Concise Review: Clinical Translation of Wound Healing Therapies Based on Mesenchymal Stem Cells

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ABSTRACT

There is enormous worldwide demand for therapies to promote the efficient resolution of hard-to-heal wounds with minimal appearance of scarring. Recent in vitro studies with mesenchymal stem cells (MSCs) have identified numerous mechanisms by which these cells can promote the process of wound healing, and there is significant interest in the clinical translation of an MSC-based therapy to promote dermal regeneration. This review provides a systematic analysis of recent preclinical and clinical research to evaluate the use of MSCs in wound healing applications. These in vivo studies provide overwhelming evidence that MSCs can accelerate wound closure by modulating the inflammatory environment, promoting the formation of a well-vascularized granulation matrix, encouraging the migration of keratinocytes, and inhibiting apoptosis of wound healing cells. The trophic effects of MSC therapy also appear to augment wound healing in diabetic tissues, thereby preventing the formation of nonhealing ulcers. Finally, a number of delivery systems have been evaluated and indicate that MSCs could be the basis of a versatile therapy to fulfill the clinical needs for dermal regeneration. However, despite the apparent advantages of MSC-based therapies, there have been only limited clinical investigations of this type of therapy in humans. Thus, our review concludes with a discussion of the translational barriers that are limiting the widespread clinical use of MSCs to enhance wound healing. STEM CELLS TRANSLATIONAL MEDICINE 2012;1:44–50

INTRODUCTION

Wound healing is usually a highly successful biological process to restore the integrity of the skin following injury. The mechanisms of wound healing typically fall along a spectrum between tissue regeneration, the end result of which would be functionally equivalent to the uninjured skin, and tissue repair, during which the skin's functional characteristics are sacrificed in favor of rapidly closing the wound with fibrotic scar tissue. Scars are an undesirable consequence of cutaneous wound healing not only because of their appearance but because of their poor mechanical strength relative to the surrounding tissue [1]. Substantial dysregulation of wound healing can result in a nonhealing wound or ulcer, as frequently observed in diabetic individuals because of their microvascular deficiencies [2]. As a result, the annual worldwide market for advanced wound care products to promote healing in hard-to-heal wounds and to reduce the appearance of scars exceeds $5 billion.

Mesenchymal stem cells (MSCs) have been proposed as a potential therapy to enhance cutaneous wound healing. MSCs are readily available from commercial, allogeneic sources or as autologous cells that can be harvested at the point of care from various tissues, which has low-

ered some of the barriers to the clinical translation of these cells for a variety of therapeutic applications [3]. As with many cell-based therapies, MSCs act through complex interactions with the endogenous cells and tissues, and they may function in the tissue via multiple mechanisms of action. MSCs are also responsive to their environment, and they can modify their activities and functions depending on the biomolecular context. Therefore, MSCs offer several advantages that make them an attractive treatment option during wound healing, including the fact that they appear to be a native constituent of the wound bed [4], where they can regulate the wound healing process by mechanisms that have been extensively studied (reviewed in Jackson et al. [5]). This review provides a systematic evaluation of recent preclinical research to evaluate the use of MSCs in wound healing applications. It will conclude by highlighting the clinical research efforts currently under way to apply MSCs to enhance the process of cutaneous wound healing.

MSCS IN WOUND HEALING BIOLOGY

Dermal wound healing is usually a highly predictable process that can be divided into four overlapping phases: (a) coagulation and hemostasis,

(b) inflammation, (c) proliferation, and (d) remodeling (reviewed in Martin [6] and Velnar et al. [7]). Hemostasis begins coincident with injury once the integrity of blood vessels below the dermal tissue layer has been compromised and escaping platelets come into contact with collagen and other extracellular matrix (ECM) molecules [8]. The stimulated platelets initiate the clotting cascade by undergoing degranulation and releasing intracellular stores of clotting factors and inflammatory cytokines [6, 9]. The inflammatory phase begins within hours of injury as neutrophils respond to these cytokines by entering the fibrin clot, followed by macrophages and leukocytes, which participate in a coordinated response to neutralize foreign antigens and achieve sterility in the wound. These immune cells then recruit reparative cells from the surrounding dermal tissue [10] and circulation [11, 12] to form a well-vascularized granulation tissue during the proliferation stage [6], and this serves as a scaffold for tissue regeneration and enables re-epithelialization of the wound to close off the wound bed. In the final phase of wound healing, endogenous dermal fibroblasts reorganize the ECM to reinforce the provisional granulation tissue matrix and produce additional ECM proteins to regenerate the dermal tissues [7].

MSCs can be found in a variety of adult tissues throughout the body, including bone marrow [13], adipose tissue [14, 15], synovial fluid [16–18], and traumatized muscle [19, 20]. They have also been derived from fetal tissues that can be collected harmlessly during or after pregnancy, such as amniotic fluid [21] and umbilical cord [22], and these have been proposed as potential sources of MSCs for cell banking applications. MSCs exhibit specific characteristics when cultured in vitro, regardless of their anatomical origin [23], and there is emerging evidence that most if not all MSCs may be derived from a common perivascular niche [24]. In vivo, MSCs appear to enhance the regenerative potential of multiple tissues types as a result of various trophic mechanisms that become activated when exposed to the biochemical factors that are characteristic of an injury environment [25]. Endogenous MSCs or a related population of endogenous cells (i.e., vascular pericytes [24, 26]) likely participate in the wound healing response by migrating to the site of cutaneous tissue damage. The inflammatory mediators interferon-γ (IFNγ) and tumor necrosis factor-α (TNFα) can regulate homing and migration of MSCs through the ECM [27], and MSCs have demonstrated chemotaxis toward a variety of wound healing cytokines in vitro [27, 28]. Engraftment of mouse [29, 30] and human [31] MSCs to regions of inflammation and tissue damage has also been observed in murine models of injury and disease. Once they are localized to the wound bed, there is evidence that MSCs can enhance dermal regeneration during multiple phases of the wound healing process.

During the inflammation phase, proinflammatory mediators, such as IFNγ, TNFα, and interleukin-1β (IL1β), can activate regulatory functions in MSCs that enable them to modulate the immune response [32]. In this state, the MSCs can inhibit the recruitment, proliferation, and biological activity of mast cells [33], T cells [32, 34], B cells [35], and natural killer cells [36], thereby attenuating the acute immune response to injury. The inflammatory wound environment of the wound also stimulates COX2 activity in MSCs, leading to upregulation of prostaglandin E$_2$ (PGE2) [37] and a transition in wound function in favor of dermal regeneration. In addition to attenuating T-cell proliferation [38], PGE2 can alter the behavior of leukocytes resident in the wound, corresponding to decreased expression of IL2 [39]

and IFNγ [40] and increased expression of IL4 [40] and IL10 [37, 41]. This shift in favor of anti-inflammatory cytokine expression encourages wound fibroblasts to upregulate their expression of matrix metalloproteinases (MMPs) and downregulate the expression of various collagen types [42, 43], resulting in the formation of a less dense, fibrotic granulation tissue in the wound bed. As a result, the activity of MSCs in the wound appears to favor wound healing over inflammation and promotes functional regeneration of the tissues by mesenchymal cells during the proliferation phase [43, 44].

There is also substantial evidence that MSCs continue to support tissue regeneration during the proliferation phase. Angiogenesis is necessary at this step of wound healing to ensure sufficient nutrient delivery to fibroblasts responsible for forming a provisional granulation matrix [33]. Inadequate microvasculature in the wound bed may inhibit wound closure and lead to a chronic nonhealing wound, as occurs frequently in diabetic patients. MSCs express several factors, including basic fibroblast growth factor (bFGF), vascular endothelial growth factor-A (VEGF-A), and adrenomedullin [45], that promote the proliferation of microvascular endothelial cells [46, 47], vascular stability [48, 49], and the development of a long-lasting functional vascular network [50]. MSCs also secrete a variety of cytokines and growth factors that have antifibrotic properties, including hepatic growth factor (HGF), IL10, adrenomedullin [45, 51, 52], and MMP-9 [53], that promote turnover of the ECM [54–56], keratinocyte proliferation [57], and inhibition of myofibroblast differentiation [58]. Therefore, MSCs in the wound bed contribute to the generation of a high-quality, well-vascularized granulation tissue, enhance re-epithelialization of the wound, and attenuate the formation of fibrotic scar tissue.

In the final stage of wound healing, dermal fibroblasts begin to remodel the wound matrix to generate new dermal tissue. ECM-generating cells from other sources may also be recruited to the tissue as it is remodeled, such as endothelial cells undergoing endothelial to mesenchymal transdifferentiation (EndoMT) [59] and circulating fibrocytes [60]. After exposure to transforming growth factor-β1 (TGF-β1) in the wound, these cells rapidly differentiate into myofibroblasts in the presence of TGF-β1 and begin expressing high levels of ECM proteins [11, 59]. This generic wound-healing response is sufficient to restore tissue integrity, but the myofibroblastic cell types produce excessive amounts of ECM compared with dermal fibroblasts, resulting in scar tissue and poor functional regeneration [61]. MSCs express HGF [62] and PGE2 [63] in the wound, which can inhibit EndoMT and myofibroblastic differentiation of fibroblasts, thus promoting wound healing functions that are specific to dermal fibroblasts [64]. Finally, MSCs can transdifferentiate into epidermal cells, keratinoctes, and microvascular endothelial in vitro under defined induction conditions [65–67] or in coculture with native epidermal cells [68]. Therefore, MSCs appear to promote the production of an ECM that more closely resembles uninjured dermal tissue.

PRECLINICAL VALIDATION OF MSCs TO PROMOTE WOUND HEALING

As described in the previous section, MSCs appear to be an attractive cell type for a cell-based therapy to promote dermal regeneration. In addition to their differentiation potential, MSCs
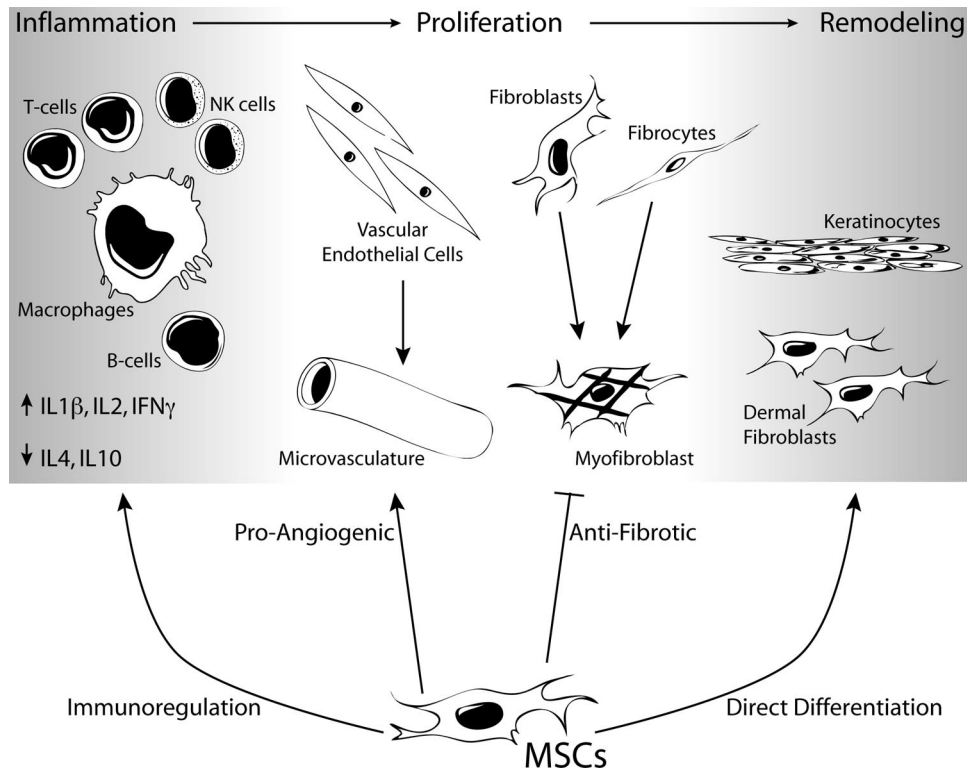
Figure 1. MSCs can enhance dermal regeneration by acting on multiple cell types throughout the phases of wound healing. Abbreviations: IFN, interferon; IL, interleukin; MSC, mesenchymal stem cell; NK, natural killer.

exhibit substantial trophic support to regenerating tissues. Autologous cells are readily available from bone marrow [3] or adipose tissue [69] using well-established procedures. Given the array of mechanisms by which the MSCs can promote wound healing (Fig. 1), there have been a number of studies to evaluate their function in vivo using a variety of cutaneous wound healing models.

Several recent studies have demonstrated the efficacy of MSCs in improving dermal wounding by direct application of the cells to the wound. Chen et al. demonstrated that the cytokines and growth factors secreted by murine MSCs are sufficient for improved wound healing by applying concentrated MSC-conditioned medium directly to a full-thickness excisional wound in mice via injection and topical administration [52]. The conditioned medium encouraged the migration of macrophages and endothelial cells into the wound space and promoted neovascularization in the regenerating tissues, thereby accelerating the rate of wound closure. In a similar study, murine MSCs were applied to excisional wounds in mice, and syngeneic and allogeneic cells demonstrated an equivalent ability to migrate through the tissue and to attenuate the local inflammatory response, as compared with the control allo-fibroblasts, which were restricted to the sites of injection [30]. This study corroborated previous findings that xenograft MSCs elicit little immunogenicity from their host [70, 71] and indicate that MSCs are immuno-privileged during cutaneous wound healing.

Following direct injection into a wound, the MSCs appear to contribute to wound healing by multiple mechanisms that could result in better dermal regeneration with less visual appearance of a scar. Nie et al. demonstrated that adipose-derived stem cells (ASCs) express VEGF-A, HGF, and fibroblast growth factor-2, and in a full-thickness excisional injury, ASCs from rats significantly

enhanced neovasculogenesis and accelerated the time to wound closure [72]. In this study, there was also evidence that the ASCs differentiated directly into endothelial and epithelial cell types and were integrated directly into the regenerated tissue. Similar results have been observed in a murine excisional injury model, such that mouse bone marrow-derived MSCs accelerated wound closure by increasing re-epithelialization, cellularity, and angiogenesis and by differentiating directly into epithelial cells expressing keratin, a keratinocyte-specific protein, that were localized to appendage-like structures [68]. Finally, in a rabbit model, human bone marrow-derived MSCs were injected into an incisional wound, and the cellular therapy resulted in improved wound closure with better wound tensile strength and with a significant reduction in the appearance of a scar [73].

The trophic functions of MSCs in the wound healing environment have been further elucidated using animal models of diabetes to evaluate their function in the context of impaired cellular metabolism and its consequences on microvascular function (reviewed in Groop et al. [2]) Using the db/db diabetic mouse model of impaired wound healing, murine bone marrow-derived MSCs were applied directly to a full-thickness excisional wound, which promoted the formation of a well-vascularized granulation tissue, more rapid re-epithelialization, and better gap closure, thereby preventing the development of a chronic nonhealing wound [74]. Similarly, in a streptozotocin-induced model of diabetes in rats, local injection of ASCs accelerated closure of full-thickness excisional wounds through mechanisms that did not require a significant increase in the expression of ECM proteins or vascular density [75]. This experiment demonstrated the importance of MSCs in inhibiting inflammation and apoptosis of wound-healing cells, and thus, MSCs may substantially improve

the wound healing in the absence of any observable effects on the wound healing fibroblasts.

MSCs have also been incorporated into three-dimensional scaffolds as a strategy to improve skin substitutes that are used to repair full-thickness wounds resulting from trauma or burns [76]. One substantial benefit of including MSCs in these tissue-engineered constructs is to promote angiogenesis throughout the scaffold, which allows for the transport of nutrients and reparative cell types that can remodel the scaffold. For example, human MSCs from the V54/2 cell line were seeded into a collagen scaffold and implanted into a full-thickness defect in mice, and the MSCs significantly improved the vascularity of the constructs during dermal regeneration [77]. There was also evidence that the MSCs were integrated into the structure of the regenerated tissue. In a similar study, allogeneic bone marrow MSCs seeded onto a nanofiber matrix and applied to a full-thickness excisional wound model in rats significantly increased the rate of wound closure [78]. The MSCs were labeled with fluorescent quantum dots prior to seeding, and quantum dots colocalized with cells expressing the epithelial cell-specific markers keratin-10 and filaggrin after wound closure, suggesting that some of the MSCs had undergone epidermal differentiation.

Alternative methods of delivering MSCs have also been evaluated as a means of facilitating the translation of a cell-based wound healing therapy into clinical practice. For example, human bone marrow-derived MSCs have been incorporated into a fibrin spray and used as a regenerative dressing for full-thickness wounds. This biologic wound dressing was sufficient to stimulate complete closure of full-thickness excisional wounds in diabetic mice, whereas controls remained in a chronic, nonhealing state [79]. Fibrin sealant has also been enriched with autograft bone marrow-derived swine MSCs and combined with either bFGF or epidermal growth factor. These therapies were sufficient to enhance the repair of excisional wounds by decreasing the time required for re-epithelialization and by increasing the thickness of the regenerated skin relative to the controls containing no cells [65]. Furthermore, the MSCs appeared to promote functional regeneration in the tissues, as there was evidence of MSC differentiation into endothelial and epidermal cell types and the formation of rete ridges extending into the dermis.

Finally, there is accumulating evidence that MSCs can be administered systemically during dermal wound healing, and they will home to the location of injury to impart their regenerative effects. In a bleomycin model of injury to lung epithelial tissues in mice, it has been established that MSCs will home to the site of inflammation, attenuate the acute inflammatory response, and decrease ECM deposition, thereby minimizing the extent of pulmonary fibrosis [29]. These findings have been extended to dermal wound healing as systemically injected murine MSCs engrafted at the site of an excisional wound model in mice and appeared to transdifferentiate into keratinocytes, endothelial cells, and pericytes and to accelerate the rate of wound healing [66]. In this study, the engraftment of MSCs was enhanced by local injection of the chemokine CCL21 into the dermal wound margin, thereby identifying a likely chemokine that might be used to promote homing of endogenous MSCs to the site of injury. An additional study has demonstrated that systemically administered allogeneic and syngeneic bone marrow-derived MSCs were effective in improving the rate and quality of ECM deposition and increasing the tensile strength of incisional wounds [80]. In the MSC-treated wounds, there appeared to be

rapid resolution of the acute inflammatory phase of the injury, followed by early formation of the granulation tissue, and as a result, the wounds could readily transition into the remodeling phase to minimize the appearance of a scar.

THE FUTURE OF MSC THERAPY FOR WOUND HEALING

In light of the extensive data demonstrating the promise of MSCs for dermal wound healing, the clinical translation of these cells remains somewhat limited. In one study, autologous bone marrow-derived MSCs were delivered via a fibrin spray to patients who suffered from acute and chronic wounds. By obtaining sequential biopsy specimens of the wound bed after MSC application, it was possible to observe the migration of the MSCs into the wound, and the MSCs appeared to stimulate elastin expression in the wound bed, which contributed to the synthesis of a dermal matrix with improved ECM composition [79]. Furthermore, there was an inverse correlation between the concentration of injected cells and size of the chronic wounds. This study demonstrated that the use of cultured MSCs was a safe therapy for dermal wounds without treatment-related adverse events. In a second study, autologous bone marrow-derived MSCs seeded on a collagen sponge were effective to facilitate the closure of ulcerated wounds [81]. Despite the success of these studies, there are currently only four active clinical trials registered at ClinicalTrials.gov pertaining to the treatment of dermal wounds with MSCs [82].

This review has highlighted the exciting preclinical research that has validated MSCs as biologic therapy to enhance dermal wound healing (Table 1). However, prior to the widespread clinical use of MSCs therapy, it will be necessary to identify and overcome the barriers to clinical translation. One limitation to MSC therapy is the method of delivering the cells. Although systemic delivery is an attractive option, the engraftment efficiency can be difficult to predict without improved methods to ensure that the cells will home to the site of injury. Local administration of the MSCs may require an appropriate carrier and/or scaffold to ensure that the cells remain viable and can efficiently migrate into the wound bed (reviewed in Sorrell and Caplan. [83]). Products are currently in development that could be adapted as a suitable carrier for MSCs, such as a novel skin substitute produced by neonatal immortalized keratinocytes (StrataGraft; StrataTech, Madison, WI, http://www.stratatechcorp.com/) [84] or a bilayered graft produced by fibroblasts and keratinocytes (VCT-01; Organogenesis, Canton, MA, http://www.organogenesis.com/). There is also evidence that committed progenitor cells derived from split thickness cutaneous biopsies can be effectively applied directly to the wounds using spray deposition [85], and commercial systems designed for spray deposition of a cellular suspension (e.g., ReCell [86]; Avita Medical, Cambridge, U.K., http://www.avitamedical.com/) might also facilitate the clinical translation of MSC-based therapies.

Further investigation is also needed to elucidate the interactions between MSCs and the various immune and wound healing cell types that are present in the wound bed, as these studies would be useful to optimize the timing and dosage of MSC delivery to the wound for maximal efficacy [3]. The use of MSCs as a therapy presents advantages over pharmaceuticals, protein/growth factors, and committed progenitor cells, as MSCs are able to interact with the surrounding cell types and biochemical environment to express in a regulated manner the appropriate

Table 1. Preclinical studies using MSC therapies to enhance cutaneous wound healing

	Cell type/source	Method of delivery	Reference
Small animal models			
Mouse excisional wound	Mouse MSCs	Topical	Chen et al. [52]
Mouse excisional wound	Mouse MSCs	Local injection	Chen et al. [30]
Mouse excisional wound	Mouse MSCs	Systemic injection	Sasaki et al. [66]
Mouse excisional wound	Human V53/2s	Collagen scaffold	Egaña et al. [77]
Rat excisional wound	Rat MSCs	3D collagen gel	Kim et al. [53]
Rat excisional wound	Rat MSCs	Systemic injection	McFarlin et al. [80]
Rat excisional wound	Human MSCs	Nanofiber graft	Ma et al. [78]
Diabetic mouse (db/db) excisional wound	Mouse MSCs	Local injection	Javazon et al. [74]
Diabetic mouse (db/db) excisional wound	Mouse MSCs	Local injection	Wu et al. [68]
Diabetic rat (STZ) excisional wound	Rat ASCs	Local injection	Nie et al. [72]
Diabetic rat (STZ) excisional wound	Rat ASCs	Local injection	Maharlooei et al. [75]
Large animal models			
Rabbit incisional wound	Human MSCs	Local injection	Stoff et al. [73]
Mini pig thermal wound	Swine MSCs	Local injection	Fu et al. [65]
Pig thermal wound	Rabbit MSCs	Fibrin spray	Mansilla et al. [76]

Abbreviations: 3D, three-dimensional; ASC, adipose-derived stem cell; MSC, mesenchymal stem cell; STZ, streptozotocin.

trophic factors for enhanced dermal wound healing. Therefore, MSCs will likely emerge as an important therapy to reduce the formation of fibrotic tissue and the appearance of scars following cutaneous injury.

AUTHOR CONTRIBUTIONS

W.M.J.: conception/design, analysis/interpretation, manuscript writing; L.J.N.: financial support, analysis/interpretation, manuscript writing; R.S.T.: conception/design, financial support, manuscript writing, final approval.

DISCLOSURE OF POTENTIAL CONFLICTS OF INTEREST

The authors indicate no potential conflicts of interest.

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