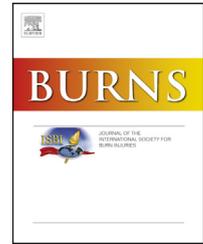


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Review

Stem cells in burn wound healing: A systematic review of the literature



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ABSTRACT

Introduction: Severe burns are often associated with high morbidity and unsatisfactory functional and esthetic outcomes. Over the last two decades, stem cells have generated great hopes for the treatment of numerous conditions including burns. The aim of this systematic review is to evaluate the role of stem cell therapy as a means to promote burn wound healing. **Methods:** Comprehensive searches in major databases were carried out in March 2017 for articles on stem cell therapy in burn wound healing. In total 2103 articles were identified and screened on the basis of pre-determined inclusion and exclusion criteria.

Results: Fifteen experimental and two clinical studies were included in the review. The majority of studies reported significant improvement in macroscopic burn wound appearance as well as a trend toward improved microscopic appearance, after stem cell therapy. Other parameters evaluated, such as re-vascularization, collagen formation, level of pro- and anti-inflammatory mediators, apoptosis and cellular infiltrates, yielded heterogeneous results across studies.

Conclusion: Stem cell therapy appears to exert a positive effect in burn wound healing. There is, therefore, justification for continued efforts to evaluate the use of stem cells as an adjunct to first-line therapies in burns.

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1. Introduction

Stem cells are undifferentiated cells characterized by their capacity for self-renewal and differentiation into various cell types. The use of stem cells to regenerate damaged tissues and organs has given rise to great hopes in the treatment of a variety of conditions, for which current therapeutic options are ineffective. The use of stem cells has attracted considerable interest also in the field of wound healing, and burns in particular, as a means to promote skin regeneration. However, such stem cell therapies remain experimental.

Burns constitute an important public health concern in the US and worldwide [1]. Advances in treatment, such as improved resuscitative management, prompt excision of burn wounds and better metabolic support, have reduced mortality due to severe burns [2–4]. However, significant challenges remain. In the acute setting, current therapeutic options can fail to provide appropriate burn wound coverage and therefore fail to prevent complications associated with disruption of the skin's protective function. In the longer term, these treatments do not achieve regeneration of fully functional skin: destruction of skin appendages, hypertrophic scarring and chronic neuropathic pain still entail significant functional and psychological morbidity for burn patients [5–7].

Studies in small and large animal models have indicated potential for improved wound healing via the use of stem cells. As a consequence, numerous reviews and editorials have stressed the potential of stem cell therapy in burns [8–19]. But no critical assessment of the available literature has yet been undertaken. In order to evaluate the experimental and clinical results achieved so far, we conducted a systematic review of the literature.

2. Methods

The study was designed according to the Cochrane Handbook for Interventional Systematic Reviews and the Preferred Reporting Items for Systematic Reviews and Meta-Analyses protocols (PRISMA-P) [20].

2.1. Literature search strategy

Comprehensive searches were carried out in EMBASE, Medline, Pubmed Publisher, Web-of Science, OvidSP, CENTRAL (The Cochrane Library 2017, issue 3) and Google Scholar. The search was conducted for articles published before April 2017 by using search terms specific to each search engine, provided in supplemental text S1. In addition, cross-referencing and related citations were used to retrieve remaining relevant literature.

2.2. Inclusion and exclusion criteria

The main inclusion criterion was the use of stem cells—either via exogenous stem cell administration or via treatment to induce mobilization of endogenous stem cells—as a means to promote wound healing and skin regeneration after thermal burn injury. Stem cells were defined as multi and pluripotent progenitor cells with the ability to self-renew and to differentiate into organ- or tissue-specific cells. Exclusion criteria were the use of oligopotent tissue-resident stem and progenitor cells (e.g. epithelial and dermal stem cells), as well as genetically modified stem cells, with the exception of labeling. Non-thermal burn injuries (e.g. chemical and radiation burns), injuries to organs other than the skin (e.g. cornea, esophagus and airways) and pre-existing burn scars were also excluded.

2.3. Literature screening

Articles were screened by two individual researchers (ARA, MC) for relevance and inclusion. Only full-text, original articles written in English were considered for inclusion in the study. All reviews, conference abstracts, book chapters, letters and editorials were excluded. The same independent reviewers screened titles, keywords and abstracts of all considered articles, according to the pre-established criteria — all articles were evaluated using the PICO method [21].

During the process of inclusion and exclusion, we noted that, in a significant number of studies, stem cells were delivered to burn wounds as part of a scaffold. Since scaffolds

varied considerably in structure and composition, we reasoned that this heterogeneity would compromise our ability to make comparisons and draw conclusions on the role of stem cells in burn wound healing. We therefore decided to retain administration via a scaffold as a further exclusion criterion. For the purpose of this review, we defined the term scaffold as a three-dimensional structure which, when implanted, serves as a temporary platform for cell survival, proliferation, and differentiation, and gradually degrades allowing for its replacement by endogenous tissue [22]. We considered therefore that fibrin glue, used for the topical application of stem cells onto burn wounds, did not constitute a ‘true’ scaffold but rather a vehicle for cell delivery. We therefore decided to retain studies using this method of stem cell delivery.

3. Results

The primary search yielded 2103 studies and the initial screening by title, key words, and abstract resulted in 40 eligible articles. The 40 articles were read in full and 23 were excluded. The PRISMA-P flow diagram for systematic reviews is presented in Fig. 1. Of the seventeen studies retained, fifteen

were experimental and two were clinical studies. Formal statistical analysis could not be performed due to the small number of studies and the heterogeneity of variables assessed. A detailed systematic review was conducted instead.

3.1. Experimental studies

The experimental studies were performed in rat (n=9), mouse (n=4) and pig (n=2) models (Table 1). A variety of techniques were used to induce either full- or partial-thickness thermal burn injuries. The extent of burn injuries varied from 5 to 30% of total body surface area (TBSA). Thirteen out of fifteen experimental studies evaluated the impact of stem cells on burn wound healing, while two studies more specifically assessed the effect of stem cells on burn wound progression by means of the comb burn model [23,24]. In this model, a brass comb is used to create several burns separated from each other by interspaces. The burns constitute the zone of necrosis, while the interspaces constitute the zone of ischemia, which is initially viable but is at risk of infarction due to burn progression.

All fifteen studies assessed the impact of exogenous stem cell administration. Mesenchymal stem cells (MSCs) were the most frequently used (n=11), followed by adipose-derived stem cells (ASCs) (n=4). MSCs were derived from either bone

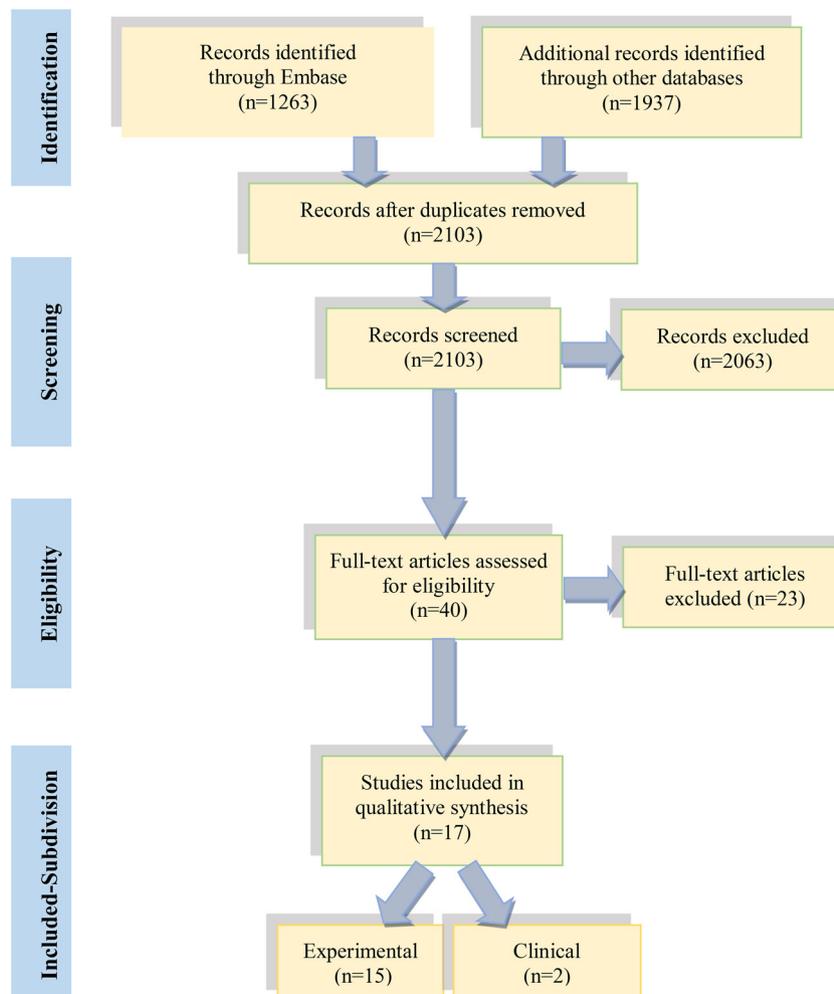


Fig. 1 – PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) flowchart of the systematic literature search.

Table 1 – Summary of the experimental animal studies.

Author & Year	Species	Burn Model	Stem Cell Type	Phenotyping	Dosage	Administration	Timing (post burn)
Xue et al. 2013	Mice	Partial-thickness (3–5% TBSA)	Human BM-MSCs	Yes	1×10^6 cells/wound	Local injection	Immediately
Karimi et al. 2014	Mice	Full-thickness	Mouse ASCs ¹	Yes	1×10^6 cells/wound	Local injection	Immediately
Loder et al. 2015	Mice	Partial-thickness (30% TBSA)	Syngenic ASCs	No ²	1×10^6 cells/wound	Local injection	1 day
Bliley et al. 2016	Mice	Full-thickness	Human ASCs	No ²	6.8×10^6 cells/wound	Local injection	24 h
Shumakov et al. 2003	Rat	Full-thickness (18–20% TBSA)	Auto + Allogenic BM-MSCs	No	2×10^6 cells/wound	Topical	2 days
Singer et al. 2013	Rat	Full-thickness, comb burn model	Rat MSCs ¹	No	1×10^6 cells/animal	Tail vein	60 min
Liu et al. 2014	Rat	Full-thickness (30% TBSA)	Human UCB-MSCs	No ²	5×10^6 cells/animal	Tail vein	3 days
Yang et al. 2014	Rat	Partial-thickness	Allogenic BM-MSCs	Yes	5×10^7 cells/wound	Topical	4 days
Caliani-Oliveira et al. 2016	Rat	Full-thickness (45.0 cm ²)	Mouse BM-MSCs	Yes	5×10^6 cells/wound	Local injection	20 min
Okusz et al. 2013	Rat	Full-thickness, comb burn model	Allogenic BM-MSCs	Yes	1×10^6 cells/wound	Local injection	30 min
Zhang et al. 2015	Rat	Full-thickness	Human UCB-MSCs	Yes	2×10^6 cells/animal	Local injection	24 h
Hosni Ahmed et al. 2017	Rat	Full-thickness	Allogenic BM-MSCs	Yes	1×10^6 cells/wound	Local injection	Unspecified
Shi et al. 2017	Rat	Partial-thickness	Human UCB-MSCs	No ²	1×10^6 cells/wound	Local injection	Immediately
Clover et al. 2015	Pig	Partial-thickness (5% TBSA)	Allogenic BM-MSCs	Yes	4.5×10^6 cells/wound	Topical	Immediately
Foubert et al. 2016	Pig	Full-thickness	Autologous ASCs	No ²	2.5×10^6 cells/wound	Local injection or Topical	2 days

¹ The authors do not specify whether stem cells are auto, syn or allogenic.

² The authors did not phenotype the stem cells, but provide references for isolation and expansion protocols used.

marrow (BM-MSCs) (n=8) or umbilical cord blood (UCB-MSCs) (n=3). From 1 to 50 million stem cells were administered either by local (n=9) or systemic injection (n=2). In three studies stem cells were delivered by topical application [25–27] (Fig. 2). One study specifically compared local injection and topical application of ASCs [28]. Timing of administration varied between 0h and 4 days post-burn.

3.2. Macroscopic assessment

Of the thirteen studies that assessed the effect of stem cells on burn wound healing, all but one included macroscopic assessment of burn wound, either by direct observation and manual measurement or by digital photography and software analysis. Results were expressed via various parameters: wound healing time (time to complete wound re-epithelialization), wound healing rate [(original wound area – wound area at defined time point)/original wound area] × 100, percentage wound re-epithelialization [(wound area at defined time point/original wound area) × 100], wound area, diameter and depth. Eleven out of twelve studies reported a positive effect of stem cells with regard to these parameters (Table 2). However, only one study specifies whether macroscopic assessment of burn wound was conducted by operators blinded to treatment group allocation [28].

3.3. Microscopic assessment

All fifteen studies included histological assessment of burn wound by hematoxylin and eosin (H&E) staining; however, the variety of histological features reported is considerable. A clear trend toward more favorable histological appearance in stem cell-treated wounds was overall evident across all studies, although most studies include only descriptions, but no quantitative measurements. The presence of hair follicles in stem cell-treated but not in control burn wounds was noted in two studies [27,29], one of which also observed the presence of ‘obviously proliferous’ sebaceous glands [27]. However, only four studies mention whether histological assessment was performed by a histopathologist blinded to treatment group allocation [28,30,31,34].

3.4. Collagen content

During the proliferative phase of wound healing, an increase in type III collagen and in type III to type I collagen ratio occurs as a result of the spurt in collagen production. During the maturation phase, type III collagen is progressively replaced by type I collagen and the normal predominance of type I over type III collagen is restored. Three studies noted a trend toward increased total collagen content on Masson’s Trichrome or Picrosirius Red staining in stem cell-treated burn wounds [26,29,31]. Bliley et al. reported significantly higher collagen type III mRNA expression in stem cell-treated burn wounds on days 4, 7, 14 and 21 post-burn, as well as significantly higher collagen type III to I ratio [29]. On the other hand, Liu et al. noted significantly higher collagen type I to III ratio via ELISA on weeks 1, 2, 3, 6, 8 and 11 post-burn [30] and Shi et al. also reported significantly higher collagen type I to III ratio via RT-PCR at 2 weeks post-burn [32].

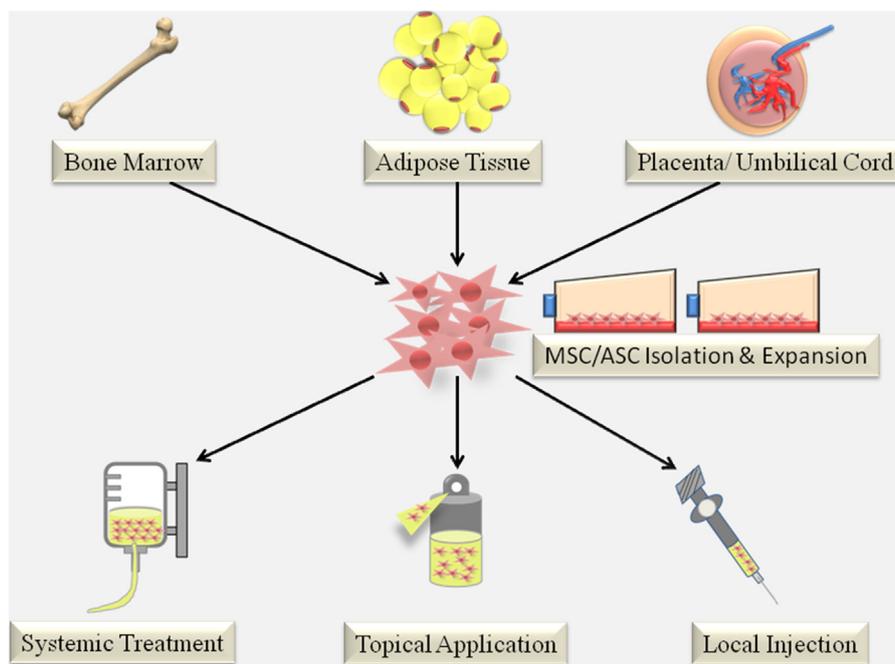


Fig. 2 – Graphical summary of different stem cell sources and administration routes.

3.5. Re-vascularization

Three studies evaluated the presence of blood vessels via H&E staining: two found significantly higher blood vessel numbers in experimental versus control groups [33,34]. Four studies performed immunohistochemistry (IHC) for the endothelial cell marker CD31: three reported significantly higher numbers of

CD31 positive vessels [28–30]. Liu et al. assessed burn wound microcirculation by means of Laser Doppler Flowmetry (LDF) and found significantly higher flow in stem cell-treated burn wounds at weeks 1, 2, 3, 6 and 8 [30]. Three studies also evaluated the presence of pro-angiogenic factors and found significantly higher levels of VEGF and/or angiopoietin-1 and 2 in stem cell-treated wounds, as assessed by ELISA or RT-PCR [30,33,35].

Table 2 – Study characteristics and macroscopic findings.

Author & Year	Species	Control (n) animals	Treatment (n) animals	Healing Control	Healing Treated	Significance
Xue et al. 2013	Mice	30	30	25 days*1	20 days	p < 0.05
Karimi et al. 2014	Mice	9	9	76.89 ± 23 mm ² *2	53.78 ± 23 mm ²	p = 0.09
Loder et al. 2015	Mice	3	3	215.2 ± 19.2 μm*3	157.1 ± 22.0 μm	p < 0.05
Bliley et al. 2016	Mice	12	12	14–21 days*4	14–21 days	p > 0.05
Shumakov et al. 2003	Rat	10	10	29 cm ² *5	5 cm ² (Auto), 11 cm ² (Allo)	p < 0.05
Liu et al. 2014	Rat	6	6	93 ± 3 days*6	74 ± 4 days	p < 0.01
Yang et al. 2014	Rat	5	5	21 days*7	51 days	p < 0.01
Caliari-Oliveira et al. 2016	Rat	7	7	76.11 ± 3.46%*8	90.81 ± 5.05%	p < 0.05
Zhang et al. 2015	Rat	6	6	35 ± 2.4 days*9	29 ± 2.8 days	p < 0.05
Hosni Ahmed et al. 2017	Rat	12	12	N/A*10	N/A	N/A
Shi et al. 2017	Rat	6	6	1/6 animals*11	5/6 animals	N/A
Clover et al. 2015	Pig	9 wounds	9 wounds	23.6 ± 4.75%*12	6 ± 2.5%	p < 0.05
Foubert et al. 2016	Pig	10	10	3.7 ± 0.5 cm ² *13	5.8 ± 0.6 cm ² (Injection)	p < 0.05
				3.7 ± 0.5 cm ² *13	4.8 ± 0.4 cm ² (Topical)	p < 0.05

*1) wound healing time; *2) wound surface area at 3 weeks; *3) wound depth at day 14; *4) wound healing time; *5) wound surface area at day 30; *6) wound healing time; *7) wound healing time: 9 out of 10 experimental wounds healed by day 21 while all control wounds healed by day 51; *8) percentage wound epithelialization at day 60; *9) wound healing time; *10) study does not report on macroscopic appearance; *11) number of animals with completely re-epithelialized wounds at two weeks; *12) percentage of wound unhealed at day 14; *13) absolute area of wound epithelialization at day 14.

3.6. Cellular infiltrates

Five studies commented on the presence and size of inflammatory infiltrates within burn wounds on H&E: one of these evidenced more abundant cellular infiltrates at day 45 post-burn, as well as a 'late neutrophil influx' detected by metalloproteinase (MPO) assay [34] while two reported the presence of smaller infiltrates in the stem cell-treated group [30,35]. Foubert et al. and Karimi et al. did not provide evidence for any difference in lymphocyte, neutrophil and macrophage counts on H&E [28,31]. However, Foubert et al. did note a significantly lower neutrophil count on IHC staining for MPO at day 7 post-treatment [28]. Similarly, Liu et al. report significantly lower numbers of neutrophils and macrophages – defined as c-ANCA and ED-1 positive cells on IHC – in stem cell-treated burn wounds [30].

3.7. Anti-inflammatory effect

Five studies measured the level of pro- and anti-inflammatory cytokines in burn wounds and/or in serum via ELISA. Oksuz et al. reported no significant difference between groups at 72h post-burn [23]. On the other hand, Liu et al. observed significantly higher IL-10 and lower IL-1, IL-6 and TNF- α levels in stem cell-treated wounds at 2 weeks [30]. Hosni Ahmed et al. reported similar findings for IL-10 and TNF- α serum levels at days 7 and 20 [35].

While Zhang et al. described significantly lower IL-6 and TNF- α serum levels they also found significantly lower IL-10 levels at days 3, 5 and 7 [36]. Caliri-Oliveira et al. reported higher IL-10, but also higher IL-6 plasma levels at day 30 [34]. Interestingly, while Zhang et al. did not find any differences in IFN- γ serum levels, they noted that local MSC administration abolished white cell count (WCC) and C-reactive protein (CRP) surge at day 2 and days 2 and 3 post-burn, respectively [36]. Overall, it appears that stem cells, whether administered via local or via intravenous injection, can modulate both wound cytokine milieu and systemic cytokine levels.

3.8. Apoptosis and viability

Oksuz et al. assessed interspace viability by scintigraphy at 72h and reported higher average percentage of viable tissue in stem cell-treated interspaces [23]. Singer et al. reported a significant reduction in both the number of interspaces and total interspace surface that underwent necrosis in the treatment group by day 7 post-burn [24]. Oksuz et al. also observed lower apoptosis count in stem cell-treated interspaces, as assessed by TUNEL, at 72h post-burn [23]. Similarly, in a standard burn wound healing model, Loder et al. reported significant lower caspase-3 staining at day 5 post-burn in ASC-treated wounds [37].

3.9. Fate of stem cells

Seven studies assessed the presence of stem cells at the site of injury via a variety of methods. Liu et al. used Bioluminescence imaging (BLI) to track the course of GFP-labeled human MSCs administered by intravenous injection: human MSCs migrated into the wound by week 1 and concentrated at the edge and

base by week 2 and 3, respectively [30]. The presence of human MSCs within wound was further confirmed by RT-PCR at weeks 1, 2 and 3. While Singer et al. administered Quantum-dot (Q-dot)-labeled MSCs also by intravenous injection, they could not detect any Q-dot-labeled MSCs in the interspaces at 24h post-burn [24].

Shumakov et al. administered BM-MSCs transfected with Lac-Z gene by topical application and confirmed stem cell presence within the wound at day 30 post-treatment via IHC [25]. Xue et al. administered GFP-labeled human MSCs by local injection: stem cells were detected in the wound via BLI at day 7 and this result was confirmed by measuring the expression of specific Human Leukocyte Antigen (HLA) genes via RT-PCR. Histology showed the absence of GFP positive cells in lungs, heart, kidneys, liver, brain and pancreas at 24 weeks [33]. Caliri-Oliveira et al. also administered GFP-labeled MSCs by local injection. The number of GFP positive cells within burn wounds was assessed by flow cytometry: it decreased from an average of 1.5% to 0.37% and 0.30% of total cells analyzed, at days 0, 15 and 60 [34]. Bliley et al. used PKH-26-labeled human ASCs, also administered by local injection: they reported migration and presence of ASCs within the wound until study end point at day 21 post-burn. The presence of ASCs was further confirmed by human PPAR- γ gene expression at days 4, 7, 14 and 21 [29].

Clover et al. used VYBRANT DiO to label MSCs administered by local injection: MSCs represented 0.33% and 0.06% of total wound cells as assessed by IHC and FACS, respectively, at 2 weeks post-burn. The authors attribute this discrepancy to the two different methodologies used: only the epidermis and upper dermis were examined by IHC, while FACS was performed on the entire wound. This is the only study assessing the occurrence of MSC trans-differentiation: co-localization of VYBRANT DiO-labeled MSCs and keratin 14 on IHC revealed that, in keratin 14 stained sections, MSC accounted for 0.39% and 0.19% of dermal and epidermal cells, respectively. This was confirmed by Z-stack analysis [26].

3.10. Human trials

In 2005, Rasulov et al. were the first group to report on their experience with stem cell treatment in a human burn patient [38]. The patient was a middle age female who sustained burn injuries to 40% of TBSA, including 30% full-thickness burns. The patient's systemic condition stabilized, but her burn wounds failed to improve despite one-month of standard therapy, including six escharectomies, and skin grafts could not be applied. Rasulov et al. cultured bone marrow cells, harvested from the iliac crest of a healthy live donor, for two weeks until formation of a cell monolayer and then cryopreserved them. The cells were defrosted and cultured for seven additional days prior to use. A suspension of 'fibroblast-like mesenchymal stem cells (FMSCs)' was obtained from plastic-adherent cells. Neither immunophenotyping nor differentiation assays were conducted. FMSCs were administered via topical application, at a concentration of 20-30 $\times 10^3$ cells/cm² of skin. In the following days, the authors reported improvement in burn wound vascularity and granulation. Skin autografting was performed

four days after stem cell treatment and excellent graft take was achieved. The authors also describe improvement in patient's pain level and overall clinical condition. The patient underwent one further skin grafting procedure and was discharged from hospital one month later.

Ten years after this initial report, Mansilla et al. described a second case: the patient was a young man, who sustained burns to 60% TBSA, including 30% full-thickness burns. Early after injury and immediately after escharotomy, he underwent MSC administration via fibrin spray, at a density of 1×10^6 cells/100cm² of skin. MSC administration was repeated two weeks after injury. They harvested bone marrow cells from the iliac crest of a cadaveric donor and cultured them for two weeks until formation of a cell monolayer [39]. The cells were detached, expanded in a second passage and cryopreserved. Immunophenotyping showed cells positive for CD105, CD73, CD44, and CD90 and negative for CD45, CD34, CD14, CD11b, CD79, CD19, and HLA-DR; this phenotype corresponds to the second MSC-defining criterion, as established by the International Society for Cellular Therapy (ISCT). The authors report performing HLA typing of both donor and recipient, but degree of histocompatibility is not commented on. No differentiation assay was conducted. The authors reported improved vascularity, as well as formation of granulation tissue. Meshed skin autografts were successfully applied at day 35 and again one week later. The patient's overall clinical condition improved, in particular with regard to pain levels. Follow-up at three years revealed good skin elasticity in burn areas.

4. Discussion

In this systematic review, we found that the majority of experimental studies report macroscopic improvement in burn wounds as a result of stem cell therapy. This finding is supported by a trend, across all studies, toward improved histological appearance. The study that specifically compared local injection and topical application of ASCs did not demonstrate any differences in therapeutic effect [28]. Similarly, our systematic review did not reveal obvious differences in therapeutic effect between routes of administration, autologous and allogeneic origin and various stem cell sources. However, these findings are limited by the small number of studies and incomplete methodological quality. Furthermore, our ability to compare results across studies and to draw conclusions is limited by the important variability in macroscopic assessment methods, microscopic features described and time points considered, as well as by the exclusive use of non-quantitative microscopic assessment methods. Stem cells were obtained from different sources, phenotyped and expanded through different methods. This complicates the comparison of study results. In order to address this major issue, The International Society for Cellular Therapies has proposed minimal criteria's to define human MSCs [40]. These criteria represent an essential set of standards in order to improve comparability between studies.

Controversy persists regarding the mechanisms through which stem cells contribute to tissue repair. Two main

mechanisms are generally described: first, trans-differentiation into healthy cells of the damaged or deficient type and second, paracrine secretion of anti-inflammatory, pro-regenerative cytokines and growth factors [41]. In the field of wound healing, several studies reported that stem cells exert their positive effect via trans-differentiation into keratinocytes [42] as well as other cell types, such as endothelial cells and pericytes [43]. Our review shows that, in most instances, stem cells were detected at the burn site regardless of attempts to increase their numbers. However, only one study evaluated the occurrence of trans-differentiation, with evidence of sporadic stem cell trans-differentiation into keratinocytes in burn wounds [29]. Two studies quantified the number of stem cells present in the burn wound: only a small fraction of the originally administered stem cells was detected at the injury site. The fate of the remaining stem cells, as well as the mechanisms through which these cells may be removed, remain unknown.

On the other hand, the majority of studies associate macro and microscopic improvements with lower pro-inflammatory and higher pro-regenerative cytokine and growth factor levels found in serum and wounds. Several studies suggest that stem cells possess systemic anti-inflammatory effects [44–46]. Experiments in LPS-induced sepsis and lung injury showed positive effects of stem cells delivered by intravenous and intra-pulmonary injection [47,48]. These findings are of particular interest in burns: extensive burns (>20% TBSA) cause a systemic inflammatory response which can lead to shock, multi-organ failure and death. In our systematic review, two studies presented evidence that stem cell therapy may indeed mitigate burn-induced systemic inflammation. Notably, stem cell therapy abolished CRP and WBC surge at days 2 to 3 post-burn [36] and, in a rat extensive burn model (45cm² dorsal full-thickness burn), significantly decreased mortality [34].

Despite positive findings in small animal models, translation into large animal models and clinical studies has been limited. Two clinical reports used allogeneic BM-MSCs administered via topical application: neither study reported adverse effects, suggesting therefore that topical application of allogeneic stem cells is safe. Both studies describe a positive effect of stem cells on burn wound vascularity and granulation, which allowed for successful skin autografting at day 4 and 35 after stem cell treatment [38,39]. Furthermore, both studies report rapid improvement in patient pain levels and overall clinical condition after stem cell treatment. However, the small number of patients and the absence of controls and objective outcome measurement limits the strength of these findings. One phase I clinical trial (NCT02104713) is currently underway in the United States to investigate the safety of allogeneic BM-MSCs for the treatment of second degree burns of less than 20% TBSA. Topical application of four different doses ranging from 2.5×10^3 to 2×10^4 allogeneic BM-MSCs/cm² of skin will be tested [49].

Tissue engineered products and scaffolds carrying stem cells or epidermal preparations are considered a promising treatment strategy to promote burn wound healing and skin regeneration. Skin substitutes can be subdivided into epidermal, dermal and composite (dermal & epidermal) constructs.

These constructs can be made of biologically natural, synthetic or a combination of both materials. Substitutes like Alloderm, Apligraf, Integra and Epicel are examples of substitutes that have reached clinical applicability [50-52]. These products have not been fully implemented as the standard of care for burns treatment as they can only provide temporary coverage, are time consuming to prepare and can be costly compared to skin allografting. Scaffolds and 3D constructs serve as extracellular matrix to stimulate cell adhesion, differentiation and proliferation to form functional skin. Current efforts are focused on incorporating stem cells and/or growth factors into scaffolds in order to promote wound healing and skin regeneration [53-56]. The application of this new generation of scaffolds is an exciting and developing field. However, biocompatibility, availability, vascularization and rejection are critical areas that will need to be addressed when developing this type of product.

As highlighted by Lau et al. 'few areas of science have generated as much public interest as stem cell research' [57]. The flip side of this positive phenomenon is the emergence of stem cell clinics, which advertise unproven stem cell treatments for financial gain, not only taking advantage of patients' distress, but also exposing them to the risk of serious adverse effects [58]. However, as remarked by Lindvakk & Hyun, so called 'stem cell tourism' should not be confused with medical innovation, defined as 'approaches where there is a scientific rationale and for which efficacy without serious side effects has been demonstrated in animal models, but the approach has not yet been established clinically' [59]. This distinction is essential to the future development and evaluation of stem cell therapies in burns.

5. Conclusion

The enthusiasm surrounding stem cell therapy in burns, expressed in numerous reviews and editorials may not be unjustified. Experimental studies conducted so far do demonstrate potential for macro and microscopic improvements of burn wounds, as well as systemic anti-inflammatory effects. This conclusion should not obscure the important limitations, such as small number of studies, imperfect methodological quality and superficial insight into the underlying mechanisms. More studies in small and, in particular, large animal models are needed in order to draw solid conclusions. However, our systematic review suggests that there is a rationale for continued efforts to evaluate the clinical efficacy of stem cells as an adjunct to first-line therapies in burns.

Authorship contributions

Ali R. Ahmadi: study conception and design, analysis and interpretation of data, manuscript composition.

Maria Chicco: study conception and design, analysis and interpretation of data, manuscript composition.

Jinny Huang: analysis of data.

Le Qi: manuscript revision.

James Burdick: manuscript revision.

George M. Williams: manuscript revision.

Andrew M. Cameron: manuscript revision.

Zhaoli Sun: study conception and design, manuscript revision, study supervision.

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Appendix A. Supplementary data

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