Adult mesenchymal stem cells (MSC) are a powerful orthopaedic tool for bone regeneration due to their ability to differentiate into osteoblasts. An MSC-containing matrix can provide all three essential bone growth properties for successful bone remodeling and repair: osteoinductive, osteoconductive, and osteogenic.

Early animal studies have shown promise and hypo-immunogenic response. In an athymic rat model for bone formation, L4-5 posterolateral spinal fusions revealed osteoblastic lining areas of new woven bone formation in 8 weeks. In a canine mid-femoral diaphyseal segmental defect both the autologous and allogenic MSCs revealed healing equivalency without any cellular immune response at 16 weeks. A baboon fibular osteoperiosteal defect model revealed various degrees of mineralization within 12 weeks of...

**Abstract:** Bone regeneration and repair via mesenchymal stem cells (MSC) are intriguing and challenging thoughts today. Many have claimed that MSCs are powerful tools for bone regeneration due to their ability to differentiate into osteoblasts. Seeking all three essential bone growth properties for successful bone remodeling and repair—osteoinductive, osteoconductive, and osteogenic—has been a complex task; however, they are essential in difficult surgical situations with poor host bone tissue. Diabetic Charcot foot and ankle neuroarthropathy is challenging due to its inherent poor bone quality and patient’s comorbidities. In this review of diabetic Charcot patients who underwent reconstructive surgery with and without MSC grafting, the radiographic healing time parameter was most striking between groups, 6.4 versus 9.2 weeks ($P < 0.024$). In both groups, there were non-unions, mal-unions, and/or delayed unions noted. Surgical application of MSC appears to be safe, and has the potential to be effective as an autograft substitute, but remains inconclusive. In these limb salvage situations, reconstructive surgeries create challenging environment for bone growth and healing. The ability to utilize the properties of MSCs to differentiate into the type of specialized cells is promising; however, it still does not support substituting autografts as the gold standard.
MSC implantation. Fluorescently labeled 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 MSCs in a rat model, revealing osteogenic activity. MSCs 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 BMP2, BMP6, BSP, and VEGF.

MSCs do not have Class II surface antigens and other co-stimulatory molecules so they may be implanted between individuals with HLA or other matches. Class II surface antigens and other co-stimulatory molecules are required for a host to mount a T-Cell reaction to foreign cells. Currently, the source of most MSCs is organ donor marrow. Bone marrow has two types stem cells: HSCs and MSCs. The majority of cells in marrow are HSCs (hematopoietic stem cells). HSCs are > 99% nucleated cells, are immunogenic, require HLA and ABO matching for allogenic transplant, and are cluster of differentiation 45+. The HSCs are not immune privileged and must be depleted from the marrow prior to implantation. The cells are identified by being positive for surface marker CD 45. MSCs are present in 1/500,000 nucleated cells, are immune privileged, lack Class II antigens, are cytokine producers—BMP-2&6, VEGF; and others—and are CD 105+ and CD 166+. MSCs, thus, are in the minority and are identified by CD makers CD 105 and CD 166. MSCs are CD 45 negative (45-). No single marker or set of markers is unique to the MSC, but CD105 and CD166 are commonly accepted. MSCs, as cytokine factories, provide two important physiologic properties: 1) anti-inflammatory and immune-modulatory cytokine production can regulate the immune system and suppress an immune reaction; 2) produces BMPs for osteoinduction. MSCs take advantage of the following properties due to the expression and/or secretion of various cytokines to diminish or prevent scar formation, to stimulate angiogenesis, and to differentiate into different connective tissue phenotypes, depending on the local environment. It is understood that MSCs are multipotential. When provided the right cues from the environment in which they are placed, they can form tissues of mesodermal origin (eg, bone, cartilage). The nature of local cues is poorly understood.

The purpose of this limited and preliminary review is to determine the clinical efficacy and potential use of stem cell derived bone grafting in diabetic Charcot foot surgery and ankle reconstructive surgery.

Materials and Methods
Eleven patients were evaluated retrospectively. Seven patients had undergone diabetic Charcot foot and ankle reconstructive surgery with a multipotential cellular bone matrix for bone remodeling and repair and four patients had not. Postoperative follow up included clinical and radiographic healing times, American Orthopaedic Foot & Ankle Society (AOFAS) scores, and complications. The pre- and postoperative AOFAS scores were measured. The Institutional Review Board at the authors’ institution approved this study. Bone surface preparation was performed via denuding of non-viable and articular tissues then subchondral fenestration until good viable bleeding bone level was achieved. The matrix was introduced into prepared sites and appropriate fixation was utilized. Clinical healing time (CHT) was defined as initial protected weight-bearing tolerance with little to no pain, no signs of inflammation, and full incision healing. Radiographic healing time (RHT) was defined as initial signs of bone consolidation with trabeculation and obliteration of the surgical site. Non-union was defined as > 6 months of no radiographic changes with or without hardware complication. Risk factors include diabetes, tobacco use, and chronic renal disease. Statistical analyses were performed using SPSS software, version 15. Numerical outcome data were analyzed using Student’s Independent Group t-tests. Categorical outcome variables were analyzed using Person’s chi-squared test of association. Results were considered statistically significant when \( P \leq .05 \).

Results
There were 7 patients in the graft group (n = 7). The mean age was 53 years (range 25–70) with a mean follow up of 11.6 months (6–17). The mean preop/postop AOFAS were 49 and 85. In the non-graft group, there were 4 patients (n = 4). The mean age was 53 years (range 24–76) with a mean follow up of 11.5 months (6–17; Table 1). The mean preop/postop AOFAS were 49 and 85. Ankle, hindfoot, and midfoot were addressed including non-unions, mal-unions, and deformity correction. The mean clinical healing time and radiographic healing times for the graft group versus non-graft group were 4.9 weeks versus 6.7 weeks, \( P < 0.123 \) and 6.4 weeks versus 9.2 weeks, respectively \( P < 0.024 \).
were non-unions and/or delayed unions in each group. Inflammatory and immunogenic rejection symptoms were not noted in the graft group. Complications in the graft group were: hardware/external fixation failure (5); non-union (1); delayed union (2) in graft group patients with tobacco use and renal disease comorbidities. The control group had two non-unions (2) in patients with comorbidities of tobacco use and renal disease.

Discussion

Historically, it was thought that MSCs were developmentally restricted to specific cell lineages. Now it is known to undergo transdifferentiation by which one committed cell type is reprogrammed into a cell of another lineage.8 These MSC are thought to be multipotent cells capable of giving rise to tissues of mesenchymal origin including bone, cartilage, fat, tendon, and muscle, thus creating promising candidates for cell-based tissue engineering to repair lost or damaged tissues.9

Clinically, it appears that both groups heal similarly. The decrease in inflammatory signs in the graft group is notable, but it is difficult to make any objective assessments at this point with our current measuring tools. The improvement in radiographic healing time between the groups may indicate increased mineralization and bone trabeculation in the surgical site of the bone graft group. It is unknown the number of MSCs required to obtain fusion. In a study done on tibial non-unions, the author noted that if there were 1500 MSCs/cc the non-unions would heal, but would not heal with lesser concentrations.10 This study demonstrates the minimum number of autologous MSCs injected into tibial non-union site required for healing.

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MSCs in humans decline with age.11 MSCs showed diminishing numbers of MSCs in marrow with age.12 Aging of MSCs is related to cellular health. The current understanding in healing of the elderly and cellular aging prompted concerns regarding the age of cells used for cell based therapy.12 There are several factors associated with cellular aging: changes in quantity, changes in quality, differentiation/regeneration capacity, changed mobilization capacity, and poor bone/tissue.

Currently, data are lacking in the literature.13 Attempts to demonstrate the cell numbers required to achieve healing of a critically sized bone defect is not yet known. Furthermore, various limitations remain: anecdotal accounts, retrospective studies, preliminary clinical outcomes, heterogenous patient population, multiple and differing procedures, fixations, lack of comparison to autografts, and synthetic/other biologic grafts. Future studies should focus on clinical and advanced imaging studies (ie, CT, MRI, nuclear medicine) to assess the properties of these viable stem cells undergoing bone regeneration and/or repair procedures.

Conclusion

The pre-clinical animal, in-vitro, and preliminary clinical data described above provide evidence that MSC application appears to be safe and has potential effectiveness as an autograft substitute, but it remains inconclusive and further validation is required. Complex non-union and mal-union reconstructive surgeries create challenging environment for bone growth and healing, especially in diabetic Charcot foot and ankle. The ability

<table>
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<th>Graft</th>
<th>N</th>
<th>F/U</th>
<th>Age</th>
<th>Pre-AOFAS</th>
<th>Post-AOFAS</th>
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<th>RHT</th>
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<td>53</td>
<td>49</td>
<td>85</td>
<td>4.9</td>
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<td>Age</td>
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<td>RHT</td>
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<tr>
<td>Charcot</td>
<td>4</td>
<td>11.5 (6–17)</td>
<td>53</td>
<td>49</td>
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<td>6.7</td>
<td>9.2</td>
</tr>
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Table 1. Patient profiles.
to utilize MSCs to differentiate into the type of specialized cells is promising, but still does not support substituting autografts as the gold standard. Further long-term and randomized studies are needed to delineate specific indications and applications.

References


