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Do Mesenchymal Stem Cells Have a Role to Play in Cutaneous Wound Healing?

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ABSTRACT: Cutaneous wounds represent a significant healthcare problem despite our increasing understanding of the molecular and cellular events governing this process. A major contributor to this problem is the lack of reliable therapies for the treatment of "hard-to-heal" wounds. Tissue engineering with the use of mesenchymal stem cells (MSCs) have emerged as a promising therapeutic tool with favorable early experimental and clinical results. This manuscript aims to provide an overview of the available approaches to up-regulate the cutaneous wound response with the use of MSCs.

KEYWORDS: mesenchymal stem cells, wound healing, growth factors

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Introduction

Wound healing is a well-orchestrated complex series of events aiming to restore the continuity of the skin.[1] It starts immediately after trauma and encompasses several distinct and overlapping phases lasting for several months to years. The principal events of wound healing have been outlined several centuries ago, when Aulus Comelius Celsus used the terms tumor, rudor, calor, and dolor to describe the cardinal symptoms of the early phase of this process.[2] Since then, these observations have been enriched with experimental data and a more sophisticated understanding is currently in place.

An inflammatory state characterizes the first stage of wound healing Figure 1. It is initiated after injury and the disruption of the soft tissue and blood vessels activates the coagulation system.[1] The resulting clot constitutes an early barrier and also the bed for the upcoming inflammation (tumor). The infiltration of leukocytes including neutrophils, lymphocytes, and macrophages together with the actions of various cytokines induces the local inflammatory reaction. In addition, these cells eliminate potential microbes and debris from the wound site.[3] Vasodilatation and increased vascular permeability follows which results in local swelling as plasma proteins and fluid are released in the interstitial space (rudor).[1,4] The local temperature (calor) is increased and the actions of prostaglandins on the peripheral nociceptors lead to increased local sensitivity and pain (dolor), which decreases the functional capacity of the affected body region.

Subsequently, the proliferative phase begins approximately the fourth day after injury. It is characterized by formation of the granulation tissue, the recruitment, and proliferation of several cell types.[4] Collagen, extracellular matrix, and several growth factors are secreted. Vascular endothelial cells and capillaries invade the area from the surrounding healthy tissue. Keratinocytes start to migrate from the wound edges and proliferate on the granulation tissue.[1,4]

The final stage of wound healing is the maturation stage.[1] The randomly deposited granulation tissue remodels into a more organized structure. The type I collagen replaces the collagen type III.[4] As a result of this process, the tensile strength increases and reaches 70–80% of the original skin.[4]

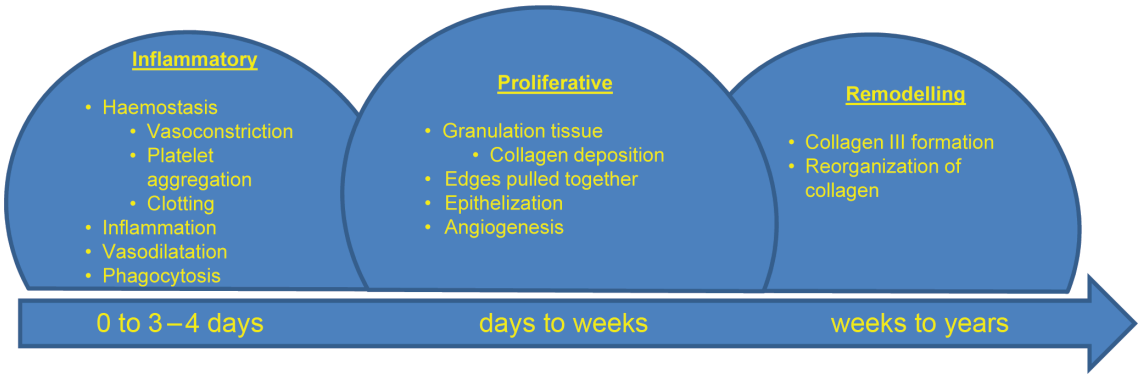

Figure 1. The phases of wound healing.

Dysregulation of the wound healing process could result in a non-healing wound or ulcer. A classic example is the diabetic foot where the peripheral vascular disease together with the decreased sensory innervation can result in chronic ulcer formation which predisposes to a high amputation rate.[5] Other risk factors for impaired wound healing include age, obesity, poor nutritional state, infection, smoking, and impaired oxygenation and the use of pharmacological agents like steroids and chemotherapeutic agents.[6]

Despite our increasing understanding of the cellular and molecular events mounted after the injury, wound healing remains a topic of vivid deliberations. This is mainly because of the fact that injured skin does not regenerate but heals with the formation of scar tissue. In addition, there is lack of reliability of the current approaches especially in chronic or "hard-to-heal" wounds. Potential novel approaches combining recent tissue engineering advances with the use of the multipotent mesenchymal stem cells (MSCs) have been proposed and are presented below.

Mesenchymal Stem Cells

Mesenchymal stem cells are non-hematopoietic stromal cells with multilineage differentiation capacity.[7] They were initially isolated from the bone marrow (BM) and the stroma of spleen and thymus.[8] Subsequently, it was noted that several other tissues harbor a population of MSCs including cartilage, synovium, fat, skin, and artery wall.[9–12] Under specific signals, they can mobilize and differentiate toward a diversity of cells like osteoblasts, chondrocytes, adipocytes, fibroblasts, and myocytes.[13] Such signals include tissue trauma, fracture, inflammation, necrosis, and tumors.[14,15] Therefore, they can be considered as reservoirs of reparative cells lacking tissue-specific characteristics.

The fate of MSCs can be influenced by chemotaxis and by interactions with the extracellular matrix through transmembrane proteins like integrins.[16,17] In addition, the local microenvironment could trigger their differentiation toward cells of the local cell population.[16] For instance, it was shown that intravenously injected MSCs have the ability to migrate and colonize distant injured sites.[18,19] Therefore, their beneficial effect has been demonstrated in conditions like myocardial infarction, fracture, ischemic cerebral disease, and spinal cord injury.[18–21] Similarly, suspended MSCs injected intra-articularly into the knee joint after injury appeared to engraft and regenerate damaged meniscus and cartilage.[22]

One of the major drawbacks in MSC research is the lack of robust phenotypic or morphologic marker to identify or characterize them. In culture, MSCs remain morphologically heterogeneous, containing cells ranging from narrow spindle shaped to large polygonal and in confluent cultures, tightly packed, slightly cuboidal cells.[23] They are able to adhere tissue culture plastics and form colonies after low-density plating. MSCs express a number of nonspecific markers, none of which individually, or in combination, has been shown to achieve high levels of enrichment.[24] Therefore, positive and negative phenotypic staining is performed, which results in a loose phenotypic definition with this being an area of ongoing controversy.[24] This task is further aggravated by the fact that MSCs share features with other types of cells including endothelial, epithelial, pericytes, and muscle cells.[25] To overcome these difficulties, the International Society for Cellular Therapy (ISCT) has proposed a collective and functional approach.[26] As per ISCT, MSCs are non-hematopoietic stromal cells with specific cell surfaces markers (positive for CD105, CD73, and CD90, and negative for CD45, CD34, CD14/CD11b, CD79-a/CD19, and Human Leukocyte Antigen-DR (HLA-DR)), capable of trilineage differentiation, ie osteoblasts, chondrocytes, and adipocytes, adhere to tissue culture plastics and form colonies of spindle-shaped cells.[26]

Despite these difficulties, MSCs have several inherent properties which make them an attractive option for tissue culture applications. First, they are able of genetic reprogramming or transdifferentiation.[27] In other words, a fully differentiated cell from one lineage is able to switch into another mature cell type. As shown by Song and Tuan, a fully differentiated osteoblast having detectable alkaline phosphatase (ALP) activity and elaboration of calcified extracellular matrix was able to de-differentiate into either fully functional

lipid-producing adipocyte or chondrocyte and vice versa.[27] A second property of MSCs is their immunosuppressive effect and the lack of immunogenicity. The immune phenotype of cultured MSCs is widely described as major histocompatibility complex (MHC) Class I^+, MHC Class II^-, $CD40^-$, $CD80^-$, and CD86.[28] In vitro co-culture of MSCs with stimulated T cells showed that the immune reaction was suppressed.[29] It merits mentioning that this suppression appeared to be independent of MHC matching between the MSCs and the T cells. This finding has become controversial with possible scenarios to include direct cell-to-cell contact or to be the result of a soluble factor produced by MSCs which increases in proportion of their number in cultures.[30,31] Furthermore, MSCs have been shown to suppress the proliferation of T cells and cytokine production in response to allo-antigens, as well as, to inhibit the function of B cells, dendritic cells, and the natural killer cells.[32–34] Additionally, MSCs have shown to down-modulate inflammation directly or by secreting factors that cause multiple anti-inflammatory effects.[35,36] Finally, MSCs were found to decrease apoptosis possibly by activation of the stannicalcin-1.[37] It is worth mentioning that these issues are currently poorly understood and the basic science research on MSC is mainly carried out either in animal models or using culture-expanded human MSCs. Therefore, significant differences in their properties in humans might exist.

MSCs in Skin and During Wound Healing

MSCs have been successfully isolated from the skin.[38] Emerging evidence suggests that MSCs reside in the dermis but not in the epidermis and that the connective tissue sheath surrounding the hair follicles is the anatomic niche for dermal MSCs.[38,39] During wound healing, MSCs play a crucial role. They control the inflammation, promote angiogenesis, upregulate the proliferation of various cells, and trigger cell migration.[40] As far as the anti-inflammatory effect is concerned, addition of MSCs in an active immune response environment decreased the secretion of TNF-α and interferon (IF)-γ and at the same time increased the levels of anti-inflammatory cytokines like interleukin-10 and interleukin-4.[41] MSCs directly interact with macrophages and regulate their phenotype.[42] Furthermore, MSCs produce antimicrobial factors like the LL-37 and promote phagocytosis and bacterial killing possibly through a paracrine mechanism.[43,44]

During the wound healing process, MSCs contribute in the reconstitution of local cell population with several ways. First, MSCs are able to differentiate toward fibroblasts, endothelial cells, and keratinocytes.[45,46] However, accumulating evidence suggests that MSCs are recruited and mobilized from distant sites toward the wound. Injury per se increases the levels of circulating MSCs in peripheral circulation.[47] During wound healing, BM MSCs tagged with a fluorescent dye were found in the wound site already differentiated into various cell lineages.[48]

MSCs influence directly and indirectly the secreted levels of a number of growth factors in the wound. These include the platelet-derived growth factors (PDGFs), fibroblast growth factor, keratinocyte growth factor, vascular endothelial growth factor (VEGF), and epidermal growth factor (EGF).[40,49] They also trigger the synthesis of VEGF, Ang-1, and EGF by other cell types.[50] These secreted mitogens have been shown to promote angiogenesis and stimulate the proliferation of keratinocytes, endothelial cells, and fibroblasts in vitro.[49,51] It worth to mention that these factors alone are capable of upregulating the wound healing response.[49] Direct application of MSC culture media on the wound in animal models promoted the formation of new blood vessels, encouraged the migration of endothelial cells and macrophages formation, and overall accelerated the wound healing process.[49]

All the above mentioned basic science evidence shows the multipotential therapeutic role of MSCs during wound healing. As a consequence, a significant number of experimental and clinical studies have emerged.

Preclinical Animal Studies

Several preclinical studies have validated the beneficial effects of application of MSCs in wound healing. The studied approaches could be divided into four main categories: (i) systemic injection of cells, (ii) local application of MSCs, (iii) manipulation of the host MSC populations, and (iv) application of genetically modified cells. All these approaches have demonstrated efficacy in improving the healing response.[49,52,53]

MSCs have the inherent property to migrate to the site of injury and contribute to the healing response. This inherent property of the cells has been used by a number of authors to study the effect of intravenously injected MSCs on wound healing.[52–54] After intravenous injection, MSCs are found recruited to the wound site, differentiate toward keratinocytes, endothelial cells, and pericytes, and accelerate the wound repair.[53] Furthermore, systemic administration of MSCs once daily for 4 days or a single treatment with 5 million MSCs 24 hours after wounding significantly increased the wound bursting strength of fascial and cutaneous wounds on days 7 and 14 after the induced trauma.[52] Liu et al studied the effect of intravenous injection of umbilical cord MSCs in rat model of severe burn.[54] They reported that MSCs migrated into the wound and contributed to the repair. A significant decrease in the quantity of infiltrated inflammatory cells and levels of IL-1, IL-6, and TNF-α was noted together with an increase in the levels of IL-10 and TNF-stimulated gene 6 protein in wound. The neovascularization and levels of VEGF as well as the collagen type I and III in wound were significantly higher in the MSC treatment group.[54]

In addition to the parenteral administration, MSCs have been either applied on the wound or injected adjacent to the wound site. Direct application of bone-derived MSCs (femur, tibia) on the wounds of diabetic mice has enhanced the epithelial gap closure and increased the angiogenesis.[55] Similarly,

BM-MSCs applied with a fibrin spray on experimentally induced murine cutaneous wounds found to accelerate the healing response.[56] MSCs utilized from more easily accessible sites like adipose tissue found to have a similar healing potential. For instance, in a mouse wound healing model, adipose tissue MSCs loaded on collagen gel resulted in acceleration of the healing, enhancement of the secretion of type I collagen and the extracellular matrix proteins.[57] Following direct transplantation in or around the wound, MSCs were also found to contribute to the repair process. Such topical transplantation of MSCs resulted in an acceleration of wound closure and increased collagen synthesis, cellular proliferation and angiogenesis.[58] The wounds treated with MSC showed decreased expression of IL-2 and interferon-γ.[58]

Maximizing the output of the host local MSCs populations could serve as an alternative to the application of culture expanded MSCs. Several authors have analyzed this avenue and the results are promising. The topical application of 1% and 3% Insulin Growth Factor (IGF)-1 creams increases the expression of myofibroblasts in the process of wound healing in rats.[59] Application of PDGF to donor site wounds in a porcine burn model showed increased epithelization rates.[60] Similarly, Gowda et al studied the effect of daily topical applications of PDGF on the healing of ischemic wounds in adult male Sprague–Dawley rats.[61] They reported that PDGF accelerated the rate of wound healing in both normal and ischemic wounds and negated the effect of ischemia. Similar results were obtained when EGF and erythropoietin were applied directly on the wound.[62] Several other constructs including the recombinant human EGF-loaded poly-lactic-*co*-glycolic-acid-alginate microspheres, fibrin-based scaffold incorporating VEGF- and Basic Fibroblast Growth Factor (bFGF)-loaded nanoparticles, PDGF on gelatin gel and recombinant human granulocyte/macrophage colony-stimulating factor hydrogel have all found capable of accelerating the wound healing response.[63–66]

Tissue engineering with the use of genetically modified cells expressing several growth factors have been analyzed as an alternative to the above mentioned approaches. Yan et al in a porcine model of radiation-induced skin injury analyzed the effect of topical transplantation of constructs composed of acellular human amniotic membrane together with autologous BM-MSCs and skin-derived keratinocytes infected by recombinant retrovirus expressing human PDGF-A.[67] Their results showed that these constructs resulted in shorter healing times through increased granulation formation and re-epithelialization rates but also up-regulated angiogenesis and collagen deposition. In another study, angiopoietin-1 gene-modified BM-MSCs were applied on a cutaneous wound healing rat model.[68] Enhanced angiogenesis and increased epidermal and dermal regeneration were noted in comparison to unmodified MSCs. Similarly, VEGF gene-modified human umbilical cord MSCs effectively improves the vascularization of tissue-engineered dermis in miniature pigs.[69] MSCs transfected with human hepatocyte growth factor and transforming growth factor-beta3 genes have also been used in similar models with beneficial results.[70,71]

Clinical Studies

Clinical trials have emerged based on the promising findings from the numerous in vitro and experimental animal studies Table 1. BM aspirates injected around the periphery of chronic wounds in three patients resulted in complete healing within 60 days.[72] A case study of a patient with chronic non-healing venous and neuro-ischemic wounds treated with direct local application of autologous BM aspirates led to a reduction of wound size, increased vascularization, and infiltration of mononuclear cells.[73] Dermal rebuilding and evidence of reduced scarring was also noted.[48] Vojtassák et al combined the application of BM aspirates and the periphery of the wound with application of culture-expanded MSCs at days 7 and 14 in a patient with chronic non-healing diabetic ulcer.[74] Complete healing of the wound was noted within a month after the treatment. Falanga et al implanted autologous BM-MSCs loaded in a fibrin spray in five patients with acute wounds from skin cancer surgery and eight patients with chronic, long-standing, non-healing lower extremity wounds.[56] The application of MSCs has stimulated the wound healing process leading to a decrease in size or healing within 20 weeks. It is of note that a strong correlation between the number of cells applied and the subsequent decrease in chronic wound size was noted. Yoshikawa et al treated 20 patients suffering from non-healing wounds of various etiologies with autologous MSCs ex vivo expanded with animal or autologous sera.[75] They reported complete healing in 18 of the 20 patients, while the remaining two patients died for reasons unrelated to the transplantation of MSCs. Dash et al conducted a level 1 randomized control trial including 24 patients with non-healing ulcers of the lower limb.[76] In the group of patients receiving autologous culture-expanded BM-derived MSCs, a significant improvement in pain-free walking distance and reduction in ulcer size was noted. Similarly, Lu et al analyzed the effect of intramuscular injections of BM-MSCs, BM-mononuclear cells or normal saline on 41 type-II diabetic patients with bilateral diabetic critical limb ischemia and foot ulcers.[77] Their results showed that the patients who received BM-MSCs had higher healing rates and limb perfusion compared with those who received the BM-mononuclear cells or those in the normal saline groups. The authors did not find any significant difference between the groups in terms of pain relief and amputation.

Discussion and Future Perspectives

The cost of treatment of over 7 million people treated annually in the United States alone for non-healing wounds has been estimated to be over $20 billion.[78,79] It is not of a surprise that the annual worldwide market for products that promote healing in such circumstances exceed $5 billion.[80] Therefore,

Table 1. Clinical studies presenting the effect of MSCs on 'hard-to-heal' wounds.

AUTHOR	NO OF PATIENTS	STUDY DESIGN	RESULTS
Badiavas & Falanga, 2003[48]	3 patients	Autologous BM cells applied in chronic wounds of more than 1-year duration	• BM-derived cells can lead to dermal rebuilding and closure of nonhealing chronic wounds.
Humpert et al, 2005[73]	Case study	Autologous BM aspirate for non-healing wound	• Reduced wound size and improved vascularity noted
Vojtassak et al, 2006[74]	Case study	BM aspirates applied directly to the wound and injected into the edges. 2 further applications of culture expanded MSCs	• Closing and healing of the non-healing diabetic ulcer was achieved
Badiavas et al, 2007[87]	4 patients	Autologous cultured MSCs	• Administration of cells to patients with chronic wounds leads to an enhanced clinical response
Falanga et al, 2007[56]	12 patients	Autologous BM-MSCs loaded in a fibrin spray	• Stimulation of wound healing • A strong correlation between the number of cells applied and the subsequent decrease in chronic wound size was found
Rogers et al, 2008[72]	3 patients	BM aspirates injected around wound	• Complete healing observed
Yoshikawa et al, 2008[75]	20 patients with non-healing wounds	Ex-vivo expanded MSCs placed in collagen sponge	• Complete healing in 18 of the 20 patients
Dash et al, 2009[76]	24 patients	Autologous cultured BM-derived MSCs and standard wound dressing versus control group	• The implantation of autologous BM-derived MSCs in non-healing ulcers accelerates the healing process and improves clinical parameters
Lu et al, 2011[77]	41 patients	Intramuscular injections of BM-MSCs, BM-mononuclear cells or normal saline	• BM-MSCs had higher healing rates and limb perfusion • No significant difference between the groups in terms of pain relief and amputation

potentially efficient and safe tissue engineering approaches involving the use of MSCs in "hard-to-heal" wounds could result in attractive and prosperous future applications.

Several obstacles should be overcome before further expansion of their use. To date, no specific marker for their isolation exists and MSCs are identified retrospectively, ie after their isolation and culture. There is still little knowledge about their niche, origin, surface markers, and definition criteria. Furthermore, the techniques used for their isolation, culture, and differentiation vary significantly between different research groups. Even the nomenclature used is sometimes different and rather confusing. To make matters worse, a significant proportion of our knowledge about these cells is based on animals, where it has to be noted that these cells have different characteristics compared to the human MSCs.

Safety-related issues to the culture ex vivo expansion techniques used is another matter still to be addressed. Following several population doublings, MSCs are subject to senescence and potential genetic instability. Modification of the telomeric structures and telomeric length, and activation of the retinoblastoma protein and p53 pathways have been associated with culture expansion and senescence of MSCs.[81] In addition, the currently available culture conditions cannot mimic the in vivo cell environment and are unable to safeguard the proliferation and differentiation of the cells. The use of tissue culture media can expose the cells to animal fetal sera. This endangers the transition of animal-derived pathogen and immunogenic reactions as MSCs were found to carry fetal calf proteins.[82] For instance, Horwitz et al reported a case of osteogenesis imperfecta where rejection of the infused MSCs occurred.[83] In addition, life-threatening arrhythmias in cardiomyoplasty with the application of MSCs have been avoided when human autologous serum was used instead of fetal calf serum.[84] Ideally, tissue culture medium should be of sound and reproducible composition, free of animal components allowing production of large homogenic populations of MSCs in a considerably short period of time.

Safety issues and cytotoxicity also exists in the genetically modified approaches. Viral proteins of adenoviral vectors could cause local toxicity, inflammation, and immune reactions.[85] Non-viral vectors have also been associated with mutagenesis, carcinogenesis, and immune reactions.[86] In fact, several clinical trials utilizing such approaches have been ceased because of these safety concerns.[86]

In conclusion, it is undisputable that MSCs play an important positive role during the wound healing process. They act on different phases of this process with effects not only limited to direct differentiation to committed cell types

but also interact and activate other cells, protect the wound, and have a significant paracrine role. Although all these attributes have been described, the underlying mechanism that links these events is yet to be unravelled. Evidence on the appropriate scaffold and the mode of application need further elucidation. Future work devoted to basic science and tissue engineering will enhance our understanding of the biological properties of MSCs and will determine efficient and safe approaches in the treatment of wounds.

Conclusion

MSCs play a vital role during the cutaneous wound healing process. Several studies have delineated that harnessing MSCs to improve wound healing could have profound clinical benefits. However, several limitations should be overcome including a better understanding of the pathways that govern these processes and the development of safe approaches for MSC isolation, ex vivo culture, and re-implantation.

Author Contributions

Wrote the first draft of the manuscript: IP. Contributed to the writing of the manuscript: MP, TG, PVG. Agree with manuscript results and conclusions: IP, MP, TG, PVG. Jointly developed the structure and arguments for the paper: IP, MP, TG, PVG. Made critical revisions and approved final version: IP, MP, TG, PVG. All authors reviewed and approved of the final manuscript.

DISCLOSURES AND ETHICS

This paper was subject to independent, expert peer review by a minimum of two blind peer reviewers. All editorial decisions were made by the independent academic editor. All authors have provided signed confirmation of their compliance with ethical and legal obligations including (but not limited to) use of any copyrighted material, compliance with ICMJE authorship and competing interests disclosure guidelines and, where applicable, compliance with legal and ethical guidelines on human and animal research participants. Provenance: the authors were invited to submit this paper.

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