

The Utility of Allograft Mesenchymal Stem Cells for Spine Fusion: A Literature Review

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Abstract

More than 50% of patients complain of postoperative donor site morbidity following iliac crest bone graft harvest, and recent discoveries have identified adverse outcomes following bone morphogenetic protein use in spine fusion. This has led the spine community to turn toward alternative methods to promote fusion following spine surgery. The present article reviews numerous studies that have shown the osteogenic potential of mesenchymal stem cells (MSCs). MSCs have been used with both in vitro and in vivo models and have involved animal studies ranging from rats to macaque monkeys to successfully induce bone regeneration in lesions of the tibia and spine. There is no fear of graft rejection, as there may be with other allograft materials, because neither undifferentiated nor differentiated MSCs elicit lymphocyte response when transplanted; they tend to alter the cytokine profile to an anti-inflammatory state. Early clinical trials are underway with various commercially available MSC formulations. Although there is much enthusiasm, it is integral that the spine surgery community carefully evaluate the use of MSCs in spine fusion through well-designed and executed studies to determine the efficacy and safety profiles in spine surgery patients.

Keywords

- ▶ mesenchymal stem cells
- ▶ allograft
- ▶ spine fusion
- ▶ osteogenic
- ▶ osteoinductive

There are ~151,000 lumbar fusions performed annually in the United States,¹ and a large number of these patients suffer from considerable morbidity due to the harvesting of autogenous iliac crest bone graft (ICBG). Fifteen to 60% of patients complain of pain at the donor site 1 to 2 years following surgery.^{2–6} Pseudarthrosis rates following ICBG fusion have been reported to occur in as many as 15% of patients, with some requiring subsequent revision surgeries, with additional associated morbidity.² Complication rates due to the iliac crest harvest have varied widely, ranging from 9.4 to 49%.⁶ In one prospective study,⁶ at 12 months following surgery, 16.5% of patients reported pain that was more severe at the harvest site than the primary surgical site, almost 30% reporting continued numbness, and 15% had some difficulty walking. Although initial studies showed promise that the use of recombinant human bone morphogenetic protein-2 in spine surgery could obviate the need for the ICBG and its associated

pain, the recent controversy surrounding its potential risks (e.g., radiculopathy, retrograde ejaculation, etc.) encourages the spine surgery community to consider alternative approaches to enhance spine fusion.

Identifying ideal spine fusion extenders (i.e., products that can be used alongside the bone graft) as well as bone graft replacements that can potentially replace ICBG may potentially eliminate the associated morbidity with the current standard procedure. The ideal graft replacement would mirror iliac crest; that is, it would have osteoconductive, osteogenic, and osteoinductive capacity.

Adult mesenchymal stem cells (MSCs) have shown potential for bone regeneration. These cells can be isolated from bone marrow, adipose, and muscle tissue, among others, and may be induced to differentiate into osteogenic cells to enhance spinal fusion.⁷ The use of MSCs to promote spinal fusion will be summarized here.

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How It Works

MSCs can be extracted from adult fat and bone marrow, as well as peripheral blood,^{8,9} and can be induced to differentiate into various mesenchymal tissues including bone, cartilage, and muscle.^{10,11} In vitro induction can be accomplished by growth factor supplementation and creating culturing conditions that are favorable for the preferred differentiation. Specifically, adipogenic differentiation from MSCs has been successfully accomplished in Dulbecco's modified Eagle medium supplemented with isobutylmethylxanthine, indomethacin, and either dexamethasone and insulin or with hydrocortisone.^{11,12} Successful induction is then verified by the identification of lipid vacuoles within the cell and various other adipose cell markers. In comparison, in vitro chondrogenic differentiation has been induced by centrifuging the MSCs into micromass pellets and culturing in a medium containing dexamethasone and transforming growth factor β -3.¹³ Chondrocytes are then detected by the presence of secreted extracellular matrix components such as type II collagen and aggrecan, among others.¹⁴ Osteogenic differentiation may be induced in a culture of dexamethasone, ascorbic acid phosphate, β -glycerol phosphate;^{11,12,14,15} successful differentiation is identified by the expression of alkaline phosphate and using cell-specific antibodies. Successful models used to expand the MSC population and induce osteogenic differentiation have also included substances such as transforming growth factor- β and fibroblast growth factor-2.^{16,17} Bone morphogenetic protein has also been shown to successfully expand the osteogenic cell population;^{7,18} however, limitations exist regarding cost and safety. The authors refer the interested reader to some of the early studies inducing MSC differentiation for additional explanations as to the nature and function of the specific growth factor supplements.^{11-14,19-21} In vivo induction occurs through site-specific differentiation and is often implanted within scaffolds.^{7,10}

In addition to requiring sufficient numbers of MSCs implanted for bone regeneration,²² an osteoconductive matrix is also necessary for osseous growth.¹⁸ The scaffold provides a structural support for cell-cell interactions, extracellular matrix formation, and new tissue formation. The osteoconductive scaffold occupies the site of the fusion, but also provides an environment conducive for the osteoinductive factors and tissue growth. Various biomaterials have been investigated as scaffolds including hydroxyapatite, tricalcium phosphate, calcium sulfate, metals, and biodegradable polymers.²³ Serving as the initial structure for tissue growth and blood vessel formation, the biodegradable types of scaffolds eventually resorb as new bone is formed.²⁴

Isolating human MSCs has traditionally been accomplished by using their selective adherence to plastic surfaces, which the hematopoietic cells less commonly do.²⁵ This method, however, typically leads to heterogeneous cell isolation, in which only ~30% of the cells are multipotent MSCs.²⁶ Although the MSCs do not express the cell surface markers normally found on hematopoietic cells, such as CD34 and CD45, there are cell markers that are unique to the MSCs.²⁷

Stro-1 was one of the earlier monoclonal antibodies developed to isolate MSCs,²⁸ and this was followed by additional cell-surface markers such as CD146, CD200, and CD271.²⁹⁻³¹ Use of these markers alone may not be sufficient to isolate pure samples of MSCs. Gronthos and colleagues²⁸ described a method whereby they used Stro-1 monoclonal antibodies and then isolated the cells using immunomagnetic cell sorting (MACS). This resulted in a milieu of cells with varying intensities of STRO-1 fluorescence intensity, which was then further purified for the high-intensity fluorescing cells using fluorescence-activated cell sorting (FACS). Further purification was accomplished using dual-color FACS to isolate those cells with the surface markers VAM-1, which are also expressed on MSCs.³² Another recent study utilized MACS to separate cells that were CD45⁻ (as this marker is not expressed on MSCs) and then subsequently used FACS to isolate cells that were CD146⁺.³⁰ The most effect cell identification technique seems to be a combination of FACS and MACS, though the cell surface markers of choice vary between laboratories. In an effort to identify the most efficient marker of MSCs, Delorme and colleagues³¹ used microarrays and flow cytometry to culture a pure sample of MSCs that expressed 113 transcripts and 17 proteins not found on other hematopoietic cells. They found that CD146 and CD200 were among the most efficient markers to purify MSCs.

Following isolation, MSCs can be cultured in either fetal calf serum or human serum, which show no difference in their effects on the cells to proliferate and differentiate.³³ For bone formation, MSCs are then directed to differentiate into osteoblast lineage cells via the aforementioned factors (e.g., transforming growth factor- β) or via selective genetic expression (e.g., *OSX*, *ZIP1*).^{34,35} Allogeneic transplantation of MSCs can be done in the site of fusion, due to their hypoinnogenic and even immunosuppressive nature. Flow cytometry experiments have shown that MSCs express intermediate levels of HLA class I and little to no HLA class II or costimulatory molecules (e.g., CD40).^{11,36} Neither undifferentiated nor differentiated major histocompatibility complexes elicit lymphocyte proliferation when transplanted,^{36,37} and in fact, they tend to alter the cytokine profile to an anti-inflammatory state by decreasing tumor necrosis factor- α and interferon-gamma and increasing interleukin-10, interleukin-4, and regulatory T cells.³⁸ MSCs have even been used for their immunosuppressive actions in treating acute graft-versus-host disease.³⁹ For these reasons, host-versus-graft disease, or graft rejection, does not appear to be a problem with MSC use in spine fusion.

The use of MSC therapy in bone regeneration has been and is currently investigated both in animal models and in the clinical setting. This is being done through both local implantation of the stem cells and via gene therapy, as well as through autologous transfer of engineered extracted MSCs.

Animal Models

Following the original discoveries of MSCs by Friedenstein et al.^{40,41} and then of Owen in 1988 that MSCs could differentiate into bone,^{42,43} several groups demonstrated successful

autologous transfer of MSCs in healing long bone lesions in various animal models.⁴⁴⁻⁴⁶ For example, Arinzeh and colleagues used allogeneic MSCs loaded in a ceramic hydroxyapatite-tricalcium phosphate scaffold to treat large femur defects in adult dogs. They showed that not only did a callus of lamellar bone fill the lesion within 8 weeks, with complete new bone form by 16 weeks, histologically, there was no immune response detected.⁴⁶

In a rat posterior spinal fusion model, Cui and colleagues showed that cloned osteoprogenitor cells implanted in the fusion bed led to successful spine fusion in all animals, compared with only 50% fusion success in animals that were implanted with mixed marrow stromal cells.⁴⁷ Similarly, Muschler et al, in a canine model of dorsal spinal fusion, demonstrated the superiority of the marrow-derived osteoblastic progenitors in promoting spine fusion versus the growth factor and cellular milieu found in a bone marrow clot.⁴⁸ The effectiveness of MSCs in promoting spinal fusion has been shown in progressively larger animals, including rabbit,⁴⁹⁻⁵³ ovine,⁵⁴⁻⁵⁷ and primate models.^{58,59} Specifically, Wang and colleagues⁵⁸ performed anterior lumbar interbody fusions in nine rhesus monkeys, with two fusion sites each, that either utilized autologous bone-marrow derived MSCs on a calcium phosphate scaffold, ICBG, or a control ceramic graft treatment. They found that the MSC group had equivalent biomechanical strength as compared with the ICBG group, and that they were both biomechanically and histologically superior to the control ceramic graft group. Orii et al⁵⁹ performed posterolateral lumbar spine fusions in nine macaque monkeys, which received either marrow-derived MSCs with a β -tricalcium phosphate graft, autogenous bone, or a control tricalcium phosphate graft treatment. Using both X-ray and manual palpation to identify fusion status, they found that the group receiving the MSCs had the highest fusion rate (83.3%) compared with the autogenous bone group (66.7%) and the control group (0% fused). The aforementioned studies used bone marrow-derived MSCs; however, another approach with potentially fewer complications and less morbidity may be the use of adipose-derived MSCs (ASCs).

ASCs were first identified in 2001 by Zuk and colleagues^{60,61} who showed that ASCs can differentiate into multiple cell types including osteogenic and chondrogenic cells, thereby providing an potential therapeutic avenue for bone regeneration. These cell types were subsequently shown to be capable of adhering to a bioengineered scaffold, as well as remaining viable, proliferating, and differentiating under various conditions.⁶² Rodbell and Jones standardized the first protocol for ASC isolation.⁶³⁻⁶⁵ Cowan et al⁶⁶ and later Levi et al⁶⁷ used ASCs to regenerate bone in large mouse calvarial defects. Lopez and colleagues⁶⁸ performed dorsolateral spinal fusions in 56 Fischer rats, with either no graft, only a scaffold, a scaffold with allogeneic ASCs, or a scaffold with syngeneic ASCs. Similar to the studies investigating marrow-derived MSCs, they found that when ASCs were used there were fewer inflammatory infiltrates compared with the control groups, as well as superior bone formation and fusion when using the ASCs. Clinical trials are currently investigating ASCs in treat-

ments ranging from type I diabetes mellitus to liver disease^{69,70} and may soon be used for spine fusion treatments as well.

Although there has been much success demonstrated in the animal models, there remain barriers prior to this therapy's translation into the clinical setting. This includes identification of the optimal number and concentration of MSCs, as well as the ideal preparation and implantation techniques needed.⁷¹ Minamide and colleagues⁵⁰ compared the use of differing number of marrow-derived MSCs in a rabbit posterolateral spine fusion model. The low dose contained one million cells per milliliter and resulted in the fusion of zero of seven spines. In contrast, the high dose was one hundred million cells per milliliter and led to the fusion of five of seven rabbit spines. Perhaps an even greater concentration would have led to 100% fusion. Wang et al⁵⁸ compared bone marrow-derived MSCs to autogenous ICBGs in rhesus monkey spine fusion models and found that although the fusions were equivalent in stiffness, the autograft produced greater bone volume. This can be explained by the fact that only three million cells per milliliter were used for the MSC-treated group, in contrast to the 100 million that was used successfully in the study by Minamide et al. Finally, Gan and colleagues,⁷² used bone-marrow-derived MSCs for posterior spine fusion in 41 patients. Using a slightly different quantification method, they recovered and implanted 16.1 million nucleated bone marrow cells per milliliter, of which they measured an average of 213 MSCs per milliliter (MSCs were defined as colony-forming units expressing alkaline phosphatase). Despite the relatively small number of implanted cells, 95% of their patients achieved complete fusion by 34 months' follow-up. It is clear that we have yet to determine the optimal number of MSCs needed for complete fusion prior to the translation of this technology to the clinical setting. Although some experimental data suggest that increased cell concentrations are required to repair bony defects,^{58,73} others have suggested that the cell concentration is not sufficient but rather the delivery type and biological environment of the graft will significantly affect success of the fusion.⁴⁸ For example, the study by Minamide et al⁵⁰ used a three-dimensional culture, as compared with the more traditional two-dimensional monolayer culture, as they believed that it enhanced proliferation and differentiation. Further studies are warranted to identify the optimal culture and delivery technique.

There are few published clinical studies reporting MSC efficacy in spine surgery. However, there is literature on the osteogenic potential of MSCs in various other skeletal defects as well as ongoing clinical investigations of MSCs in spine surgery with various commercial products such as NeoFuse[®] (Mesoblast Ltd., Melbourne, Australia) and Osteocel[®] (NuVasive, San Diego, CA, USA) among others (see clinicaltrials.gov, including studies No. NCT00996073, NCT01290367, NCT00810212).

Clinical Studies

The earliest clinical studies involving MSCs involved small case studies of autologous MSCs used for bone regeneration.

Quarto et al⁷⁴ showed successful and abundant callus formation in three patients with tibial, ulnar, or humeral fractures using autologous MSCs; Lendeckel and colleagues⁷⁵ reported a case study where autologous ASCs were successfully used to treat a large calvarial injury. Subsequent trials, including some larger ones, involved autologous bone marrow successfully used to promote bone fusion in tibial nonunions,^{76,77} autologous MSCs for femoral head osteonecrosis,^{78–81} and allogeneic MSCs to treat osteogenesis imperfecta,⁸² all of which showed the clinical feasibility of therapeutic application of MSCs to promote bone growth.

The 2011 study by Goldschlager and colleagues,⁵⁵ which demonstrated superior bone formation in ewes with the use of allogeneic ovine MSCs (Mesoblast Limited, Melbourne, Australia) compared with autograft or stand-alone scaffold, was the precursor study to the ongoing clinical trials (NCT01290367, NCT00996073, NCT01097486, NCT00810212, NCT00549913, NCT01106417) using this MSC allograft product in both cervical and lumbar spine fusions. Results on safety and preliminary efficacy in patients will likely be revealed in the coming years.

Current Challenges and Future Directions

Much of the preliminary research done in animal models has shown potential for the use of MSCs, both bone marrow and adipose derived, for bone regeneration. Although there have been limited systematic clinical trials, small case studies have shown that MSC use in humans may have successful bone growth and long-term durability.⁷⁴ Some current limitations include decreased bone growth compared with autograft,⁵⁸ weaker mechanical stability of the implanted graft and poor resorption of the bioceramic constructs,⁷⁴ and ambiguity surrounding the optimal cell concentration and delivery method. More studies examining optimal MSC concentrations are needed in larger animals, which are more comparable to humans, considering the fact that there is decreased potential for bone growth as compared with smaller animals (e.g., rabbits, rats, etc.).^{58,83,84} This also demonstrates the need for methods to maximize the number of MSCs collected, as well as techniques that can be feasible in the operating room setting.⁸⁵ Other options can include obtaining somatic cells and converting them into pluripotent cells,⁸⁶ using minimally invasive approaches to collect and culture bone marrow- or adipose-derived MSCs prior to surgery, and potentially even using recombinant forms of MSCs. The present hurdles to clinical use include optimization of osteoinductive and osteoconductive properties of MSCs in bone grafts. Vascularization of the implant and integration of the vasculature with the host will prove to be important; additionally the long-term mechanical strength and durability, particularly at the load-bearing sites such as the lower lumbar spine regions will need to be comparable to native bone.⁸⁷

Conclusion

Allogeneic MSC therapy for spine fusion and other skeletal treatments is still in its infancy. There has been a surge of

interest in the various MSC formulations commercially available for clinical use in spinal surgery. This enthusiasm, and clinical use, must be tempered with the understanding that there are no clinical data that had defined the efficacy or safety profiles in spine surgery patients. Therefore, it is imperative that the spine surgery community carefully evaluate the use of MSC in spine fusion through well-designed and executed studies. Although more than a decade of preclinical animal research that has shown promising results, the safety and efficacy of these products in randomized controlled trials must be ascertained. With the rapidly growing number of spine fusion surgeries performed annually, further study into fusion-enhancing compounds becomes increasingly necessary. MSC therapy remains an interesting and important avenue of research.

Disclosures

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