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Review

Review

Mesenchymal stem cell-based therapy for burn wound healing

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Abstract

Burns, with their high incidence and mortality rates, have a devastating effect on patients. There are still huge challenges in the management of burns. Mesenchymal stem cells (MSCs), which have multidirectional differentiation potential, have aroused interest in exploring the capacity for treating different intractable diseases due to their strong proliferation, tissue repair, immune tolerance and paracrine abilities, among other features. Currently, several animal studies have shown that MSCs play various roles and have beneficial effects in promoting wound healing, inhibiting burn inflammation and preventing the formation of pathological scars during burn healing process. The substances MSCs secrete can act on peripheral cells and promote burn repair. According to preclinical research, MSC-based treatments can effectively improve burn wound healing and reduce pain. However, due to the small number of patients and the lack of controls, treatment plans and evaluation criteria vary widely, thus limiting the value of these clinical studies. Therefore, to better evaluate the safety and effectiveness of MSC-based burn treatments, standardization of the application scheme and evaluation criteria of MSC therapy in burn treatment is required in the future. In addition, the combination of MSC pretreatment and dressing materials are also conducive to improving the therapeutic effect of MSCs on burns. In this article, we review current animal research and clinical trials based on the use of stem cell therapy for treating burns and discuss the main challenges and coping strategies facing future clinical applications.

Highlights

- This article reviews the present animal research and clinical trials of mesenchymal stem cell-based therapy for burn wound healing.
- We analyze the potential mechanism during the therapy process then point out the pros and cons.
- Finally, we provide improvement strategies for the further application of mesenchymal stem cells for burn wound healing.

Key words: Burn, Wound healing, Stem cell-based therapy, Clinical trial, Regenerative medicine, Proliferation, Tissue repair, Inflammation

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Background

Burns remain a severe public health problem associated with considerable incidence as well as high mortality. Based on records from the World Health Organization, the number of burn-related deaths is nearly 180,000 each year globally. Although burn-related deaths have decreased in recent years due to technological improvement, great challenges remain in the management and treatment of burn wounds [1,2].

Burn severity is divided by the percentage of total body surface area burned, depth, location and patient age [3]. Burns are commonly classified as first-degree and superficial second-degree burns when only the epidermis and part of the dermis are damaged [1, 4, 5]. For wound healing, rapid healing occurs in 1 to 2 weeks, and scar formation is uncommon in these cases [3]. However, it takes much longer for severe burns, such as deep second-degree and third-degree burns, to heal, and these patients are more susceptible to infections, organ dysfunction and hypertrophic scarring [1]. Severe thermal burns can cause devastating consequences, both functionally and cosmetically [4], and can involve damage to muscles, bones, vasculature and dermal and epidermal tissues, as well as extreme pain due to nerve injuries [5]. The pathophysiological cascades caused by the increased capillary permeability, vascular resistance and changed platelet aggregation after burns may increase the risk of death and burn shock [1,5]. A series of treatment strategies, including surgical management, intensive care, wound nursing, adapted nutrition and sterile confinement, have been attempted, but great challenges still need to be overcome.

Stem cells, which have multidirectional differentiation potential, have been applied to cure various intractable diseases (Table 1) (Figure 1) and have also been considered a potential alternative for burn treatment. Stem cells in skin tissue occupy a significant position in normal wound healing and skin homeostasis regulation, and their capabilities for healing burn wounds have also been demonstrated in recent studies. Furthermore, several animal researches have investigated the therapeutic potential of mesenchymal stem cells (MSCs), as well as of their secretions, such as exosomes. Comprehension of the repair mechanisms not only benefits the combination of stem cell therapy with other strategies in burn wound healing but also is conducive to transferring the stem cell-based therapy for treating burns from the bench to the bedside. In this article, we review preclinical and clinical trials concerning different types of stem cells used in burn wound treatment and introduce the principles of burn care, the biology of MSCs and the mechanisms by which stem cells repair burn wounds. Additionally, we detail the application of MSCs for treating burn wounds and suppressing inflammation and scar formation and outline the feasibility and effectiveness of stem cell therapy in animal studies. In particular, we focus on the progress of the clinical application of stem cells in burn treatment. Finally, we discuss and analyze the main challenges that need to be overcome before MSCs can be used widely in burn wound treatment and try to propose a reference strategy for stem cell treatment of burns.

Review

Burn treatment

Generally, burn treatment includes 5 strategies: surgical management, intensive care, wound nursing, adapted nutrition and sterile confinement [1]. While extensive strategies have been developed for treating severe burns, barriers concerning healing time and scar suppression are still difficult to overcome. Innovative cell therapies, such as stem cell therapy, might offer great hope for burn wound management in the future.

Roles of allogeneic MSCs in burn wound treatment

Allogeneic MSCs, which have the potential of multidirectional differentiation, can be extracted and isolated from many tissues. With the expansion of stem cell-based therapy, MSCs have begun to perform a significant role in the treatment of a variety of difficult diseases. Bone marrow [6] and adipose tissue are considered to be the most easily available and richest sources of MSCs [7], while tissues such as cartilage, mouth, synovial membrane, synovial fluid, muscle, tendon, foetal tissue, placenta, and cord blood [8,9] can also be regarded as appropriate sources of MSCs. MSCs not only have a strong proliferative capacity but also exhibit a variety of lineage differentiation capabilities. Generally, MSCs can produce various tissues (including bone, cartilage, adipose tissue, tendons, and muscles): *in vitro*, they contain growth factors or other substances, such as pedestal-specific media, indomethacin, hydrocortisone, and transforming growth factor β (TGF-β), which can guide them to proliferate and differentiate along multiple phenotypic pathways; *in vivo*, stimulus signals, such as tissue damage, including trauma, fractures, inflammation, necrosis and tumours [10], can directly mobilize MSCs to differentiate into connective tissue cells.

In recent years, MSCs have been widely applied to promote wound healing in various skin diseases [11], especially skin burns [12]. The therapeutic potential of MSCs is due to their exosome secretion ability [13]. MSC exosomes range from 10–100 nm in size and contain bioactive molecules, like proteins, mRNA and microRNA (miRNA) [14]. Exosomes derived from MSCs can be transferred to cells and the microenvironment, and also regulate cells and modulate targeted gene expression.

The contributions of MSCs to burn wound healing mainly include 3 phases: inflammation, proliferation and maturation [15]. By regulating the phases of burn wound healing, MSCs accelerate the healing process and inhibit pathological scar formation.

Inflammation phase Delayed immune dysregulation may prolong burn wound healing, promote fibrosis and form hypertrophic scars. MSCs are capable of immunomodulation

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Table 1. Animal studies of MSC-based therapy for burn wound healing

Cell type	Year	Animal	Cell source	Dose (cells)	Delivery/application route	Condition	Outcome
BM-MSCs	2003	Rat	Rat	2×10^6	i.w.		Improved vascularization and quicker wound healing [62]
	2004	Minipig	Porcine	1×10^7	i.v.		Improved wound healing with MSC plus bFGF [63]
	2007	Mouse	Human	1×10^6	i.v.		Improved wound healing (radiation burn) [65]
	2008	Rat	Rat	1×10^7	s.t.	hPDGF-A + hBD2 transfected	Improved granulation tissue formation and reduced bacterial load (radiation burn) [68]
	2010	Rat	Rat	1×10^6	i.w.	Ad-HGF transfected	Increased re-epithelialization [69]
	2010	Minipig	Porcine	1.5×10^8	i.w.		Improved wound healing [71]
	2011	Minipig	Human	1.2×10^5	i.w.	hPDGF-modified	Improved granulation, re-epithelialization and angiogenesis [70]
	2013	Mouse	Mouse	5×10^5	i.v.		Reduced inflammation and fibrosis [73]
	2013	Mouse	Human	1×10^6	s.c.		Improved wound healing and neovascularization (non-lethal scald of forelimb) [74]
	2013	Mouse	Mouse	1×10^6	i.v.		MSCs home in to the site of injury, CXCL12/CXCR4 axis crucial for mobilization [75]
	2013	Rat	Rat	1×10^6	s.c.		Reduced apoptosis and improved vital tissue percentage [76]
	2013	Mouse	Mouse	1×10^6	i.v.		MiR-27b may be a unique signature of the stem cell niche in burned mouse skin and can suppress the directional migration of mMSCs by targeting SDF-1α by binding directly to its 3′UTR [77]
	2014	Pig	Porcine	Unknown	With collagen	CBD-E7 transinfected	Rapidly healing skin wounds [82]
	2015	Pig	Porcine	4.5×10^6	In fibrin matrix		Improved wound healing [88]
	2015	Rat	Rat	2×10^6	s.c. (with polysaccharide hydrogel)		Increase in the expression of anti-inflammatory cytokines (TGF-β), antiangiogenic cytokines (TSP-1) and decrease in those promoting inflammation (TNF-α), chemotaxis (MIP-1α and MCP-1) and angiogenesis (VEGF and MMP-2) [89]
	2016	Rat	Rat	5×10^5	i.i.	GFP labelled	Reduced inflammation and improved survival
	2016	Rat	Rat	4.5×10^6	i.p. (with SIS)		Repair of the large and deep burn wounds and the loaded MSCs possessed positive effects to accelerate the wound closure in a rat model [92]
	2017	Rat	Rat	Unknown	i.w. (PRP loaded)		BM-MSCs with PRP gel was a promising treatment for corneal alkali burns and may be applicable for other types of corneal disorders [94]

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Table 1. Continued

Cell type	Year	Animal	Cell source	Dose (cells)	Delivery/application route	Condition	Outcome
	2018	Mouse	Mouse	1×10^5	i.w. (ACgel loaded)		MSC-seeded ACgels have potential use as a novel adjuvant therapy for severe burns to complement commonly used skin grafting and, thus, minimize the downsides of grafting [96]
	2018	Rat	Rat	1×10^6	s.c.		The protective effects of MSCs are mediated by the inhibition of apoptosis through immunomodulatory, antioxidative and angiogenic actions [97]
	2018	Mouse	Mouse	5×10^5	i.v. (with Fluc)		MSCs might assist burn wound healing and MSCs expressing Fluc could be a useful tool for optimizing MSC-based therapeutic strategies for burn wound healing [98]
ADSCs	2012	Minipig	Porcine	5×10^7	i.i.		Improved wound healing [72]
	2013	Mouse	Mouse	1×10^5	i.i.		Down-modulate TNF-α-dependent inflammation, increase anti-inflammatory M2 macrophage and myofibroblast numbers and induce TGF-β1-dependent angiogenesis [78]
	2014	Mouse	Mouse	1×10^6	i.w.		Burn wound revealed no significant improvement [85]
	2017	Rat	Rat	2.5×10^5	s.c.		Differentiated adipocytes (+IBMX[D1–5] + INSULIN) and fat grafting accelerate early healing relative to ADSC [95]
	2019	Rat	Rat	2×10^6	s.c.		Autologous ASC transplantation accelerated the burn wound healing process and promoted blood vessel regeneration. ASCs could potentially be used in burn wounds [99]
	2019	Rat	Rat	3×10^5	i.w.	With honey	Provide a nutrient media for the ASCs and enhance the ability of regeneration of the ASC-based therapies [100]
	2019	Rat	Rat	8×10^5	i.w.		ADSCs influence the healing of total-thickness burns in rats.
UC-MSCs	2014	Rat	Human	5×10^6	i.v.		Improved wound healing and neovascularization; reduced inflammation at the injury site [79]
	2015	Rat	Human	2×10^6	s.c.		Reduced circulating inflammation; enhanced wound healing [86]

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Table 1. Continued

Cell type	Year	Animal	Cell source	Dose (cells)	Delivery/application route	Condition	Outcome
UCB-MSCs	2007	Mouse	Human	30 eGFP+ PKH26+ cells	i.v.		Contribute to skin tissue regeneration *in vivo* and may be an ideal cell source for therapy of skin epithelial tissue injury, including burns [66]
K-MSCs	2014	Mouse	Mouse	Unknown	With nano-collagen	PDLLA associated	Groups with the scaffolds showed better cicatrization in comparison [83]
AM-MSCs	2014	Rabbit	Human	1×10^6	s.c.		Enhance epithelial healing and reduce corneal opacification and neovascularization in corneal alkali wounds [84]
HFSCs	2020	Rat	Rat	1×10^6	Unknown		Accelerate burn wound healing as well as tensile strength in rats
BM-MSCs/ADSCs/LSCs	2016	Rabbit	Rabbit	Unknown	With nanofiber scaffold		Accelerated regeneration of the corneal epithelium, restored the antioxidant protective mechanism and renewed corneal optical properties [93]
AECs/UC-MSCs	2019	Rat	Human	3×10^4	i.w. (with paraffin gauze covering)		Improved severe burn wounds in a rat model of burn injury [101]

AECs amniotic epithelial cells, *AM-MSCs* amniotic membrane-derived mesenchymal stem cells, *BM-MSCs* bone marrow-derived mesenchymal stem cells, *CXCL12* chemokine (C-X-C motif) ligand 2, *CXCR4* C-X-C chemokine receptor type 4, *HFSCs* hair follicle stem cells, *i.i.* intradermal injection, *i.p.* intraperitoneal injection, *i.v.* intravenous, *i.w.* wound injection directly, *K-MSCs* kidney mesenchymal stem cells, *LSCs* limbal epithelial stem cells, *MCP-1* monocyte chemoattractant protein-1, *MSCs* mesenchymal stem cells, *PDLLA* poly-D,L-lactic acid, *TNF-α* tumour necrosis factor α, *UCB-MSCs* umbilical cord blood mesenchymal stem cells, *UC-MSCs* umbilical cord mesenchymal stem cells, *VEGF* vascular endothelial growth factor, *s.c.* subcutaneous, *s.t.* subcutaneously transplanted. *ADSCs* adipose derived stem cells, *bFGF* basic fibroblast growth factor, *eGFP* enhanced green fluorescent protein, *hBD2* Human beta-defensin-2, *+IBMX* 3-isobutyl-1-methylxanthine, *Ad-HGF* adenovirus hepatocyte growth factor, *hPDGF* human platelet-derived growth factor, *hPDGF-A* Human platelet-derived growth factor A, M*iR-27b* microRNA 27b, *PRP* platelet-rich plasma, *SDF-1α* stromal cell-derived factor 1α, *mMSCs* marrow mesenchymal stem cells, *3'UTR* three prime untranslated region, *Fluc* firefly luciferase, *ACgel* Acgel-CL Nano Gel, *CBD-E7* collagen-binding domain-E7 peptide, *TSP-1* thrombospondin 1, *MIP-1α* macrophage inflammatory protein-1 alpha, *TGF-β* transforming growth factor beta, *NNT* number needed to treat

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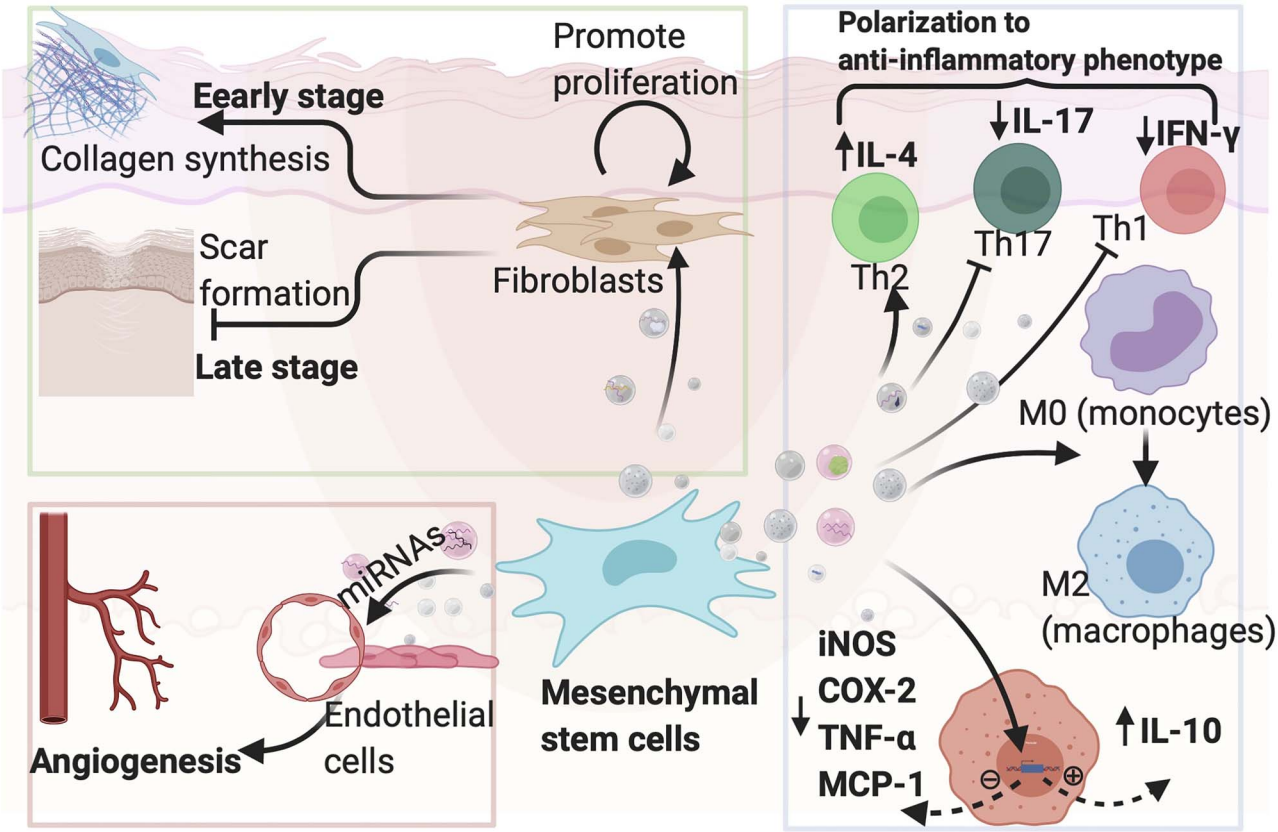


Figure 1. MSCs roles in burns wound healing. *MSC* mesenchymal stem cells, *IL* interleukin, *INF-γ* interferon γ, *iNOS* inducible nitric oxide synthase, *COX*-2 cyclooxygenase-2, *TNF-α* tumour necrosis factor-α, *MCP*-1 monocyte chemoattractant protein-1

and can modulate innate and adaptive immune responses [16,17]. Macrophages present a pro-inflammatory M1 phenotype or an anti-inflammatory M2 phenotype and play important roles in skin tissue regeneration [18]. MSC exosomes promote the conversion of macrophages towards the M2 phenotype [19]. In addition to innate immunity, MSCs also have an inhibiting effect on the proliferation of activated helper T (Th) cells, thus leading to decreased production of interferon γ (IFN-γ) and interleukin (IL)-17, which are secreted by Th1 and Th17 cells, respectively, and to increased production of IL-4 secreted by Th cells, indicating that T cells polarize from a pro-inflammatory to an anti-inflammatory phenotype [20–22]. Additionally, MSC exosomes can mitigate the inflammatory response by downregulating pro-inflammatory enzymes, cytokines and chemokines, including inducible nitric oxide synthase, monocyte chemoattractant protein-1, tumour necrosis factor andcyclooxygenase-2 [14,18,23,24]. In the burned rat model reported by Li *et al.*, MSC exosomes upregulated IL-10, [14] which is an anti-inflammatory cytokine. Overall, MSCs can suppress the inflammatory response by regulating immune cells, as well as bioactive molecules expressed through MSC exosomes.

Proliferation phase The proliferation phase is characterized by angiogenesis, collagen deposition, granulation tissue formation, re-epithelialization and wound contraction [18]. This phase features fibroblast and keratinocyte activation and migration [25,26].

Normal control of angiogenesis and wound contraction in wound healing is important to avoid chronic wound emergence [1]. MSC exosomes can deliver miRNAs (miRNA-30b, miRNA-30c, miRNA-424 and let-7f) into endothelial cells and promote angiogenesis [27]. The miRNA regulatory landscape of MSC exosomes constructed by Nguyen *et al.* revealed that miRNAs secreted from MSC exosomes can target several pathways and biological processes, some of which are involved in angiogenesis and vascular development [28]. In addition, studies have shown that MSC exosomes can regulate fibroblast proliferation and migration by modulating the expression of growth factors and their related genes [29]. Li *et al.* found that adipose stem cell (ASC)-derived exosomes can be taken up by fibroblasts, in which adipose MSCs can stimulate cell proliferation and collagen synthesis by upregulating the expression of related genes, such as N-cadherin and cyclin-1 [30].

Keratinocytes play a vital role in wound closure [25] and skin re-epithelialization [31]. Keratinocytes start to migrate from the edge to the gap at a very early stage of wound healing [31]. A small number of studies have described the effect of mesenchymal stem cell-exosomes (MSC-EVs) on keratinocytes during the burn wound healing process. A

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study found that ASC exosome treatment promoted the proliferation and migration of HaCaT skin keratinocyte cells both *in vitro* and *in vivo*. The underlying mechanism of this process is that the ASC exosome treatment upregulated the proliferative markers (cyclin A2 and cyclin D2) [32]. Another study showed that exosomes derived from iMSCs (induced pluripotent stem cell-derived MSCs) increased the proliferation and migration of HaCaT keratinocyte cells by stimulating extracellular signal-regulated kinase-1/2 [33]. Moreover, a preclinical study using a rat skin burn model found that human umbilical cord MSC-derived exosomes can significantly increase wound re-epithelialization. Wnt4 derived from human umbilical cord MSC-derived exosomes promoted the nuclear translocation and activity of β-catenin so that the proliferation and migration of keratinocytes were enhanced [34].

Maturation phase In the final phase of wound healing, the wound matures as fibroblasts differentiate into myofibroblasts with transforming growth factor β1 (TGF β1) and express high levels of extracellular matrix (ECM) proteins [25,35].

The remodelling of an acute wound is tightly controlled to sustain the balance between the collagen degradation and synthesis [26]. MSCs have the ability to enhance specific ECM events while healing wounds [36]. Conditioned media from MSCs promote fibroblast cells to secrete collagen, elastin and fibronectin. Moreover, conditioned medium suppresses matrix metalloprotease-1 expression [37]. However, excessive myofibroblast aggregation and ECM sedimentation usually lead to scar formation, which affect both the appearance and functions [38]. A study found that ASC-derived exosomes can stimulate collagen synthesis in the early phase of wound healing while inhibiting collagen expression to reduce scar formation in the late stage. This study indicated that ASC-derived exosomes can promote wound healing by promoting fibrosis. Additionally, studies have indicated that bioactive proteins and miRNAs contained in MSC exosomes play a significant role in inhibiting scar formation. Fang *et al.* found that umbilical cord-derived MSCs were enriched in several miRNAs (miR-21, -23a, -125b, and -145) that can suppress myofibroblast formation by inhibiting the transforming growth factor-β2/SMAD2 pathway [39]. Zhang *et al.* found that at high cell density, human umbilical cord MSC exosome 14–3-3ζ mediated the binding of yes-associated protein (YAP) and phospho- large tumor suppressor (LATS) proteins and promoted the phosphorylation of YAP. Phosphorylated YAP then repressed Wnt/β-catenin signalling pathway activation and inhibited excessive fibroblast expansion [38,40].

In conclusion, by secreting exosomes containing various functional proteins, mRNAs and microRNAs, MSCs occupy an important position in all burn wound healing stages, including the inflammation phase, angiogenesis, re-epithelialization, wound closure and collagen remodelling. Notably, MSCs possess immunomodulatory characteristics, while their activities mainly rely on the regulation of inflammatory cytokines secreted by neighbouring immune cells. Therefore, when MSCs are applied in preclinical and clinical studies, cell pretreatment is a good strategy to improve their immunomodulatory effect.

MSC-based therapy in burn wound healing

Preclinical trials Many animal studies have been conducted to evaluate the therapeutic potential of stem cells for treating different types and degrees of burns (Table 1), including radioactive burns [41–49], alkali burns [50–53], general burns [54–67] and deep burns [68–77]. Although these studies were conducted on different cell sources, in different animal models and with different cell pretreatments and injection methods, high consistency was observed with respect to their effectiveness.

Stem cell injection not only promotes burn wound healing but effectively suppresses inflammation and promotes the recovery of skin function by cytokines secreted by stem cells, which can create a favourable repair microenvironment. Small animal (such as rats and mice) and large animal models (rabbits, sheep, pigs, etc.) are also widely used in stem cell-based treatments for wound healing research due to their availability and the feasibility of experimental operations. However, we must admit that there is a large difference between the healing mechanisms of human wounds and animal wounds. In previous studies, we considered the pig skin wound burn model to be the best model for human burns among all existing research models.

In terms of cell sources, stem cells, especially MSCs, from different sources have been carefully investigated in animal studies for their use in burn repair, such as bone marrow-derived MSCs (BM-MSCs) [41–44,47–49,52,53,55,60,68–72,74,75,78], umbilical cord blood MSCs [54], porcine MSCs [44,69,70,74], adipose-derived stem cells, [45,46,58,65,79] adipose tissue-derived MSCs [61], umbilical cord MSCs [64,73], amniotic membrane-derived MSCs [50], amniotic epithelial MSCs [57] and hair follicle stem cells [59]. However, BM-MSCs and adipose-derived stem cells are the most widely used stem cells in burn repair. Studies have shown that transplantation of BM-MSCs and other transplants promote vascularization by secreting cytokines, including fibroblast growth factors and vascular endothelial growth factor [51,69,80], chemokine (C-X-C motif) ligand 2/C-X-C chemokine receptor type 4 and other factors [71], to recruit cells to promote the healing of burn wounds. Furthermore, the function of stem cells of increasing the expression of tumour necrosis factor [51,61] to inhibit the expression of prostaglandin E2, TGF-β1 and other factors [48] could reduce the inflammatory response (Table 3).

The application of MSCs in burn wound treatment is usually combined with traditional methods, among which the combination of MSCs and scaffolds performs outstandingly. Tissue scaffolds can provide a suitable environment for stem cells, allowing them to obtain nutrients and perform gas exchange [81]. The first hint of the combined use of tissue scaffolds and stem cells was in 2014, when Daniela *et al.* [63] reported MSCs seeded onto nanofibers as new substitutes for

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split-thickness autologous skin grafting. In this study, they proved that the scaffolds prolonged MSC function and promoted cicatrization. Coincidentally, various scaffolds, such as collagen [62], fibrin matrix [74], hydrogel [51,77] and small intestine submucosa (SIS), work well in burn wound healing when combined with MSCs. Clinically, in a case reported in 2015, MSCs together with thrombin were sprayed onto burn wound surfaces after deep escharotomy, and burn wounds were covered with a sterile polymeric transparent film [82]. In this way, MSCs could adhere to the injured surface within the fibrin matrix, which was formed by thrombin.

Clinical trials In 2005, the first transformation of BM-MSCs was successfully performed in a 45-year-old female patient with extensive skin burns (percentage of burn area: 40%, percentage of second degree burn area: 30%). This study proved the safety of BM-MSC treatments, which activated new vessel formation, led to rapid granulation tissue formation and reduced scar formation [83]. Since 2005, several studies on stem cell-based therapy for treating burn injury have been reported (Table 2) and there are only 12 related cases listed on the public website of the Clinical Trials Data Bank at the National Institutes of Health (www.clinicaltrials.gov). In 2015, Mansilla *et al.* published an article about their first attempt to use cadaveric BM-MSCs to treat large burns. The treatment outcome was excellent because the patient had a fast and significant improvement in clinical conditions, with reduced pain in the burn areas [82]. It seems that cadaveric BM-MSCs have potential in clinical use with respect to tissue regeneration and wound healing.

MSC-based therapy has been demonstrated to be safe [82,83] and capable of ameliorating granulation formation [82,83] and causing new vessel formation [83] and re-epithelialization, [82,84] reducing scar formation [83,84] and decreasing side effects (pain, infection and blistering) [81,82,85] (Tables 2 and 3). Although the outcomes of previous studies showed excellent results of MSC-based therapy in burn wound healing, only one study investigated the immunomodulatory effect of MSCs [84], which were underlined in preclinical studies. Only one study employed BM-MSCs for the treatment of skin graft contraction, which showed good performance in a 2-year follow-up interval [81]. Notably, follow up of a patient treated with MSCs and skin grafts for a period of time showed limited hair growth but good elasticity [82]. In addition, among all 12 studies listed in the Clinical Trials Data Bank website, few study results are available—only one study presented its result of reduced side effects on the website. Therefore, the poor efficiency reported may be due to insufficient patients and the short follow-up time.

Outcome measures and delivery Outcome indicators are usually the time and rate of wound healing, the number of surgical procedures performed, the proportions of wound contraction and re-epithelialization, the Vancouver scar scale score, functional sequelae and the incidence of treatment-related side effects (pain, bleeding and infections), among other factors. The safety of stem cell treatment must be confirmed in a phase I clinical trial. However, even though stem cell treatment has been proven safe, some studies have shown no significant effects on patients and have even been withdrawn [86]. Long-term follow-up studies after burn treatments are common, mainly because hypertrophic scars [81] and systemic complications [87–89] probabilistically occur in burn patients, such as cachexia resulting from skeletal muscle waste, hypermetabolism and inflammation. Subsequent studies are usually used to analyze the shrinkage of skin grafts [81], the degree of scar formation [84] and the incidence of functional sequelae [82].

Clinically, efficient burn wound healing requires full-thickness regeneration of the skin and its appendages [90]. However, efficient burn wound healing is difficult to achieve in conventional burn management. The application of stem cells in burn treatment is promising and different strategies have been applied for improving its efficiency. Here, we not only summarize and then classify the abovementioned studies using cell sources, as shown in Table 1, but also analyze different therapy improvements.

Pretreatment in MSC-based treatment

Pretreatment of MSCs is also a promising strategy for burn wound healing. When combined with cell therapy, pretreatment can adapt MSCs to the pathological environment by stimulating protective and survival pathways [1]. Upon burn injury, resident and recruited cells are primed by mechanical and biological stressors, thus initiating the wound repair process [91]. The environment at burn sites changes continuously at different stages of wound healing. Therefore, the treatment of burns must adapt to the stage of the disease to achieve high efficacy. In this way, MSC pretreatment according to the time of cell transplantation is an ideal method to meet this demand. Gene transfection (such as hPDGF-A [43,44], hBD2 [43] and Ad-HGF [55]) and physical treatments (such as poly-D,L-lactic acid [63] and honey [56]) lead to great improvements in MSC treatments. However, the priming dose, duration and protocol need to be determined and tested in both animal studies and clinical research.

Application of MSC exosomes in burn wound healing

As a novel type of cell-free therapy, MSC exosomes may be safe, efficient and easily prepared [92, 18]. The application of MSC exosomes can avoid the problems caused by cell transplantation, such as tumorigenicity, immune compatibility, infection transmission and complicated material storage [93]. MSC exosomes can secrete various biologically active molecules, including cytokines, chemokines, growth factors and lipid mediators [94], which can regulate the responses of surrounding cells [95]. Also, by inhibiting pro-inflammatory processes and suppressing fibrosis, MSC exosomes may promote tissue regeneration by creating a pro-regenerative environment that allows MSCs to successfully repair wounds [96]. Thus, MSC exosomes are a promising approach in burn wound treatment. The promoting effect of MSC exosomes on burn wounds has been proven in many animal experiments

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[14,34,40]. However, clinical trials are limited in cell-free therapy of burns, and only one clinical trial (NCT04235296) used MSC-conditioned media containing insulin-like growth factor 1 (IGF-1), vascular endothelial growth factor and TGF-β1 to treat residual burn wounds.

Future preclinical research

Although there are many factors that affect the results of preclinical research in the clinic, in general, the following 3 points play significant roles in evaluating the efficacy of stem cell therapy.

Cell source MSCs have various sources; strong self-renewal, differentiation, migration and factor-secretion abilities; and play important roles in inflammation suppression and tissue damage repair. It is worth noting that the functions of MSCs from different tissues and organs from different sources vary, and the subtle regulatory effects of proteases and the hypoxic microenvironment, as well as miRNA regulation, also affect the steady state and fate of transplanted MSCs [97]. Although research has shown that among the most widely studied sources, MSCs derived from the umbilical cord or cord blood have more advantages than bone marrow-derived MSCs (BMSCs) [98], their abilities in burn treatment remain to be discussed. The identification of the stem cell population used and comparative experiments on the efficacy of MSCs from different sources is of great significance for analysing the types of MSCs most suitable for burn treatment.

Cell safety Although MSCs occupy a significant position in burns treatment, we still need to realize that the application of MSCs involves risk. First, the genetic stability of intermediate MSCs during *in vitro* expansion is difficult to guarantee. The rational application of ultra-low-temperature storage and serum is considered to be an effective measure to improve the genetic stability of MSCs during preparation and expansion [99]. Second, the orderly homing of transplanted MSCs can effectively ensure that the cells reach the site that needs to be repaired and reduce the induction of unnecessary immune responses. By deepening the understanding of MSC migration, MSC homing signals can be enhanced so that they can achieve their repair effect and ensure the safety of transplantation [100].

Experimental design of standard protocol for stem cell-based therapy As with stem cell-based therapy for other diseases, the protocol for treating burns, including stem cell production, number of transplanted cells, administration route, treatment times or cycles and other considerations, remains unelucidated. Although there are still differences between the protocols used in preclinical and clinical trials, it has been shown that these factors can make a difference in the therapeutic effect. Unknowns regarding cell characteristics and their great complexity and specificity may be responsible for this slow process. Few experiments have revealed the exact impact of the protocol of stem cell therapy for treating burns and more research is needed.

Future clinical trials

Challenges In clinical studies, it is essential to improve the engraftment and survival rates of transplanted MSCs. In this way, MSC-based therapy can have long-term benefits for burn patients. However, some studies have shown that implanted cells cannot survive long-term [101]. Therefore, MSC-based treatment requires the combination of MSCs and scaffolds. In one case, a MSC suspension was injected into a scar excisional wound and covered with a piece of decellularized allogeneic dermal matrix that functioned as a scaffold to help reduce cell loss [81]. In all clinical studies, the effectiveness of various scaffolds in improving the engraftment rate and survival rate of MSCs has rarely been tested. More studies need to be performed to improve the engraftment and survival rates of transplanted MSCs, which can provide information regarding the dosage and protocol for MSC-based treatment.

Despite the convincing findings in animal models, conversions to clinical research have been limited. Only 12 related studies are listed in the Clinical Trials Database, and only a few case reports with limited patients have been published. These studies proved the safety of MSC-based treatment, and some showed good efficacy in improving burn wound healing. However, due to the limited number of patients, lack of regulation, great variabilities in treatment protocols and inconsistent outcome evaluations, the value of these clinical studies has been limited. In the research, experimental and control groups usually include the same patient. In one case, a patient with a severe burn was treated with MSC dermis composite grafts on the left side of their chest and received only artificial dermis on the left thigh. The results showed milder inflammatory cell infiltration after 5 days and more aesthetically favourable findings after one year on the left side of the chest [84]. It is difficult to evaluate the outcome of MSC-based treatments in clinical studies without control groups. Additionally, no consensus regarding cell dose, source or application time has been reached in any clinical trials. Moreover, the variability in macro- and microscopic assessment methods makes it difficult to draw conclusions from different clinical studies [102].

It takes a long time to culture enough cells *in vitro* for use in autologous MSC transplantation, which may lead to an adverse conversion to necrotic tissues in burn patients. Although limited amounts of MSCs can be isolated from adult tissues [103], cadaver-derived MSCs may be a promising source [82]. Therefore, large-scale MSC expansion is needed in the future development of MSC-based therapies. Conversion from benchmark research to guanosine monophosphate (GMP)-compliant, clinical-grade, large-scale MSC expansion requires the standardization of all parameters, such as the cell source, culture technology and device, harvesting methods and cell seeding density [104]. In addition, cell culture may subject cells to potential damage, such as oxidative and mechanical stress, which can lead to possible mutations and senescence [105]. Methods for large-scale MSC expansion and ways to avoid possible cell damage need to be developed in the future.

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Table 2. Case reports of MSC-based therapy for burn wound healing

Study	Year	%TBSA or burn degrees	Sex, age and numbers	MSC therapy			
				Source	Dose	Treatment protocol	Outcomes
First experience in the use of bone marrow mesenchymal stem cells for the treatment of a patient with deep skin burns	2005	40%	Female, 45 years, 1	BM-MSCs	$20–30 \times 10^3$ cells/cm^2	After necrectomy, BM-MSC suspension was applied to burn wounds and the first skin graft transplantation was carried out in 4 days. Additional transplantations of MSCs on to wounds and space between skin grafts was immediately made.	Proved safety, improved new vessel and granulation formation and reduced coarse cicatrices [109].
Wound therapy by marrow mesenchymal cell transplantation	2008	Second- or third-degree burns	All, average 64.8 years, 2	BM-MSCs	Not mentioned	Artificial dermis (Pelnac) was cut to match the shape of skin wound, and the cultured marrow mesenchymal cells were combined in the artificial dermis.	Confirmed skin regeneration and epithelialization in 2 to 4 weeks. One year after graft, scarring degree was milder [110].
Autologous transplantation of bone marrow–derived mesenchymal stem cells: a promising therapeutic strategy for prevention of skin-graft contraction	2011	More than 80%	Male, 19 years, 1	Autologous BM-MSCs	2,100,000 cells/ml	The autologous split-skin graft was spread over the dermal matrix sheet to cover the wound.	No pain, infection or other side effects during wound healing and reduced skin contraction after 2 years [111].
Cadaveric bone marrow mesenchymal stem cells: first experience treating a patient with large severe burns	2015	Full-thickness burn area: 30%	Male, 26 years, 1	Allogeneic BM-MSCs	1×10^4 cells/cm^2	Spraying the MSCs onto the burn wound surface after deep escharotomy using 2 sterile conical tubes. Then the surface was dressed with a sterile polymeric transparent film and keep changing the film.	Good blood supply without infection, better granulation tissue formation and better re-epithelialization [112].
Role of cord blood and bone marrow mesenchymal stem cells in recent deep burn: a case–control prospective study	2017	10 to 25%	All, 15 to 50 years, 60	Autologous BM-MSCs and allogenic UC-MSCs	100,000 cells/cm^2	Two days after surgical excision of deep burned tissue, stem cells were injected in the burned area and the application repeated in 10 days.	Study showed improved rate of healing and reduced hospitalization period in both BM-MSC and UC-MSC groups [113].

%TBSA percentage of total body surface area burned, *MSCs* mesenchymal stem cells, *BM-MSC* bone marrow-derived mesenchymal stem cells, *UC-MSC* umbilical cord mesenchymal stem cells

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Table 3. Clinical trials of MSC-based therapy for burn wound healing registered in www.clinicaltrials.gov

Study	Condition	Status	Phase	Outcomes	NCT number
Mesenchymal stem cell conditioned medium-derived pleiotropic factor in treating residual burn wound	MSC-derived epidermal growth factors	Recruiting	Phase I	NYR	NCT04235296
Allogenic stem cell therapy in patients with acute burn	hUC-MSCs	Unknown status	Phase I/II	NYR	NCT01443689
Autologous keratinocyte suspension versus adipose-derived stem cell-keratinocyte suspension for post-burn raw area	Adipose-derived stem cell-keratinocyte suspension	NYR	NYR	NYR	NCT03686449
A study to evaluate the safety of ALLO-ASC-DFU in the subjects with deep second-degree burn wound	Allogeneic AD-MSCs combined with a hydrogel sheet	Completed	Phase I	NYR	NCT02394873
A clinical trial to evaluate the safety and efficacy of ALLO-ASC-DFU for second deep degree burn injury subjects	Allogeneic AD-MSCs combined with a hydrogel sheet	Unknown status	Phase II	NYR	NCT02619851
Allogeneic ADSCs and platelet-poor plasma fibrin hydrogel to treat the patients with burn wounds (ADSCs-BWs)	Allogeneic AD-MSCs combined with a platelet-poor plasma fibrin hydrogel	Unknown status	Phase I/II	NYR	NCT03113747
Safety and exploratory efficacy study of collagen membrane with mesenchymal stem cells in the treatment of skin defects	Medical collagen membrane with UC-MSCs	Unknown status	Phase I/II	NYR	NCT02672280
A follow-up study to evaluate the safety of ALLO-ASC-DFU in ALLO-ASC-BI-101 clinical trial	Allogeneic AD-MSCs combined with a hydrogel sheet	Completed	Phase I	NYR	NCT03183622
A follow-up study to evaluate the safety of ALLO-ASC-DFU in ALLO-ASC-BI-201 clinical trial	Allogeneic AD-MSCs combined with a hydrogel sheet	Unknown status	Phase II	NYR	NCT03183648
Stem cell therapy to improve burn wound healing	Allogeneic MSCs	Completed	Phase I	Reduction of number of adverse events, such as blistering, inflammation and rash per participant	NCT02104713

MSCs mesenchymal stem cells, *AD-MSC* adipose tissue-derived mesenchymal stem cells, UC-MSCs umbilical cord mesenchymal stem cells, *ADSCs* adipose-derived stem cells, *NYR* not yet reported, *hUC-MSCs* human umbilical cord mesenchymal stem cells, *NCT* national clinical trial

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Skin tissue engineering is considered as a promising treatment strategy for burn wound healing. Skin-tissue-engineered products include scaffolds carrying stem cells or healing-promoting factors. The application of these products is capable of promoting wound healing, reducing pain and correcting poor treatment [5]. In clinical studies of MSC-based therapies, several studies have proven the safety and effectiveness of scaffolds. Also, platelet-rich plasma is regarded as a potential medical treatment in deep burn wound treatment [106].

Outlooks Although the mechanism of MSCs promoting burn wound healing remains controversial, most studies have emphasized the paracrine effect of MSCs. Thus, in recent years, cell-derived product therapies, including MSC exosomes and conditioned media, have drawn much attention. One of the advantages of using cell-derived products is that we can avoid using stem cells that may induce tumorigenesis, immunogenicity and pathogen transmission [1]. Moreover, these products are easier to produce and bring to the market at a lower cost. The challenges of MSC exosomes still lie in their isolation and purification [107], which may result in too few functional exosomes for treatment. In addition, it has been reported that MSC-derived products from different donors have different immunomodulatory effects [108], which indicates the variability of MSC-derived products.

Conclusions

MSCs have shown promise in burn wound care. Extensive animal studies show that they play key roles in wounds. Healing and paracrine signalling are considered to be the main repair mechanisms. Consistent with preclinical studies, clinical trials have also shown that wound healing is significantly improved after using MSCs. However, some limitations should be noted, particularly the low number of patients and the imperfect methods used. Additional research, especially that employing large-scale controlled multicentre clinical trials, is recommended to optimize and standardize the cell source, dose, timing and route of administration. However, based on current data, it is undeniable that MSC-based treatment provides a promising option for burn treatment.

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Conflict of Interest

No competing financial interests exist.

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Authors' contributions

MYW and XXX contributed equally to the review and should be viewed as co-first authors; they drafted and revised the manuscript and did the bulk of literature search and categorization of references. XXL, JT and HQX contributed by guiding the framework, placing the points in context and constantly giving invaluable feedback. All authors read and approved the final manuscript.

Conflicts of interest

None declared.

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