Review Article

Mesenchymal stromal cells in spinal fusion: Current and future applications

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ABSTRACT

Mesenchymal stromal cells (MSCs) have been a promising area of study for regenerative medicine. These cells can be harvested from bone marrow, adipose tissue, and other areas allowing for autologous transplantation of these cells into the area of degeneration or injury. With the proper signals, these cells may be able to regenerate healthy tissue. Recent studies have yielded promising evidence supporting translational mesenchymal stromal cell applications particularly in spinal fusion surgery.

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1. Introduction: what are mesenchymal stromal cells?

Mesenchymal stromal cells (MSCs) are non-blood adult multipotent cells that originate from various adult tissues, including bone marrow, adipose tissue, periosteum, and skeletal muscle.^{1,2} Initially referred to as mesenchymal stem cells in the 1990s,² MSCs are now referred to as mesenchymal stromal cells. The nomenclature has recently changed due to a lack of evidence confirming these plastic-adhering, self-renewing multipotent cells to definitively demonstrate the characteristics of stem cells.^{2,3}

Unlike embryonic stem cells, which have been the subject of significant ethical debates, MSCs can be found in adult tissues, allowing for autologous transplantation of these cells into the area of damage.¹ The ability of mesenchymal stromal cells to both proliferate, renew, and differentiate into many different cell types, including osteocytes, chondrocytes, and adipocytes makes it possible for directed application of MSCs to locally differentiate in response to cues from growth factors and the extracellular matrix.⁴ Recent studies of MSCs have yielded promising evidence supporting their application in the orthopedic procedures such as spinal fusion surgery and treatment of degenerative disk disease.² This brief review will focus specifically on the current and future applications of MSCs in spinal fusion surgery.

2. Review

2.1. Challenges remaining

Prominent challenges remain in the translation of mesenchymal stromal cell therapies to clinical trials, namely that the control of survival and differentiation of MSCs has proved challenging.⁵ However, genetic engineering has made it possible for the MSCs to express the growth factors needed to specify differentiation and tissue reconstruction.^{6–8} Additionally, a combination of materials engineering and tissue engineering has improved scaffolds for the cells, integrating a three-dimensional environment with the important physical, chemical, and biological cues needed for appropriate localization and differentiation of MSCs.^{9–11}

2.2. Spinal fusion surgery

Arthrodesis, or vertebral fusion, is performed to fuse vertebrae that cause pain, deformity, or neurologic deficits due to spinal deformity, trauma, degenerative disk disease, spinal tumor, spondylolisthesis, and other indications.^{1,4} Through the fusion in conjunction with decompression, the patient is expected to have reduced pain or instability from movement of the two vertebrae. Over the past thirty years, both the number and cost of spinal fusion procedures have increased by over 111% between 1998 and 2009.¹⁰ Spinal fusion was the 37th most common procedure in 1998, and became the 16th most common procedure in 2008.¹²

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Despite the increasing utilization and technology, reoperation rates have not declined. Fusion failure, or pseudarthrosis, is a common issue which can occur in 13–41.4% of patients.¹³ Pseudarthrosis most commonly affects patients who are older, female, smoke, have diabetes mellitus, metabolic bone disease, and thoracolumbar kyphosis.^{14–16} The combination of an aging population and increased utilization of vertebral fusion surgery necessitates newer clinical techniques to increase the success rate of spinal fusion. One such introduction since 2005 has been internal fixation for spinal fusion, but this technique has not been entirely successful at significantly reducing pseudarthrosis rates.^{4,16} Thus, much attention has turned toward mesenchymal stromal cells as a potential solution.

2.3. Use of MSCs in spinal fusion

Currently, the technique for spinal fusion surgeries involves using an autograft from the iliac crest, donor allograft, or allograft substitute allowing the vertebrae to grow together and keep the spine stable. Although the iliac crest autograft has been the gold standard for the past decade, obtaining iliac crest autograft has been associated with increased morbidity, and may not contain enough material for fusing more than one intervertebral disk space.⁴ Despite the association of iliac crest autograft with increased morbidity, it has been shown that the pain classically attributed to harvesting for allografts may not be entirely attributable to the harvesting, and may be more causally associated with the back pain associated with surgery.¹⁷ Furthermore, local autograft from the vertebral bodies is now routinely done. Surgeons may obtain a high yield of stromal cells without significantly increased morbidity.¹⁸ Instead of autograft or allograft, a third option includes using bone substitutes, which do not have living, osteogenic cells. There is limited evidence supporting either their efficacy, and much more attention has focused upon grafts including living, osteogenic cells.¹⁹ Further studies are required to elucidate the relative advantages and disadvantages of the various grafting techniques. Since procuring bone grafts increases time in surgery (17–49.4 additional minutes^{20,21}), furthering risk of infection and bleeding, graft success as well as time required for the graft must be taken under careful consideration.

To attain a successfully consolidated spinal fusion, three factors are necessary for the transplant: osteogenesis (cells forming bone), osteoinduction (growth factors inducing growth), and osteoconduction (scaffold directing growth). Thus, attention has turned to MSCs cells due to their ability to improve osteogenesis.¹³

2.4. Osteogenesis and osteoinduction

Osteogenesis involves differentiation of mesenchymal stromal cells and preosteoblasts into osteoblasts. These osteoblasts synthesize osteoid matrix, which eventually envelops them, allowing the cells to terminally differentiate into osteocytes. This mineralized combination of cells and osteoid constitutes bone. Given the proper signal molecule, such as bone morphogenetic protein (BMP), MSCs differentiate into preosteoblasts. If given a different signal molecule or combination of growth factors, the MSC could differentiate into chondrocytes, adipocytes, or other mesodermal cell types.¹³

It is important to note the controversy surrounding the use of BMP. Retrospectively reviewing the initial industry-sponsored prospective studies, independent investigators found BMP use was associated with increased rates of implant displacement, subsidence, infection, retrograde ejaculation, radiculitis, ectopic bone formation, osteolysis, and possible new malignancy.^{22,23}

2.5. Bone marrow MSCs

There is confirmation of differentiation of bone marrow mesenchymal stromal cells into osteoblasts *in vivo* and *in vitro* in both animals and humans.^{24,25} Numerous studies have been published suggesting that bone marrow-derived mesenchymal stromal cells can enhance spinal fusion. In 2006, Risbud et al. demonstrated that adult human MSCs of the marrow in the lumbar spine respond similarly to progenitor cells of the marrow in the iliac crest, expressing similar immunophenotypes.²⁵ Hu et al. in 2015 demonstrated bone marrow-derived MSCs assembled with low-dose bone morphogenetic protein 2 (BMP-2) enhances posterolateral spinal fusion in syngeneic rats.²⁴ In a rabbit model bone marrow-derived mesenchymal stem cells expressing baculovirus-engineered BMP-2 and VEGF enhanced posterolateral spinal fusion.⁶ Despite the successes in animal models, it is essential to consider that accessing bone marrow in awake patients is painful and requires an extended time of cell culture expansion due to the limited numbers of MSCs cells in bone marrow.

2.6. Adipose-derived MSCs

Research has focused on adipose-derived mesenchymal stromal cells (ADSCs) due to the ease of deriving these cells from fat pads or through liposuction *versus* painful bone marrow aspirates. Additionally, it is possible to derive more MSCs from relatively small amounts of adipose tissue compared to bone marrow.²⁶ Between 2000 and 2001, adipose-derived stromal cell harvesting and processing has previously required 8 h of strenuous work in the early.^{27,28} However, recent studies suggest isolation times of only 30 min,²⁹ and total harvest and processing times of 2–3 h.^{30,31}

Novel studies investigating the use of ADSCs in spinal fusion suggest that there is a possibility to take advantage of the more easily accessible mesenchymal stromal cells. Tang et al. in 2011 demonstrated increased fusion rates due to new bone-like tissue formation in posterolateral spinal fusion with a nano-hydroxyapatite-collagen/PLA composite and autologous adipose-derived MSCs in a rabbit model.⁹ Compared to bone marrow-derived MSC research, much work remains to be done to characterize, functionally define, and more generally understand adipose-derived MSCs before clinical applications are pursued.

2.7. Osteoconduction

Many studies have involved genetic engineering to further the success of mesenchymal stromal cells by inducing expression of growth factors, such as the bone morphogenetic proteins (BMPs). BMPs can help guide the MSCs along osteogenic differentiation. Studies investigating expression of BMPs have led to conflicting results as to whether bone marrow derived MSCs or adipose-derived MSCs are more useful. For example, Mizrahi et al. in 2012 found that both bone marrow MSCs (BMSCs) and adipose-derived MSCs (ADSCs) expressing rhBMP-6 were more successful at inducing bone formation than the BMSCs and ADSCs expressing rhBMP-2.⁷ Furthermore, the BMSCs expressing rhBMP-6 more efficiently induced osteogenic differentiation than the ASCs expressing rhBMP-6.⁷ However, Lin et al. in 2006 described evidence supporting ADSCs over BMSCs. Adult rabbit BMSCs and ADSCs expressing BMP-4 were autologously transplanted *ex vivo* to repair calvarial defects in a rabbit model. The ADSCs expressing BMP-4 demonstrated a higher rate of calcium deposition and a higher rate of proliferation than the BMSCs expressing BMP-4. However, the use of undifferentiated ADSCs did result in fat tissue structures in the control groups, which is an important issue to consider when choosing a type of cell for clinical application.³²

Further inquiry is needed to clarify which combination of cells and growth factors proves most successful in spine fusion, though initial animal studies seem hopeful.³³

3. Conclusions and future directions

As mesenchymal stromal cell transplants are continually evaluated for safety and efficacy in animal models and clinical trials, numerous factors will need to be optimized: area of stromal cell harvest, intrinsic expression or extrinsic expression of certain growth factors and proinflammatory cytokines such as BMP, TGF- β , and lipopolysaccharides, and material and construction of scaffolds on which the cells are supported.^{34–38}

If a successful solution is found in a clinical trial that could replace currently used autograft, allograft, and allograft substitute options, a cost-benefit analysis will be a necessary step in determining whether mesenchymal stromal cell transplants will become the new standard of care. Time invested in harvesting from bone marrow or adipose tissue, time for expansion of MSCs in culture, maintaining staff highly knowledgeable in the fields of tissue engineering and material science are all critical factors that play into this decision.

Mesenchymal stromal cells have a strong possibility of improving patient outcomes after spinal fusion, and may also be useful in many other applications in spine surgery, including treatment of degenerative disk disease, spinal cord injury, and peripheral nerve damage.¹

Conflicts of interest

AEME, CJS have none to declare. AHD – DePuy, A Johnson & Johnson Company: Paid consultant; Globus Medical: Paid consultant; Orthofix, Inc.: Paid consultant and Research support; Osseus: Unpaid consultant; Stryker: Paid consultant.

References

- Schroeder J, Kueper J, Leon K, Liebergall M. Stem cells for spine surgery. *World J Stem Cells*. 2015;7(1):186–194.
- Spencer ND, Gimble JM, Lopez MJ. Mesenchymal stromal cells: past, present and future. *Vet Surg*. 2011;40(2):129–139.
- Horowitz EM, Keating A. Nonhematopoietic mesenchymal stem cells: what are they? *Cytotherapy*. 2000;2:387–388.
- Werner BC, Li X, Shen FH. Stem cells in preclinical spine studies. *Spine J*. 2014;24(3):542–551.
- Sheyn D, Rütthemann M, Mizrahi O, et al. Genetically modified mesenchymal stem cells induce mechanically stable posterior spine fusion. *Tissue Eng Part A*. 2010;16(12):3679–3686.
- Fu TS, Chang YH, Wong CB, et al. Mesenchymal stem cells expressing baculovirus-engineered BMP-2 and VEGF enhance posterolateral spine fusion in a rabbit model. *Spine J*. 2015;25(9):2036–2044.
- Mizrahi O, Sheyn D, Tawackoli W, et al. BMP-6 is more efficient in bone formation than BMP-2 when overexpressed in mesenchymal stem cells. *Gene Ther*. 2013;20(4):370–377.
- Kaito T, Johnson J, Ellerman J, et al. Synergistic effect of bone morphogenetic proteins 2 and 7 by ex vivo gene therapy in a rat spinal fusion model. *J Bone Joint Surg Am*. 2013;95(17):1612–1619.
- Tang ZB, Cao JK, Wen N, et al. Posterolateral spinal fusion with nano-hydroxyapatite-collagen/PLA composite and autologous adipose-derived mesenchymal stem cells in a rabbit model. *J Tissue Eng Regen Med*. 2012;6(4):325–336.
- Wheeler DL, Fredericks DC, Dryer RF, Bae HW. Allogeneic mesenchymal precursor cells (MPCs) combined with an osteoconductive scaffold to promote lumbar interbody spine fusion in an ovine model. *Spine J*. 2016;26:389–399.
- Niu CC, Lin SS, Chen WJ, et al. Benefits of biphasic calcium phosphate hybrid scaffold-driven osteogenic differentiation of mesenchymal stem cells through upregulated leptin receptor expression. *J Orthop Surg Res*. 2015;10:111.
- Rajae SS, Bae HW, Kanim LE, Delamarter RB. Spinal fusion in the United States: analysis of trends from 1998 to 2008. *Spine (Phila Pa 1976)*. 2012;37(1):67–76.
- Guerado E, Andrist T, Andrades JA, et al. Spinal arthrodesis. Basic science. *Rev Esp Cir Ortop Traumatol*. 2012;56(3):227–244.
- Hermann PC, Webley M, Bornemann R, et al. Influence of smoking on spinal fusion after spondylolysis surgery: a comparative clinical study. *Technol Health Care*. 2016;24:737–744.
- Buchlak QD, Yanamadala V, Leveque JC, Sethi R. Complication avoidance with pre-operative screening: insights from the Seattle spine team. *Curr Rev Musculoskelet Med*. 2016;9:316–326.
- Shen FH, Samartzis D, An HS. Cell technologies for spinal fusion. *Spine J*. 2005;15(6 Suppl):231S–239S.
- Pirris SM, Nottmeier EW, Kimes S, O'Brien M, Rahmathulla G. A retrospective study of iliac crest bone grafting techniques with allograft reconstruction: do patients even know which iliac crest was harvested? *J Neurosurg Spine*. 2014;21(4):595–600.
- McLain RF, Boehm CA, Rufo-Smith C, Muschler GF. Transpedicular aspiration of osteoprogenitor cells from the vertebral body: progenitor cell concentrations affected by serial aspiration. *Spine J*. 2009;19(12):995–1002.
- Buser Z, Brodke DS, Youssef JA, et al. Synthetic bone graft versus autograft or allograft for spinal fusion: a systematic review. *J Neurosurg Spine*. 2016;27:1–8.
- Radcliff K, Hwang R, Hilibrand A, et al. The effect of iliac crest autograft on the outcome of fusion in the setting of degenerative spondylolisthesis: a subgroup analysis of the Spine Patient Outcomes Research Trial (SPORT). *J Bone Joint Surg Am*. 2012;94(18):1685–1692.
- Dawson J, Kiner D, Gardner 2nd W, Swafford R, Nowotarski PJ. The reamer-irrigator-aspirator as a device for harvesting bone graft compared with iliac crest bone graft: union rates and complications. *J Orthop Trauma*. 2014;28(10):584–590.
- Carragee EJ, Hurwitz EL, Weiner BK. A critical review of recombinant human bone morphogenetic protein-2 trials in spinal surgery: emerging safety concerns and lessons learned. *Spine J*. 2011;21(6):471–491.
- Walker B, Koerner J, Sankarayanarayan S, Radcliff K. A consensus statement regarding the utilization of BMP in spine surgery. *Curr Rev Musculoskelet Med*. 2014;7(3):208–219.
- Cuenca-López MD, Andrades JA, Gómez S, et al. Evaluation of posterolateral lumbar fusion in sheep using mineral scaffolds seeded with cultured bone marrow cells. *Int J Mol Sci*. 2014;15(12):23359–23376.
- Hu T, Abbah SA, Toh SY, et al. Bone marrow-derived mesenchymal stem cells assembled with low-dose BMP-2 in a three-dimensional hybrid construct enhances posterolateral spinal fusion in syngeneic rats. *Spine J*. 2015;25(12):2552–2563.
- Risbud MV, Shapiro IM, Guttapalli A, et al. Osteogenic potential of adult human stem cells of the lumbar vertebral body and the iliac crest. *Spine (Phila Pa 1976)*. 2006;31(1):83–89.
- Zuk PA, Zhu M, Ashjian P, et al. Human adipose tissue is a source of multipotent stem cells. *Mol Biol Cell*. 2002;13:4279–4295.
- Zuk PA, Zhu M, Mizuno H, et al. Multilineage cells from human adipose tissue: implications for cell-based therapies. *Tissue Eng*. 2001;7:211–228.
- Francis MP, Sachs PC, Elmore LW, Holt SE. Isolating adipose-derived mesenchymal stem cells from lipoaspirate blood and saline fraction. *Organogenesis*. 2010;6(1):11–14.
- Yoshimura K, Sato K, Aoi N, et al. Cell-assisted lipotransfer for facial lipoatrophy: efficacy of clinical use of adipose-derived stem cells. *Dermatol Surg*. 2008;34(9):1178–1185.
- Yoshimura K, Shigeura T, Matsumoto D, et al. Characterization of freshly isolated and cultured cells derived from the fatty and fluid portions of liposuction aspirates. *J Cell Physiol*. 2006;208:64–76.
- Lin L, Shen Q, Wei X, et al. Comparison of osteogenic potentials of BMP4 transduced stem cells from autologous bone marrow and fat tissue in a rabbit model of calvarial defects. *Calif Tissue Int*. 2009;85(1):55–65.
- Piccirilli M, Delfinis CP, Santoro A, Salvati M. Mesenchymal Stem cells (MSCs) in lumbar spine surgery: a single institution experience about red bone marrow and fat tissue derived MSCs, Clinico radiological remarks on a consecutive series of 22 patients. *J Neurosurg Sci*. 2015. [Epub ahead of print].
- Olabisi R. Cell-based therapies for spinal fusion. *Adv Exp Med Biol*. 2012;760:148–173.
- Aslan H, Sheyn D, Gazit D. Genetically engineered mesenchymal stem cells: applications in spine therapy. *Regen Med*. 2009;4(1):99–108.
- Gottfried ON, Dailey AT. Mesenchymal stem cell and gene therapies for spinal fusion. *Neurosurgery*. 2008;63(3):380–391. discussion 391–2.
- Barba M, Cicione C, Bernardini C, et al. Spinal fusion in the next generation: gene and cell therapy approaches. *ScientificWorldJournal*. 2014;2014:406159.
- Croes M, Oner FC, Krut MC, et al. Proinflammatory mediators enhance the osteogenesis of human mesenchymal stem cells after lineage commitment. *PLOS ONE*. 2015;10(7):e0132781.