Comprehensive review of the clinical application of autologous mesenchymal stem cells in the treatment of chronic wounds and diabetic bone healing

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ABSTRACT

Chronic ulcerations are a physical and financial burden to the health and economic establishment in the United States and Worldwide. Improvements in biotechnology and knowledge in stem cell applications have progressed and basic science results are making their way slowly into the clinical arena. Chronic wounds and diabetic bone healing are the key components in the limb salvage of the common diabetic foot. We have examined the current available literature and present the latest on stem cells applications as a novel clinical technique in the treatment of chronic wound and diabetic bone healing and their impact in the treatment paradigm of patients.

Key words: Bone healing • Chronic wounds • Stem cell • Tissue repair • Ulcer

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CHRONIC WOUNDS

Chronic ulcerations are a physical and financial burden to the health and economic establishment in the United States and Worldwide. Lower extremity wounds occur in 4–10% of people with diabetes, with a lifetime risk of up to 25% and a 20–80% recurrence rate. The cost of treating an ulcer may range up to $48 000 per year, not including the financial burden of potential amputations or secondary problems. There is no financial amount that can be associated with the mental and physical effect on an individual's decreased quality of life and consequence on society in general. Expediting closure of wounds while decreasing associated complications would have a significant impact on quality of life, rate of morbidity

Key Points

- lower extremity wounds occur in 4–10% of people with diabetes, with a lifetime risk of up to 25% and a 20–80% recurrence rate
- expediting closure of wounds while decreasing associated complications would have a significant impact on quality of life, rate of morbidity and mortality and cost of care associated with this population

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Key Points

- the currently available limited evidence suggests that autologous stem cells derived from bone marrow have potential to treat many disorders given their plasticity and ability to differentiate into various types of tissues, including endothelium, liver, muscle, skin, bone, cartilage, brain, fibroblasts and keratinocytes
- however, this may not necessarily represent the biological mechanism underlying MSC-augmented tissue repair
- upon initial application of mesenchymal stem cells (MSCs) into a chronic wound environment,it has been suggested that they work primarily to amplify the signals of surrounding cells via mRNA production and release
- several published case reports indicate the clinical effectiveness of bone marrow aspirate (BMA) or MSC for healing chronic wounds

and mortality and cost of care associated with this population (1–4).

There are several factors that contribute to delayed wound healing; macro- and microvascular disease, hyperglycaemia, infection, pressure, increased inflammatory proteases and cellular senescence. In chronic wounds, the senescent cells because of the inhibition of fibroblast proliferation are unable to divide and become unresponsive to growth factors. An ulcer with more than 15% of senescent cells is more difficult to heal (5). Ulcers that have been present for more than 1 year have generally proven more difficult to manage, and several studies have shown the inverse relationship between duration and healing (1,6). It is not unusual for patients to present to hospitals and wound clinics with ulcers that have been present for more than 1 year. Aggressive debridement, in the absence of contraindicating factors including inadequate vascular flow, may not be sufficient to induce wound closure.

The currently available limited evidence suggests that autologous stem cells derived from bone marrow have potential to treat many disorders given their plasticity and ability to differentiate into various types of tissues, including endothelium, liver, muscle, skin, bone, cartilage, brain, fibroblasts and keratinocytes. They are believed to assist with the tissue repair process by secreting large amounts of growth factors and cytokines (7–10). However, this may not necessarily represent the biological mechanism underlying MSC-augmented tissue repair.

Upon initial application of mesenchymal stem cells (MSCs) into a chronic wound environment, it has been suggested that they work primarily to amplify the signals of surrounding cells via mRNA production and release (8). Further studies and evidence are needed to substantiate these beliefs. MSCs are very resilient and persist in wounds for extended periods acting as only modulators of cellular signalling initially (10). Full differentiation into keratinocytes, myofibroblasts (11) and epidermal stem cells is seen; however, this is not until later in the wound healing process (8,9). MSCs furthermore play a large role in angiogenesis when applied or injected locally. Secreted chemokines attract pericytes which potentially differentiate into fibroblasts, smooth muscle or macrophages and regulate endothelial microvasculature at the capillary level (12). A large down regulation of wound degrading matrix metalloproteinases is also observed following MSC introduction into a wound (13) (Table 1).

Table 1 Current clinical studies published using MSC therapy in chronic wound care

	No. of patients	Clinical level of evidence	Study design
Badiavas *et al*. (14)	4	2	Randomised controlled trial
Badiavas and Falanga (15)	3	4	Case series
Dash *et al*. (16)	24	1	Randomised controlled trial
Falanga *et al*. (10)	13	3	Case control
Humpert *et al*. (5)	1	5	Case report
Lataillade *et al*. (17)	1	5	Case report
Ichioka *et al*. (18)	1	5	Case report
Kirana *et al*. (19)	1	5	Case report
Mulder *et al*. (23)	8	4	Case series
Rogers *et al*. (20)	3	4	Case series
Vojtassak *et al*. (21)	1	5	Case report
Yoshikawa *et al*. (22)	20	3	Case control

CLINICAL DATA

Several published case reports indicate the clinical effectiveness of bone marrow aspirate (BMA) or MSC for healing chronic wounds. Humpert *et al*. (5) applied BMA topically on a neuro-ischaemic chronic diabetes mellitus (DM) wound and showed reduced wound size, increased wound vascularity without any systemically observed effects within 7 days of application. Although this was a single case presentation, the result should be considered for future study designs. The weakness is that of any single case presentation.

Vojtassak *et al*. (21) reported complete wound resolution of a 25-year open wound within 4 weeks of application of marrow-derived MSCs and fibroblast collagen membrane. As with the other case studies, the single patient report is limited in validity. Ichioka *et al*. (18) reported complete wound closure after stem cell therapy with >1-year open wound. The Ichioka study was based on mouse models. While this data is encouraging, animal studies are very limited in their ability to mimic the complexity of a diabetic, venous

or other chronic wound. Kirana *et al*. (19) reported a positive result with topical application on a neuro-ischaemic diabetic ulceration. Again, the presentation is of a single patient. It is important to note the result of bone marrow mononuclear cells when applied in an ischaemic environment. Lataillade *et al*. (17) have shown promise in conservatively treating severe radiation burns with MSCs. A single patient was presented with notable results. As with chronic wound studies, larger randomised trials are needed in the bone population.

In 2008, Rogers *et al*. (20) injected BMA topically into the wound periphery in three patients with differing aetiologies and suggested this procedure as useful and safe adjunct to wound closure. These ulcers healed in 47, 50 and 60 days, respectively. Safety is supported but not significantly confirmed with the small number of patients who were reviewed. Similar results were achieved by Badiavas and Falanga (15) in which three patients had complete closure of their year-long ulcers with use of BMA and cultured cells. The purpose of this study was to determine effects of dermal rebuilding. This was seen in all the patients thereby providing insight into possible effects of bone marrow-derived cells. All healed within 3 months, although one patient required additional application of bioengineered skin. In 2007, Badiavas *et al*. (14) conducted a randomised trial applying cultured MSCs versus autologous bone marrow aspirate (ABMA) into the wounds of four subjects, only one healed completely, but a positive clinical response was seen in all patients. As with the previous study, the numbers analysed were small.

Falanga *et al*. (10) applied up to three applications of autologous culture-expanded MSCs with a fibrin glue system to acute and chronic wounds. This delivery system was proven to allow MSC persistence to stimulate wound healing for prolonged periods of time. The acute wounds secondary to excision of non melanoma skin cancers healed within 8 weeks. The chronic year-long lower-extremity wounds significantly decreased or healed in 16–20 weeks. This study showed a strong correlation between the number of MSCs per square centimetre surface area and reduction in ulcer size. The study was larger than most trials yet was non randomised with a small patient population. The value of the results are in their similarity in positive outcomes compared to other small trials, as well as the data on cell concentration and its affect on outcomes. The comparison with mouse models lends credibility to other animal studies using stem cells.

In 2008, Yoshikawa *et al*. (22) performed a larger study which included 20 subjects with non healing wounds of various aetiologies. The authors reported nearly complete healing in 18 patients and showed fibrous and vascular regeneration of native tissue by immunohistochemical examination. In our institution, we reviewed eight patients with chronic wounds of several aetiologies who received ABMA collected from the ipsilateral calcaneal bone and xenograft application. Out of the eight patients, only three showed significant wound size reduction during the 12 week follow up. The low numbers attaining significant results may have been due to the small population number, the non equivalent demographics and the retrospective nature of the review (23).

The largest study to date using bone marrow-derived MSCs in extremity based wounds was published by Dash *et al*. (16). In this level 1 randomised controlled trial, the MSC therapy significantly reduced wound size and increased several clinical parameters as compared with controls. MSC treatment groups were also shown to lack any ill effects of the body's normal biochemical parameters. To date, this is the largest trial conducted with MSC lending support to the use of the cells to expedite wound closure.

DIABETIC BONE HEALING

Lower-extremity deformity, joint and muscular instability and ulcerations are constant factors in diabetic Charcot neuroarthropathy when dealing with limb salvage situations. Often, there are unstable and misaligned lower extremities with or without ulceration, which become a major clinical challenge. Because of the nature of poor bone stock and quality in the diabetic patient, this type of deformity is a complex disorder, which is due to the lack of number of treatments and availability of adequate surgical technology, lacks evidence-based, universally agreed upon treatment protocols. The use of stem cells in this scenario is novel, and there is lack of literature on this

Key Points

- the largest study to date using bone marrow-derived MSCs in extremity based wounds was published by Dash et al
- in this level 1 randomised controlled trial, the MSC therapy significantly reduced wound size and increased several clinical parameters as compared with controls
- MSC treatment groups were also shown to lack any ill effects of the body's normal biochemical parameters
- to date, this is the largest trial conducted with MSC lending support to the use of the cells to expedite wound closure.

Key Points

- although there is strongly suggestive evidence that MSCs assist in wound healing, there is a paucity of validated clinical research with adequate number of subjects and well designed clinical studies to prove the efficacy of this wound therapy using MSCs
- large level 1 studies are needed to determine the value of MSCs in wound therapy

topic. There seems to be sufficient basic science papers on this topic, but there is truly a lack of clinical literature on this very important topic nowadays.

Bone healing assistance with the application of stem cells is powerful orthopaedic tool for bone regeneration because of their ability to differentiate into osteoblasts (24–27). Recently, MSCs were used in a type 2 diabetic rat model for osteogenic analysis implanted into the right tibia. After 4–8 weeks, MicroCT analysis showed that bone volume ratio and trabecular thickness increased significantly ($P < 0{\cdot}05$), and trabecular separation decreased significantly ($P < 0{\cdot}05$) compared with the titanium implants in diabetic rats. Histological examination showed a greater amount of new bone tissue forming around the MSC-implant complexes and a higher bone implant contact (BIC) rate than the titanium implants (28).

Stem cells can provide all three essential bone growth properties for successful bone remodelling and repair: osteoinductive, osteoconductive and osteogenic. Early animal studies have shown promise and hypoimmunogenic response (29–31). In an athymic rat model for bone formation, L4-5 posterolateral spinal fusions showed osteoblastic lining areas of new woven bone formation in 8 weeks. In a canine midfemoraldiaphyseal segmental defect, both the autologous and allogenic stem cells showed healing equivalency, and no cellular immune response. In a high mammal, a baboon fibular osteoperiosteal defects, within 12 weeks of stem cell implantation, showed various degrees of mineralisation. Fluorescently labelled cells were found within the areas of newly forming bone and not in the host marrow spaces or cortical resected segment margins. Bone formation was also noted in an ectopic site using human stem cells (HSCs) in a rat model, showing osteogenic activity. Stem cells were noted to be differentiating directly into osteoblastic to form bone, with no evidence of endochondral osteogenesis and induced (osteoinduction) host cells noted to differentiate along an osteoblastic lineage expressing for bone morphogenic protein (BMP)-2, BPM-6, bone sialoprotein (BSP) and vascular endothelial growth factor (VEGF).

Current ability to bring stem cells containing products is based on the ability and evolution on what is known about the MSC. Because MSCs do not have Class II surface antigens and other costimulatory molecules, they can be implanted between individuals with human leukocyte antigen (HLA) or other matching. Class II surface antigens. Other costimulatory molecules are required for a host to mount a T-cell reaction to foreign cells. The source of most MSC today is organ donor marrow. Bone marrow has two types of stem cells: HSCs and MSC. The majority of cells in marrow are HSCs (haematopoietic stem cells). HSCs are >99% nucleated cells, are immunogenic, require HLA and ABO matching for allogenic transplant and are cluster of differentiation (CD) 45+. The HSCs are not immune privileged and must be depleted from the marrow before implantation. The cells are identified by being positive for surface marker CD45. MSCs are present in 1/500 000 nucleated cells, are immune privileged, lack Class II antigens, are cytokine producers, including BMP-2 and BMP-6, VEGF and others; and are CD 105+, CD 166+. MSCs, thus, are in the minority and are identified by CD markers, CD 105 and CD 166. MSCs are CD 45 negative. No single marker or set of markers are unique to the MSC but CD105 and CD166 are commonly accepted. MSCs, as cytokine factories, provide two important physiologic properties: anti-inflammatory and immune-modulatory cytokine production can regulate the immune system and suppress an immune reaction; and produce BMPs for osteoinduction.

Amputation is always an option. As such, there are many ways to salvage the lower extremity. Saving the lower extremity will keep the patient alive and functional and prevent another amputation and ultimately their lives. Functional foot and ankle surgery and minimally invasive amputations are key in the survival of the patient and the limb. MSC takes advantage of the following properties because of the expression and/or secretion of various cytokines to diminish or prevent scar formation, to stimulate angiogenesis, to differentiate into different connective tissue phenotypes depending on the local environment. It is understood that MSCs are multipotential. When provided by the right cues from the environment in which they are placed they can form tissues of mesodermal origin such as bone, cartilage. The nature of local cues is poorly understood.

Although there is strongly suggestive evidence that MSCs assist in wound healing, there is a paucity of validated clinical research with adequate number of subjects and well-designed clinical studies to prove the efficacy of this wound therapy using MSCs. Large level 1 studies are needed to determine the value of MSCs in wound therapy.

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REFERENCES

1 Calam MJ, Ruckley CV, Harper DR, Dale JJ. Chronic ulceration of the leg: extent of the problem and provision of care. Br Med J (Clin Res Ed) 1985;290:1855–6.
2 Phillips TJ. Chronic cutaneous ulcers: etiology and epidemiology. J Invest Dermatol 1994;102:38S–41S.
3 Brem H, Kirsner RS, Falanga V. Protocol for the successful treatment of venous ulcers. Am J Surg 2004;188(1A Suppl):1–8.
4 Morrell CJ, Walters SJ, Dixon S, Collins KA, Brereton LM, Peters J, Brooker CG. Cost effectiveness of community leg ulcer clinics: randomized controlled trial. BMJ 1998;316:1487–91.
5 Humpert PM, Bärtsch U, Konrade I, Hammes HP, Morcos M, Kasper M, Bierhaus A, Nawroth PP. Locally applied mononuclear bone marrow cells restore angiogenesis and promote wound healing in a type 2 diabetic patient. Exp Clin Endocrinol Diabetes 2005; 113:538–40.
6 Skene AI, Smith JM, Dore CJ, Charlett A, Lewis JD. Venous leg ulcers : a prognostic index to predict time to healing [see comments]. BMJ 1992;305:1119–21.
7 Bianco P, Riminucci M, Gronthos S, Satomura K, Bianco P, Robey PG. Circulating skeletal stem cells: nature biology and potential applications. Stem Cells 2001;19:180–92.
8 Borue X, Lee S, Grove J, Herzog EL, Harris R. Bone marrow-derived cells contribute to epithelial engraftment during wound healing. Am J Pathol 2004;165:1767–72.
9 Deng W, Han Q, Liao L, Li C, Ge W, Zhao Z, You S, Deng H, Murad F, Zhao RC. Engrafted bone marrow-derived flk-(1) mesenchymal stem cells regenerate skin tissue. Tissue Eng 2005;11:110–9.
10 Falanga V, Iwamoto S, Chartier M, Yufit T, Butmarc J, Kouttab N, Shrayer D, Carson P. Autologous bone marrow-derived cultured mesenchymal stem cells delivered in a fibrin spray accelerate healing in murine and human cutaneous wounds. Tissue Eng 2007; 13:1299–312.
11 Yamaguchi Y, Kubo T, Murakami T, Takahashi M, Hakamata Y, Kobayashi E, Yoshida S, Hosokawa K, Yoshikawa K, Itama S. Bone marrow cells differentiate into wound myofibroblasts and accelerate the healing of wounds with exposed bones when combined with an occlusive dressing. Br J Dermatol 2005;152:616–22.
12 Sorrell JM, Baber MA, Caplan AI. Influence of adult mesenchymal stem cells on in vitro vascular formation. Tissue Eng Part A 2009;15:1751–61.
13 Smith AN, Willis E, Chan VT, Muffley LA, Isik FF, Gibran NS, Hocking AM. Mesenchymal stem cells induce dermal fibroblast responses to injury. Exp Cell Res 2010;316:48–54.
14 Badiavas EV, Ford D, Liu P, Kouttab N, Morgan J, Richards A, Maizel A. Long-term bone marrow culture and its clinical potential in chronic wound healing. Wound Repair Regen 2007;15:856–65.
15 Badiavas EV, Falanga V. Treatment of chronic wounds with bone marrow-derived cells. Arch Dermatol 2003;139:510–6.
16 Dash NR, Dash SN, Routray P, Mohapatra S, Mohapatra PC. Targeting nonhealing ulcers of lower extremity in human through autologous bone marrow-derived mesenchymal stem cells. Rejuvenation Res 2009;12:359–66.
17 Lataillade JJ, Doucet C, Bey E, Carsin H, Huet C, Clairand I, Bottollier-Depois JF, Chapel A, Ernou I, Gourven M, Boutin L, Hayden A, Carcamo C, Buglova E, Joussemet M, de Revel T, Gourmelon P. New approach to radiation burn treatment by dosimetry-guided surgery combined with autologous mesenchymal stem cell therapy. Regen Med 2007;2:785–94.
18 Ichioka S, Kouraba S, Sekiya N, Ohura N, Nakatsuka T. Bone marrow-impregnated collagen matrix for wound healing: experimental evaluation in a microcirculatory model of angiogenesis, and clinical experience. Br J Plast Surg 2005;58:1124–30.
19 Kirana S, Stratmann B, Lammers D, Negrean M, Stirban A, Minartz P, Koerperich H, Gastens MH, Götting C, Prohaska W, Kleesiek K, Tschoepe D. Wound therapy with autologous bone marrow stem cells in diabetic patients with ischaemia-induced tissue ulcers affecting the lower limbs. Int J Clin Pract 2007;61:690–2.
20 Rogers LC, Bevilacqua NJ, Armstrong DG. The use of marrow-derived stem cells to accelerate healing in chronic wounds. Int Wound J 2008;5:20–5.
21 Vojtassák J, Danisovic L, Kubes M, Bakos D, Jarábek L, Ulicná M, Blasko M. Autologous biograft and mesenchymal stem cells in treatment of the diabetic foot. Neuro Endocrinol Lett 2006;27Suppl 2:134–7.
22 Yoshikawa T, Mitsuno H, Nonaka I, Sen Y, Kawanishi K, Inada Y, Takakura Y, Okuchi K, Nonomura A. Wound therapy by marrow mesenchymal cell transplantation. Plast Reconstr Surg 2008;121:860–77.
23 Mulder GD, Lee DK. Autologous Bone Marrow-Derived stem cells for chronic wounds of the

lower extremity: a retrospective study. Wounds 2010;22:219–24.
24 Jaiswal N, Haynesworth SE, Caplan AI, Bruder SP. Osteogenic differentiation of purified, culture-expanded human mesenchymal stem cells in vitro. J Cell Biochem 1997;64:295–312.
25 Bruder SP, Fink DJ, Caplan AI. Mesenchymal stem cells in bone development, bone repair, and skeletal regeneration therapy. J Cell Biochem 1994;56:283–94.
26 Livingston TL, Gordon S, Archambault M, Kadiyala S, McIntosh K, Smith A, Peter SJ. Mesenchymal stem cells combined with biphasic calcium phosphate ceramics promote bone regeneration. J Mater Sci Mater Med 2003:14:211–8.
27 Rush SM, Hamilton GA, Ackerson LM. Mesenchymal stem cell allograft in revision foot and ankle surgery: a clinical and radiographic analysis. J Foot Ankle Surg 2009;48:163–9.
28 Yu M, Zhou W, Song Y, Yu F, Li D, Na S, Zou G, Zhai M, Xie C. Development of mesenchymal stem cell-implant complexes by cultured cells sheet enhances oseointegration in type 2 diabetic rat model. Bone 2011;49:387–94.
29 Le Blanc K, Tammik C. Rosendahl K, Zetterberg E, Ringdén O. HLA expression and immunologic properties of differentiated and undifferentiated mesenchymal stem cells. Exp Hematol 2003;31:890–6.
30 Arinzeh TL, Peter SJ, Archambault MP, van den Bos C, Gordon S, Kraus K, Smith A, Kadiyala S. Allogeneic mesenchymal stem cells regenerate bone in a critical-sized canine segmental defect. J Bone Joint Surg Am 2003;85-A:1927–35.
31 Bruder SP, Jaiswal N, Ricalton NS, Mosca JD, Kraus KH, Kadiyala S. Mesenchymal stem cells in osteobiology and applied bone regeneration. Clin Orthop Relat Res 1998;(355 Suppl):S247–56.