral commissures and 0.1-0.2 ml aliquots of fat were injected. 2) Hands: small aliquots injected over the dorsum of hand	Fat	Not reported
Jeon, 2020 [7]	10	Abdomen	Upper and lower lips, nasolbial folds, cheeks and chin	No comparison	cannula connected to a 10 mL Luer Lock syringe	Centrifugation at 3000 rpm for 3 mins, oil and blood discarded.	Fat injected using cannula connected to 1 ml syringes	Fat	Not reported
Pignatti, 2020 [8]	16	Flanks or trochanteric area	Mouth, hands	No comparison	Tumescent infiltration of donor areas with modified Klein solution (50 ml saline solution, 0.5 ml 1:1.000 adrenalin and 10 ml 2% mepivacain) through epidural 23 guage needle attached to a 20 ml Luer-lock syringe.	Centrifugation at 3 mins at 3000 rpm/1900 RCF	19 gauge needle to introduce a Coleman injection cannula connected to 2.5 ml syringes	Fat	Not reported

					Harvesting cannulaconnected to 10 ml Luer lock syringe				
Gheisari, 2018 [9]	27	Trochanteric area, flank, periumbilical, buttock	perioral, upper lip, lower lip, buccal, malar, periorbital	No comparison	Entry points for the infiltration cannula were anesthetised with 1 ml of pure lidocaine with a 30-gauge needle. Then, 500 ml of tumescent solution (normal saline, 25 ml lidocaine 2%, 0.5 ml epinephrine 1:1000) was infiltrated in the selected donor area with a 1.5 mm cannula. 3 mm blunt cannula connected to 10 ml a Luer-lock syringe	Sedimentation by gravity for 10 minutes. Oil and blood excess were eliminated and the remaining fat was collected.	1 ml syringe directly injected into the face using disposable 18-gauge cannulas	Fat	Not reported
Blezien 2017 [10]	3	Abdomen	Face (lips)	No comparison	Multi-perforated cannulas (around 0.5–0.7 mm).	Sedimentation by gravity for 10 minutes, oil and blood excess eliminated and then platelet rich plasma was added.	Multi-perforated cannulas (around 0.5–0.7 mm)	Fat	Not reported
Papa, 2015 [11]	12	Trochanteric area, preiumbilical abdominal region	upper lip, lower lip, mouth corner	No comparison	Tumescent infiltration of 150 ml of local modified Klein solution (containing 100 ml of saline, 20 ml of mepivacaine 2%, 20 ml of ropivacaine 7.5 mg/ml, 1 ml of epinephrine, and 5 ml of sodium bicarbonate solution 1 mEq/ml). Cannula was connected with a luer-lock syringe	Centrifugation at 700 × g for 3 min	Blunt cannula (Coleman Style II, 9 cm × 17 ga)	Fat	Not reported

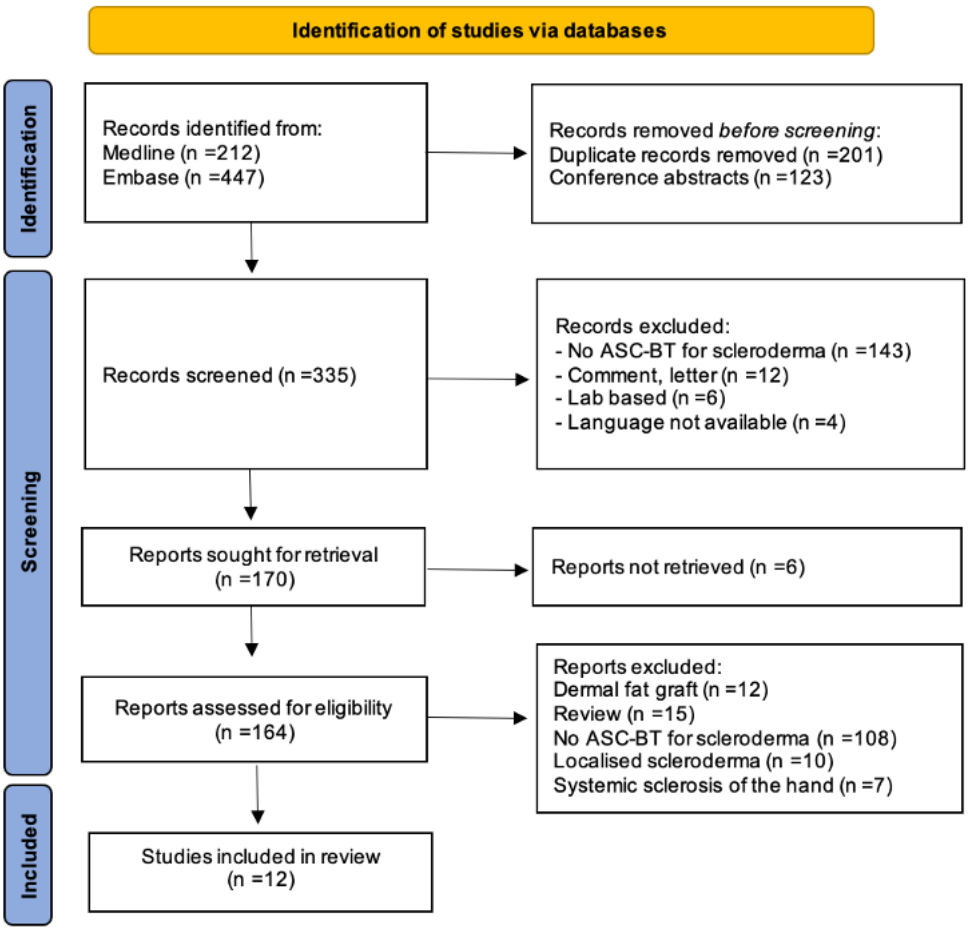
Onesti, 2015 [12]	16	periumbilical abdominal region	upper and lower perioral region	Fat transplatation and adipose-derived stromal cells - (5 treated with fat, 5 with ASCs)	Local modified Klein solution, 1 liter of sodium chloride 0.9%, 20 mL of lidocaine 2%, and 1 mL of epinephrine 1:200,000 at donor site. 3mm blunt cannula attached to a 10 cc Luer- lock syringe	1) Fat transplantation: decantation for 15 minutes and only the layer containing adipocytes was used for fat injection; 2) ADSC: Lipoaspirate cell cultivation within 1 hour of isolation. Primary cultures of ADSC expanded following guidelines of current GMP.	1) Fat transplantation: blunt injection cannula of 2 mm in diameter; 2) ADSC: 2 ml syringe with 30-guage 1/2 needle, cell transferred by 4 ml of hyaluronic acid for each patient	Fat, adipose derived stromal cells	Not reported
Virzi, 2017 [13]	24		Perioral and malar areas	Adipose-derived mesenchymal stem cells (AD-MSCs) and PRPs	Local infiltration of 150 ml of Klein solution. 10-gauge cannula connected to a 10-ml syringe with luer-loc	Centrifugation for 5 min at 2700 rpm	After 10 min, in the same PRP injection spot, the lipotransfer was performed with a 15-gauge infiltration cannula. Patients remained under observation for 24 hours.	Adipose-derived mesenchymal stem cells	Not reported
Ramon, 2005 [14]	4	lower abdomen	perioral area	na	o.5% lidocaine, adrenaline (1:100.000), 0.8% bicarbonate for infiltration	not described.	not described	fat	Not reported
Philandrianos, 2017 [15]	19,25	INNER thighs	cheekbones, the nasogenian grooves, superior and inferior lips and the chin	na	lidocaine mixed with NaCl 0.9% solution. 14-Gauge cannula with eight 600 µm orifices from St'rim kit® (Thiebaud Biomedical Device, France).	A closed system was used with anti-return valves, 10 mL syringe and a 50 mL PureGraft® filtration technology device (Pure- graft LLC, USA). Fat was mixed with prp - PRP preparation was performed using the SkinPras® de- vice (Soluciones Biogeneratives, Spain). Briefly, peripheral blood was collected by venipuncture using a 20 mL syringe anticoagulated with 10% ACD-A (9). Blood was transferred in a secondary device and centrifuged (Omnigrafter, Soluciones Biogeneratives) at 3200 rpm over 10 minutes. PRP was col- lected in a 10 mL	21-gauge (0.8mm) needle, which was then replaced by a cannula of the same diameter	microfat + prp (26% PRP and 74% microfat containing 53 million of platelets/ mL in case 1 and 30% PRP and 70% microfat containing 17.6 million of platelets/	Not reported

						syringe. A 0.5 mL sample was used for qual- ity controls.		mL in case 2)	
Sauterau, 2016 [16]	16.3	inner side of the knees, abdomen and hips.			In local anesthesia entry points for the infiltration cannula were anesthetized with pure 1% adrenaline and lidocaine with a 30-gauge (0.25mm) needle. An infiltration was then carried out in the area with a 14-gauge cannula with 10ml of 1% adrenaline and lidocaine diluted in 20ml of physiological salt solution, with an injected volume at each entry point of 0.5ml. 14-gauge (2-mm cannula height holes of less than 1-mm blunt tip) connected to a 10-ml syringe	closed-circuit PureGraft 50 ml system filtration pocket (Puregraft, San Diego, Calif.).	21-gauge (0.8mm) needle, which was then replaced by a cannula of the same diameter.	microfat	60 to 90 minutes

Table 5. Level of Evidence

Author, Year	Study Design	Loe	Stenght of Recommendations	Risk Of Bias Rate
Almadori, 2019 [1]	Retrospective	3	Moderate	Moderate
Strong, 2021 [6]	Retrospective	3	Low	Serious
Jeon, 2020 [7]	case report	5	Moderate	Moderate
Pignatti, 2020 [8]	Prospective	4	Moderate	Moderate
Gheisari, 2018 [9]	Open-label study, prospective	4	Moderate	Moderate
Blezien 2017 [10]	Prospective	4	Low	Low
Papa, 2015 [11]	Prospective	4	Moderate	Moderate
Onesti, 2015 [12]	Prospective	4	Moderate	Moderate
Virzi, 2017 [13]	Prospective	4	Moderate	Moderate
Ramon, 2005 [14]	Case report	5	low	high
Philandrianos, 2017 [15]	case series	5	moderate	Moderate
Sauterau, 2016 [16]	open-label study (?)	4	moderate	Moderate

Figure 1. Prisma Flowchart



The flowchart illustrates the screening process to select the papers to be included in this systematic review.