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REVIEW ARTICLE

Adipose-derived stem cells in wound healing of full-thickness skin defects: a review of the literature*†

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ABSTRACT

The complex process of wound healing can be delayed in circumstances when the natural niche is extremely altered. Adipose-derived stem cells (ADSC) seem to be a promising therapy for these type of wounds. We aim to describe the studies that used ADSC for wound healing after a full-thickness skin defect, the ADSC mechanisms of action, and the outcomes of the different ADSC therapies applied to date. We performed a review by querying PubMed database for studies that evaluated the use of ADSC for wound healing. The Mesh terms, adipose stem cells AND (skin injury OR wound healing) and synonyms were used for the search. Our search recorded 312 articles. A total of 30 articles met the inclusion criteria. All were experimental in nature. ADSC was applied directly (5 [16.7%]), in sheets (2 [6.7%]), scaffolds (14 [46.7%]), skin grafts (3 [10%]), skin flaps (1 [3.3%]), as microvesicles or exosomes (4 [13.3%]), with adhesives for wound closure (1 [3.3%]), and in a concentrated conditioned hypoxia-preconditioned medium (1 [3.3%]). Most of the studies reported a benefit of ADSC and improvement of wound healing with all types of ADSC therapy. ADSC applied along with extracellular matrix, stromal cell-derived factor (SDF-1) or keratinocytes, or ADSC seeded in scaffolds showed better outcomes in wound healing than ADSC alone. ADSC have shown to promote angiogenesis, fibroblast migration, and up-regulation of macrophages chemotaxis to enhance the wound healing process. Further studies should be conducted to assure the efficacy and safety of the different ADSC therapies.

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Introduction

Repair is defined as the physiologic change of an organ to try to recover its tissue after injury without exact replacement of the damage [1]. Regeneration refers to the ability to replace the damage entirely and recover morphology and functionality [2]. Wound healing and regeneration is a complex process consisting of four stages: hemostasis, inflammation, proliferation, and remodeling [3]. Although healing is a natural process, in some circumstances, wounds are difficult to heal, such as those caused by removal of full-thickness of the skin such in surgical resections due to skin cancer. In these situations, skin grafts or flaps are considered; however, several limitations have been identified with the use of these two therapies. Adverse events related to skin grafting consist in graft dehiscence, partial graft loss, donor site morbidity, poor color matching, pain, discomfort, and hypertrophic scar [2,4]. Whereas, the skin flap adverse events include hematomas, infection, necrosis, and dehiscence [5]. Limitations on the rotation arch and size in skin flaps are also present, besides the need for microsurgery to keep the flap vascularized. For these reasons, cell therapies appear to be a potential treatment to decrease the morbidity of these procedures.

Cell therapies for skin tissue replacement after surgical resections have taken advantage of the properties of stem cells. These undifferentiated cells are characterized for the ability to self-renew and differentiate into functional cells [6]. Mesenchymal stem cells are the focus of studies on skin regeneration; between them, bone marrow-derived stem cells are the multipotent adult stem cells most often studied [7]. However, current studies are evaluating the use of adipose-derived mesenchymal stem cells (ADSC) due to their easier method of extraction, cost-effectiveness, lack of ethical issues, and properties of differentiation into osteoblasts, chondrocytes, adipocytes, myocytes, and skin cells [8–10].

To date, the efficacy of these stem cells on cutaneous wound healing of full-thickness defects has not been well defined. In our review, we aimed to describe all the preclinical studies that used different ADSC therapies to promote wound healing of full-thickness skin defects. In addition, we sought to describe the mechanisms of actions and outcomes after the application of these ADSC therapies.

Methods

Study selection

Our review included experimental *in vitro*/*in vivo* studies about the use of ADSC in surgical wound healing of full-thickness skin defects. Studies were included if they met following criteria: (1) original experimental, prospective, or retrospective design focused on ADSC as a primary resource for skin tissue regeneration; (2) application of ADSC in any form of administration to surgical skin wound healing of full-thickness defect; and (3) compared their

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results with a control group. Studies were excluded if they evaluated the use of fat grafting, stromal vascular fraction, keratinocytes, growth factors, platelets, and derivatives as a primary source for wound healing; analyzed the use of ADSC specifically in radiated or burned skin, scars, chronic pressure ulcers, diabetic models or patients, and other causes of skin injury; were reviews, case reports, and noncomparative descriptive studies of the topic, or were written in other language than English.

Data sources and search strategy

A review was conducted by one author (MTH) on March 30, 2020 in the PubMed database searching for articles reporting on the use of ADSC in wound healing for full-thickness skin defect. The Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) guidelines were followed. The Mesh Terms used for the search strategy were the following: (Adipose derived Stem Cells[Title/Abstract] OR Adipose-Derived Mesenchymal Stem Cells[Title/Abstract] OR Adipose Derived Mesenchymal Stem Cells[Title/Abstract] OR Mesenchymal Stem Cells, Adipose-Derived[Title/Abstract] OR Adipose Derived Mesenchymal Stromal Cells[Title/Abstract] OR Adipose-Derived Mesenchymal Stromal Cells[Title/Abstract] OR Adipose Derived Mesenchymal Stromal Cells[Title/Abstract] OR Adipose Tissue Derived Mesenchymal Stromal Cells[Title/Abstract] OR ((mesenchymal stem cells[Title/Abstract] OR adult stem cells[Title/Abstract] OR stem cells[Title/Abstract]) AND (adipose[Title/Abstract] OR adipocyte[Title/Abstract]))) AND (skin injury[Title/Abstract] OR ((wound healing[Title/Abstract] OR Healing, Wound[Title/Abstract] OR wound repair[Title/Abstract]) AND (skin [Title/Abstract] OR cutaneous[Title/Abstract])))). Identified studies were uploaded into EndNote (Clarivate). Two independent reviewers selected the final studies. Manuscripts were screened manually by the first author and selected according to the inclusion and exclusion criteria in a two-steps process. First, studies were reviewed based on the title and abstract. Second, the full text of the selected studies was screened for the final selection. If the first author doubted selecting an article, the second author reviewed the article according to the selection criteria and both reviewers came to a consensus for the final decision.

Data pooling and data analysis

Relevant data were extracted and pooled as description of experimental studies. The variables selected to describe experimental study results included author, year of publication, and the type of ADSC therapy. All data were compiled and compared. We classified the articles into the following six categories: ADSC directly, ADSC in sheets, ADSC in scaffolds, ADSC in skin grafts and flaps, ADSC-derived microvesicles or exosomes, ADSC with adhesives for wound closure, and ADSC in concentrated hypoxia-preconditioned medium.

Study quality assessment

Risk bias assessment was done according to a modified version of SYRCLE's guidelines (Table 1) [11]. Quality assessment was difficult in most cases due to the lack of information about possible bias risks. Fifteen (50%) studies had a total bias free score of 5 or more [9,12–25], and so, were considered to have a relatively low risk of bias.

Results

We found 312 articles in our PubMed search, and looked for articles inside the citations that could meet our inclusion criteria. In total, 30 articles were included in our review (Figure 1). All included studies were experimental and were published between 2011 and 2020. Five (16.7%) studies analyzed the effectiveness of ADSC directly [8,9,18,25,26], two (6.7%) studies tested the effects of ADSC in sheets [17,27], fourteen (46.7%) studied ADSC in combination with scaffolds [15,19–21,23,24,28–34], three (10%) in skin grafts [13,14,22], one (3.3%) in skin flaps [33], four (13.3%) as derivatives (e.g. microvesicles or exosomes) [12,35–37], one (3.3%) in adhesives for wound closure [38], and one (3.3%) in a concentrated hypoxia-preconditioned medium [16] (Tables 2 and 3). Biomaterials used as scaffolds for the ADSC in skin injury were platelet-rich plasma hydrogels with polyethylene glycol [31], gelatin-based hydrogel [23,32], fibrin gel [33], fibrin matrix [34], hyaluronic acid gel with added vitamins and minerals [15], dermal substitute [19,24,28,30], collagen gel [21,29], and matrigel [20].

In general, most of the studies demonstrated better outcomes in wound healing after using ADSC compared to other groups of therapy. In addition, when ADSC were placed along with ECM, cytokines or keratinocytes, wound healing was improved [8,9]. However, these outcomes varied according to the type of ADSC therapy applied. For instance, Zomer et al. [26] in an *in vitro* study found a greater wound closure rate for dermal stem cells compared with ADSC. This may be explained probably due to the lack of growth factors that influence the action of ADSC. On the other hand, ADSC therapy when seeded in scaffolds, showed better outcomes such as faster wound closure, decrease of inflammation and neovascularization compared with controls and even with ADSC only groups [21,23,24,30–32,34].

ADSC effects in wound healing

Overall, the principal mechanisms described of ADSC were angiogenesis, migration, differentiation of fibroblasts, and up-regulation of macrophage chemotaxis (Figure 2). Production of growth factors involved in ADSC mechanism of action was reported in 4 (13.3%) articles [12,13,22,31] and suggested in 2 (6.6%) [21,32].

Studies have described the paracrine function of ADSC to increase angiogenesis and generate growth factors that stimulate neovascularization, although they still have an unidentified role over the endothelial cells and pericytes [13–17,19–22,26,28,32–34,37]. Samberg et al. [31] studied ADSC seeded in platelet-rich plasma hydrogels and found expression of the specific genes involved in neoangiogenesis, including *α-smooth muscle actin, VEGF, Angpt-1, and Angpt-2*. Equally important, an upregulation of Angpt-1 and Angpt-2 was described to increase the concentration of platelets in wound healing when ADSC were cultured within platelet-rich plasma hydrogels [31].

On the other hand, vascular endothelial growth factors (VEGF) and transforming growth factors (TGF-β1) were found to be secreted by the ADSC and associated with increased expression of angiogenesis [12,13,21,31,32]. Releasing of these factors along with the fibroblast growth factor, favor the reconstitution of dermal fibroblasts promoting wound healing [27,32]. Stromal cell-derived factor 1 (SDF-1) is a potent chemotactic cytokine commonly produced by bone marrow stromal cells, with special functions in stem cell mobilization, inflammatory cell infiltration, and angiogenesis. SDF-1 has been found to regulate and control migration of ADSC during wound healing [8].

In addition, ADSC has been found to differentiate into fibroblasts and, therefore, to migrate and promote skin wound healing

Table 1. Quality assessment by modified SYRCLE's risk of bias tool.

	1. Adequate sequence allocation	2. Similar groups at baseline	3. Concealed allocation to different groups	4. Animals randomly housed	5. Investigators blinded for intervention	6. Animals selected at random for outcome assessment	7. Outcome assessor blinded	8. Incomplete outcome data adequately addressed	9. Free of selective outcome reporting	10. Free of other problems	Total bias free score (0-10)	Suspected bias score (0-10)
Yang et al. [35]	Unclear	Unclear	Yes	Unclear	Unclear	Yes	Unclear	Unclear	Unclear	Yes	3	–
Kallmeyer et al. [25]	**Yes**	**Yes**	**Yes**	**Unclear**	**Unclear**	**Yes**	**Unclear**	**Unclear**	**Unclear**	**Yes**	**5**	–
Zomer et al. [26]	Yes	Unclear	Yes	–	Unclear	–	Unclear	Unclear	Unclear	Yes	3	–
Mirzaei-Parsa et al. [24]	**Yes**	**Yes**	**Yes**	**Unclear**	**Yes**	**Yes**	**Yes**	**Unclear**	**Unclear**	**Yes**	**7**	–
Ma T et al. [36]	Yes	Unclear	Yes	Unclear	Unclear	Yes	Unclear	Unclear	Unclear	Yes	4	–
Gao et al. [23]	**Yes**	**Yes**	**Yes**	**Unclear**	**Unclear**	**Yes**	**Unclear**	**Unclear**	**Unclear**	**Yes**	**5**	–
Doornaert et al. [28]	Yes	Unclear	Yes	Unclear	Unclear	Yes	Unclear	Unclear	Unclear	Yes	4	–
Zhou et al. [9]	**Yes**	**Yes**	**Yes**	**Unclear**	**Unclear**	**Yes**	**Unclear**	**Unclear**	**Unclear**	**Yes**	**5**	–
Samberg et al. [31]	Yes	Yes	Yes	Unclear	Unclear	Unclear	Unclear	Unclear	Unclear	Yes	4	–
Ren et al. [12]	**Yes**	**Yes**	**Yes**	**Unclear**	**Unclear**	**Yes**	**Unclear**	**Unclear**	**Unclear**	**Yes**	**5**	–
Hsu et al. [32]	No	Yes	Yes	Unclear	Unclear	Yes	Unclear	Unclear	Unclear	Yes	4	1
Yu et al. [27]	Yes	Yes	Yes	Unclear	Unclear	Unclear	Unclear	Unclear	Unclear	No	3	1
Vidor et al. [13]	**Yes**	**Yes**	**Yes**	**Unclear**	**Unclear**	**Yes**	**Yes**	**Unclear**	**Unclear**	**Yes**	**6**	–
Zhang et al. [37]	Yes	Unclear	Yes	Unclear	Unclear	Yes	Unclear	Unclear	Unclear	Yes	4	–
Zhang et al. [29]	Yes	Unclear	Yes	Unclear	Unclear	Yes	Unclear	Unclear	Unclear	Yes	4	–
Zeng et al. [33]	Yes	Yes	Yes	Unclear	Unclear	Unclear	Unclear	Unclear	Unclear	Yes	4	–
Yucel et al. [14]	**Yes**	**Yes**	**Yes**	**Unclear**	**Unclear**	**Unclear**	**Yes**	**Unclear**	**Unclear**	**Yes**	**5**	–
Ozpur et al. [34]	Yes	Yes	Yes	Unclear	Unclear	Unclear	Unclear	Unclear	Unclear	Yes	4	–
Nowacki et al. [38]	Yes	Yes	Yes	Unclear	Unclear	Unclear	Unclear	Unclear	Unclear	Yes	4	–
Wu et al. [8]	Yes	Yes	Yes	Unclear	Unclear	Unclear	Unclear	Unclear	Unclear	No	3	1
Rodriguez et al. [15]	**Yes**	**Yes**	**Yes**	**Unclear**	**Unclear**	**Yes**	**Unclear**	**Unclear**	**Unclear**	**Yes**	**5**	–
Sun et al. [16]	**Yes**	**Yes**	**Yes**	**Unclear**	**Unclear**	**Yes**	**Unclear**	**Unclear**	**Unclear**	**Yes**	**5**	–
Lin et al. [17]	**Yes**	**Yes**	**Yes**	**Unclear**	**Unclear**	**Yes**	**Unclear**	**Unclear**	**Unclear**	**Yes**	**5**	–
Hong et al. [18]	**Yes**	**Yes**	**Yes**	**Unclear**	**Unclear**	**Unclear**	**Yes**	**Unclear**	**Unclear**	**Yes**	**5**	–
Meruane et al. [19]	**Yes**	**Yes**	**Yes**	**Unclear**	**Unclear**	**Unclear**	**Yes**	**Unclear**	**Unclear**	**Yes**	**5**	–
Huang et al. [30]	Yes	Unclear	Yes	Unclear	Unclear	Yes	Unclear	Unclear	Unclear	Yes	4	–
Zografou et al. [22]	**Yes**	**Yes**	**Yes**	**Unclear**	**Unclear**	**Yes**	**Yes**	**Unclear**	**Unclear**	**Yes**	**6**	–
Lee et al. [21]	**Yes**	**Yes**	**Yes**	**Yes**	**Unclear**	**Unclear**	**Unclear**	**Unclear**	**Unclear**	**Yes**	**5**	–
Natesan et al. [20]	**Yes**	**Yes**	**Yes**	**Unclear**	**Yes**	**Yes**	**Yes**	**Unclear**	**Unclear**	**Yes**	**7**	–
Lee et al. [21]	Yes	Yes	Yes	Unclear	Unclear	Unclear	Unclear	Unclear	Unclear	Yes	4	–

The bold text is highlighting all the studies with lower risk of bias.

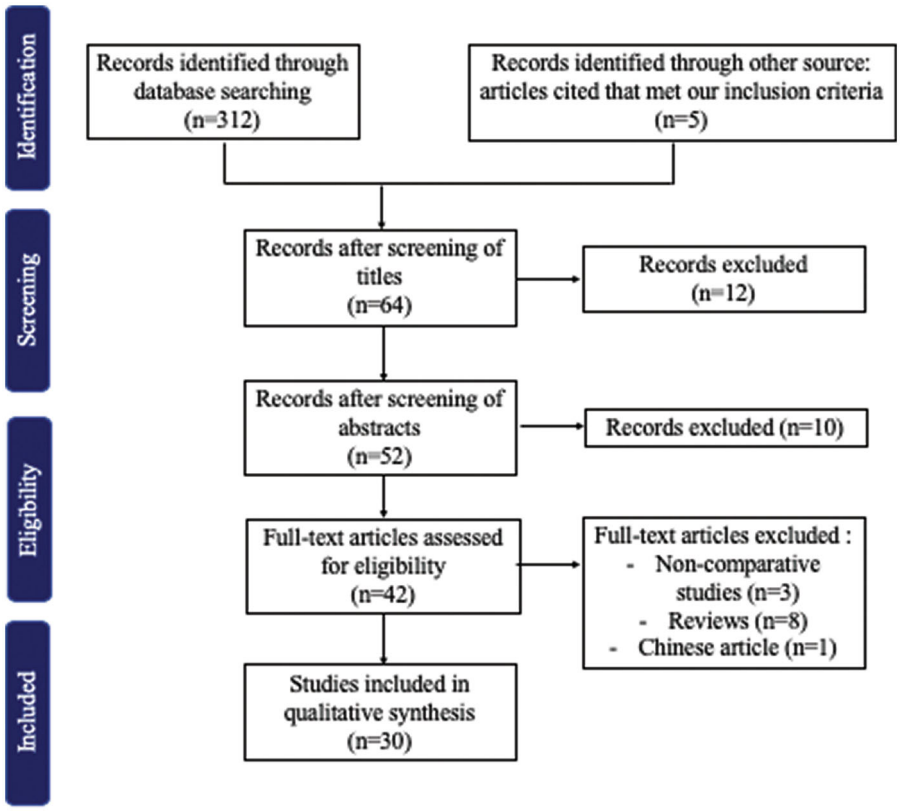

Figure 1. Inclusion and exclusion criteria.

by increasing the production of collagen [9,12,16,21,23,33,34]. Zhou et al. [9] proposed that this differentiation is caused by an increased expression of CK19, vimentin and collagen I and III, which is probably induced by the adipose extracellular matrix (ECM). Interestingly, recruitment of macrophages and enhanced granulation tissue formation in wounds were found to be stimulated by ADSC [18]. However, at the same time, ADSC in sheets seemed to promote wound healing through the suppression of macrophages and modulation of tumor necrosis factor-α expression [27]. Regarding this, ADSC were associated with an anti-scarring effect by increase secretion of hepatocyte growth factor, inhibition of differentiation of fibroblast to myofibroblast, and decrease of macrophage chemotaxis [27]. Consequently, ADSC can regulate macrophages migration in different stages of wound healing.

Discussion

Our review described the mechanisms of action of ADSC in the preclinical stage, the potential applied ADSC therapies and their outcomes to date after their use for the wound healing process. ADSC applied directly to the wounds demonstrated a greater wound healing and an increased granulation tissue formation compared with the control groups. This result was even better than the effects of the therapy with Bone Marrow Mesenchymal Stem cells and dermal fibroblasts that did not show these changes when applied directly to wounds [18]. However, throughout the reported studies the ADSC action was enhanced by cells and other factors that surround their niche such as the ECM and stromal cell-derived factor 1 (SDF-1). ADSC and adipose ECM applied directly to the wound showed a faster healing rate and better expression of collagen I and II compared to control groups [9]. In a like manner, Wu et al. evaluated the intracarotid injection of ADSC action alone and after an injection of SDF-1 directly to the wound [8]. They found that levels of ADSC exhibited a significantly higher number of ADSC located in the injured skin after an intradermal application of SDF-1, compared with the control group.

Regarding the type of ADSC administration, Kallmeyer et al. [25] have tested the action of ADSC through an injection directly to the wound site and intravenously in rats. They reported that the local administration of the ADSC has demonstrated a faster wound closure than the systematic administration probably related with the short time of life of the ADSC in blood [25].

On the other hand, depending on the type of therapy applied, the ADSC treatment for wound healing has resulted in different outcomes as we analyze in the following sections.

ADSC in sheets

Cell sheets of ADSC are scaffold-free culture systems that have been applied with the promise of decreasing the adverse responses to exogenous biomaterials [39]. During cell sheet formation, the ECM is preserved and it continuously stimulates the harvested ADSC, keeping their properties intact [40]. The disadvantages of the use of ADSC sheets are their fragility and poor mechanical properties as they easily contract, resulting in reduced graft sizes. However, these limitations may be alleviated by the use of external supports [40]. In addition, the sheet process formation is complicated and time-consuming requiring special materials. Yu et al. studied the ADSC sheet formation after the induction with L-ascorbate 2-phosphate [27]. Through this, they demonstrated a higher survival rate of ADSC 14 days after treatment, and also identified their antifibrotic effect in the last stage of wound healing. Indeed, they noted that ADSC in wounds remained only until day 28 post-treatment, which may limit the

Table 2. Articles to date classified according to the adipose-derived stem cells therapy.

ADSC directly		
Kallmeyer et al. [25]	2020	ADSC
Zomer et al. [26]	2019	ADSC
Zhou et al. [9]	2019	ADSC
Wu et al. [8]	2015	ADSC
Hong et al. [18]	2013	ADSC
ADSC in sheets		
Yu et al. [27]	2018	ADSC in sheets
Lin et al. [17]	2013	ADSC and multilayer sheet
ADSC with scaffolds		
Mirzaei-Parsa et al. [24]	2019	ADSC in nanofiber-acellular dermal matrix
Gao et al. [23]	2019	ADSC seeded in polyvinyl alcohol (PVA) hydrogel dressing
Doornaert et al. [28]	2019	ADSC in decellularized dermal matrix
Samberg et al. [31]	2019	ADSC seeded within Platelet-rich plasma (PRP) hydrogels
Hsu et al. [32]	2019	ADSC and gelatin-based hydrogel (GBH) wound dressing
Zhang et al. [29]	2018	ADSC in collagen scaffold
Zeng et al. [33]*	2017	Compound ADSC and Fibrin Gel in skin flaps
Ozpur et al. [34]	2016	ADSC and fibrin matrix
Rodriguez et al. [15]	2015	ADSC and hyaluronic acid gel with added vitamins and minerals (Cytocare 532).
Meruane et al. [19]	2012	ADSC and a Dermal substitute (Integra)
Huang et al. [30]	2013	ADSC in acellular dermal matrix
Lee et al. [21]	2011	ADSC and collagen gel
Natesan et al. [20]	2011	ADSC and matrigel
Lee et al. [21]	2011	ADSC seeded in collagen gel
ADSC in skin grafts and flaps		
Vidor et al. [13]	2018	Heterologous ADSC in skin grafts
Yucel et al. [14]	2016	ADSC in chondrocutaneous composite graft
Zeng et al. [33]*	2017	Compound ADSC and Fibrin Gel in skin flaps
Zografou et al. [22]	2011	ADSC in skin grafts
ADSC-derived microvesicles and exosomes		
Yang et al. [35]	2020	Exosomes from ADSC
Ma et al. [36]	2019	Exosomes from ADSC
Ren et al. [12]	2019	Isolated microvesicles from ADSC
Zhang et al. [37]	2018	Exosomes from ADSC
ADSC and adhesives for wound closure		
Nowacki et al. [38]	2016	ADSC and octyl blend cyanoacrylate adhesive (a topical skin adhesive for wound closure)
ADSC in concentrated hypoxia-preconditioned medium		
Sun et al. [16]	2014	ADSC in concentrated hypoxia-preconditioned medium

*Corresponded to the same study.

uncontrolled cell differentiation of these cells, increase the safety of the therapy and decrease the likelihood of malignancy. Similar results were found by Lin et al., who also pointed out that the number of sheets was directly related to the improvement in wound healing. They identified a significantly better wound healing in the triple-layer ADSC sheet compared to the single-layer ADSC group [17].

ADSC with scaffolds

In general, wound healing was enhanced when ADSC were seeded in scaffolds compared with the other groups ADSC or scaffolds alone [21,23,24,30–32,34]. Tissue engineering strategies involve the construction of 3-dimensional natural or synthetic scaffold biomaterials that are seeded with ADSC. Platelet-rich plasma (PRP) is the plasma obtained after removing blood cells through centrifugation, and contains sequestered platelets with high concentrations of cytokines, self-growth factors, and other molecules [41]. Polyethylene glycol (PEG) has been used to produce a crosslinked hydrogel in different biomaterials for wound healing like PRP that allows the release of those factors [42]. Therefore, PEGylation PRP hydrogels were shown to be an excellent scaffold for ADSC that promotes their growth and differentiation over 14 days post-treatment [31]. When compared to platelet free plasma treatment with ADSC and alone, ADSC in PEGylated PRP hydrogels showed higher neovascularization by day 8 post-treatment. However, improvement of other wound healing parameters such as epidermal thickness, epithelialization, and collagen remodeling was not found. These results were suggested to be caused by limitations of the study regarding the low number of animals studied per group, the short follow-up, and the utilization of immunosuppressed rats that were not the ideal for the study of the wound healing process. An important issue to consider this therapy in the clinical setting would be the lack of a standardized protocol and regulations for PRP generation, and the lack of identification of the specific factors in the PRP that enhance the action of ADSC in wound healing [43].

Alternatively, gelatin-based hydrogel wound dressing combined with ADSC has demonstrated to facilitate matrix formation and fast angiogenesis in injured skin also through the release of growth factors and cytokines [32]. Fibrin gel with ADSC seeded and applied in skin flaps showed better results in wound healing than other groups [33]. ADSC-seeded fibrin matrix was found to have less wound contracture, more epithelization, and minimal ulceration [34]. However, a greater vascularization was found when keratinocytes were added to the ADSC-seeded fibrin matrix, which means that keratinocytes can also enhance the regenerative properties of ADSC. Similarly, hyaluronic acid gel with added vitamins and minerals (Cytocare 532, Revitacare) was observed to shorten the time of wound healing and potentiate the ADSC effect [15]. On the other hand, Meruane et al. studied the use of dermal substitute (Integra) with ADSC and showed an increase of the microvascular density and synthesis of collagen I compared with the group using only scaffold [19]. However, the authors did not find an increase in the epithelial differentiation at sites with ADSC. These partial results may be explained due to the short period of the study, because they observed the changes in the epithelial tissue only until the 21st day after treatment and the

Table 3. Summary of articles to date that studied the use of adipose-derived stem cells for wound healing.

Authors	Year	Type of study	ADSC therapy	Number of animals /animal model	Groups of comparison	Form of administration	Source of cells	Quantity of ADSC	Outcomes
Yang et al. [35]	2020	In vitro/ In vivo	Exosomes from ADSC	BALB/c mice	3 groups: ADSC, exosomes and control groups	Injection in the injured site	Adipose tissue from patient	2 ml of suspension of 1×10^7 ADSC mixed in PBS or 2 ml of isometric cell extraction of exosome dissolved in PBS	Both ADSC and exosomes could improve wound healing at 7th postoperative day compared to control group. Exosomes enhanced the migration and proliferation of keratinocytes cells through the expression of miR-21.
Kallmeyer et al. [25]	2020	In vitro/ In vivo	Only ADSC	57 Wistar rats	27 rats treated with ADSC systemically, 21 rats treated with ADSC locally and 9 rats treated with NaCl systemically (control group)	Systemically injection into the tail vein and local injection	Inguinal subcutaneous adipose tissue from rats	2×10^6 ADSC systemically and 2×10^5 ADSC in two sides of each wound.	No ADSC were detected in the wound site when systemically administered. Locally administered ADSC were detectable for 7 days at the injection site with an increase at 72 h. Wound closure was significantly faster when ADSC were administered locally (19 days, $p = 0.01$) and systematically (21 days, $p < 0.01$) compared with the control group (26 days).
Zomer et al. [26]	2019	In vitro	Only ADSC	–	2 groups: Dermal stem cells group and ADSC group	–	Subcutaneous adipose tissue or dermis from healthy patients who underwent abdominoplasty.	–	The in vitro wound closure are was greater for dermal stem cells compared with ADSC 24 h after the scratch. Both demonstrated to promote angiogenesis.
Mirzaei-Parsa et al. [24]	2019	In vitro/ In vivo	ADSC in nanofiber-acellular dermal matrix	56 Wistar rats	7 groups (8 rats/ group): Control, acellular dermal matrix, acellular dermal matrix with ADSC, nanofiber, nanofiber with ADSC, bilayer scaffold, bilayer scaffold with ADSC.	Application in the injury site	Adipose tissue from rats	Scaffolds seeded with ADSC at a density of 3×10^5/ well	The wound closure rate was higher when ADSC were seeded into scaffolds and the healing process was significantly accelerated. Also it was found a decrease in the inflammation at day 14 with the bilayer-ADSC group in comparison to the bilayer scaffold without cells.
Ma et al. [36]	2019	In vitro	Exosomes from ADSC	–	3 groups: Exosomes, PBS and Control groups	–	–	–	Exosomes could more efficiently cause the proliferation, migration and inhibit the apoptosis of keratinocytes. Also, it was found an increase of the expression of B-catenin in the exosomes group.

(*continued*)

Table 3. Continued.

Authors	Year	Type of study	ADSC therapy	Number of animals /animal model	Groups of comparison	Form of administration	Source of cells	Quantity of ADSC	Outcomes
Gao et al. [23]	2019	In vitro/ In vivo	ADSC seeded in polyvinyl alcohol (PVA) hydrogel dressing	Sprague-Dawley rats	2 groups: ADSC in PVA hydrogel and only PVA hydrogel groups	Application in the injury site	Adipose tissue from mice	Dressings seeded with 400 μL of ADSC suspension in 48-well plates at 1×10^5 cells/mL	The *in vitro* experiment showed that ADSC seeded in PVA hydrogel promoted fibroblast proliferation and migration. ADSC in PVA hydrogel performed faster wound healing compared with PVA hydrogel without ADSC.
Doornaert et al. [28]	2019	In vitro/ In vivo	ADSC in decellularized dermal matrix	15 T-cell deficient nude mice (BALB/c-nude)	Left side with only decellularized dermal matrix, the right side with ADSC seeded in the decellularized dermal matrix	Application in forms of discs the injury site	Adipose tissue from abdominal liposuction in a patient	Discs seeded with 1×10^5 ADSC	Re-epithelization on the right side was faster and increased in the right side compared to the left side. Granulation thickness and neovascularization on the right side was greater than the left side.
Zhou et al. [9]	2019	In vitro/ In vivo	Only ADSC	30 BALB/c male mice	3 groups (10 mice/ group): a phosphate-buffered saline (PBS) group treated with an injection of 1 ml PBS at the wound base and wound edge, a control group treated with an injection of 1 ml ADSC, and an extracellular matrix group (ECM) injected with 1 ml of ADSC-conditioned media with ECM	Injection at the wound base and edge	Adipose tissues from five healthy adult women admitted for liposuction from abdomen and thigh	1 ml of ADSC suspension, 5×10^5 cells/ml	More fibroblasts were found in the group with induction of ADSC with adipose ECM, also they showed an increased expression of CK19 and vimentin, greater degree of fibrosis and positive effect of the adipose ECM on the differentiation of ADSC into fibroblasts. Upregulated expression of the wound healing factors Col I and Col III in differentiated fibroblasts, and increase in collagen fibers.
Samberg et al. [31]	2019	In vitro/ In vivo	ADSC seeded within Platelet-rich plasma (PRP) hydrogels	40 male athymic rats	5 groups (8 rats/ group): saline control (150ul of saline), Platelet Free Plasma (PFP) gel, PFP + ADSC gel, PRP gel, and PRP + ADSC gel treatments, PRP was defined as 200,000 platelets/uL	Injection into the injured sites	Discarded Human abdominoplasty skin tissues.	2 ml of total hydrogel with 200 μL (1×10^6 cells) of ADSC mixed with polyethylene glycol modified (PEGylated) platelet-rich plasma solution	After 14 days of incubation in vitro, increased platelet concentration resulted in higher ADSC proliferation, vascular gene and protein expression. In the *in vivo* model, wound treated with PRP + ASC hydrogels increased the number of vessels in the wound by day 8(80.2 vs 62.6 vessels/ mm2) compared to controls.
Ren et al. [12]	2019	In vitro/ In vivo	Isolated microvesicles from ADSC (ADSC-MV)	18 male BALB/ c mice	2 group (9 mice/ group): 50 μl Phosphate-buffered saline (PBS) group and 50 μl ADSC-MV (1.0 μg/μl)	Injection in the injured skin at five sites once the wound was created.	From the subcutaneous fat of patients.	–	The local injection of ADSC-MV at wound sites significantly increased the re-epithelialization, collagen deposition, neovascularization and led

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Table 3. Continued.

Authors	Year	Type of study	ADSC therapy	Number of animals /animal model	Groups of comparison	Form of administration	Source of cells	Quantity of ADSC	Outcomes
									to accelerated wound closure. ADSC-MV could be internalized by Human Umbilical Vein Endothelial Cells (HUVEC), human immortalized keratinocyte cell lines (HaCAT), and fibroblast, and promote proliferation, migration and angiogenesis.
Hsu et al. [32]	2019	In vitro/ In vivo	ADSC and Gelatin-Based Hydrogel (GBH) wound dressing	18 FVB mice and 18 pigs	3 groups for mice and 3 groups for pigs: (6 animals/group): the GBH wound dressing (control group), the GBH wound dressing combined with ADSC and PBS, and the GBH wound dressing combined with ADSC and its culture medium.	Application on the injury site	Adipose tissue collected from mice and pigs.	1×10^6 ADSC	Morphology of both ADSC in mouse and pigs was spindle-shaped fibroblast. ADSC of the pigs induced a higher growth rate and increased colony units as compared with the ADSC of mice. GBH wound dressing combined with ADSC and its culture medium proved to be superior in promoting wound healing and had a smaller wound area of healing compared to the control and GAP wound dressing samples.
Yu et al. [27]	2018	In vitro/ In vivo	ADSC in sheets	9 Nude mice	3 groups (3 mice/ group): 100 μL phosphate-buffered saline (PBS) group, Monolayer ADSC group, ADSC sheets group.	Application of the monolayer and ADSC sheets in one of the two dorsal wounds in every mouse. Contralateral wound injected with 100 μL of PBS as control	Subcutaneous fat tissue of 4 nonsmoking, nondiabetic female donors.	7.5×10^5 ADSC in 100 μL of PBS comparable number for the sheets group and the monolayer group.	ADSCs sheets promote wound healing and ameliorate neoskin quality. ADSC sheets at 14 days after injury, showed more engrafted in the wound tissue significantly. The neoskin formed in the presence of ADSC sheets exhibited a comparable thickness to normal skin with high organized collagen structure.
Vidor et al. [13]	2018	In vivo	Heterologous ADSC in skin grafts	15 male Wistar rats	3 groups (5 rats/ group): ADSC resuspended in saline solution (200 μL) administered in skin graft, control group with only saline solution (200 μL), and negative control group that did not received any treatment (only grafted).	Subcutaneous injection in the skin graft	Inguinal fat of 4 Wistar rats	1×10^6 ADSC in 200 μL 0.9% saline solution	The presence of epidermolysis or epidermal necrosis was significantly lower in the ADSC group than in the other groups. ADSC group presented lower ulceration rates than the other groups, but without any statistical significant differences. Epidermal inflammatory infiltrate was statistically significant lower in the ADSC group than in the saline solution group.

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Table 3. Continued.

Authors	Year	Type of study	ADSC therapy	Number of animals /animal model	Groups of comparison	Form of administration	Source of cells	Quantity of ADSC	Outcomes
Zhang et al. [37]	2018	In vitro./ In vivo	Exosomes from ADSC	20 male BALB/ c mice	2 groups: Control (PBS-treated) and ADSC exosomes groups	Subcutaneously and intradermally in injury site	Adipose tissue from human liposuction	ADSC exosome group: 200 μg of ADSC exosomes suspended in 200 μl of PBS	Wound closure was faster in mice who were treated with ADSC exosomes compared with the control group. Enhanced re-epithelization and angiogenesis were also greater in ADSC exosome group.
Zhang et al. [29]	2018	In vitro/ In vivo	ADSC in collagen scaffold	24 nude mice	4 groups: ADSC in collagen scaffold, only collagen scaffold, only ADSC, control group	Application in the injury site	Adipose tissue from patients	2×10^5/well into a 24-well plate	The thickness of regenerative tissues and vascularization were greater in the group with ADSC seeded in the collagen scaffold compared with all the other groups.
Zeng et al. [33]	2017	In vitro/ In vivo	Compound of ADSC and Fibrin Gel in skin flaps	24 pure green fluorescent protein (GFP) transgenic rats	4 groups (6 rats/ group): Model group (autologous skin flap transplantation), ADSC transplantation group, fibrin gel transplantation group and compound transplantation group.	Transplantation in the site of injury	1 ml of adipose tissue from groin of the healthy pure green fluorescent protein (GFP) transgenic rats.	5×10^6 ADSC / ml in 0.15 ml of the membrane gel	The skin flap survival rate of the compound group 7 and 21 d after transplant was significantly higher than the ADSC group, followed by the model group and the fibrin gel group, respectively. *In vitro*: a large number of GFP-ADSC positive cells differentiated into dermal fibroblasts and transformed into endothelial cells to form tube-like structures in compound group. Time in wound healing was significantly shorter for the compound group. Increased angiogenesis was found in the compound group statistically higher than other groups.
Yucel et al. [14]	2016	In vitro/ In vivo	ADSC in chondro cutaneous composite graft	36 Wistar Albino rats	6 groups (6 rats/ group): Groups 1, 2, and 3 were the groups with implanted grafts immediately after the defect was formed; and Groups 4, 5, and 6 were those in which grafts were adapted 4 days after the defect was formed.	Injection of the cutaneous edges of the defects at sites 3, 6, 9, 12 lines and muscle tissue on the floor of the wound.	Inguinal area of the rats.	Group 1 and 4 with 1 ml of GFP tagged ADSC containing 1×10^6 cells	Composite graft survival areas of the group treated with ADSC increased significantly, in comparison with control and medium groups. Immunofluorescence staining studies showed less apoptosis and fewer GFP (β) ADSC in the composite grafts of the stem cell group. Apoptosis was more severe in the

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Table 3. Continued.

Authors	Year	Type of study	ADSC therapy	Number of animals /animal model	Groups of comparison	Form of administration	Source of cells	Quantity of ADSC	Outcomes
					Groups 1 and 6 were treated with ADSC, and Groups 2 and 5 were injected with 1ml medium solution. Groups 3 and 6 did not receive any injection.				control and medium groups which also had decreased vascularity in the graft.
Ozpur et al. [34]	2016	In vitro/ In vivo	ADSC and fibrin matrix	30 Wistar albino rats	5 groups (6 rats/ group): Group 1, no graft and secondary wound healing model; group 2, only fibrin matrix; group 3, a keratinocyte-coated fibrin matrix; group 4, ADSC-seeded fibrin matrix; and group5, a keratinocyte-coated and ADSC-seeded fibrin matrix.	Application in the injury site	1 ml fat tissue from the inguinal region of the rats	–	Ulcerated areas were presented in the center of the lesions, and severe contracture and less epithelialization were observed in groups 1, 2, and 3. Less contracture, more epithelialization, and minimal ulceration were observed in group 4. Vascular size of group 5 was significantly greater than those of groups 3 and 2. The collagen volume measurement in group 5 was significantly greater than those of group 3. Epithelial progression was greater in group 4, and nearly complete in group 5.
Nowacki et al. [38]	2016	In vivo	ADSC and octyl blend cyanoacrylate adhesive (a topical skin adhesive for wound closure)	40 nude athymic RNU rats(35 for the in vivo experiments, 5 used as donors of ADSC)	6 groups: Study group (10 rats) ADSC combined with adhesive application for wound closure, 5 control groups I-V (5 rats each), different wound closure. Control I: only adhesive, Control II: adhesive + Nexcare™ Steri-Strip™, Control III: Nexcare™ Steri-Strip™, Control IV: sutures, Control V: no closing material applied.	Multipoint injection of six surrounding intrawound areas	Retroperitoneal space of 5 donor rats.	–	In the study group, healing was mild but not palpable, linear, symmetric scar formation with good cosmetic appearance.
Wu et al. [48]	2015	In vitro/ In vivo	Only ADSC labeled with Green Fluorescent Protein (GFP-ADSC)	Number not described /Sprague Dawley rats	Group 1 (traumatic rats) received intradermal injection of Stromal cell-derived factor 1	Intracarotid artery injection	Inguinal fat pads of rats	Green Fluorescent Protein- ADSC 0.5 ml, 1×10^7 cells	In SDF-1 treated rats the levels of ADSC in plasma increased as the time passed, and reached to the peak value in 21 days.

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Table 3. Continued.

Authors	Year	Type of study	ADSC therapy	Number of animals /animal model	Groups of comparison	Form of administration	Source of cells	Quantity of ADSC	Outcomes
					(SDF-1) 1 μg in wound sites at 0,1,2,3,and 4 days; group 2 (traumatic rats received PBS); group 3(normal rats received PBS). All groups were treated with GFP-ADSC.				In SDF-1 treated rats exhibited a significantly increased number of ADSC in injured skin compared with PBS treated rats.
Rodriguez et al. [15]	2015	In vitro/ In vivo	ADSC and Cytocare® 532 (hyaluronic acid gel with vitamins and minerals).	30 nude mice	3 groups: group 1 (6 mice, spontaneous healing), group 2 (12 mice, Cytocare® 532), and group 3 (12 mice, ADSC in Cytocare® 532)	1 ml of the final product intradermally in four injection sites, and other 500 μL applied into the wound surface.	Surgical residue harvested from human patients donors.	1×10^6 ADSC/ml of Cytocare®532 (500 μL Cytocare®532 containing 0.5×10^6 cells)	ADSC on wound healing shortened the time to complete healing for more than six days when compared to the spontaneous healing. Cytocare® 532 has been shown to potentiate the ADSC effect. No defect in vascular smooth muscle function suggested that the enhanced endothelial response observed in the ADSCs groups could produce an improved endothelial function.
Sun et al. [16]	2014	In vitro/ In vivo	ADSC in a concentrated conditioned medium of hypoxia-preconditioned	30 female Sprague-Dawley rats	3 groups(10 rats/ group): group 1 (concentrated hypoxia-preconditioned ADSC-conditioned medium group), group 2 (ADSC-conditioned medium group), group 3 (control group with 0.4% rat serum medium)	Injection in the injured sites.	Abdominal subcutaneous adipose tissue collected from female Sprague-Dawley rats	$1.4 \pm 0.5 \times 10^6$/mL from abdominal fat cultured for 7 days	Wound closure after treatment with Concentrated Hypoxia-preconditioned ADSC-conditioned medium showed well-organized epidermis, thick cuticular layer, and increased collagen content, whereas wound areas treated with ADSC-conditioned medium group showed immature epidermal regeneration, weak cuticular layer, and less collagen content.
Lin et al. [17]	2013	In vitro/ In vivo	ADSC and multilayer sheet	18 athymic nude mice	3 groups (6 mice/ group): non-treated control group; one-layer ADSC sheet treatment, and three-layer ADSC sheet treatment group.	Direct application to the wounds immediately after wounding.	Discarded human abdominal subcutaneous adipose tissue	1×10^6 human ADSC were seeded into a surface plate to form a confluent layer.	The injury area of the ADSC sheet group was significantly smaller than the non-treated control group at all time points except day 21. The triple-layer ADSC sheet treatment significantly enhanced wound healing compared to the single-layer ADSC sheet at 7, 10 and 14 days. The density of blood vessels showed

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Table 3. Continued.

Authors	Year	Type of study	ADSC therapy	Number of animals /animal model	Groups of comparison	Form of administration	Source of cells	Quantity of ADSC	Outcomes
									that ADSC cell sheet treatment slightly enhanced total vessel proliferation compared to the empty wound injury treatment.
Hong et al. [18]	2013	In vitro/ In vivo	Only ADSC	10 Rabbit ear model	6 wounds were created in both ears of each rabbit. Contralateral ear of each rabbit was used as control. 6 rabbits were used to evaluate ADSC and 4 rabbits to evaluate dermal fibroblast and Bone Marrow-Mesenchymal Stem Cells.	Direct application into the wounds	Inguinal fat pads from female New Zealand White rabbits	–	ADSC topically delivered increased granulation tissue formation in wounds compared to saline control groups, whereas Bone Marrow Mesenchymal Stem Cells and dermal fibroblasts did not.
Meruane et al. [19]	2012	In vitro/ In vivo	ADSC and a Dermal substitute (Integra)	8 male Sprague Dawley rats	ADSC seeded into a piece of dermal substitute for 48 hours, and then implanted in rats. Comparison with contralateral implant without ADSC in the same rats.	ADSC seeded into a piece of dermal substitute and then applied in the dorsal wounds.	Inguinal region of 8 Sprague-Dawley adult rats	1.5×10^5 ADSC seeded in parallel onto wells containing or not the scaffold	ADSC adhered well to the dermal matrix, and autologous tissue integration in the rat was good. ADSCs significantly increased microvascular density and synthesis of collagen I compared to the scaffold alone. There was a tendency for migration of keratinocytes from the edges of the wound to increase, but this difference was not significant.
Huang et al. [30]	2012	In vitro/ In vivo	ADSC in acellular dermal matrix	BALB/c-nu mice	4 groups: silicon only, silicon with ADSC, acellular dermal matrix only, ADSC seeded in acellular dermal matrix	Application in the injury site	Adipose tissue from human liposuction of the abdomen	1×10^5 ADSC/well into 96-well plate	The greater wound closure rate, granulation thickness and neovascularization was found in the ADSC seeded in acellular dermal matrix group.
Zografou et al. [22]	2011	In vitro/ In vivo	ADSC in skin grafts	20 male Sprague-Dawley rats	2 groups (10 rats/ group): ADSC and control groups	Injection into the fascial layer of the wound before skin-graft	Inguinal fat pads of each rat	1×10^6 ADSC resuspended in PBS	The ADSC group showed a lower area of skin graft necrosis compared with the control group. A greater angiogenesis and increased VEGF and TGFb3 expression were found in the ADSC group compared with the control group.

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Table 3. Continued.

Authors	Year	Type of study	ADSC therapy	Number of animals /animal model	Groups of comparison	Form of administration	Source of cells	Quantity of ADSC	Outcomes
Lee et al. [21]	2011	In vitro/ In vivo	ADSC seeded in collagen gel	12 BALB/c nude mice	3 groups (4 mice/ group): ADSC in collagen gel, human dermal fibroblasts in collagen gel, and collagen gel only groups.	Application in the injury site	Subcutaneous adipose tissue from liposuction of healthy patients	1×10^6 ADSC in 300 μl of collagen solution	The size of the wound in the ADSC group was significantly smaller than in the collagen gel only group. However, dermal fibroblasts showed a faster wound healing than ADSC and collagen gel groups alone.
Natesan et al. [20]	2011	In vitro/ In vivo	ADSC and matrigel	15 *rnu* male nude athymic rats	3 groups (5 rats/ group): saline control (250 μL), Matrigel (1ml) and Matrigel (1ml) + 1 × 105 ADSC.	Direct application into the wound	Discarded human burned skin samples	1×10^5 cells of ADSC	ADSC in the hypodermis are preserved during and after severe thermal injury. ADSC -Matrigel mixture engrafted into the granulation wound bed after 8 days. ADSC were able to form tube-like networks across the three dimensional Matrigel, but became unstable after several days.
Lee et al. [21]	2011	In vitro/ In vivo	ADSC and collagen gel	12 BALB/c nude mice	3 groups (4mice/ group): Group 1, human ADSC-populated collagen gel; group 2, human dermal fibroblast (DF) populated collagen gel; group 3, collagen gel alone.	Direct application into the wound	Human subcutaneous adipose tissues from healthy female donor's elective liposuctions.	1×10^6 ADSC were added to 300 μl of collagen solution mixture.	ADSC promoted healing of full-thickness wounds in nude mice, and unexpectedly, DF closed wound faster than ADSC.

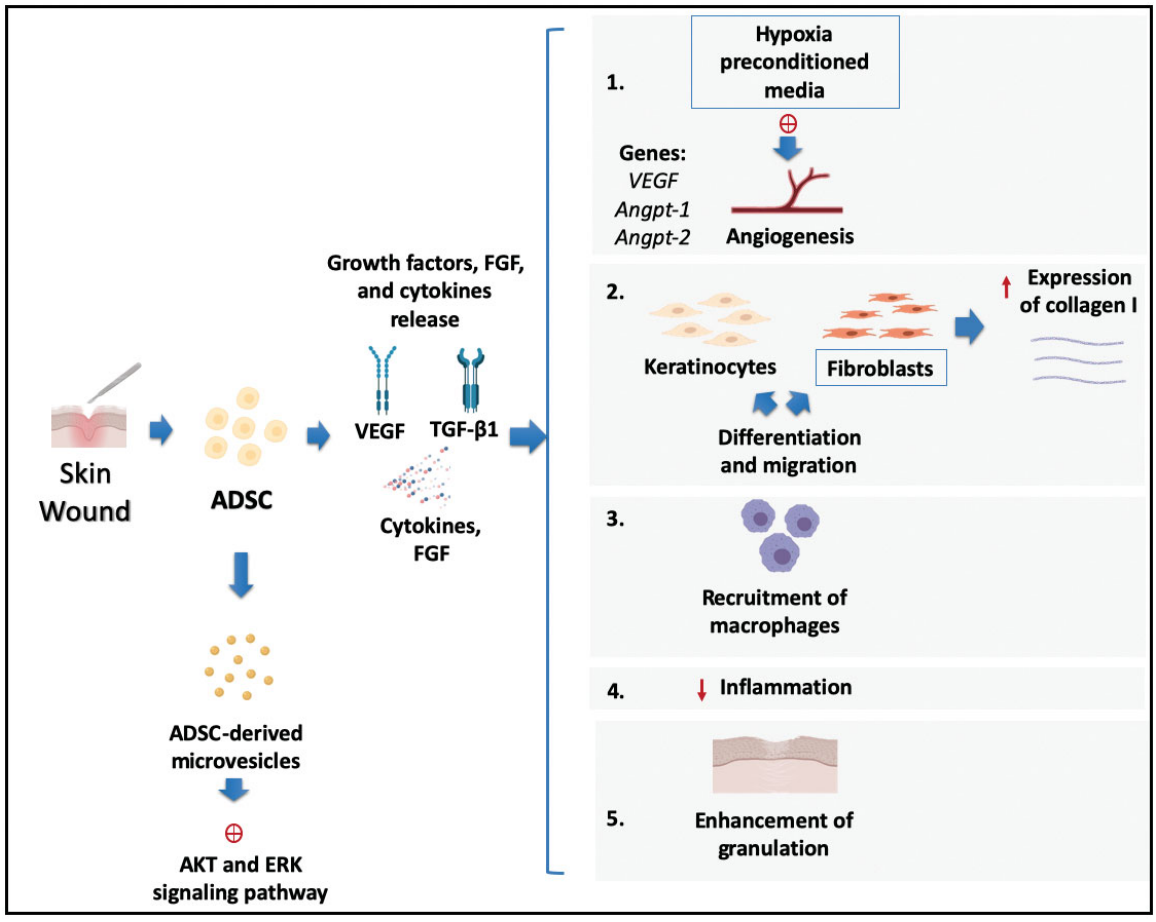

Figure 2. Mechanism of action suggested for adipose-derived stem cells in wound healing model. Abbreviations: ADSC: adipose derived stem cells; FGF: fibroblast growth factor; VEGF: vascular endothelial growth factor.

epithelial changes seem to appear at fourth week after treatment. Similarly, ADSC seeded on matrigel showed signs of microvascularization after the application within 10 days, but became unstable after 12 days which means that although matrigel can induce ADSC into an endothelial phenotype, there might be other factors that regulate the action of these cells [20]. Finally, human ADSC-seeded in collagen gel was found to promote wound healing, although the comparison group of dermal fibroblasts populated in the collagen gel closed the wound faster than the group with ADSC [21]. Despite this finding, ADSC therapy is still preferred for wound healing over fibroblasts, due to the disadvantages of self-skin isolation to harvest autologous fibroblasts and the fibroblasts' immunogenicity that could reduce their effects on wound healing that makes them less attractive.

ADSC in skin grafts and flaps

Skin grafts and flaps are used to reconstruct skin defects caused by tumor excision, as well as congenital defects and chronic wounds [13]. However, skin grafts and flaps have common adverse effects, including partial or complete necrosis and contraction, which lead to deformity of structures [44]. Added as a measure to obtain better results in the use of skin grafts and flaps, ADSC have been shown to protect them from ischemia. We found three studies that used ADSC in skin grafts [13,14,22], and one in skin flap [33]. Zografou et al. [22] reported a lower area of skin graft necrosis when ADSC were used, compared with the control group. Similarly, Vidor et al. [13], after studying the use of ADSC in skin grafts, identified a significantly lower epidermal necrosis, ulceration rates, and epidermal inflammatory infiltrate in the ADSC group, compared to the controls groups [13]. Interestingly, this study used heterologous ADSC to prove their effects, suggesting that due to the low immunogenicity, these cells could be applied in the clinical setting safely when performing skin grafts. However, regarding this, more preclinical studies with a larger sample size are needed to confirm the safety of the procedure. On the other hand, Yucel et al. [14] studied ADSC in chondrocutaneous composite grafts and found significantly less apoptosis and more survival areas of the graft in the ADSC group compared to other groups when applied ADSC four days before of the graft. However, when applied the ADSC and grafts simultaneously, total necrosis of the graft was found. This study concluded that a short period of time is needed before placing the graft to allow the ADSC settling in the tissue and maximize their neovascularization properties. Regarding the timing, Vidor et al. [13] applied the ADSC simultaneously with the grafts; however, they did find positive results in the ADSC and graft group. The differences in the results between these two studies may be explained by the type of graft used in each of the studies. A composite graft involving two types of tissue such as cartilage and skin used by Yucel et al. [14] would have needed more vascularization for viability and, as a result, a short period between the application of ADSC and the graft. Moreover, it has been known that neovascularization effects of ADSC start after day three of aplication [45].

Regarding ADSC in skin flaps, Zeng et al. [33] seeded ADSC in scaffolds of fibrin gel and found better results in the survival rate of the flap in this group compared to others.

ADSC-derived microvesicles and exosomes

Microvesicles and exosomes are mediators that allow intercellular communication and exchange of DNA, RNA, proteins, and lipids between cells [46]. ADSC derivatives in wound healing are an important new area to study due to their advantages to act directly without the need for any further processing and acting through the release of paracrine factors that contribute to tissue repair. We found promising outcomes in wound healing with the use of ADSC derivatives; although, its efficacy was not compared to any group using ADSC cellular therapy. Ren et al. [12] found that local injection of ADSC-derived microvesicles increased re-epithelialization, collagen deposition, neovascularization and accelerated wound closure. They suggested that this function was mediated by the activation levels of Akt and ERK signaling pathways in keratinocytes, fibroblasts, and endothelial cells. Upregulation of the PI3K/Akt signaling pathway was previously found to enhance wound healing. In fact, Zhang et al. [37] suggested that this signal pathway produced the internalization of the ADSC-derived microvesicles in human dermal fibroblasts and the increase in production of collagen type I and III. Moreover, increased phosphorylation and activation of Akt and ERK in endothelial cells were found to enhance endothelial cell proliferation and migration, explaining the potential angiogenic role of ADSC-derived microvesicles [47].

Regarding the studies evaluating the action of ADSC exosomes, it was reported that ADSC caused a proliferation and migration of keratinocytes, as well as, an enhanced re-epithelization and angiogenesis that improved wound healing compared with the control group [36,37]. When compared with ADSC alone, Yang et al. [35] found that both experimental groups (ADSC alone and ADSC exosomes alone) applied in the wound site in mice demonstrated a similar improvement in wound healing at the 7th postoperative day compared to the control group.

ADSC and adhesives for wound closure

The use of ADSC in adhesives for wound closure was also studied as a new therapy. Our review identified one study that found better cosmetic and aesthetic outcomes in the ADSC group compared with other types of topical skin adhesives [38].

ADSC in concentrated hypoxia-preconditioned medium

ADSC applied in a concentrated hypoxia-preconditioned medium was proposed as a possible treatment for wound healing. Sun et al. [16] studied its use in a rat model suggesting that this medium could enhance the wound healing through the angiogenesis, increase of collagen, migration of fibroblasts and recruiting of stem cells. This study found that the concentrated medium showed a better-organized epidermis, an increase in collagen content, a shorter healing time, and a better wound healing from day 7 after surgery, compared to the nonconcentrated. There was not a comparison between the use of ADSC with and without hypoxia-preconditioned medium, as a result, further studies should be conducted to measure the additional benefit of the low oxygen concentration.

Strengths and limitations

In this review, we reported the manuscripts to date studying the use of ADSC in wound healing of full-thickness defects. We summarized mechanisms of action, the type of therapies, and outcomes of the ADSC therapy studied to promote skin regeneration.

Limitations of our study included the description of reported data with considerable heterogeneity due to the different protocols presented in each manuscript. In addition, there are inherent limitations in the review methodology because of the search, selection and publication biases that should be considered. As a result, this review is descriptive in its results, in alignment with the purpose of the study.

Conclusion

Evidence has demonstrated that ADSC favor the neovascularization, migration, differentiation of fibroblasts and the upregulation of macrophage chemotaxis in wound healing. Application of ECM, SDF-1, or keratinocytes along with ADSC and ADSC therapy in scaffolds have shown to improve their effects in wound healing compared with ADSC alone. ADSC therapy in sheets, grafts, flaps, in microvesicles, exosomes, adhesives, and hypoxia media have shown different outcomes in the preclinical stage and need further studies to clarify their potential benefits. Cell therapy based on ADSC is a promising solution for wound healing of full-thickness skin defects; however, the absence of a standardized protocol of ADSC extraction makes their use difficult in a clinical setting. In addition, the number of cells and methods of application with higher numbers of animals should be taken into consideration to evaluate the results in a more generalized manner and to better understand the biology of these cells in wound healing. In the future, clinical studies should be performed after assuring the safety and efficacy of this treatment.

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