Mesenchymal stem cells for intervertebral disc repair and regeneration

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1. Introduction

Low back pain (LBP) is the leading cause of activity limitation and work absence throughout much of the world, causing an enormous economic burden to affected individuals, their families, communities, industry, and governments.1 In the United States alone, the total costs of LBP exceed $100 billion per year.2 According to a recent public health report—the Global Burden of Disease Study—LBP is the leading cause of disability, with current estimates of 632 million people affected worldwide.3 Although the exact cause of LBP has not been established, several studies point to degenerative disc disease (DDD) as the primary etiology of LBP.4–7

The current treatment options for DDD rely on conservative symptomatic relief including core muscle stabilization and surgical interventions aimed at removal of the intervertebral disc (IVD) followed by spinal fusion or artificial disc replacement.8 There are currently no treatment modalities targeting the reversal of disc degeneration and IVD repair.9

Recent advances in the fields of stem cell technology and bioengineering have stimulated research efforts aimed at cellular treatments of DDD. Bone marrow-derived mesenchymal stem cells (MSCs) have demonstrated the ability to arrest IVD degeneration in early rabbit and canine animal model IVDs and have emerged as a promising technology for the treatment of DDD and LBP.10

1.1. The normal IVD

The human IVD is a fibrocartilaginous structure whose primary function is to act as a shock absorber, transmitting compressive loads between adjacent vertebrae. The IVD is composed of three main structures: the nucleus pulposus (NP), the annulus fibrosus (AF), and the cartilaginous endplates.

The NP lies between adjacent endplates and forms the core of the disc. The NP contains a three-dimensional network of randomly organized type II collagen fibers (with smaller
amounts of type VI, IX, and XI collagen fibers) surrounded by radially oriented elastin fibers embedded in a highly hydrated aggrecan-containing gel.\textsuperscript{11} Aggrecan, the primary proteoglycan composing the NP, is highly hyrophilic and provides the osmotic properties needed to resist compression.\textsuperscript{12} Dispersed at low density are chondrocyte-like NP cells that synthesize proteoglycans and collagens in response to changes in hydrostatic pressure.\textsuperscript{13} NP cells decline in number after birth and eventually become undetectable at the age of 4–10 years.\textsuperscript{14}

The AF surrounds the NP and is composed of 15–25 concentric rings (lamellae) consisting of highly organized collagen type I and type III fibers. The primary function of the AF is to contain the NP and maintain its pressurization under compressive loads. The tensile properties of the AF allow the NP to recover its original shape and position once a compressive load is removed.\textsuperscript{15}

The cartilaginous endplate is a thin horizontal layer of avascular hyaline cartilage approximately 1-mm thick located at the interface of the IVD and the vertebral body.\textsuperscript{16} The endplates are permeable to small molecules and are the main route for nutrients to reach the IVD cells. IVD cells reside within the extracellular matrix (ECM) where they are adapted to an anaerobic environment, resulting from low oxygen tension associated with high lactate and low pH levels.\textsuperscript{7} The function of the IVD depends in large part on the properties of the ECM. The ECM consists of a complex network of macromolecules synthesized and maintained by a small population of cells (9000 cells/mm\textsuperscript{3} in the AF and 500 cells/mm\textsuperscript{3} in the NP), occupying less than 1% of the IVD volume.\textsuperscript{15} The IVD cells synthesize various cytokines, growth factors, and proteases to maintain a steady state between the rates of ECM synthesis and degradation.\textsuperscript{15}

1.2. IVD degeneration

IVDs degenerate earlier and at a faster pace than do other musculoskeletal tissues. The first unequivocal finding of IVD degeneration in the lumbar spine is seen as early as 11–16 years of age.\textsuperscript{17} About 20% of teenagers have IVD with signs of mild degeneration. IVD degeneration is associated with increased age and male sex.\textsuperscript{18} Approximately, 10% of 50-year-old discs and 60% of 70-year-old discs are severely degenerated.\textsuperscript{19}

The most physiologically important changes of disc degeneration start within the NP with increased degradation of aggrecan and other proteoglycans. The accumulation of degraded proteoglycan end products leads to an impairment in oxygen and nutrient diffusion through the disc. This results in decreased synthesis of glycosaminoglycans that leads to a decreased osmotic pressure of the ECM, disc dehydration, and loss of disc height.\textsuperscript{20} IVD degeneration also leads to a disorganization and destruction of the collagen network, resulting in marked alterations in IVD biomechanics, increasing the potential for structural damage.\textsuperscript{21}

As the IVD continues to degenerate, the distinction between the AF and the NP becomes less defined and disc height continues to decrease.\textsuperscript{19} In advanced degeneration, there is evidence of loss of lamellae with the AF, fissuring of the AF, and discoloration and solidification of the NP.\textsuperscript{22,23} These changes are accompanied by ingrowth of blood vessels and nerves into the IVD, a process that has been associated with LBP.\textsuperscript{15} These structural changes lead to severe alterations in the biomechanical properties of the IVD.

IVD degeneration has been associated with a progressive loss of IVD cells, leading to further decrease in the available ECM.\textsuperscript{24} Previous studies have shown that programmed cell death (apoptosis) may also be responsible for many of the features of IVD degeneration.\textsuperscript{25} Recent studies have described clustering of cells adjacent to areas of neovascularization, which may be an attempt to offset the progressive destruction and loss of the ECM.\textsuperscript{26}

1.3. Cell-based approaches to IVD restoration

Our current medical and surgical therapies for DDD are only capable of symptomatic relief, and no therapies exist addressing the underlying IVD degenerative process.

Initial attempts at IVD regeneration were approached by way of signaling existing IVD cells in situ to secrete increasing quantities of proteoglycans and other critical ECM proteins. Growth factors including osteogenic protein-1 (OP-1), tissue growth factor (TGF), fibroblast growth factor (FGF), growth and differentiation factor-5 (GDF-5), bone morphogenetic protein-2 (BMP-2), as well as others have been either successfully injected directly into the IVD or their production in IVD cells has been stimulated with gene therapy.\textsuperscript{27–29} Even though promising results have been reported with the use of these techniques, the relative acellularity of degenerated IVDs has raised concerns that the patient’s own disc cells may not be present in sufficient numbers to mount a therapeutic repair response.\textsuperscript{30} For this reason, the introduction of cells capable of surviving and proliferating within degenerated IVDs that are capable of producing functional ECM has become an area of intense research focus in the treatment of DDD.

1.4. MSCs

MSCs are multipotent adult stem cells that have the capacity to self-renew. MSCs cultured ex vivo have shown to be able to replicate up to 38 times before undergoing degeneration.\textsuperscript{31} MSCs give rise to all the cells of the mesoderm, including bone, cartilage, fat, nerve, muscle, tendon, and mature stromal cell lineages.\textsuperscript{32} The differentiation of these cells is dependent on both intrinsic and extrinsic factors in their local environment as well as on neighboring cells.\textsuperscript{33} Most MSCs are isolated from the bone marrow; however, they can be isolated from adipose tissue, placenta, umbilical cord blood, connective tissue, skin, synovial fluid, fat, and teeth.\textsuperscript{34}

In vitro experiments have demonstrated that the application of specific growth factors in predetermined culture conditions can guide MSCs to differentiate into particular cell phenotypes.\textsuperscript{33} Steck et al.\textsuperscript{35} demonstrated that MSCs cultured in the presence of tissue growth factor-beta (TGF-β) were able to express a phenotype very similar to that of NP cells with the production of type II collagen and aggrecan. Risbud et al.\textsuperscript{36} demonstrated that under conditions of hypoxia in vitro, the hypoxia contributed to maintain a NP-cell phenotype. Furthermore, the combination of hypoxia and TGF-β/1 advanced
differentiation of the MSCs into a NP phenotype. Based on these experiments, it was hypothesized that the hypoxic environment of the degenerated IVD may promote differentiation of an implanted MSC toward a NP-like phenotype in vivo. Le Visage et al. evaluated the in vitro effect of cocultures of MSCs with AF and NP cells obtained from human-degenerated IVDs in three-dimensional pellet cultures. The authors demonstrated that the co-culture led to an increased proliferation in both the AF/MSC and NP/MSC cultures, but a higher proteoglycan production was found only in the AF/MSC co-culture. This study suggested that MSCs can be implanted into the degenerated IVD without needing to be differentiated into AF- or NP-like phenotypes.

1.5. MSCs and IVD regeneration and repair

The regenerative potential of MSCs for disc degeneration has been studied experimentally in a variety of in vivo animal models (Table). Sakai et al. were the first in 2003 to evaluate the possible potential of bone marrow-derived MSCs in the treatment of DDD in a rabbit model. This study suggested that MSC transplantation was effective in decelerating disc degeneration in their experimental model. Crevensten et al. reported on the viability of allograft MSCs implanted in a rat IVD. The authors described the proliferation of MSCs as well as a trend toward increased IVD height after 4 weeks, suggesting an enhanced production of proteoglycans. Zhang et al. transplanted bone marrow-derived MSCs into New Zealand white rabbits and found that the cells survived, proliferated, and differentiated into native NP-like phenotype cells. The authors followed up the animal for 6 months and found that in the rabbits treated with MSCs, the IVDs contained a greater amount of type II collagen along with increased proteoglycan concentrations.

MSC transplantation into the IVD of large animal models has also been confirmed. In a canine model of IVD degeneration, transplantation of bone marrow-derived MSCs resulted in evidence of IVD regeneration. This study also revealed that MSC transplantation may contribute to the maintenance of IVD immune privilege by the differentiation of transplanted MSCs into cells expressing the Fas-ligand (FasL), a transmembrane protein found only in tissues with isolated immune privilege also found in high concentrations in the NP. In porcine IVD models, bone marrow-derived MSC survival was described up to 6 months after implantation, with evidence of chondrocyte surface marker expression in the implanted MSCs.

Two studies have involved the utilization of adipocyte-derived MSCs (AD-MSCs) for implantation into degenerated IVD animal models. Chun et al. assessed the possibility of disc regeneration by treatment with AD-MSCs in a rabbit model after percutaneous IVD injury. The authors found that the AD-MSC-injected discs exhibited elevated ECM secretion and little ossification of damaged cartilage in the NP compared with degenerative control discs. Liang et al. investigated whether implantation of AD-MSCs seeded into three-dimensional microspheres into the rat IVD could regenerate the degenerated disc. The authors found that at 24 weeks after implantation, there were increased levels of ECM and evidence of partially improved disc heights. Murrell et al.

### Table: Results of in vivo animal studies on MSC transplantation for the treatment of degenerated IVDs.

<table>
<thead>
<tr>
<th>Author</th>
<th>Year</th>
<th>Source of MSCs</th>
<th>Animal model</th>
<th>Finding</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sakai et al.39</td>
<td>2003</td>
<td>Bone marrow</td>
<td>Rabbit</td>
<td>MSCs survived and differentiated into IVD-like cells</td>
</tr>
<tr>
<td>Crevensten et al.40</td>
<td>2004</td>
<td>Bone marrow</td>
<td>Rat</td>
<td>MSC proliferation and trend toward increased disc height</td>
</tr>
<tr>
<td>Zhang et al.41</td>
<td>2005</td>
<td>Bone marrow</td>
<td>Rabbit</td>
<td>MSCs survived and increased proteoglycan levels</td>
</tr>
<tr>
<td>Sakai et al.42</td>
<td>2006</td>
<td>Bone marrow</td>
<td>Rabbit</td>
<td>MSCs survived and regenerated IVD</td>
</tr>
<tr>
<td>Hiyama et al.43</td>
<td>2008</td>
<td>Bone marrow</td>
<td>Canine</td>
<td>MSCs survived and led to IVD regeneration</td>
</tr>
<tr>
<td>Yang et al.10</td>
<td>2009</td>
<td>Bone marrow</td>
<td>Murine</td>
<td>Arrested degeneration of notochondral NP and augmentation of ECM</td>
</tr>
<tr>
<td>Henriksson et al.44</td>
<td>2009</td>
<td>Bone marrow</td>
<td>Porcine</td>
<td>MSCs survived for 6 months and expressed typical chondrocyte markers</td>
</tr>
<tr>
<td>Wei et al.45</td>
<td>2009</td>
<td>Bone marrow</td>
<td>Rat</td>
<td>MSCs survived and differentiated to express chondrocyte markers</td>
</tr>
<tr>
<td>Murrell et al.46</td>
<td>2009</td>
<td>ON Stem cells</td>
<td>Rat</td>
<td>Differentiated into NP-like cells</td>
</tr>
<tr>
<td>Yang et al.47</td>
<td>2010</td>
<td>Bone marrow</td>
<td>Rabbit</td>
<td>MSCs inhibited apoptosis and slowed rate of decrease of DHI</td>
</tr>
<tr>
<td>Bendtsen et al.48</td>
<td>2011</td>
<td>Bone marrow</td>
<td>Porcine</td>
<td>Partial regeneration of IVD and protection of endplate function</td>
</tr>
<tr>
<td>Acosta et al.49</td>
<td>2011</td>
<td>Bone marrow</td>
<td>Porcine</td>
<td>No evidence of MSC differentiation</td>
</tr>
<tr>
<td>Chun et al.50</td>
<td>2012</td>
<td>Adipose derived</td>
<td>Rabbit</td>
<td>Elevated ECM secretion</td>
</tr>
<tr>
<td>Barczewska et al.51</td>
<td>2013</td>
<td>Bone marrow</td>
<td>Porcine</td>
<td>MSCs survived and were readily detected</td>
</tr>
<tr>
<td>Yuan et al.52</td>
<td>2013</td>
<td>Bone marrow</td>
<td>Rabbit</td>
<td>MSCs survived and produced ECM</td>
</tr>
<tr>
<td>Liang et al.53</td>
<td>2013</td>
<td>Adipose derived</td>
<td>Rat</td>
<td>Increased ECM at 24 weeks and partly improved disc height</td>
</tr>
<tr>
<td>Leung et al.54</td>
<td>2014</td>
<td>Bone marrow</td>
<td>Rabbit</td>
<td>Suppress abnormal deposition of collagen type I in NP</td>
</tr>
<tr>
<td>Subhan et al.55</td>
<td>2014</td>
<td>Bone marrow</td>
<td>Rabbit</td>
<td>MSCs reduce rate of IVD degeneration</td>
</tr>
<tr>
<td>Omlor et al.56</td>
<td>2014</td>
<td>Bone marrow</td>
<td>Porcine</td>
<td>MSCs metabolically active at 7 days</td>
</tr>
<tr>
<td>Yi et al.57</td>
<td>2014</td>
<td>Bone marrow</td>
<td>Rabbit</td>
<td>MSCs increased ECM content</td>
</tr>
<tr>
<td>Sun et al.58</td>
<td>2014</td>
<td>Bone marrow</td>
<td>Rabbit</td>
<td>Sox9 gene transfer significantly enhances repair effect of MSCs</td>
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implanted olfactory neurosphere-derived MSCs into rat IVDs and found that these cells successfully differentiated into NP-like cells. The results of these studies suggested that MSCs derived from a variety of primary sources could be used in cell-based IVD therapy.

MSCs possess special immunologic properties. These cells do not express the human leukocyte antigen (HLA) Class II molecules that are essential for the activation of the cellular immune response or the accessory molecules (CD40, CD80, and CD86) that are necessary for T-cell activation and immune system recognition in vitro.53–56 Several xenogeneic transplantation studies of human MSCs into porcine, murine, or rabbit discs have revealed MSC survival without immunosuppressant administration.54,49,50,62

Despite the encouraging results from in vivo animal studies, the effect of MSC implantation into human-degenerated IVDs has yet to be established. There are currently only three studies conducted in humans evaluating stem cell implantation into IVDs for the treatment of low back pain. Haufe and Mork63 prospectively analyzed 10 patients who underwent intradiscal injection of hematopoietic precursor stem cells (HSCs) obtained from pelvic bone marrow. The patients had confirmed discogenic pain via provocative discography and were followed up at 6- and 12-month intervals to determine their degree of pain relief from the procedure. Of the 10 patients, none (0%) achieved any improvement of their discogenic low back pain after 1 year. The authors concluded that although animal studies suggested possible regeneration of disc via HSC injections, living human studies reveal that this effect does not correlate with reduced pain, and thus intradiscal HSC injection appeared to be of little value.

Yoshikawa et al.64 analyzed the regenerative ability of autologous MSCs in markedly degenerated IVDs. The study included two subjects: both the subjects presented with LBP, leg pain, and numbness and were found to have evidence of lumbar spinal stenosis on MRI and vacuum phenomenon with instability on plain radiographs. Marrow fluid was collected from the ilium of each patient, and MSCs were cultured using the medium containing autogenous serum. In surgery, following spinal canal decompression, pieces of collagen sponge containing autologous MSCs were grafted percutaneously into the degenerated IVDs. At the 2-year follow-up, radiographs and computed tomography scans demonstrated improvements in the vacuum phenomenon in both the patients, as well as symptomatic improvement. The authors concluded that MSC disc regenerative therapy appears to be a promising minimally invasive treatment.

Orozco et al.65 designed a pilot study to assess the feasibility and efficacy of treatment of DDD with MSCs. A total of 10 patients with chronic LBP diagnosed with DDD were treated with autologous expanded bone marrow MSCs injected into the NP area. The patients were followed up clinically for 1 year, and magnetic resonance imaging measurements of disc height and fluid content were performed. The authors established feasibility and safety of the intervention and found that injected patients exhibited rapid improvement of pain and disability (85% of maximum in 3 months) that approached 71% of optimal efficacy. The authors found that although disc heights were not recovered, water content appeared significantly elevated at 12 months. The authors concluded that MSC therapy may be a valid alternative treatment for chronic back pain caused by DDD.

There is currently an ongoing prospective, randomized, blinded, and controlled trial evaluating the clinical use of allogenic MSCs obtained from bone marrow of healthy donors for the treatment of DDD.66 The results from this clinical trial will not be made publically available until December 2015.

2. Conclusion

LBP secondary to DDD is a major health problem in the developed world and the leading cause of disability. As more is understood about the pathophysiology of IVD degeneration, new treatment modalities have emerged focusing on IVD regeneration and repair. MSC-based cellular therapy for DDD appears to be a promising new technology; however, there is a lack of data regarding the safety and effectiveness of this therapeutic modality in humans. As such, there is currently no clinical evidence that supports the use of this technology in patients with DDD and LBP.

References


