Mesenchymal Stem Cells in Chronic Wounds: The Spectrum from Basic to Advanced Therapy

Marta Otero-Viñas^{1,2} and Vincent Falanga^{1,*}

¹Dermatology Department, Boston University School of Medicine, Boston, Massachusetts.

²The Tissue Repair and Regeneration Laboratory, Department of Systems Biology, Universitat de Vic—Universitat Central de Catalunya, Vic, Spain.

Significance: Almost 7 million Americans have chronic cutaneous wounds and billions of dollars are spent on their treatment. The number of patients with nonhealing wounds keeps increasing worldwide due to an ever-aging population, increasing number of obese and diabetic patients, and cardiovascular disease.

Recent Advances: Advanced treatments for difficult wounds are needed. Therapy with mesenchymal stem cells (MSCs) is attractive due to their differentiating potential, their immunomodulating properties, and their paracrine effects.

Critical Issues: New technologies (including growth factors and skin substitutes) are now widely used for stimulating wound healing. However, in spite of these advances, the percentage of complete wound closure in most clinical situations is around 50–60%. Moreover, there is a high rate of wound recurrence.

Future Directions: Recently, it has been demonstrated that MSCs speed up wound healing by decreasing inflammation, by promoting angiogenesis, and by decreasing scarring. However, there are some potential limitations to successful MSC therapy. These limitations include the need to improve cell delivery methods, cell viability, heterogeneity in MSC preparations, and suboptimal wound bed preparation. Further large, controlled clinical trials are needed to establish the safety of MSCs before widespread clinical application.

Vincent Falanga, MD, FACP

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*Correspondence: Dermatology Department,
Boston University School of Medicine, Boston,
MA 02118 (e-mail: vfallanga@bu.edu).

SCOPE AND SIGNIFICANCE

IN THE WESTERN world, about 1–2% of people will develop a chronic wound during their lifetime.¹ These numbers will increase with the aging population and with the rapid increase in the incidence of diabetes and obesity, as well as vascular disease, worldwide.^{2–5} The cost of caring for chronic wounds is 2–3% of health budgets in developed countries.³ Moreover, chronic wounds are related with psychosocial issues from loss of mobility, decreased bodily function, social problems, poor quality of life, and loss of participation in the workforce.³

TRANSLATIONAL RELEVANCE

Treatments for chronic wounds have addressed (1) identification and attempts to correct factors of chronic wounds; (2) optimal management of the wound bed; and (3) contribution to developing different phases of the wound healing process. Yet, these efforts are often unsuccessful. Therefore, it is critical to find more effective and efficient treatments to reduce health costs and the social impact of chronic wounds. Mesenchymal stem cell (MSC)-based therapy has shown beneficial effects on enhancing tissue repair

and regeneration in different diseases and could be a major breakthrough in wound healing.

CLINICAL RELEVANCE

Preclinical and clinical trials show that MSC therapy accelerates wound closure.⁶ This therapy is promising for treating wounds with delayed healing. MSC treatment promotes different stages of the wound repair process.⁷ New studies with cell-based therapies to treat venous leg ulcers, diabetic foot ulcers, and pressure ulcers—the three main types of chronic wounds—are a major effort. However, there are some potential limitations to successful MSC therapy, and further research is needed. We will now review the use of bone marrow-derived MSCs as a therapy for chronic nonhealing wounds.

BACKGROUND

Stem cells promise an emerging opportunity for advancing tissue repair and regeneration. MSCs have shown benefits for the treatment of diabetes mellitus, Crohn's disease, and graft-versus-host

disease. MSC therapy also reduces tissue damage after injury in the heart, lung, kidney, liver, brain, and skin.⁸ These results have promoted cell-based therapy as a solution for chronic nonhealing wounds.^{6,9} MSCs regulate the main phases of normal wound healing. Although MSCs may differentiate in the wound, it has been shown that MSCs enhance wound healing through multiple effects, including modulation of inflammation, promotion of angiogenesis, and stimulation of cell movement during epithelial remodeling (Fig. 1).⁷ The immunosuppressive properties of MSCs allow their potential use in allogeneic therapy.

Wound healing is a dynamic complex process involving the reconstitution of several skin layers. Wound healing progresses through to different and overlapping phases of hemostasis, inflammation, proliferation, and remodeling.^{9,10} Hemostasis starts when blood components extravasate into the site of injury. Platelets then are exposed to collagen and other extracellular matrix components. This exposure leads the platelets to release clotting factors and also essential growth factors and cytokines.¹¹ In

Figure 1. Proposed MSC mechanism(s) of action in wound healing. MSCs could affect different stages of the wound healing process. MSC mechanisms of action may include acceleration of wound healing, immunomodulation, differentiation into epidermal cells, and paracrine signaling pathways. Transdifferentiation remains controversial. Through these mechanisms, MSCs contribute to the reduction of inflammation in the wound bed, allow use of allogeneic MSCs, reduce excessive scar formation, reconstitute epidermal and dermal components, reduce the inflammatory response, and enhance angiogenesis.

the inflammatory phase, neutrophils and macrophages infiltrate the wound bed, remove pathogenic organisms, and secrete cytokines to recruit fibroblasts, endothelial cells, and keratinocytes. The inflammatory phase is critical because it leads to the subsequent steps in the healing process.^{9,10} The next wound healing phase, that is, proliferation, consists of several subphases: fibroplasia, wound matrix deposition, angiogenesis, and reepithelialization. The granulation tissue formed during this phase provides volume to the wound and facilitates closure by driving wound contraction; this phase also enables healing by promoting reepithelialization.^{10,12} Once the wound is filled with new granulation tissue, angiogenesis stops and many of the newly formed blood vessels may undergo apoptosis.¹⁰ During the last phase, which is remodeling, the wound undergoes constant alterations. Collagen is degraded and deposited in an equilibrium-producing manner. This new extracellular matrix is then cross-linked.^{10,13}

In pathological conditions, such as severe trauma, pressure, diabetes, vascular dysfunction disease, and burn injury, the ideal process of wound healing is lost and failure to heal develops. The reason why wounds become chronic includes other conditions, aging, hypoperfusion, poor nutrition, excessive pressure to the wound, immunosuppression, malignancy, infection, and obesity.⁹ Typically, patients with nonhealing wounds have several of these factors and comorbidities at the same time, making single therapeutic approaches less successful. A continuous state of inflammation within the wound, with neutrophil infiltration, reactive oxygen species, and metalloproteinases may perpetuate a nonhealing state. One common characteristic of nonhealing wounds is a sustained inflammation.⁹ Healing generally takes place after the inflammation is decreased. On the other site of the spectrum, fibrosis/sclerosis are characterized by increased extracellular matrix deposition and decreased remodeling.¹³ Another potential explanation for impaired healing is dysfunctional or senescent cells that not are responding to normal biochemical signals.^{14,15}

Therapies for nonhealing wounds have mostly focused on correcting factors involved in chronic wounds. Over the last 25–30 years, a spectrum of advanced therapeutic approaches has evolved (Fig. 2) while even basic wound care has continued to improve (Table 1). These therapies include antibiotic treatment of cellulitis, removal of possible biofilms, revascularization of ischemic limbs, off-loading of decubitus (pressure) ulcers, negative pressure to remove fluid, and the use of compression wraps for venous ulcers.⁴ Appropriate ulcer care principles also include optimizing the wound

Figure 2. Evolution of healing approaches to chronic wounds. The figure shows the evolution of advanced wound healing therapies over the last several decades. Presently, there is increased emphasis of stem cell therapy.

bed by debridement, facilitating reduction of edema, decreasing bacterial burden, and providing the right balance of moisture.¹⁶ Some therapies tend to accentuate the development of different stages of the wound healing process, but the overlap of various treatments is substantial (Tables 1 and 2).¹⁷ However, despite the fact that these therapies may improve outcomes, there is only a 50–60% complete wound closure in most chronic wounds.

COMMONLY USED TREATMENTS FOR CHRONIC WOUNDS

To date, the treatment of chronic wounds is directed at correcting precipitating and perpetuating factors and optimizing the wound bed.^{18–20} Many ulcers heal when the basic principles of wound healing are implemented. However, some wounds do not heal or heal very slowly. Thus, innovative approaches are needed. Over three decades, the field of cutaneous regeneration and tissue repair has seen many advances resulting from molecular biology, biomedical and tissue engineering, and improved understanding of wounds and their pathophysiology (Fig. 2). Because of these therapies, clinicians have more sophisticated treatments for patients with acute wounds, chronic wounds, burns, and other types of injuries.

In this study, we review several existing treatments for chronic wounds classified according to their characteristics: (1) general therapeutic modalities, (2) growth factors and other mediators, and (3) skin substitutes, matrix, and/or cellular therapy.

General therapeutic modalities

Table 1 summarizes some of the basic and general approaches and therapeutic modalities.

Debridement. An important aspect of wound management is debridement, which is the elimination of foreign material and nonviable tissues from a wound bed, to allow healthy tissues to be exposed.

Table 1. General therapeutic modalities for chronic wounds

<i>Treatment</i>	<i>Proposed Mechanism(s) of Action</i>	<i>Level of Evidence*</i>
Debridement (variety of methods)	Removed foreign debris and devitalized or contaminated tissues from a wound bed	Level III _a ²³
(a) Surgical		Biosurgical debridement, Level I _a ²²
(b) Chemical		
Compression	Decreased venous backflow and capillary leakage	Level II _b ²⁴
Pressure-relieving devices and surfaces	Prevention and offloading	Level III _c ^{27,29,30}
Negative pressure	Remove exudate, reduce edema, increase local perfusion, decrease bacterial count, and enhance granulation tissue formation	Level III _a ³⁵
Electrical stimulation	May promote migration of various cell types to the wound, reducing the size of venous leg ulcers	Level III _a ^{38,39,42}
Hyperbaric oxygen therapy (HBOT)	Improve neovascularization, reduce production of proinflammatory cytokines, and increase synthesis of growth factors and collagen	Level III _a ^{46,47,48,49}
Lasers, phototherapy, shockwaves, and ultrasound	Decrease inflammatory cells, increase fibroblast proliferation, stimulate angiogenesis, promote formation of granulation tissue, and increase collagen synthesis	Level III _a ^{50,52}
Antimicrobials		
Silver	Antimicrobial	Level III _a ^{53,56}
Cadexomer iodine	Antimicrobial	Level II _a ⁵³
Others (povidone-iodine, peroxide-based preparations, ethacridine lactate, chloramphenicol, framycetin, mupirocin, ethacridine, or chlorhexidine)	Antimicrobial	Level III _a ^{53,56}
Dressings (alginate, foam dressing, hydrocolloids, hydrogels)	Provide appropriate moist wound environment, which contributes to faster wound reepithelization	Level III _a ⁶¹⁻⁶⁴

*Levels of evidence according to Oxford Centre for Evidence-based Medicine 2011 (available from www1.wfh.org/publications/files/pdf-1502.pdf).

Level I, systematic review of randomized trials; Level II, randomized trial; Level III, nonrandomized controlled cohort/follow-up study. Level of evidence was graded down on the basis of study quality, imprecision, because of inconsistency between studies, small sample size, high risk of bias, unclear randomized method used, or because the absolute effect size is very small.

_aLevel of evidence in promoting wound healing.

_bLevel of evidence in decreasing recurrence.

_cLevel of evidence in preventing ulcer development/reduce pressure.

Regular maintenance debridement has been proposed to stimulate the wound and the process of healing.²¹ Debridement may be accomplished by a variety of methods, including surgery, biosurgical (larvae) debridement, mechanical debridement such as hydrosurgery, autolytic debridement, chemical agents, and enzymatic means. A 2014 systematic review has shown evidence that biosurgical debridement shortened the healing time and improved the wound healing rate.²² Currently, there is not enough evidence to favor a specific debridement

technique or agent for surgical wounds above others. The present debridement methods should be compared in future trials.²³

Compression. Compression therapy, achieved with compression bandages, is the mainstay of treatment for stasis leg ulcers. A 2014 Cochrane analysis has shown evidence that compression stocking decreases the rates of recurrence of stasis ulcers.²⁴ The data show that recurrence is lower with high-compression stockings. However, we

Table 2. Representative growth factors and other mediators of wound healing

<i>Treatment</i>	<i>Mechanism(s) of Action</i>	<i>Level of Evidence*</i>
Growth factors		
PDGF-BB	For diabetic foot ulcers, providing a scaffold on which new cells can migrate and attach	Level I _a ⁶⁷⁻⁶⁹
bFGF	Promoted the proliferation of fibroblasts and capillary formation and accelerates tissue regeneration	Level III _a ^{67,69,72}
rhEGF	For both diabetic foot ulcers and venous chronic wounds. Increased granulation tissue formation and reduced wound discharge and cellulitis in the surrounding area.	Level I _a in burn wounds ⁷² Level III _a ^{67,69,72,75}
Platelet-rich plasma	Rich in many growth factors and cytokines	Level III _a ⁷⁹
Pentoxifylline	Reduced blood viscosity, decreased thrombus formation	Level I _a ^{81,82}

*Levels of evidence according to Oxford Centre for Evidence-based Medicine 2011 (available from: www1.wfh.org/publications/files/pdf-1502.pdf).

Level I, systematic review randomized trials; Level II, randomized trial; Level III, nonrandomized controlled cohort/follow-up study. Level of evidence was graded down on the basis of study quality, imprecision, because of inconsistency between studies, small sample size, high risk of bias, unclear randomized method used, or because the absolute effect size is very small.

_aLevel of evidence in promoting wound healing.

PDGF-BB, platelet-derived growth factor BB; bFGF, basic fibroblast growth factor; rhEGF, recombinant human epidermal growth factor.

presently cannot recommend one type of compression over another.²⁴ Compression bandages require patient adherence. Our recent pilot trial showed that tubular elastic bandages are a good compromise in stasis ulcers requiring frequent dressing changes.²⁵

Pressure-relieving devices and surfaces. Pressure-relieving devices and surfaces (including beds, mattresses, and cushions) are meant to redistribute pressure and are used as treatments for pressure ulcers.^{26,27} Their effectiveness is not totally clear.^{27–30} Further studies are needed to study these challenges and to improve.

Negative pressure. Negative pressure wound therapy (NPWT) removes wound exudate by applying dressing connected to a vacuum device. It is generally not used alone in the healing of stasis leg ulcers. NPWT also reduces edema, increases local perfusion, decreases bacterial burden, promotes angiogenesis, and enhances granulation tissue formation.^{31,32} The success of NPWT in increasing healing is established, as shown by several studies.^{33,34} In contrast, two systematic reviews have reached opposite conclusions. More studies are needed to understand the mechanism of action.^{35–37}

Electrical stimulation. Electrical stimulation (ES) uses pulsing currents of electromagnetic energy applied to the body to treat wounds and it is an adjuvant effective agent found to promote stasis leg ulcer and pressure ulcer healing.^{38–40} ES and local dry heat may work synergistically to heal chronic diabetic ulcers.⁴¹ Several hypotheses may explain how ES may be helpful. However, a 2013 Cochrane review showed no statistically significant increase in rates of wound closure using electromagnetic treatment.⁴²

Hyperbaric oxygen therapy. Hyperbaric oxygen therapy (HBOT), by exposure to 100% oxygen at a pressure > 1 atmosphere, has been used in the treatment of chronic wounds.⁴³ Its efficacy may involve improved neovascularization, reduced proinflammatory cytokines, and increased production of growth factors and collagen.⁴⁴ Our hypothesis is that the sudden decrease in oxygen levels after the patient leaves the HBOT chamber could stimulate wound cells and increase the production of growth factors or the recruitment of stem cells.⁴⁵ A 2012 Cochrane review analyzed HBOT for chronic wounds and concluded that there was a statistically significant benefit at 6 weeks in ulcer healing. Longer-term follow-up analyses are needed.^{46–49}

Lasers, phototherapy, shockwaves, and ultrasound therapy. Some studies have shown that the biological effects of lasers, phototherapy, shockwaves, and ultrasound therapies are related to the decrease in inflammatory cells, increased fibroblast proliferation, angiogenesis stimulation, formation of granulation tissue, and increased collagen synthesis.⁵⁰ However, the biological effects are dependent on such parameters as light wavelength and dose, thus highlighting the importance of determining an appropriate treatment protocol.⁵¹ There is insufficient evidence that these laser therapies have a significant effect on wound healing.^{17,50,52}

Antimicrobials. Many venous leg ulcers are colonized by bacteria or may be infected. Clinical infection in stasis leg ulcers is treated with systemic antibiotics and topical antibiotics or antiseptics, but the regular use of systemic antibiotics is ineffective.⁵³ Topical cadexomer iodine is helpful.⁵⁴ Silver-releasing dressings are also commonly used.^{55,56} Honey is probably ineffective in chronic wounds.^{57,58} In contrast, some studies have reported a broad range of effects by honey in burn treatment.⁵⁹ In spite of the commonly held view that debridement of wounds and aggressive treatment of the bacterial burden are beneficial, definite prospective interventions addressing these topics have proved to be challenging. Further research is needed before we can promote the routine use of povidone–iodine, peroxide-based preparations, ethacridine lactate, chloramphenicol, framycetin, mupirocin, ethacridine, or chlorhexidine. Current guidelines suggest that antibacterial preparations must be used only with clinical infection, not colonization.⁵³

Dressings. Over the last 30–40 years, there has been increasing acceptance of wound dressings that promote moist wound healing. In general, these dressings include the following: transparent films, hydrocolloids, foams, alginates, gels, and collagen-based products. A moist wound microenvironment accelerates wound reepithelization by facilitating keratinocyte migration, although the mechanisms for this are largely unknown.⁶⁰ Presently, there is not enough evidence favoring a specific wound dressing.^{61–65} Smart dressings may be helpful. Biopolymers may provide opportunities to synthesize matrices for stimulating wound healing. In addition, nanobiotechnology allows the production of nanoparticles of copper, silver, and zinc. These agents may serve as excellent antimicrobial agents.⁶⁶

Growth factors and other mediators

There are very few systemic agents that have been shown to facilitate wound healing. Oddly,

systemic corticosteroids, which normally interfere with healing because of their immunosuppressive properties, actually accelerate the healing of certain types of chronic wounds. These wounds include pyodema gangrenosum and those due to vasculitis. Growth factors have largely been used topically (Table 2).

Growth factors. Topical cytokine growth factors continue to be studied as a treatment for stasis leg ulcers. Among the various human growth factors, the epidermal growth factor has various effects on cell regeneration, proliferation, keratinocyte motility, granulation tissues, and stimulation of fibroblast migration. Platelet-derived growth factor BB (PDGF-BB) is the only FDA-approved topically applied recombinant growth factor in the United States of America. It is approved for use in the treatment of diabetic neuropathic ulcers of the foot.^{67–69} Currently, there are no FDA-approved growth factors for venous leg ulcers.⁷⁰ In Japan, human recombinant basic fibroblast growth factor (bFGF) (Kaken Pharmaceutical, Tokyo, Japan) is in use since 2001. bFGF stimulates fibroblasts and blood vessel formation.^{71,72} Several studies have shown the effectiveness of recombinant human epidermal growth factor (rhEGF) in both diabetic foot ulcers and venous chronic wounds.^{69,72–75}

Platelet-rich plasma. Recent findings suggest that platelet concentrates contain multiple functional growth factors and cytokines and may be used as cell therapy.⁷⁶ Platelets are rich in many growth factors, including PDGF, transforming growth factor- β 1 and affiliated molecules, vascular endothelium growth factor (VEGF), epithelium growth factor (EGF), bFGF, brain-derived neurotrophic factor, and hepatocyte growth factor.^{11,77} However, growth factors in platelets are generally in an inactive form and they need to be activated. A platelet gel is prepared by activating platelet-rich plasma with thrombin or CaCl_2 to polymerize fibrinogen into a fibrin gel and activate platelets to release growth factors into the wound.⁷⁸ A 2012 Cochrane systematic review on the topic application of autologous platelet gel in wounds found no conclusive evidence for its effectiveness.⁷⁹

Pentoxifylline. It has been demonstrated that pentoxifylline, an oral methylated xanthine derivative, improves blood flow by decreasing blood viscosity and may be effective in accelerating healing of venous leg ulcers.⁸⁰ Faster healing with combined pentoxifylline and compression therapy was shown in a 2014 Cochrane systematic review.^{81,82}

Skin substitutes, matrix, and/or cellular therapy

These constructs are often a combination of sophisticated matrix products and cell therapy. Many of the constructs are acellular. Those that include cellular components may be classified as living or nonliving, depending on whether the cells are viable or not.

Skin substitutes. Skin substitutes range from purely synthetic compounds to both cellular and acellular constructs derived from human and animal sources. Anatomically, skin substitutes may comprise a dermal layer, an epidermal layer, or are bilayered. Recently, we published a review of the several skin substitutes, which have been used in the last two decades.⁸³

The use of skin substitutes has improved prognosis and reduced morbidity in the treatment of chronic wounds. Allografts from human placenta have several angiogenic growth factors capable of stimulating angiogenesis and amplifying the angiogenic response from human endothelial cells *in vitro*. Randomized clinical trials have shown some substitutes to be effective for healing chronic wounds.⁸⁴ Only two types of skin substitutes have been FDA approved. A human fibroblast-derived dermal living substitute is approved for diabetic neuropathic ulcers. A bilayered living cellular construct is approved for both venous and diabetic ulcers.^{85–87} The exact mechanisms of action of these skin substitutes are not known. These constructs stimulate the host to produce cytokines and growth factors and may therefore act as pharmacological agents.^{86,88} For subjects with longstanding chronic wounds, the success rate of the bilayered living cellular construct is about 50%.⁸⁵ For all skin substitutes to be used optimally, good wound care needs to be maintained, patients with chronic wounds should be identified in a timely manner, and adequate patient selection and use of the construct also need to be employed.⁸⁶

In addition, both Europe and Japan have been active in producing in the area of 3D *in vitro* reconstructed human skin models; these products are commercially available and include Reconstructed Human Epidermis (SkinEthic, Lyon, France), Full-Thickness Skin Model, OS/Rep model (Open Source Reconstructed Epidermis) (Henkel, Düsseldorf, Germany), the Straticell model (Straticell, Les Isnes, Belgium), and Labcyte model (Gamagori, Japan). In general, bioengineered skin has been used to successfully treat several types of wounds.

On the other hand, worldwide economic realities point to achieving useful outcomes in a cost-effective patient-centered manner. So, further research

is required to fully explore the role of these new technologies.^{89,90}

Stem cells. Stem cells have great potential and their main feature is the ability of self-renewal and of differentiating into a number of different cell types. Generally, stem cells have been classified as being totipotent, pluripotent, multipotent, oligopotent, and unipotent, depending on all their differentiation potential.⁹¹ However, it is becoming increasingly clear that such a classification may be too static or inflexible. In fact, we now know that stem cells can be reprogrammed.^{92,93} This classification challenge is very evident with the issue of pluripotency. In this study, we propose to subdivide pluripotent stem cells into two categories of A and B (Fig. 3). In this new classification, there is recognition that even without reprogramming some stem cells are more capable of pluripotent behavior.

One must recognize that stem cells can undergo asymmetrical division, whereby one of the two daughter cells continues as a stem cell, while the other daughter cell can go on and differentiate. This mechanism allows the retention of a reservoir of stem cells. A useful concept, which continues to be controversial, is that of stem cell plasticity. This mechanism would allow stem cells to transdifferentiate.⁹¹ However, the possibility that plasticity is wholly, or in part, the result of cell fusion has not been excluded.

In the past several years, bone marrow-derived MSCs have promoted acceleration of wound closure. MSCs orchestrate the main phases of wound healing, are involved in inflammation, and promote cell motility. Currently, clinical research is testing MSCs as a therapy for difficult-to-heal wounds.⁹⁴ In addition, due to MSC properties of immunosuppression and promotion of angiogenesis, this cell therapy approach shows promise.⁷

MESENCHYMAL STEM CELLS

MSC characterization

MSCs are multipotent cells with a resemblance to fibroblasts. MSCs were first isolated from bone marrow, but are now known to be present in other tissues. It is important to use a homogenous and well-characterized MSC population. In 2008, the Mesenchymal and Tissue Stem Cell Committee of the International Society for Cellular Therapy proposed minimal criteria to define human MSCs: (1) they must be plastic adherent when maintained in standard culture conditions, (2) they must be lineage negative and express CD105, CD73, and CD90 and lack expression of CD45, CD34, CD14 or CD11b, CD79 α or CD19, and HLA-DR surface

Figure 3. Modification of stem cell classification. Through the differentiation pathway, stem cells can be categorized as totipotent, pluripotent, multipotent, oligopotent, and unipotent, depending upon all their possible reversible, progressively acquired characteristics. The figure proposes that in the absence of reprogramming, there may be different levels of pluripotency.

molecules, and (3) they must have the ability to differentiate to at least osteoblasts, adipocytes, and chondroblasts *in vitro*.⁹⁵

Another distinguishing characteristic of MSCs is their capacity to expand *in vitro* under usual culture conditions using suitable tissue culture media. The clinical feasibility of culture-expanded MSCs has been validated in a number of trials. Thus, a small amount of bone marrow aspirate is adequate for the expansion into large number of MSCs for transplantation.⁹⁶ MSCs are relatively simple to isolate, but extended expansion has been shown to adversely alter their properties.⁹⁷ Human MSCs undergo senescence and exhibit decreased differentiation capacity.^{98,99} On the other hand, MSCs are proving to be multifunctional in their differentiation and immunomodulatory properties (Fig. 1).

MSCs can differentiate into mesodermal lineage cells, including osteoblasts, chondrocytes, adipocytes, cardiomyocytes, hepatocytes, endothelial cells, smooth muscle cells, and neuronal cells, under appropriate culture conditions.¹⁰⁰ These findings have led to the evaluation of MSC potential for treating diseases and the birth of MSC-based therapy.⁹⁶ Clinical trials for diseases, such as osteogenesis imperfecta, graft-versus-host disease, and myocardial infarction, have shown some promise, demonstrating the safe use of both allogeneic and autologous MSCs.⁹⁶ Preclinical trials have shown the successful use of MSCs for delivering therapeutic proteins and repairing defects in several disease models. However, lack of knowledge of MSC behavior and responses *in vitro* and *in vivo* requires basic and animal studies before bringing these therapies to humans.⁹⁶

A possible limitation of obtaining MSCs from bone marrow in terms of yield and differentiation abilities

has led to considering adipose-derived stem cells (ASCs) as a new possibility to cell therapy. ASCs are an abundant supply of multipotent adult stem cells, which are easily harvested from subcutaneous adipose tissue through minimally invasive procedures, such as limited liposuction. Whereas only 0.001–0.002% of cells found in the bone marrow are MSCs, up to 1% of adipose cells are estimated to be stem cells.¹⁰¹ The abundance and accessibility of ASCs and their ability to differentiate along multiple lineages have made them promising candidates for stem cell therapies in regenerative medicine.¹⁰⁰ In addition, it was proposed that ASCs delivered in specific tissues through acellular matrices act as structural elements to increase wound healing; this may be due to differentiation into endothelial and epithelial cells.¹⁰²

For cutaneous wound healing, bone marrow-derived MSCs are the most often used source in most clinical trials.⁹⁴ However, ASCs and umbilical cord-derived cells have shown promise in clinical trials of diabetic ulcers.⁷

Strategies for delivering MSCs

Depending on the disease process and wound, different strategies involving specific cell delivery systems, genetic modification, and the use of scaffolds have been developed. Different strategies for MSC delivery to cutaneous wounds are used: direct topical/spray, scaffold loaded, subcutaneous injection, or systemic delivery.⁹

Work performed by Shimizu and colleagues showed that intravenously injected MSCs transdifferentiated into keratinocytes, endothelial cells, and pericytes at the wound site, thus speeding up the repair process. Those investigators identified chemokine receptor, CCR7, as playing a main role as its ligand, SLC/CCL21, induced MSC motility.¹⁰³ Expression of keratin by transplanted MSCs and the formation of glandular structures were reported by Wu *et al.* upon injection of MSCs around excisional wounds in diabetic mice.¹⁰⁴ Our own investigative group used subcutaneous injections of bone marrow and topical application of whole bone marrow-derived cells in an early human study.¹⁰⁵ Subsequently, we focused on MSCs. We showed acceleration of wound closure in both human and diabetic mouse models by topical delivery of MSCs with a modified fibrin spray system that we developed.¹⁰⁶ This work, for the first time, supported the concept of MSC wound engraftment.¹⁰⁶ Initially, we focused on chronic lower extremity ulcers. Subsequently, we developed data suggesting that this approach could help other types of wounds. Figure 4 shows that MSCs delivered in a fibrin construct

system promote healing of scleroderma (systemic sclerosis) digital ulcers within 4 weeks.

Javazon *et al.* demonstrated improvement in wound healing in a diabetic mouse model by using a topical application of stem cells.¹⁰⁷ It has been shown that topically applied allogeneic MSCs increase healing of experimental full-thickness wounds and show less inflammation.¹⁰⁸ In one of the only two reported randomized clinical trials using MSCs, Dash *et al.* treated ischemic and diabetic ulcers and noted both accelerating wound healing and decreased pain. Those experiments used intramuscular injections of MSCs and showed an increase in immature cells, blood vessels, and reticulin fibers.¹⁰⁹ In contrast, Jain *et al.* found no significant improvement in healing with the clinical use of topically and subcutaneously injected MSCs.¹¹⁰ Another investigative group noted MSC differentiation into epidermal cell lines in addition to greater angiogenesis through paracrine signaling in a diabetic mouse model.¹⁰⁴ A bidirectional cross talk between MSCs and macrophages may be operative. In addition, ASCs injected directly into excisional wounds differentiated directly into endothelial and epithelial cell types.¹¹¹

Animal models have shown that stem cells accelerate wound healing and also synergize with scaffolds. Rustad *et al.* found that stem cells delivered with a hydrogel remained viable longer; enhanced engraftment efficiency was demonstrated. Wounds treated with MSC-seeded hydrogels showed significantly enhanced angiogenesis, which was accompanied by greater wound levels of VEGF and other angiogenic cytokines.¹¹² In addition, ASCs loaded on biological scaffoldings showed increased viability when compared with topical application. Therefore, scaffold-loaded stem cells may increase stem cell efficacy.¹¹³

MSC persistent engraftment might be a limitation of MSC therapy when treating wounds. In nonwound injury models, engraftment appears to be affected by the delivery protocol of MSCs into the site of the wound. From those studies, it is apparent that timing of delivery, cell number, and the delivery site all affect the efficiency of MSC engraftment.⁸ The donor immunogenicity and the wound microenvironment may also impact engraftment. It remains to be seen whether persistent engraftment is absolutely necessary in wound healing.

MSCs: mechanism(s) of action

MSCs derived from either the bone marrow or adipose tissue have been demonstrated to accelerate cutaneous wound healing. There are several potential mechanisms by which MSCs could signifi-

Figure 4. Systemic sclerosis (scleroderma) digital ulcers treated by MSCs delivered in fibrin. Scleroderma digital ulcers treated with MSCs. Ulcer on the index and a pitting scar on the middle fingertips, respectively. MSCs were delivered in a fibrin construct system, appearing as a gel on the fingertips: **(A)** digital ulcers and pitting scars at baseline; **(B)** MSCs in fibrin being applied to the digital ulcers; and **(C)** healed digital ulcers by 4 weeks. (Copyright Vincent Falanga, MD, 2010).

cantly contribute to wound healing, but the exact mechanism is still under investigation. Different studies propose that MSC mechanisms of action include immune modulation, differentiation into epidermal and dermal cells to replace the damaged skin, and paracrine signaling pathways (Fig. 1).

One of the most important barriers to treatment in chronic wounds is the presence of excessive inflammation. As observed in fetal wound healing, the optimal healing environment may be when inflammation is minimized.⁹ MSCs have recently been demonstrated to hold several immunomodulatory effects on host immune cells in both wound healing and transplant biology contexts. MSCs release immunosuppressive factors that inhibit proliferation of immune cells such as T cells, B cells, and natural killer cells.^{114,115} These features make MSCs an attractive cell type for cell therapy in nonhealing wounds; they exert pleiotropic effects on the inflammatory mechanisms to move the wound past the state of persistent inflammation.⁷ To release immunosuppressive factors, MSCs can downregulate the immune response, making them immune privileged and more ideal for allogeneic transplantation.⁹⁶ ASCs also release growth factors critical for healing, modulate the immune system, downregulate inflammation, and home to wounded tissues.¹¹⁶

Present research into the immune response to allogeneic MSCs has demonstrated that in most systems, the donor MSCs are immunoprivileged and do not induce a significant host response. These findings suggest that allogeneic cells may be used for chronic wound therapy and could be a useful strategy for situations when the host's endogenous MSC population may possibly be defective, as in diabetes. The use of allogeneic MSCs may be critical in the chronic wound microenvironment, where excessive inflammation already leads failure to heal. However, several studies have demonstrated that this immunoprivileged property is lost as the MSCs differentiate; this effect leads to a host response to the cells.⁷ MSC low immunogenicity and immunosuppressive features, combined with their availability, ease of propagation, and storage, allow allogeneic grafting from healthy donors. This aspect is particularly important for treating chronic wounds in older individuals, patients with diabetes, and those with autoimmune diseases and compromised MSCs.¹¹¹ Economy of scale for the production of ready-to-use allogeneic stem cells would also make treatment less expensive than the use of autologous treatment. Recently, it has been reported that MSCs may have an antimicrobial effect, which reduces excess inflammation. The combination of the MSC antimicrobial effect with

silver-based dressings might decrease the inflammation associated with healing.⁷

Another consideration in wound repair is scarring caused by deposition of excessive extracellular matrix. The anti-inflammatory effects of MSCs may decrease fibrosis and therefore reduce scar formation.⁷ Present reports show that MSCs can decrease fibrosis in a mouse model.¹¹⁷ Systemic therapies aimed at global immune suppression can improve the healing process, but the quality of healing may be compromised through a weakened scar formation. It is possible that MSCs can sense the degree of inflammation in the microenvironment and respond by releasing of growth factors, cytokines, and other mediators to reduce inflammation using real-time biochemical cues. If effective, reducing inflammation to an appropriate level to allow healing to proceed should also result in improved tensile strength and scar quality, thereby reducing wound recurrence.⁹

In vitro and *in vivo* experiments have shown that MSCs can differentiate into keratinocyte, fibroblast, endothelial cell, and adipocyte phenotypes when cultured under specific conditions.^{4,104} MSCs injected directly into excisional wounds were shown to differentiate into a number of skin cell types and therefore repopulate the wound bed.¹⁰⁴ In tissues with a high rate of cell turnover, such as skin, differentiation or transdifferentiation rather than cell fusion is the principal mechanism.¹¹⁸ However, MSC transdifferentiation ability has been questioned.^{91,96} MSCs are also rapidly mobilized in response to hypoxia, which is commonly found in acute wounds and chronic wounds with poor vascularization.¹¹¹ MSC differentiation into epidermal cells and their migratory capacity contribute to the cell repopulation of the wound bed.

Revascularization of the wound bed is also a crucial event of wound healing.⁷ Wu *et al.* reported a decrease in the number of donor-derived cells in the wound during the 4-week follow-up injection of MSCs, suggesting that the effects of MSCs are temporary.¹⁰⁴ MSCs secrete paracrine factors, including VEGF- α , EGF, keratinocyte growth factor, stromal cell-derived factor 1, insulin-like growth factor-1, and angiopoietin-1, which enhance the recruitment of macrophages, keratinocytes, dermal fibroblasts, and endothelial cells to the wound site, facilitate angiogenesis, stimulate collagen production from dermal fibroblasts, and reduce apoptosis, inflammation, and scar formation at the site of the wound.^{9,8,96,119–121} Conditioned medium from MSC cultures can modulate wound healing.^{120,122} There is also evidence that MSCs enhance neovascularization during wound healing.⁴ MSC-secreted mediators may play a critical role in MSC regulation of blood vessel

formation in wounds. In addition, paracrine effects of ASCs in several skin cells have been studied for initiating the tissue regeneration process.^{116,123} In one animal study, the use of ASCs seeded on an acellular dermal matrix found that it enhanced wound healing, promoted angiogenesis, and contributed to newly formed vasculature in murine mouse models.¹²⁴ Finally, MSC treatment of wounds reduces inflammation, accelerates wound healing, promotes granulation tissue, and increases angiogenesis.²

MSC safety has been evaluated in a present meta-analysis and at this time appears to be safe for clinical use.¹²⁵ However, one must always consider the potential for MSC transformation and tumor formation.^{126,127} Thus, further research into the immunosuppressive mechanisms and other systemic effects of MSCs and new, large, controlled clinical trials are needed to ensure the safety of MSCs.

PRESENT LIMITATIONS OF MSCs AND FUTURE CHALLENGES TO USE IN CHRONIC WOUND REPAIR

Cell therapy for improving wound healing is very timely, particularly for chronic wounds. These difficult wounds can benefit from more effective wound care approaches. There are several reasons why MSCs provide novel and effective stimulation of wound healing. Finally, MSCs have the ability to suppress excessive inflammation and decrease scarring while simulating *de novo* angiogenesis in the wound bed, all leading to promising outcomes in chronic wound repair. However, in spite of these advances, there are some potential limitations to MSC successful treatment. Such limitations include cell delivery methods, cell viability, heterogeneity in MSC preparations, suboptimal wound microenvironment, and the need for a long-term safety profile of the use of MSCs.

The sources of MSCs can create a great deal of variation among therapeutic cells. There are complex and sometimes large differences among MSCs harvested from bone marrow, adipose tissue, and other sources, ranging from cell surface marker expression to differentiation limitations and, importantly, immunomodulatory ability.¹¹⁵ In terms of the cell-based therapy strategy, this makes selection of a cell source an important consideration and/or limitation for any given cell therapy.

The heterogeneity of MSCs may present a serious challenge in terms of changes in cell proliferation and differentiation.¹²⁸ There are no reliable guidelines for MSC expansion and general use in cell therapeutic purposes. Another possible limitation to the use of MSCs for treating chronic

wounds is the varying degrees of cell survival following implantation, which might adversely affect long-term treatment.^{7,113}

We need a better mechanistic understanding of MSCs, including investigations to define the interactions between MSCs and the other cell types present in the wound. We also need to identify the MSC-derived factors responsible for tissue responses to injury for the understanding of how MSCs are affected by the wound environment.⁶ Determining whether MSC production of cytokines and growth factors is regulated during wound healing will explore whether the MSC supernatant can be used to enhance healing. Treatment of chronic wounds in diabetic patients with autologous MSCs is of clinical significance, but its value is restricted by deficient functions of the MSCs due to diabetes. Compared with MSCs isolated from nondiabetic mice, MSCs from diabetic mice were significantly less effective at accelerating epithelialization, increasing granulation tissue formation, and increasing angiogenesis in healing diabetic wounds. In addition, the diabetic wound microenvironment has a direct impact on the function of MSCs.¹²⁹

The process of developing cell-based therapies is complex and must be done correctly to ensure a safe application of MSCs. Safety measures to consider include the use of Good Manufacturing Practice facilities required for manipulating and manufacturing human cells *in vitro*,¹³⁰ animal protein-free freezing solutions, cryoprotectants, freezing and thawing protocols, viability assays, and packaging and distribution. Recent developments and ongoing research with the use of MSCs in cutaneous wounds have led interest in the regulation of MSCs as potentially useful therapies.¹³¹

Other considerations for the use of MSCs may be (1) cost-effectiveness of acquisition, testing, storage, and subsequently use in humans; (2) stem cell delivery must be easily accomplished and without the use of sophisticated laboratory techniques; (3) the treatment must be such that it can be used in the clinic, and not just within the confines of a laboratory-associated clinical research team; (4) having competent laboratory personnel; and (5) the availability of off-the-shelf stem cell product for immediate use in cases involving burns and/or trauma. Thus, it is urgent to resolve these limitations to support cell-based therapeutic commercialization.

NEW STRATEGIES TO ENHANCE THE THERAPEUTIC EFFECTIVENESS OF MSCs IN CHRONIC WOUNDS

We have developed two novel paradigms and concepts, supported by laboratory and clinical in-

Figure 5. A didactic strategy to render stem cells more capable of the desired differentiation. The diagram proposes the concept that didactic strategies, using both stem cells and an additional tissue therapy, may guide stem cells toward the desired pathway of differentiation.

vestigation, to enhance the effectiveness of MSCs and, possibly, other stem cell approaches. One consideration or concern is that once placed within the wound, MSCs may not know what to do and how to best mediate a healing response. Therefore, we have developed the concept that a didactic component is also required (Fig. 5). In the context of this paradigm, the delivery of MSCs would be accompanied by the administration of a more mature tissue component, such as autologous grafting or bioengineered skin. We have preliminary data supporting this concept (not shown; V. Falanga, 2014). It seems to us that this didactic step would induce the stem cells toward the goal of restoring tissue and, although not proven, along different lines of differentiation.

In addition, recently, we have found that a priming step is required just before the administration of cell therapy. In that work, we have shown that a living bioengineered skin construct would produce up to 200-fold increases in wound repair mediators when preincubated in ideal tissue culture conditions.¹³² We believe that a similar approach may be required with stem cell therapy.

SUMMARY

The incidence of chronic wounds keeps increasing. However, in spite of the number of advances in technology (including growth factors, cytokines, and skin substitutes), clinical outcomes are generally suboptimal. Wound recurrences remain high, as do the costs of managing chronic wounds.

Current studies into MSC immunomodulation and wound support in other diseases provide

additional insights into the beneficial properties of MSCs. Numerous experimental animal studies have shown the therapeutic potential of MSCs and their safety and efficacy *in vivo*. In addition, several clinical trials have demonstrated that MSCs enhance wound closure, increase tensile strength within the wound, promote angiogenesis, and decrease the inflammatory response. However, the regenerative ability of MSCs in human wounds rests on limited human studies. Further research is needed to clarify the optimal methods for cell isolation, harvesting, and characterization and expansion. We need to define optimal timing and route of administration, understand MSC mechanisms of action in tissue repair and regeneration, and prove the efficacy in the setting of chronic wounds. Large-scale multicenter trials designed with great stringency need to be performed for complete validation of MSC-based therapy.

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TAKE-HOME MESSAGES

Advantages of MSCs for use in chronic wound repair

- Easily available source (bone marrow, adipose tissue)
- Easy to isolate and characterize
- Modest number of cells are required
- Cell delivery methods are being established
- Cell survival in culture is acceptable

Limitations of MSCs for use in chronic wound repair

- Mechanisms of action need to be identified
- Long-term safety profile must be investigated
- Differentiation into multiple cell types is somewhat limited
- A didactic component may be needed
- Bone marrow mobilization by granulocyte colony-stimulating factor (G-CSF) is limited

written by the authors listed. No ghostwriters were involved in writing of this article.

ABOUT THE AUTHORS

Marta Otero-Viñas, PhD, is Professor at the Department of Systems Biology and Director of the Laboratory of Tissue Repair and Regeneration at the University of Vic-Central University of Catalonia, Spain. She is currently working as a Research Scholar at the Boston University School of Medicine, Boston, MA. She is involved in several projects for wound healing. **Vincent Falanga, MD, FACP**, is Professor of Dermatology and Biochemistry at Boston University and Vice-Chair for research at Boston University School of Medicine, Boston, MA. His primary research interest is in wound healing and fibrosis.

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Abbreviations and Acronyms

ASCs = adipose-derived stem cells
 bFGF = basic fibroblast growth factor
 EGF = epithelium growth factor
 ES = electrical stimulation
 FDA = Food and Drug Administration
 HBOT = hyperbaric oxygen therapy
 MSCs = mesenchymal stem cells
 NPWT = negative pressure wound therapy
 PDGF-BB = platelet-derived growth factor BB
 rhEGF = recombinant human epidermal growth factor
 VEGF = vascular endothelium growth factor