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Original Article

Autologous fat grafting and adipose-derived stem cells therapy for acute burns and burn-related scar: A systematic review

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Abstract

Objectives: The objective of this study was to analyze all available research on the application of autologous fat grafting (AFG) and adipose-derived stem cells (ADSC) to present evidence-based recommendations, particularly in the clinical treatment of acute burns and burn-related scars. **Materials and Methods:** We conducted a systematic search of PubMed, COCHRANE, and EMBASE, as well as a manual search of previous reviews' reference lists up. The risk of bias (RoB) was assessed using RoB 2.0 and ROBINS-I, where appropriate. **Results:** Six eligible studies were selected (2 randomized clinical trials [RCT], 1 retrospective cohort, and 3 experimental studies) with subjects ranging from 3 to 100. Only one study evaluated the use of AFG for acute burns. Improvements in wound healing, vascularization, scar characteristics, and tissue architecture were generally observed in some studies, supported by molecular markers, while one study reported nonsignificant results. Subjective patient satisfaction was reported to have improved. Functional outcomes improvement in the treated regions was minimal. However, study heterogeneity arose mainly from treatment protocols. Cautious results interpretation due to potential bias, especially in selection and confounding domains, and limited clinical trials are important to note. More studies are needed to evaluate. **Conclusion:** AFG and ADSC hold potential as valuable treatment options for burn-related scars, supported by a body of evidence, but further well-designed RCT are needed. The efficacy of acute burn settings is yet to be further evaluated since evidence is limited.

Keywords: *Acute burns, Adipose-derived stem cells, Autologous fat grafting, Burn wound, Burn-related scars*

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Introduction

Autologous fat grafting (AFG) has become a widely utilized method for addressing deficiencies in volume and contour in both aesthetic and reconstructive surgical procedures. It encompasses the autologous transfer of fat from one anatomical region to another within the same individual. Beginning in the early 1900s, AFG has gained significant popularity since 1974 due to the era of liposuction techniques, which increased the availability of fat for grafting purposes [1,2].

The main purpose of AFG is to promote the restoration and enhancement of tissue through harnessing the regenerative potential of fat cells. The stromal vascular fraction (SVF) derived from processed fat grafts comprises multipotent stem cells that exhibit gene expression patterns affiliated with diverse tissue development processes, including the formation of fat cells (adipogenesis), osteogenesis, and chondrogenesis. These cells have demonstrated angiogenic, immunomodulatory, and antiapoptotic properties, making them valuable in the rejuvenation of tissues. Furthermore, adipose-derived stem cells (ADSC) contained in AFG contribute to the process of opposing aging and promoting skin rejuvenation through the formation of tissue layers comprising the hypodermis, dermis, and epidermis [3].

The AFG has demonstrated practical applications beyond mere aesthetic enhancements. It is used successfully in cases such as improving radiation-induced skin damage [4], facilitating wound healing, promoting scar remodeling [5],

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and managing burn injuries. However, while ample evidence supports the efficacy of AFG in aesthetic and reconstructive cases, there is a need for a comprehensive analysis of its specific utility in the context of burn wound treatment. Burn trauma itself results in over seven million incidents of serious injury and exceeds 265,000 mortalities every year worldwide [6]. The most recent systematic review on the subject was conducted over 5 years ago [7], highlighting the necessity to update our understanding of the current state of the art in employing AFG for burn injuries. Moreover, the majority of prior investigations were conducted in preclinical settings (animal studies), thereby presenting an inadequate amount of clinical evidence despite the potential of AFG and ADSC. The objective of this study is to carry out a comprehensive analysis that will serve as an invaluable reference for health-care professionals and researchers involved in the clinical implementation of AFG and ADSC to treat burn wounds and facilitate burn-related scar remodeling.

Materials and methods

This systematic review was reported in accordance with the Preferred Reporting Items for Systematic Review and Meta-Analysis 2020 statement [detailed checklist in Supplementary File S1]. Ethical clearance was exempt due to the nature of this systematic review, which did not include patient-level data.

Searching strategy

A thorough and systematic search was carried out across three databases: MEDLINE (via PubMed), CENTRAL (via Cochrane), and EMBASE up until July 2023 (The search was conducted on July 1, 2023). Additional articles were manually searched from the reference list of previously available reviews. The search queries for each database are described in Supplementary File S2. Two reviewers (FEL and CAW) did the search process independently, while the other two reviewers (IDS and LH) subsequently did a double-check to ensure accuracy. Any disagreements were solved in discussion until an agreement was reached.

Eligibility criteria

Any clinical studies evaluating the use of any types of AFG and ADSC on burn injury (acute and chronic wound, as well as burn-related scars) treatment were included. The exclusion criteria are as follows: wound or scar unrelated to burn injury, burn injury other than skin, nonoriginal articles, single patient on a case report, no full-text access, and written in a language other than English or Indonesia.

Selection process and data extraction

Four assessors in pairs (FEL with IDS, CAW with LH) separately reviewed titles and abstracts. Any inconsistencies were solved by discussion until consensus was obtained. The same process was used for full-text assessment. Extracted data included (1) identification: author, year of publication, study design, location; (2) subject characteristics: number of subjects, age, sex proportion, burn degree, burn area, type of wound (acute/chronic) or scar; (3) treatment: type of therapy, methods, administration, dosage; and (4) outcomes. From our preliminary analysis of the included studies, we observed inconsistencies and a variety of outcomes in every report; therefore, we categorized and described outcomes into clinical and nonclinical outcomes. No quantitative analysis was done due to the heterogeneity of reported outcomes. For each recorded outcome, GRADE level of certainty of evidence was applied.

Risk of bias assessment

Risk of bias (RoB) for clinical studies was assessed by two teams of impartial assessors. Cochrane RoB 2.0 was implemented for randomized clinical trials (RCT), while ROBINS-I was intended for non-RCT [8].

Results

A total of ninety articles were identified through a systematic search, with the addition of one article obtained from manual searching. In contrast to the most recent systematic review conducted in 2015, a greater number of clinical trials have been initiated; however, only a limited number of trials have disseminated their findings through publication. Although more studies were accessible, unfortunately, those were documented in Chinese. The final study flowchart is depicted in Figure 1. At the end, six articles were included in the systematic review. A meta-analysis did not take place due to the heterogeneity of study design, treatment modalities, and reported outcomes. Out of the six studies included in the analysis, only two were RCT [9,10], three were experimental, and the remaining was a retrospective cohort design [11-13]. The detailed characteristics and outcomes of the conducted studies are outlined in Table 1. Table 2 shows the depiction of AFG harvesting sites, harvesting methods, processing procedures, and administration.

Five studies focused on the Western population, and only one took place in Egypt, with a diverse sample size ranging from 3 to 100 subjects. One study was conducted exclusively on pediatric subjects [10]. Not all studies reported the degree and total body surface area (TBSA) of burn injuries. Only one study went ahead to assess the impact of AFG on acute burn wounds [9], whereas the other studies evaluated the efficacy of AFG on chronic wounds spanning various stages of burn-related scar development.

The aforementioned studies demonstrated various outcomes related to AFG treatment. In relation to patient satisfaction and scar improvement, Bruno's study showed a significant increase in satisfaction scores following AFG intervention, while Padula's study reported a decrease in scar pigmentation, thickness, and pain. Both studies emphasized the positive impact of AFG on patient satisfaction and scar characteristics.

Regarding functional outcomes, Byrne's study observed a rise in overall active movement; nevertheless, no significant improvement in grip strength was observed after AFG treatment. Yet, Gal's study did not find any significant disparities between AFG and normal saline (NS) on physical examination. Abouzaid's randomized controlled trial (RCT) showed a reduced length of stay (LoS), frequency of surgery in the operating room, need for further skin grafting, and improved scar texture with AFG treatment compared to

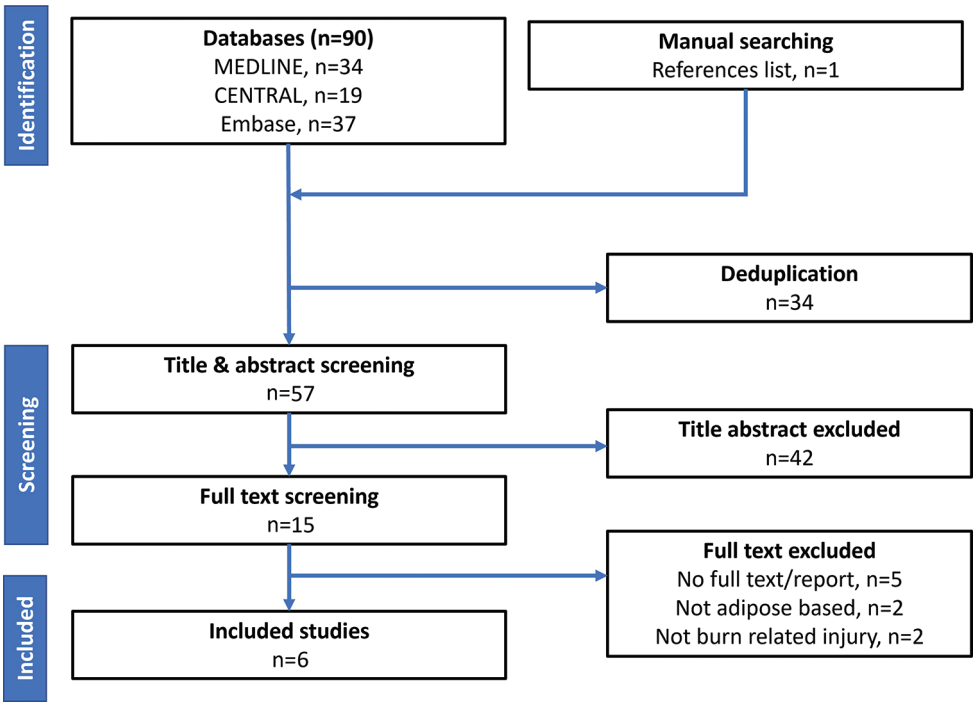

Figure 1: Preferred reporting items for systematic review and meta-analysis flowchart

conventional dressing. A summary of outcomes' GRADE level is presented in Table 3.

While all studies employed anesthetic infiltration before gentle lipoaspiration with minimal negative pressure to harvest fat, variations were noted in harvesting sites, processing methods, and transfer techniques. The harvesting sites mostly included the abdomen in addition to the hips, trochanter, inner thigh, medial knee, and lateral thigh. Still, some studies lacked specific information regarding the exact locations. Centrifuge speeds ranged from 1200 to 3000 rpm, and processing times varied from 3 to 5 min. The central layer of purified fat was transferred to syringes for injection in all cases, with syringe sizes ranging from 1 to 3 ml. One study explained handling excess oil using cotton patties during centrifugation [11]. Abouzaid *et al.* [9] applied topical nano fat dressing additionally, processing it by passing the fat back-and-forth using a Luer-lock connector and two syringes.

Risk of bias assessment

The detailed RoB for each study is portrayed in Figure 2, while the summary is portrayed in Figure 3. While available RCT studies have a low RoB, other studies (non-randomized) have some issues, especially in terms of confounding bias, due to no adjustment of probable confounders from patients' selection or statistical analysis. Some studies did not mention the recruitment method, although some suggest consecutive sampling, which raises the problem of selection bias.

Discussion

Advantages and downsides of using adipose-derived modalities for burn therapy

Adipose-derived modalities, such as AFG and ADSC, are readily available sources of tissue without the need for external grafts or synthetic materials, minimizing the risk of immune reactions or graft rejection. Thus, it may promote a more natural and integrated healing process. ADSC, which is naturally contained in harvested fat, has shown remarkable regenerative capabilities, promoting angiogenesis, immunomodulation, and tissue remodeling [14,16]. Besides that, adipose-derived modalities have the advantage of possessing a rich source of mesenchymal stem cells within the SVF. These multipotent cells can differentiate into various cell types, including adipocytes, osteoblasts, and chondrocytes, facilitating tissue repair and regeneration. Adipose tissue acts as a circulatory network, a cushion, and an insulator, supporting tissue viability and fostering wound healing. These processes are critical in wound healing, either acute or chronic [17].

This review indicated that, alongside the biomolecular improvement as demonstrated by the rise in proliferative markers and lowered inflammation, AFG improved clinical results and even patients' satisfaction, as Bruno's study [13] reported. Yet, we should note that in wound healing, the balance between proliferation and cell rest is important and dynamically changes over time and over layers. Histological examination also found the better architecture of the skin (collagen deposit, neoangiogenesis, and hyperplasia of the dermis), as in Padula's [12] report. Abouzaid's RCT further confirmed these findings and other improvements in major hospitalization-related clinical outcomes [9]. A small case series on three patients that was done by Klinger *et al.* [15] demonstrated mild asymmetry on MRI evaluation compared to healthy, unaffected area, suggesting the healing process after AFG on a scar is almost unnoticeable even on imaging.

Bryne's study gave insights into the improvement of some functional components of the affected hands.

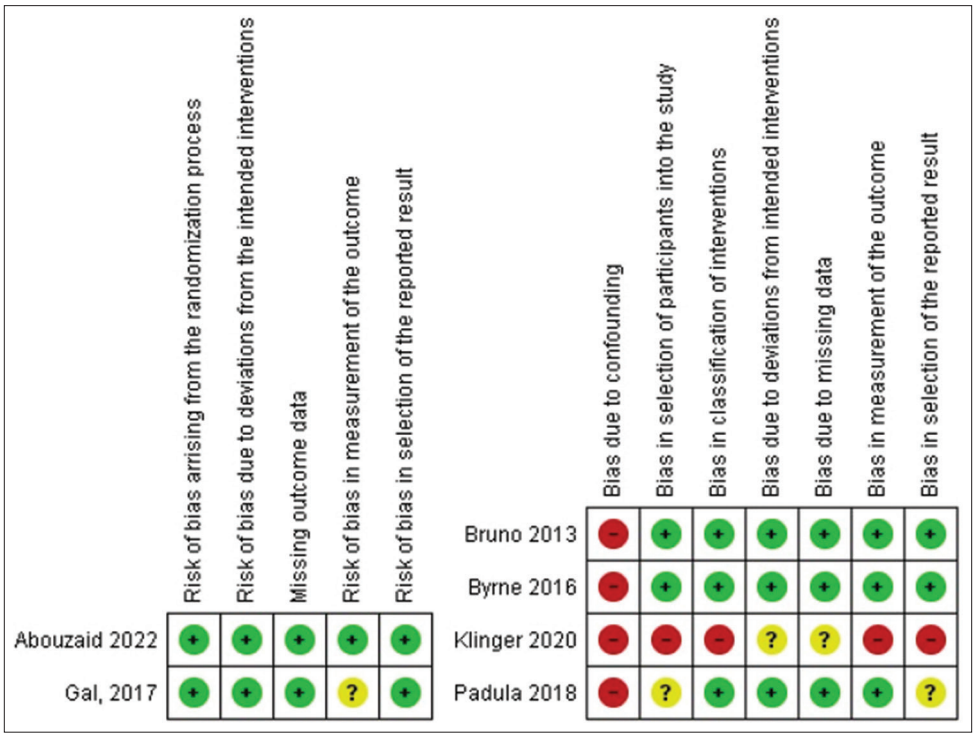

Figure 2: Risk of bias of individual studies (left: Randomized clinical trial; right: Non randomized intervention)

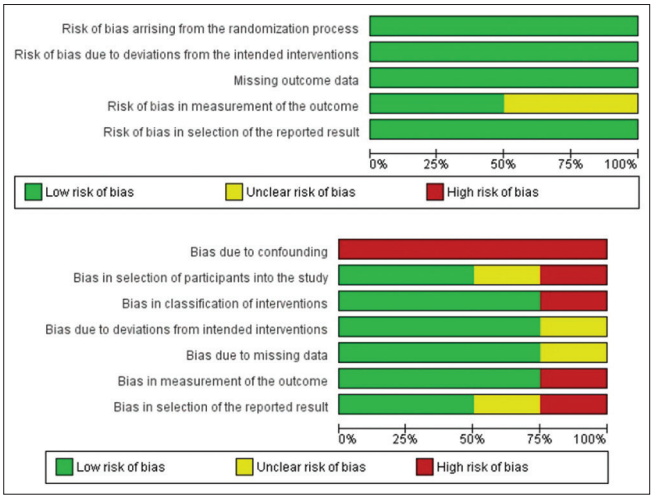

Figure 3: Summary of risk of bias assessment (top: Randomized clinical trial; bottom: Nonrandomized intervention)

Unfortunately, not all studies supported Bryne's suggestion that scar characteristics may be improved. However, Byrne's study's level of evidence is diminished by the fact that this was a single-arm experimental trial with a small sample size (13 subjects) [11]. The same caution should be applied to the nonsignificant result from Gal's study [10] due to the small sample size.

Several downsides are to be considered. The potential for donor site morbidity during adipose tissue harvesting for grafting (infection, bleeding, or irregular contours), the requirement of specialized techniques and expertise for processing and administration, which lead to increased complexity and cost, as well as the issue of longevity and long-term outcomes of adipose-derived modalities in burn therapy [3]. According to this analysis review, the mean follow-up durations range from 6 to 13.5 months, a relatively short time in the case of chronic wounds. It is important to note that the 1-year fat graft survival was only 72% in a study by Scotto di Santolo *et al*. Although it was not exclusively performed on burn-related scars [18]. Further investigation is needed to determine their efficacy over extended periods.

While the available studies demonstrated promising results, more studies are still required since only one large RCT is available to date. More RCTs are still running (NCT03686449, EUCTR2018-002870-27-DK, NCT02619851, and IRCT201202169044N1). Patient selection may serve as an important factor affecting treatment outcomes since the pilot study that was studied by Gal *et al*. [10] showed no difference between AFG and placebo (NS). Note that this study was centered on pediatrics; different wound healing processes may occur in addition to only a single AFG session, a small dose given, and surgeons' experiences. Burn degree and TBSA are related to future scar formation, either keloid (8%–67%) or contractures (38%–54%) [19]. These factors affect the treatment plan and efficacy.

Uniqueness of burn injuries and rationale of treatment

Wounds related to burn injury differ fundamentally from other traumatic wounds due to (1) a dysregulated immune and inflammatory response (characterized by elevated proinflammatory mediators and hypermetabolic state) and (2) the extensively involved skin surface, thus resulting in a higher chance of scarring [20,21]. Furthermore, it has a unique dimension of space (skin thickness involved) and time (evolution of burn injury). Irreversible coagulative

Table 1: Study characteristics and outcomes

Author, year; design (location)	Subject characteristic			Treatment		Evaluation period (month)	Outcomes	
	n; age; sex (male %)	Burn degree; % TBSA	Wound/scar age (years)	Modalities; dosage	Administration		Clinical	Nonclinical
Bruno *et al*., 2013; experimental (Italy) [13]	93; 43 (mean); NI	NI; NI	2.3 years scar	AFG versus none; NI	Subscar injection	Before treatment; 3 and 6 after treatment	The mean score of satisfaction increased from 31 to 64 to 95 (before, 3, and 6 months after treatment) Improvement in the Vancouver scar scale from 41 to 29 to 15 (before, 3, and 6 months after treatment)	Negative expression of S-100, langerin, VEGF, β-catenin, TGF-β, and P63 6 months after treatment Positive expression of Ki-67 and P-53 6 months after treatment
Byrne *et al*., 2016; Cohort retro (UK) [11]	13; 40.2 (mean); 30.70%	Mixed to full thickness; 4.5%–85%	2.3 years scar, with history of previous surgery	AFG; NI	Subdermal injection	Average of 9.1	Improvement in total active movement: 147–170; no significant improvement in grip strength No improvement for all components of the MHQ of the same hand after treatment No significant differences in MHQ compared to healthy hands No change in DASH score No change in scar characteristics of itch and pain	NA
La Padula *et al*., 2018; experimental (France) [12]	1424; 33.7 (mean); 62.50%	Deep to full thickness; NI	2–39 years scar, with history of previous surgery (mean of 4.3 times)	AFG and CO_2 laser; 30 ml (mean) divided in 2 sessions	Dermal-hypodermal injection	13.5	Reduction of thescar pigmentation, thickness, and pain (questionnaire) Better postoperative versus preoperative score (34.6 *vs*. 22.6; $P<0.00001$)	New collagen deposit, neoangiogenesis, dermal hyperplasia
Gal *et al*., 2017; RCT (USA) [10]	8; 13 (mean); 37.50%	NI; NI	Chronic wound and scars (unspecified age)	AFG versus NS; 1 ml per 3.5 cm^2	Intrascar injection	8	No difference between NS and AFG (pigmentation, vascularity, and height of scar)	NA
Klinger *et al*., 2020; experimental (Italy) [15]	3; NI; NI	NI; 5%–40%	4–33 years scar	AFG versus none/ healthy area; NI (two separated procedure sper 3 months)	Dermal-hypodermal junction	6	Improvement on mimic features, skin texture, softness, thickness, and elasticity	Micros: Preserved architecture, collagen deposit, hypervascularity, and dermal hyperplasia MRI: Mild asymmetries (*vs*. healthy), no increase irregularity signal
Abouzaid *et al*., 2022; RCT (Egypt) [9]	100; 26.2 (mean); 58.00%	Superficial, deep and full thickness; 15.9% (case); 17.3% (control)	Acute	AFG versus conventional dressing; 177.2 ml (intra and postoperative) divided in 5 sessions	Subdermal injection and nanofat dressing	3	Less: Hospital LoS, frequency to OR, further skin grafting, outpatient visit, contracture, hypertrophic, or keloid in AFG group Improved scar texture in the AFG group	Micros: Better structure and arrangement of epidermis, dermis, and collagen in treatment versus control Morphometry: increase collagen deposition

AFG: Autologous fat grafting, DASH: Disabilities of Arm, Shoulder and Hand, LoS: Length of stay, MHQ: Michigan Hand Outcome Questionnaire, MRI: Magnetic resonance imaging, NA: Not available, NI: Not informed, NS: Normal saline, OR: Operation room, RCT: Randomized controlled trial, TBSA: Total body surface area, TGF-ß: transforming growth factor beta, VEGF-β: Vascular endothelial growth factor beta

Table 2: Autologous fat grafting procedure: Harvesting site, methods, processing, and administration

Author, year (location)	Procedure			
	Harvesting site	Harvesting method	Processing	Administration
Bruno *et al*., 2013 (Italy) [13]	Abdomen, hips, trochanter, inner thigh, medial of knee	Super wet technique with anesthetic infiltration (RL, ropivacaine 2%, epinephrine 1:500,000) Lipoaspiration: 2-hole blunt cannula 3 mm connected to a 10 mL Luer-lock syringe, minimal negative pressure	Centrifuge 1250 rpm, 3 min Central layer (purified fat) transferred to 1 or 3 mL Luer-lock syringes	Infiltration in the subscar layer, using sharp angiographic cannula
Byrne *et al*., 2016 (UK) [11]	Abdomen; (lateral thigh in one patient)	Anesthetic infiltration (NS, lidocaine 0.2%, epinephrine 1:1,000,000) with a 26 G needle in a ratio of 1 mL solution per cm^3 fat Lipoaspiration: cannula 3 mm connected to a 10 mL Luer-lock syringe, minimal negative pressure	Centrifuge 1200 rpm, 3 min Cotton patties were positioned in the barrel syringe to absorb excess oil Central layer transferred to a 1 mL syringe	1 mm incision at an adjacent site to the scar. V-shaped introducer to create a tunnel Subdermal plane 0.1–−0.2 mL each pass Subdermal injections using 18 G needles where necessary Scar riggotomies to release thick scars Fat is delivered in radial motion using a 1 mL syringe
La Padula *et al*., 2018 (France) [12]	Abdomen	No anesthetic solution is mentioned, only NS at 4°C Lipoaspiration: cannula through a 3 mm incision connected to 10 mL Luer-lock syringe, minimal negative pressure	Centrifuge 1300 rpm 5 min Central layer (purified fat) transferred to 2 mL Luer-lock syringe with a 17–18 G cannula	Multiple sites of injection at the dermal-hypodermal junction of the scar 2 sessions with a 3 months interval
Gal *et al*., 2017 (USA) [10]	Abdomen; thigh (one patient)	Anesthetic infiltration: NI Lipoaspiration: 18 G cannula of a 1 mL syringe	Centrifuge 3000 rpm, 3 min Central layer transferred to a 1 mL syringe	Using 1 mL syringe with 18 G needle, inject 5 mL of fat in each scar half
Klinger *et al*., 2020 (Italy) [15]	Abdomen	Anesthetic infiltration: NI Lipoaspiration: NI	No centrifugation was mentioned Obtained central layer, no further details	Using 0.1–0.2 mm cannula injected at the dermal-hypodermal junction of the scar 2 sessions with a 3 months interval
Abouzaid *et al*., 2022 (Egypt) [9]	NI	Anesthetic infiltration (NS, depocaine 2%, epinephrine 1:500,000, 6 mL $NaHCO_3$) Lipoaspiration: cannula 4 mm, minimal negative pressure	No centrifugation was mentioned; purified fat by gravitational separation transferred to syringes For topical application: Small syringes with a connector through which fat is passed back and forth~30 times until color changes and it gets more fluid	1–2 mm stab incision, using a 1–2 mm cannula; fanning fashion; subdermal and subcutaneous under the eschar. 1 mL fat over 10 cm line Nanofat is topically applied two times with a 48 h interval over the wound surface, covered with a standard dressing

NI: Not informed, NS: Normal saline, RL: Ringer lactate, rpm: Revolution per minute

Table 3: GRADE certainty of evidence for recorded outcomes

Outcomes	GRADE
AFG improved scar characteristics, including - less contracture or hypertrophic scar	Moderate
AFG improved patients' satisfaction	Low
AFG did not improve the functional status of the burn-affected region	Low
AFG results in a shorter LoS, fewer needs for skin graft, OR admission, and outpatient visits	High

AFG: Autologous fat grafting, LoS: Length of stay

necrosis with varying degrees of surrounding ischemic area is observed in burns, implying the dynamic spatiotemporal nature of burns [22].

Scarring (and further contracture), which can be as high as 72% [23], is an issue even in the modern era of burn management. In the beginning, AFG was used to increase the volume (such as filling), which was found to gradually improve the skin's quality, not to mention the resolution of the atrophic scar along with the changes in consistency [24]. It is proposed that immediate improvement on the scar is related to the mechanical release of fibrosis and the restoration of the subcutaneous plane. While the changes in scar characteristics as a result of tissue remodeling are driven by ADSCs, which promote angiogenesis and fibrinolysis through the mediation of pro- and anti-fibrotic agents, some of the most well-known profibrotic agents are TGFB1, ACTA1, and COLIA1 genes. While interferon alpha-2 is widely recognized for its antifibrotic properties [25].

Profound immunosuppression in extensive burns is related to the decline of both polymorphonuclear and mononuclear leukocytes' functions, in addition to depressed humoral immune activity. This explains the reality that infections are

the most common problem due to burning injuries. Although systemic inflammation developed in burn injury due to released DAMPs and PAMPs and their subsequent cytokines, an ineffective response only leads to sepsis instead of infection clearance [20,21,26]. The reality that only the latest study by Abouzaid *et al.* demonstrated how AFG also has plays a role in acute burns [9] implies that AFG usage in acute settings is limited. This is understandable since the available preclinical studies did not agree with each other. Loder *et al*. showed improved wound depth and size along with apoptotic activity (caspase staining) while having no significant effect on angiogenesis (assessed by CD31) in an acute setting [27], in contrast to a chronic setting [28]. In burns cases, vasodilatation is impaired [29], so using AFG is justified as it has angiogenic properties.

Although stem cells have been recognized for their anti-inflammatory and anti-fibrotic properties, we should be aware that the effect might be dose-dependent in a nonlinear fashion. Chen *et al*. reported that a high number of ADSC (10^8/mL) results in worse retention, higher inflammation, and fibrosis compared to a lower ADSC concentration (10^4/mL) [30]. These findings suggested that balancing immune responses (pro- and anti-inflammatory) is crucial, and we have not found any comparison of different dosages in the available clinical studies. Acute and chronic burns may not respond similarly, even though they were treated with similar doses.

Lack of standardized treatment protocol and heterogeneity of studies

The biggest challenge is the heterogeneity of the included studies [Tables 1 and 2], which affects the comparability and generalizability of the results. Differences in patient demographics, burn injury characteristics (such as degree, area, and duration-both acute and chronic), treatment protocols (including graft harvesting, administration techniques, and dosage), follow-up durations, and outcome measures make it difficult to draw definitive conclusions; thus, a case-by-case decision should always be made. Regarding the treatment protocol, the notable limitations identified in the current review are the lack of standardization in harvesting, preparing, and administering procedures.

Variability in techniques for harvesting adipose tissue can influence the quality and quantity of the harvested cells, potentially impacting treatment outcomes [31]. Harvesting methods vary between studies. The selection of harvesting sites and processing methods may also have an impact on the quality and viability of the harvested fat cells, and the amount of SVF and stem cells, which could, in turn, affect the regenerative capacity and tissue integration at the recipient site [16,31]. While there is no consensus on the best harvesting site, the abdomen and trochanteric region are the most preferred, with better cell viability from the superficial layer [31].

Harvesting methods have more varied differences: excision to liposuction, manual or tools-assisted. While it differs across studies, certain principles were set as guidelines: the use of lower negative pressure and a blunt cannula with a larger inside diameter maximized cell yield and viability, although these principles might not parallel the clinical results [16,31]. Even though 54% of surgeons employed the standardized Coleman technique to harvest fat, there were inconsistencies in the graft retention field [32]. Regarding cell viability of SVF and ASC content, there was no agreement on the most effective method for preparation before harvesting, whether it be dry or wet (Klein's solution containing NS, epinephrine, and local anesthetic) [16,31].

The methods and duration of processing are capable of having a significant effect on the purification and enrichment of the ADSC, which is crucial to the process of tissue healing and regeneration. However, there were distinctions between preclinical and clinical trials since, in the latter, centrifugation led to superior graft outcomes than gravity sedimentation in clinical trials. Furthermore, different centrifugation speeds and durations are related to the different harvesting methods. Another method that was not used in the included studies is gauze rolling. A study found this method achieved higher levels of vascular endothelial growth factor (VEGF) and platelet-derived growth factor (PDGF) [31].

Different transfer techniques for injecting purified fat may affect the graft's dispersion and viability, thereby potentially influencing the AFG procedure's overall efficacy. Several factors must be considered: the size of the cannula, the injection site, and the injection speed, which should achieve a balance between minimal trauma and fat globule deposition [31]. To establish the optimal AFG protocol for burn injury (acute and chronic wound, also burn-related scars) treatments, it is necessary to run additional research projects that explicitly compare the techniques and parameters implemented in the different studies. Following a standard AFG procedure could contribute to the consistency and reproducibility of results.

While several studies have referenced the Coleman technique for injection, certain variations have been noted that might pertain to the outcomes, such as the experience of the surgeons. The dosage of the treatment is another concern, as not all studies provided information regarding the quantity of lipoaspirate extracted and injected or the dosage administered per unit per area. Previous studies suggested a dose of 1 ml per 3.5 cm^2 for aesthetic and functional improvement [15]. Aside from the dose, the number of sessions and the interval between sessions determine the outcomes, as the study suggested serial AFG for scar improvement [33].

Every study reported different outcomes: clinical and nonclinical. Standardized clinical assessment using established questionnaires for objective physician assessment or patient subjective satisfaction may reduce bias. The reported outcomes should be explored not only in terms of appearance but also functionality and their effect on future medical needs (LoS, further grafting, inpatient or outpatient admission). For nonclinical parameters, standard histological and immunohistochemistry examinations can be carried out objectively using software (e.g., ImageJ). In this review, collagen deposition is an important parameter that was found to be improved, as it is natural in the wound healing process to undergo an increment of collagen deposit to repair skin structure and increase the tensile strength of the tissue [34].

The composition of collagen changes along the wound healing period, from type III to type I, as the wound matures. Although the available studies did not detail the type of collagen fibers observed, an increase in collagen content is favorable. Increased collagen fibers after fat graft have been supported in past preclinical studies, even from the past decades [35,36].

Molecular marker examination has not been widely carried out since we only found one study [13] that reported the parameters. However, the results were not clearly explained by Bruno *et al*. since there are inconsistent results (e.g., negative expression of P63, which is a proliferation marker, while positive for cell growth inhibition marker P53, yet positive Ki-67 as a proliferation marker). Molecularly, AFG and ADSC, which contain platelet-rich plasma and SVFs, promote and regulate wound healing in burn injuries through various pathways because they deliver abundant growth factors such as VEGF, TGF, FGF, and PDGF. The activation of numerous pathways (Ras-Raf-ERK, PI3K-PIP3-Akt, PLC-PIP2-IP3, and JAK-STAT) leads to cell proliferation, survival, and differentiation, which increases collagen synthesis, angiogenesis, cell motility, and adhesion and, in the end, result in better wound healing [37,38].

S100, as a cell differentiation and melanin marker chosen by Bruno, has been described elsewhere as having dual properties: pro- and anti-inflammatory [39,40]. Langerin also acts differently across studies: downregulation increases the healing of skin wounds [41] and upregulation increases the healing of diabetic ulcers [42]. These are not ideal markers to evaluate wound healing, as it is a dynamic process. The negative expression of ß-catenin, which upregulates wound healing theoretically and leads to a smaller scar surface area [43], was observed in the end of Bruno's study period [13]. Further studies should use markers that have a specific role and are expressed in contrast in the wound healing process or markers that act as major drivers of the intended histological changes, such as collagen deposit, for example, Discoidin Domain Receptors [34]. VEGF, which is an angiogenesis marker, may be a relevant marker; however, serial observation may be needed to understand the dynamic process of angiogenesis during wound healing.

Standardized outcome reporting would facilitate better comparison and pooling of data, allowing for more comprehensive meta-analyses and systematic reviews. Controlling for important confounders is also critical in future studies. The aforementioned variables that act as confounding factors should be adjusted. Furthermore, our systematic reviews did not include Chinese articles, which are substantial, composed of studies on this topic. Thus, further review of Chinese studies is needed.

Conclusions

AFG and ADSC demonstrate promise for the management of burn injuries with potential advantages in terms of tissue restructuring in wound healing, scar remodeling, functional outcomes, and patient satisfaction. However, the evidence is limited to chronic burn-related scars, while its efficacy in acute burn settings needs more studies. Nevertheless, given the possibility of confounding bias and selection bias, it is imperative to approach the findings of the non-randomized research with caution. To pursue comprehensive studies into the effectiveness of AFG and ADSC in the burn wound healing and scar remodeling processes, it is mandatory for future research endeavors to prioritize the implementation of carefully designed RCTs accompanied by rigorous assessments of potential biases. Standardizing harvesting techniques, addressing differences in study designs, and controlling for important variables will make it possible to learn more about how adipose-derived modalities help heal burn wounds and remodel scars.

Data availability statement

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

References

1. Costanzo D, Romeo A, Marena F. Autologous fat grafting in plastic and reconstructive surgery: An historical perspective. Eplasty 2022;22:e4.
2. Hanson SE, Kapur SK, Hwang RF, Dryden MS. Autologous fat grafting in breast reconstruction: Implications for follow-up and surveillance. Gland Surg 2021;10:487-93.
3. Mahoney CM, Imbarlina C, Yates CC, Marra KG. Current therapeutic strategies for adipose tissue defects/repair using engineered biomaterials and biomolecule formulations. Front Pharmacol 2018;9:507.
4. Nepon H, Safran T, Reece EM, Murphy AM, Vorstenbosch J, Davison PG. Radiation-induced tissue damage: Clinical consequences and current treatment options. Semin Plast Surg 2021;35:181-8.
5. Saeed K, Khan FA, Qudus SB, Javed S. Autologous fat grafting – A step forward in wound management. Int J Low Extrem Wounds 2022;21:647-50.
6. Wardhana A, Basuki A, Prameswara AD, Rizkita DN. The epidemiology of burns in Indonesia's national referral burn center from 2013 to 2015. Burns Open 2017;1:67-73.
7. Condé-Green A, Marano AA, Lee ES, Reisler T, Price LA, Milner SM, et al. Fat grafting and adipose-derived regenerative cells in burn wound healing and scarring: A systematic review of the literature. Plast Reconstr Surg 2016;137:302-12.
8. Higgins JP, Thomas J, Chandler J, Cumpston M, Li T, Page MJ, et al. Cochrane handbook for systematic reviews of interventions. 2nd ed. Hoboken, NJ: Wiley-Blackwell; 2020.
9. Abouzaid AM, El Mokadem ME, Aboubakr AK, Kassem MA, Al Shora AK, Solaiman A. Effect of autologous fat transfer in acute burn wound management: A randomized controlled study. Burns 2022;48:1368-85.
10. Gal S, Ramirez JI, Maguina P. Autologous fat grafting does not improve burn scar appearance: A prospective, randomized, double-blinded, placebo-controlled, pilot study. Burns 2017;43:486-9.
11. Byrne M, O'Donnell M, Fitzgerald L, Shelley OP. Early experience with fat grafting as an adjunct for secondary burn reconstruction in the hand: Technique, hand function assessment and aesthetic outcomes. Burns 2016;42:356-65.
12. La Padula S, Hersant B, Meningaud JP, D'Andrea F. Use of autologous fat graft and fractiononal co_2 laser to optimize the aesthetic and functional

results in patients with severe burn outcomes of the face. J Stomatol Oral Maxillofac Surg 2018;119:279-83.

13. Bruno A, Delli Santi G, Fasciani L, Cempanari M, Palombo M, Palombo P. Burn scar lipofilling: Immunohistochemical and clinical outcomes. J Craniofac Surg 2013;24:1806-14.
14. El-Husseiny HM, Kaneda M, Mady EA, Yoshida T, Doghish AS, Tanaka R. Impact of adipose tissue depot harvesting site on the multilineage induction capacity of male rat adipose-derived mesenchymal stem cells: An *in vitro* study. Int J Mol Sci 2023;24:7513.
15. Klinger M, Marazzi M, Vigo D, Torre M. Fat injection for cases of severe burn outcomes: A new perspective of scar remodeling and reduction. Aesthetic Plast Surg 2020;44:1278-82.
16. Simonacci F, Bertozzi N, Grieco MP, Grignaffini E, Raposio E. Procedure, applications, and outcomes of autologous fat grafting. Ann Med Surg (Lond) 2017;20:49-60.
17. Al-Ghadban S, Artiles M, Bunnell BA. Adipose stem cells in regenerative medicine: Looking forward. Front Bioeng Biotechnol 2021;9:837464.
18. Scotto di Santolo M, Sagnelli M, Tortora G, Santoro MA, Canta PL, Molea G, et al. The utility of the high-resolution ultrasound technique in the evaluation of autologous adipose tissue lipofilling, used for the correction of post-surgical, post-traumatic and post-burn scars. Radiol Med 2016;121:521-7.
19. van Baar ME. Epidemiology of scars and their consequences: Burn scars. In: Téot L, Mustoe TA, Middelkoop E, Gauglitz GG. eds. Textbook on scar management: State of the art management and emerging technologies. Cham (CH): Springer; 2020. Available from: https://www.ncbi.nlm.nih.gov/books/NBK586079/. [Last accessed on 15 Jul 20].
20. Tiwari VK. Burn wound: How it differs from other wounds? Indian J Plast Surg 2012;45:364-73.
21. Jeschke MG, van Baar ME, Choudhry MA, Chung KK, Gibran NS, Logsetty S. Burn injury. Nat Rev Dis Primers 2020;6:11.
22. Lanier ST, McClain SA, Lin F, Singer AJ, Clark RA. Spatiotemporal progression of cell death in the zone of ischemia surrounding burns. Wound Repair Regen 2011;19:622-32.
23. Lawrence JW, Mason ST, Schomer K, Klein MB. Epidemiology and impact of scarring after burn injury: A systematic review of the literature. J Burn Care Res 2012;33:136-46.
24. Bellini E, Grieco MP, Raposio E. The science behind autologous fat grafting. Ann Med Surg (Lond) 2017;24:65-73.
25. Walter AS, Volkmer E, Gauglitz G, Böcker W, Saller MM. Systematic review of molecular pathways in burn wound healing. Burns 2023;49:1525-33.
26. Kamolz LP, Hecker A. Molecular mechanisms related to burns, burn wound healing and scarring. Int J Mol Sci 2023;24:8785.
27. Loder S, Peterson JR, Agarwal S, Eboda O, Brownley C, DeLaRosa S, et al. Wound healing after thermal injury is improved by fat and adipose-derived stem cell isografts. J Burn Care Res 2015;36:70-6.
28. Sultan SM, Barr JS, Butala P, Davidson EH, Weinstein AL, Knobel D, et al. Fat grafting accelerates revascularisation and decreases fibrosis following thermal injury. J Plast Reconstr Aesthet Surg 2012;65:219-27.
29. Romero SA, Moralez G, Jaffery MF, Huang M, Crandall CG. Vasodilator function is impaired in burn injury survivors. Am J Physiol Regul Integr Comp Physiol 2018;315:R1054-60.
30. Chen X, Chen Y, Wang Z, Dong Z, Yao Y, Li Y, et al. Adipose-derived stem cells regulate CD4+T-cell-mediated macrophage polarization and fibrosis in fat grafting in a mouse model. Heliyon 2022;8:e11538.
31. Nemir S, Hanson SE, Chu CK. Surgical decision making in autologous fat grafting: An evidence-based review of techniques to maximize fat survival. Aesthet Surg J 2021;41:S3-15.
32. Gause TM 2nd, Kling RE, Sivak WN, Marra KG, Rubin JP, Kokai LE. Particle size in fat graft retention: A review on the impact of harvesting technique in lipofilling surgical outcomes. Adipocyte 2014;3:273-9.
33. Strong AL, Cederna PS, Rubin JP, Coleman SR, Levi B. The current state of fat grafting: A review of harvesting, processing, and injection techniques. Plast Reconstr Surg 2015;136:897-912.
34. Mathew-Steiner SS, Roy S, Sen CK. Collagen in wound healing. Bioengineering (Basel) 2021;8:63.
35. Mojallal A, Lequeux C, Shipkov C, Breton P, Foyatier JL, Braye F, et al. Improvement of skin quality after fat grafting: Clinical observation and an animal study. Plast Reconstr Surg 2009;124:765-74
36. Nolan GS, Smith OJ, Jell G, Mosahebi A. Fat grafting and platelet-rich plasma in wound healing: A review of histology from animal studies. Adipocyte 2021;10:80-90.
37. Karina S, Samudra MF, Rosadi I, Afini I, Widyastuti T, Sobariah S, et al. Combination of the stromal vascular fraction and platelet-rich plasma accelerates the wound healing process: Pre-clinical study in a Sprague-Dawley rat model. Stem Cell Investig 2019;6:18.
38. Sujana RS, Sulmiati S, Mariana N, Josh F, Laidding SR, Zainuddin AA, et al. The effect of stromal vascular fraction and platelet-rich plasma combination on basic fibroblast growth factor serum level during anal trauma healing in a Wistar rat model. Ann Med Surg (Lond) 2022;76:103375.
39. Su W, Wang P, Dong Q, Li S, Hu S. S100A8 accelerates wound healing by promoting adipose stem cell proliferation and suppressing inflammation. Regen Ther 2022;21:166-74.
40. Xia C, Braunstein Z, Toomey AC, Zhong J, Rao X. S100 proteins as an important regulator of macrophage inflammation. Front Immunol 2017;8:1908.
41. Rajesh A, Stuart G, Real N, Ahn J, Tschirley A, Wise L, et al. Depletion of langerin$^+$ cells enhances cutaneous wound healing. Immunology 2020;160:366-81.
42. Stojadinovic O, Yin N, Lehmann J, Pastar I, Kirsner RS, Tomic-Canic M. Increased number of Langerhans cells in the epidermis of diabetic foot ulcers correlates with healing outcome. Immunol Res 2013;57:222-8.
43. Choi S, Yoon M, Choi KY. Approaches for regenerative healing of cutaneous wound with an emphasis on strategies activating the Wnt/β-catenin pathway. Adv Wound Care (New Rochelle) 2022;11:70-86.

Supplementary Material

Supplementary File S1: Preferred Reporting Items for Systematic Review and Meta-Analysis checklist

Section and topic	Item number	Checklist item	Page
		Title	
Title	1	Identify the report as a systematic review	1
		Abstract	
Abstract	2	See the PRISMA 2020 for Abstracts checklist	2
		Introduction	
Rationale	3	Describe the rationale for the review in the context of existing knowledge	3
Objectives	4	Provide an explicit statement of the objective(s) or question (s) the review addresses	3
		Methods	
Eligibility criteria	5	Specify the inclusion and exclusion criteria for the review and how studies were grouped for the syntheses	4
Information sources	6	Specify all databases, registers, websites, organizations, reference lists, and other sources searched or consulted to identify studies. Specify the date when each source was last searched or consulted	4
Search strategy	7	Present the full search strategies for all databases, registers, and websites, including any filters and limits used	4, S1
Selection process	8	Specify the methods used to decide whether a study met the inclusion criteria of the review, including how many reviewers screened each record and each report retrieved, whether they worked independently, and, if applicable, details of automation tools used in the process	4
Data collection process	9	Specify the methods used to collect data from reports, including how many reviewers collected data from each report, whether they worked independently, any processes for obtaining or confirming data from study investigators, and if applicable, details of automation tools used in the process	4
Data items	10a	List and define all outcomes for which data were sought. Specify whether all results that were compatible with each outcome domain in each study were sought (e.g., for all measures, time points, analyses), and if not, the methods used to decide which results to collect	4
	10b	List and define all other variables for which data were sought (e.g., participant and intervention characteristics, funding sources). Describe any assumptions made about any missing or unclear information	4
Study risk of bias assessment	11	Specify the methods used to assess the risk of bias in the included studies, including details of the tool(s) used, how many reviewers assessed each study and whether they worked independently, and if applicable, details of automation tools used in the process	4
Effect measures	12	Specify for each outcome the effect measure(s) (e.g., risk ratio, mean difference) used in the synthesis or presentation of results	4
Synthesis methods	13a	Describe the processes used to decide which studies were eligible for each synthesis (e.g., tabulating the study intervention characteristics and comparing against the planned groups for each synthesis (item #5))	4
	13b	Describe any methods required to prepare the data for presentation or synthesis, such as handling of missing summary statistics, or data conversions	4
	13c	Describe any methods used to tabulate or visually display the results of individual studies and syntheses	4
	13d	Describe any methods used to synthesize results and provide a rationale for the choice (s). If meta-analysis was performed, describe the model(s), method(s) to identify the presence and extent of statistical heterogeneity, and software package(s) used	NA
	13e	Describe any methods used to explore possible causes of heterogeneity among study results (e.g., subgroup analysis, meta-regression)	NA
	13f	Describe any sensitivity analyses conducted to assess the robustness of the synthesized results	NA
Reporting bias assessment	14	Describe any methods used to assess the risk of bias due to missing results in a synthesis (arising from reporting biases)	NA
Certainty assessment	15	Describe any methods used to assess certainty (or confidence) in the body of evidence for an outcome	NA
		Results	
Study selection	16a	Describe the results of the search and selection process, from the number of records identified in the search to the number of studies included in the review, ideally using a flow diagram	5
	16b	Cite studies that might appear to meet the inclusion criteria but which were excluded, and explain why they were excluded	5
Study characteristics	17	Cite each included study and present its characteristics	5, 14–17
Risk of bias in studies	18	Present assessments of risk of bias for each included study	5
Results of individual studies	19	For all outcomes, present, for each study: (a) summary statistics for each group (where appropriate) and (b) an effect estimate and its precision (e.g., confidence/credible interval), ideally using structured tables or plots	6,7,8

Contd...

Supplementary File S1: Contd...

Section and topic	Item number	Checklist item	Page
		Results	
Results of syntheses	20a	For each synthesis, briefly summarize the characteristics and risk of bias among contributing studies	NA
	20b	Present results of all statistical syntheses conducted. If meta-analysis was done, present for each the summary estimate and its precision (e.g., confidence/credible interval) and measures of statistical heterogeneity. If comparing groups, describe the direction of the effect	NA
	20c	Present results of all investigations of possible causes of heterogeneity among study results	NA
	20d	Present results of all sensitivity analyses conducted to assess the robustness of the synthesized results	NA
Reporting biases	21	Present assessments of risk of bias due to missing results (arising from reporting biases) for each synthesis assessed	NA
Certainty of evidence	22	Present assessments of certainty (or confidence) in the body of evidence for each outcome assessed	18
		Discussion	
Discussion	23a	Provide a general interpretation of the results in the context of other evidence	12
	23b	Discuss any limitations of the evidence included in the review	13
	23c	Discuss any limitations of the review processes used	14
	23d	Discuss implications of the results for practice, policy, and future research	6-9
		Other information	
Registration and protocol	24a	Provide registration information for the review, including register name and registration number, or state that the review was not registered	NA
	24b	Indicate where the review protocol can be accessed, or state that a protocol was not prepared	NA
	24c	Describe and explain any amendments to information provided at registration or in the protocol	NA
Support	25	Describe sources of financial or nonfinancial support for the review, and the role of the funders or sponsors in the review	10
Competing interests	26	Declare any competing interests of review authors	10
Availability of data, code, and other materials	27	Report which of the following are publicly available and where they can be found: template data collection forms; data extracted from included studies; data used for all analyses; analytic code; any other materials used in the review	NA

NA: Not available, PRISMA: Preferred Reporting Items for Systematic Review and Meta-Analysis

Supplementary File S2: Search queries

Database	Search queries	Hit
MEDLINE	(“nanofat”[Title/Abstract] OR “fragmented fat”[Title/Abstract] OR “mechanical svf”[Title/Abstract] OR “autologous fat graft”[Title/Abstract] OR “svf gel”[Title/Abstract] OR “fat transplant”[Title/Abstract] OR “adipose stem cell”[Title/Abstract] OR “lipofilling”[Title/Abstract] OR “lipoinjection”[Title/Abstract] OR “lipotransfer”[Title/Abstract]) AND (“burn scar”[Title/Abstract] OR “burn wound”[Title/Abstract] OR “burn injury”[Title/Abstract] OR “burn”[Title/Abstract] OR “thermal injury”[Title/Abstract])	34
CENTRAL	#1 (nano?fat):ti, ab, kw OR (“mechanical svf”):ti, ab, kw OR (“autologous fat graft”):ti, ab, kw OR (svf gel):ti, ab, kw OR (“mechanical stromal vascular fraction”):ti, ab, kw #2 (‘fat transplant*’):ti, ab, kw OR (‘adipose stem cell*’):ti, ab, kw OR (lipotransfer*):ti, ab, kw OR (lipofilling):ti, ab, kw OR (lipoinjection):ti, ab, kw #3 #1 OR #2 #4 (“burn injury”):ti, ab, kw OR (“thermal injury”):ti, ab, kw OR (burn):ti, ab, kw #5 #3 AND #4	19
Embase	#1 nano?fat: ti, ab, kw OR ‘mechanical svf’:ti, ab, kw OR ‘autologous fat graft’:ti, ab, kw OR ‘mechanical stromal vascular fraction’:ti, ab, kw OR ‘svf gel’:ti, ab, kw OR ‘fat transplant’:ti, ab, kw OR ‘adipose stem cell’:ti, ab, kw OR ‘lipofilling’:ti, ab, kw OR ‘lipoinjection’:ti, ab, kw OR ‘lipotransfer’:ti, ab, kw #2 ‘burn’:ti, ab, kw OR ‘thermal injury’:ti, ab, kw OR ‘burn scar’:ti, ab, kw #3 #1 AND #2	37