Review article

Regeneration potential and mechanism of bone marrow mesenchymal stem cell transplantation for treating intervertebral disc degeneration

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Abstract Intervertebral disc degeneration is a primary cause of low back pain and has a high societal cost. The pathological mechanism by which the intervertebral disc degenerates is largely unknown. Cell-based therapy especially using bone marrow mesenchymal stem cells as seeds for transplantation, although still in its infancy, is proving to be a promising, realistic approach to intervertebral disc regeneration. This article reviews current advances regarding regeneration potential in both the in vivo and vitro studies of bone marrow mesenchymal stem cell-based therapy and discusses the up-to-date regeneration mechanisms of stem cell transplantation for treating intervertebral disc degeneration.

Introduction

Disorders in relation to chronic low back pain (LBP) are the common causes of morbidity or life quality deterioration. Recent studies have established a causal association between lumbar intervertebral disc degeneration and LBP.[1,2] Lumbar intervertebral discs (IVDs) degenerate with normal aging, and most lumbar IVDs show some evidence of degeneration by the fifth decade of life.[3] Degenerative disorders of IVDs are generally characterized by disequilibrium between extracellular matrix repair and degradative processes.[4]

With aging and degeneration, IVDs undergo substantially morphological and cellular changes,[5–8] including altered cellularity in the annulus fibrosus (AF) and the nucleus pulposus (NP), increased cell density but decreased cell viability,[7] increased cell senescence[5,8] and apoptosis,[7] elevated matrix metalloproteinase and aggrecanase activity, and increased expression of catabolic cytokines.[5–8] These changes lead to progressive loss of proteoglycans and water content in the NP, filling of the NP space with fibrocartilage, derangement/delamination of the AF,[9] narrowing of the disc space,[10] and mechanical failure of the disc.[6,11]

Susceptible gene-mediated familial predisposition, acute or chronic biomechanical injuries, inflammatory factors involved in unbalanced anabolic and catabolic metabolism, a reduced nutrient supply after chondrocyte endplate trauma or ossification, loss of the immune privilege stage in the NP space, cell senescence with aging, and IVD apoptosis are considered to be the main causes or a secondary response of the triggering element in the process of IVD degeneration.[5,12–14]

An ideal solution to managing disc degeneration would be to repair the IVDs, producing a matrix with similar or improved biological and biomechanical properties compared with the original.[15] Recent approaches to biological repair and regeneration of the disc function are under investigation, including gene therapy,[16,17] growth factor injections,[18,19] cell therapy, and cell-based tissue engineering.[9,15,20] Cell transplantation is a new therapy that is based on the supplementation/replenishment of matrix-producing cells because a decrease of matrix components, primarily proteoglycan and collagen — whether resulting from their declining numbers, change in phenotype, replacement by less effective cells, or other factors — is the major initial trigger for disc degeneration.[9,20] Several seed cells for transplantation — e.g., AF cells,[21] elastic cartilage-derived chondrocytes,[22] autologous or allologous NP,[23,24] immortalized human nucleus pulposus cell line,[25,26] mesenchymal stem cells (MSCs),[9,15,20] embryonic stem cells[27] — have been widely tested both in vivo and ex vivo.

Although autologous disc chondrocyte transplantation has been in clinical trials with more than 112 patient participations and encouraging 2-year interim analysis outcomes,[28–30] MSCs still have several theoretical and practical advantages over mature cells for cell therapy and tissue engineering applications. First,

MSCs are available from many autologous sources that can be harvested and isolated without significant donor site morbidity,[31,32] whereas the NP harvesting procedure damages the already degenerated IVD. Second, MSCs can easily be expanded in culture to produce adequate numbers of cells for transplantation strategies; and the preconditioning use of gene therapy to guide and stimulate MSCs toward the desired phenotype may be easier than that for the terminally differentiated mature cells.[8,15,20,33,34] Third, MSCs, as primitive cells, may have better potential to survive and produce significant quantities of matrix than the terminally differentiated cells (e.g., chondrocytes or disc cells), which tend to be relatively metabolically quiescent.[33,34] Furthermore, and of most importance, MSCs that share the same cell surface makers as thymic epithelium and express low levels of HLA-I/II with no co-stimulatory molecules such as CD80/86 can increase the potential application of allogeneic MSCs as a therapeutic cell source.[35] Because IVD degeneration is thought to take a multifactorial course, it is becoming more and more reasonable to utilize allogeneic stem cells for the sake of off-the-shelf availability, thereby eliminating potential autogenic precipitating factors such as genetic predisposition[36–40] or the diminished potency of stem cells due to natural aging.[41–43] Ever since Sakai et al.,[33] in 2003, reported their pioneering results of transplanting bone marrow-derived MSCs (BMSCs) into rabbit degenerated IVDs and showed the regeneration effects, other sources of MSCs from bone marrow,[15,20] adipose,[37,44,45] muscle tissue,[46] and olfactory mucosa[47] have been widely researched, with BMSCs being the most focused seed cells.

In the current review, we discuss recent findings regarding the regeneration potential and mechanism of BMSC transplantation for treating IVD degeneration.

Potential of BMSCs as a source for transplantation therapy

Cells of the IVD are responsible for maintaining tissue homeostasis.[48] The extracellular matrix provides physical and biochemical cues that regulate processes from tissue-specific stem cell differentiation in the development to stem cell-mediated repair or breakdown in mature or aging IVDs.[48,49] When an appropriate spatiotemporal dialogue occurs (e.g., certain signals for growth or repair of an injury in the tissue or a nearby tissue is activated), adult tissue-specific stem cells located in stem cell niches can accomplish cell renewal to fulfill lifelong demands for differentiated cells.[49,50] Indeed, Risbud et al.[51] demonstrated that the pathologically degenerated human disc contained cells with MSC properties.

The MSCs exist postnatally, are uncommitted precursor cells, have extensive renewal potential, and have shown an ability to migrate and engraft in various tissues as well as to exert beneficial effects on other cell types.[52] In an interesting study, Henriksson et al.[49] found that cells expressing progenitor and stem cell markers were detected in the AF border to the ligament zone and the perichondrium region in mammals (rabbit, rat, porcine, human), indicating the presence of stem cell niches in the disc. Based on these studies, it seems possible that progenitor cells are recruited and migrate from these regions into the disc and, together with solitary stationed progenitor cells, maintain the disc's regeneration processes possibly in cooperation with BMSCs from the bone marrow niche. In other words, it is probably feasible that BMSCs can be a cell source for treating degenerated discs. Indeed, some recent in vivo and in vitro studies have shown that BMSCs are capable of surviving and proliferating within the microenvironment of degenerated discs and can potentially retard, or regenerate, their normal structure and function.[53]

In vivo studies

Several in vivo studies have been conducted using isogenetic or xenogeneic BMSCs in small and larger animals[9,20,33,54–59] (Table 1). Previous work from our laboratory[60] showed that autologous BMSCs, transplanted into a rabbit normal intervertebral disc and labeled with superparamagnetic iron oxide (SPIO) for in vivo tracking dynamically by means of magnetic resonance imaging (MRI), migrated from the nucleus regions to the AF inner border about 4 weeks after BMSC transplantation (Fig. 1). Our further studies showed, with safranin-O staining, that rabbit IVD degeneration induced by nucleotomy and treated with BMSCs for 4 weeks was severe in the nucleotomy-only group, whereas no significant degeneration was seen in the BMSC-transplanted group (Fig. 2).

The results of these animal model pilot studies are promising and imply that it is feasible to retard the process of disc degeneration or to regenerate IVDs with the use of BMSCs. In the future, the use of BMSC-seeded absorbable three-dimensional (3D) scaffolds could also provide a feasible, minimally invasive, clinically viable treatment for regeneration of degenerated IVDs. Nonetheless, it is worth noting that the benefits of transplanted BMSCs reviewed here are limited to restoring disc structure and function. Whether disc repair from BMSCs will result in pain relief for patients with symptomatic IVD degeneration remains to be tested further in a clinical setting, as there is no animal model for LBP to date.[61]

Table 1. Relevant in vivo animal experimental studies on BMSC transplantation therapy for degenerated intervertebral discs

Cell type	Cellular scaffolds	Animal model	Result	Study	Year
Autologous BMSCs, genetic marking with LacZ	Atelocollagen gel	Rabbit (nucleus aspiration)	Improved annular structure and proteoglycan preservation	Sakai[33]	2003
Allogeneic BMSCs	Hyaluronan gel	Rat (no injury)	Increased disc height and matrix synthesis	Crevensten[54]	2004
Allogeneic BMSCs, genetic marking with LacZ	No	Rabbit (no injury)	Increased proteoglycan and collagen type II synthesis	Zhang[55]	2005
Autologous BMSCs, genetic marking with GFP	Atelocollagen gel	Rabbit (nucleus aspiration)	Proliferation and site-dependent differentiation	Sakai[20]	2005
Allogeneic BMSCs, genetic marking with LacZ	No	Rabbit (no injury)	Transplanted BMSCs migration and engraftment into the inner annulus fibrosus	Sobajima[9]	2008
Autologous BMSCs, genetic marking with GFP	No	Canine (nucleus aspiration)	Suppression of disc degeneration and preservation of immune privilege	Hiyama[56]	2008
BMSCs from EGFP transgenic mice	No	Murine (annular puncture)	Increased matrix synthesis by both autonomous differentiation and stimulatory action on endogenous cells	Yang[57]	2009
Human BMSCs	Hydrogel	Porcine (nucleus aspiration)	Cells survival and disc-like differentiation	Henriksson[58]	2009
Human BMSCs labeled with cell tracker orange	No	Rat (no injury)	Cells survival and chondrocytic differentiation	Wei[59]	2009

BMSC, bone-marrow-derived mesenchymal stem cell; EGFP, enhanced green fluorescent protein

Currently, to the best of our knowledge, there have been two inspiring reports on IVD regeneration therapy with somatic stem cells in clinical trials, although they present different results. Haufe and Mork[62] inserted hematopoietic precursor stem cells (HSCs), which are similar to the precursor BMSCs, into problematic IVDs of 10 patients with disc pain confirmed via provocative discograms and then determined the degree of pain relief from this procedure. They found that none of the patients achieved any alleviation of their discogenic LBP after 1 year and so concluded that the possible regeneration of disc via HSC injections did not correlate with reduced pain. The possible mechanism of this result is that HSCs cannot survive in the oxygen-poor environment of the disc in living human subjects, even with hyperbaric oxygen therapy. In contrast, Yoshikawa et al.[63] recently performed therapeutic IVD regeneration therapy with autologous BMSCs in two patients with lumbago, leg pain, and numbness and obtained favorable findings. They grafted a collagen sponge containing autologous BMSCs to degenerated IVDs percutaneously and found that lumbar disc instability diminished and LBP and neurological symptoms were alleviated in both patients 2 years after surgery.

In vitro studies

The IVD is comprised of a central gelatinous NP surrounded by a more highly organized AF.[64] Evidence from studies investigating the pathogenesis of IVD degeneration shows that it originates from the NP; thus, NP cells comprise the target for BMSC-based repair of the IVDs according to various investigative groups worldwide. Some in vitro studies have been successfully performed using a co-culture system of BMSCs and NP cells[9,15,64–68] (Table 2).

In any case, all of these animal studies demonstrated that BMSCs can generate a large population of differentiated cells and up-regulate the viability of disc cells in the microenvironment of degenerated discs. These finding support the feasibility of BMSC-based tissue engineering therapy for regeneration of the degenerated IVDs. Limited clinical trials also confirm that pain
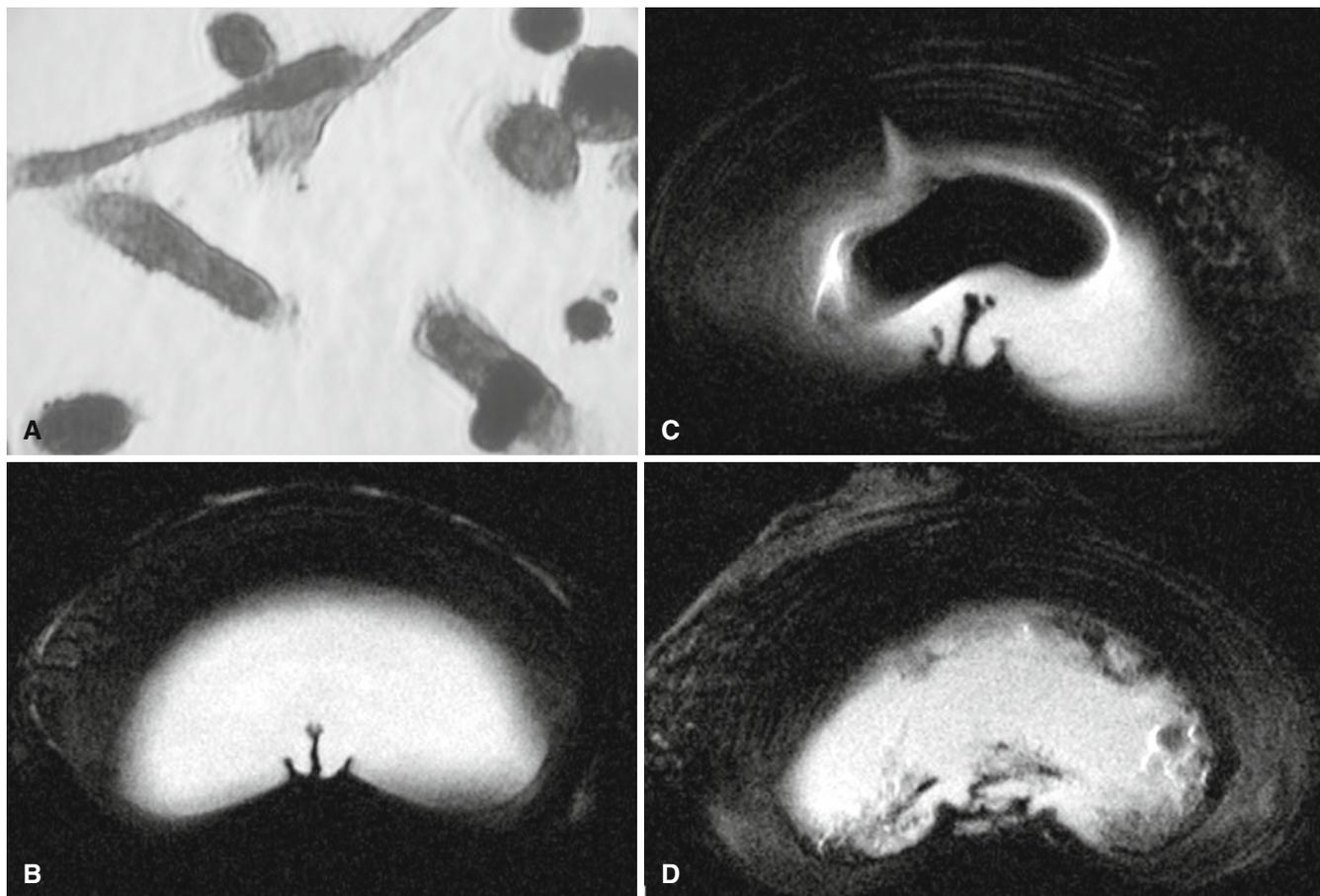
Fig. 1. Magnetic resonance imaging (MRI) monitoring of implanted bone marrow-derived mesenchymal stem cells (BMSCs) labeled with superparamagnetic iron oxide (SPIO). **A** Most of the BMSCs stained positive for SPIO. (Prussian blue stain, ×400) **B–D** T2-weighted MRI scans of rabbit nucleus pulposus (NP). **B** Normal NP. **C** Image obtained within 1 h of normal NP injection of labeled BMSCs. Low signal intensity indicates the presence of SPIO-labeled BMSCs. **D** Image obtained 4 weeks after the injection. Low signal intensity indicates the migration of implanted BMSCs from the nucleus regions to the annulus fibrosus inner border

relief can be achieved by the grafted BMSCs, although further research is required for future clinical application.

## Possible mechanism of BMSC transplantation for IVD regeneration

The exact etiopathogenesis of IVD degeneration is not understood completely, with the cell-based therapy still in its infancy. To date, however, some regeneration mechanisms of BMSC transplantation can be proposed based on the increasing in vitro and vivo BMSC regeneration studies.

Transplanted MSCs differentiate into disc-like cells to make up the loss of IVDs and functional extracellular matrix

Advances in stem cell biology have shown that differentiation of MSCs depends primarily on the environment in which they are placed.[69] Several groups worldwide have illustrated that MSCs have the ability to differentiate into an NP-like or AF-like phenotype when appropriately stimulated.

MSCs differentiate into NP-like cells
Sakai et al.[20] demonstrated that BMSCs transplanted into rabbit IVDs displayed a chondrocyte-like pheno-
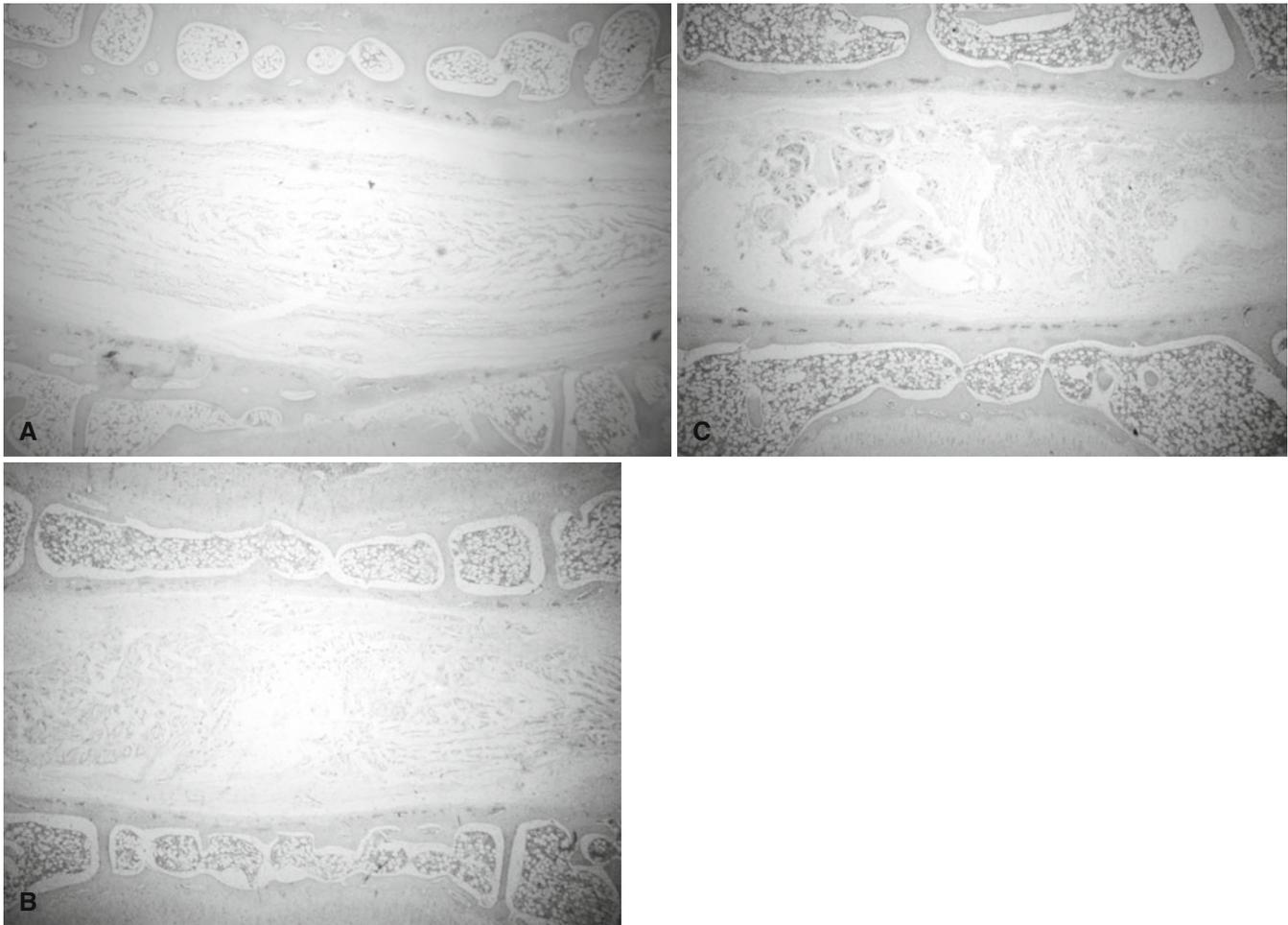
Fig. 2. Rabbit intervertebral discs with degeneration induced by nucleotomy and treated with BMSCs for 4 weeks. (safranin-O, ×40) **A** Normal group. **B** Group with transplanted BMSCs. **C** Nucleotomy-only group. Safranin-O staining was minimal in the nucleotomy-only group, whereas there was intense staining in the group with transplanted BMSCs. These findings indicated that BMSC transplantation effectively suppressed a decrease in the proteoglycan content of degenerated discs

type, typical of NP cells, with expression of proteoglycans and type II collagen 2 weeks after transplantation. Richardson et al.[70] seeded human BMSCs onto a temperature-sensitive hydrogel chitosan–glycerophosphate gel and cultured them for 4 weeks in standard medium. They found that BMSCs retained high cell viability, demonstrated a rounded morphology, and expressed the NP marker genes SOX-9, type II collagen, and aggrecan with relatively low levels of type I collagen, a phenotype that had been described as being consistent with that of NP cells. Le Maitre et al.[64] found that BMSCs in all injected NP tissue samples displayed cellular staining for SOX-9 and displayed cellular and matrix staining for aggrecan and type II collagen that increased during culture. Similar results were also found after injecting CD34-negative human BMSCs into rat IVDs.[59] All of these studies provided the scientific basis for BMSCs differentiating into NP-like cells in the local IVD tissue niche composed of native cells, matrix, and growth factors,[64] although currently there is no commonly accepted marker for disc cells.

It is well known that the microenvironmental niche has a strong influence on BMSC behavior and differentiation, and the harsh microenvironment of IVDs can influence resident cells negatively. Therefore, the mild and moderate stages of degenerative conditions, which are crucial for BMSC survival, proliferation, and differentiation, are considered critical and reasonable because early intervention is likely to offer the greatest promise for cell therapy in the clinical setting.[71] Furthermore, the close proximity of BMSCs to native disc cells plays an important role in the differentiation of the BMSCs.[64] Yang et al.[67] suggested that BMSCs must be in an environment containing numerous NP cells for differentiat-

Table 2. Relevant in vitro experimental studies on BMSC transplantation therapy for degenerated intervertebral discs

Cell and tissue type	Co-culture system	Cell ratio (%)	Result	Study	Year
Rabbit normal NP cells and autologous BMSCs	Direct cell-to-cell contact (PET track-etched tissue culture inserts)	NP cells/BMSCs (50:50)	Significant up-regulation in viability of NP cells; enhancement of growth factor secretion	Yamamoto[65]	2004
Human normal NP cells and allogeneic BMSCs	Direct cell-to-cell contact (PET track-etched tissue culture inserts)	NP cells/BMSCs (75:25, 50:50, 25:75)	NP-like differentiation under optimum cell ratio (75:25 NP cells/BMSCs)	Richardson[15]	2006
Human degenerative NP cells, AF cells, and allogeneic BMSCs	Direct cell-to-cell contact (pellet aggregates)	NP cells/BMSCs (50:50), AF cells/BMSCs (50:50)	Increased matrix synthesis, especially in AF cells/BMSCs pellets	Le Visage[66]	2006
Human degenerative NP cells and allogeneic BMSCs	Without cell-to-cell contact (PET track-etched tissue culture inserts)	Various ratios of NP cells/BMSCs	NP cells proliferation and BMSCs NP-like differentiation under optimum cell ratio through paracrine stimulation	Yang[67]	2008
Human degenerative NP cells and allogeneic retroviral-LacZ infected BMSCs	Direct cell-to-cell contact (pellet aggregates)	NP cells/BMSCs (75:25, 50:50, 25:75)	Greatest increases in extracellular matrix production under 75:25 and 50:50 NP cells/BMSCs	Sobajima[9]	2008
Bovine normal NP tissue and human Ad-GFP infected BMSCs	Direct cell-to-cell contact (BMSCs injected into NP tissue explants)	Injected BMSCs density of 1178 cells/mm^3	NP-like differentiation without additional stimulation	Le Maitre[64]	2009
Human degenerative NP cells and autologous BMSCs	Direct cell-to-cell contact (PET track-etched tissue culture inserts)	NP cells/BMSCs (50:50)	Enhanced biological properties of NP cells	Watanabe[68]	2009

NP, nucleus pulposus; AF, annulus fibrosus; Ad-GFP, adenoviral vectors carrying the green fluorescent protein transcript; PET, positron emission tomography

ing into NP-like cells with higher expression of collagen II and that populations of BMSCs and NP cells possibly influence each other by exchanging cellular components and/or signaling by direct cell-to-cell contact or soluble factors. Richardson et al.[15] showed significant increases in NP marker genes in BMSCs when BMSCs and NP cells were co-cultured with contact for 7 days and noted that this change was regulated by the cell ratio. However, no significant change was observed when cells were cultured without contact, regardless of the cell ratio. Vadalà et al.[72] co-cultured female NP cells with male BMSCs in 3D culture; after 2 weeks, they found that the BMSCs showed a significant increase in collagen type II and aggrecan but a decrease in collagen type I mRNA levels compared with BMSCs cultured alone. A fluorescence in situ hybridization (FISH) assay for the X and Y chromosomes demonstrated that all co-cultured cells analyzed were euploid, and cell fusion did not occur, indicating that differentiation of BMSCs per se, as opposed to cell fusion, was responsible for the observed

beneficial interaction between BMSCs and NP cells. They concluded that the BMSC–NP cell interactions induced a change in the *BMSC* gene expression profile toward a more chondrogenic genotype, indicating BMSC differentiation.

MSCs differentiate into AF-like cells

During the past decade, cell therapy and cell-based tissue engineering strategies worldwide have been developed mainly targeted at the regeneration of the NP of the degenerated IVDs. For increasing the potential of NP repair, techniques that deal with the damaged AF are now increasingly recognized.[73] Hiyama et al.[74] reviewed the in vitro induction of AF-like cells from BMSCs, which further implied that stem cell therapy would possibly become a major option in the treatment of IVD degeneration. Meanwhile, the differentiation of BMSCs into AF-like cells had also been evidenced in some in vivo studies. Kimelman et al.[75] molded a hybrid of fibrin gel and a filamentous poly lactide-glycolide

acid (PLGA) scaffold into the shape of a rat IVD and then suspended BMSCs overexpressing the brachyury transcription factor in the central fibrin gel, whereas the BMSCs expressing Smad8/BMP-2 genes were seeded on the PLGA, forming the outer ring of the construct. The grafts were implanted subcutaneously in C3H/HeN mice or used to replace caudal discs of nude rats and were harvested 3 weeks later. the authors found that the Smad8/BMP-2-expressing BMSCs formed an AF-like tissue in the outer portion of the graft; and the BMSCs expressing the brachyury gene gave raise to cartilaginous NP-like tissue in the center of the construct. Sobajima et al.[9] reported that the lacZ-marked allogeneic adult rabbit BMSCs that migrated into the transition zone and inner AF area 24 weeks after transplantation showed a more spindle shape, similar to native AF cells, which provided morphological evidence that the transplanted BMSCs underwent differentiation into AF-like cells. This homing potential of the transplanted BMSCs to the AF region might be of value in the development of an annular repair strategy.

Transplanted BMSCs as trophic mediators up-regulate disc cells' viability, protect disc cells from apoptosis, inhibit increased cell senescence, and suppress the local immune system

The BMSCs can secrete a variety of growth factors and cytokines,[76–78] including transforming growth factor β1 (TGFβ1), endothelial growth factor (EGF), insulin-like growth factor-1 (IGF-1), platelet-derived growth factor (PDGF), vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), bone morphogenetic proteins (BMP-2, BMP-4, BMP-6, BMP-7), interleukin-1 (IL-1), granulocyte-macrophage colony-stimulating factor (GM-CSF), leukemia inhibitory factor (LIF), IL-6, IL-10, IL-11, prostaglandin E_2 (PGE$_2$), and stem cell factor, among others. The functional secretions of bioactive factors[76,78] — which stimulate mitosis and differentiation of tissue-intrinsic reparative or stem cells, inhibit apoptosis and senescence, and suppress local immune system — can have profound effects on local cellular dynamics and tissue regeneration. These effects of BMSCs are referred to as trophic effects[78] because they do not themselves differentiate but, rather, their secreted bioactive factors mediate the functional tissue outcomes.

**MSCs up-regulate disc cells' viability**

The stimulation of proliferative capacity and matrix synthesis of disc cells by cytokines or growth factors alters IVDs' homeostasis by shifting cellular metabolism to the anabolic state.[18,79,80] So far, several bioactive factors — e.g., TGFβ1,[81] EGF,[82] IGF-1,[83–85] PDGF,[84,85] growth and differentiation factor-5 (GDF-5),[86] osteogenic protein-1 (OP-1),[87] and BMP-2 and BMP-12[88,89] — have been shown to be an effective stimulator on matrix metabolism and cell proliferation during biological repair of IVDs. These bioactive factors can also be secreted by IVD cells themselves and BMSCs.[79] Therefore, the autocrine and paracrine production of bioactive factors, which assist the degenerated disc cells in regenerating and maintaining viability, should be considered one of the major regulatory mechanisms in BMSCs-based IVD regeneration.

Yang et al.[67] found that the proliferation ability of NP cells was significantly induced when co-cultured with even a few BMSCs, and the increased expression of aggrecan in NP cells required more trophic effects from BMSCs. Mochida[90] reported that NP cells from an adult beagle dog were activated by a co-culture system with autologous BMSCs that included direct cell-to-cell contact. Recently, Watanabe et al.[68] also reported that human NP cells significantly enhanced biological properties in a co-culture system with direct cell-to-cell contact with autologous BMSCs. The mechanism behind BMSCs serving as feeder or nursing cells for NP cells was most likely to be the contribution of the secreted growth factors listed above, although it was not sure which cells, BMSCs or NP cells initially triggered the co-stimulating effects between them.

Yamamoto et al.[65] co-cultured rabbit BMSCs with NP cells and reported that NP cells showed more biological properties (e.g., cell proliferation, DNA synthesis, proteoglycan synthesis) in a co-culture system than when cultured alone. They also tested the growth factors' concentrations in the co-cultured medium and found that the BMSCs–NP cells co-culture resulted in more IGF-1, PDGF, EGF, and TGFβ1. Furthermore, if the BMSCs and NP cells were co-cultured with direct cell-to-cell contact, there were more synergistic trophic effects.

Our group transplanted green fluorescent protein (GFP)-labeled BMSCs into early degenerated rabbit IVDs and observed that the transplanted stem cells survived, reproduced, and migrated.[91] An increased production of aggrecan and collagen type II were also seen, and the GFP-positive BMSCs started to express TGFβ1 (Fig. 3) and IGF-1, which was a sign of the trophic effects with the peak effect occurring about 2 weeks after transplantation. We further used a co-culture system with trans-wells to study the activation of NP cells by autologous BMSCs; we found that the TGFβ1, IGF-1, EGF, and PDGF concentrations in the co-cultured medium were significantly higher than in the control group at different time points after co-culturing and the viability of NP cells was up-regulated.
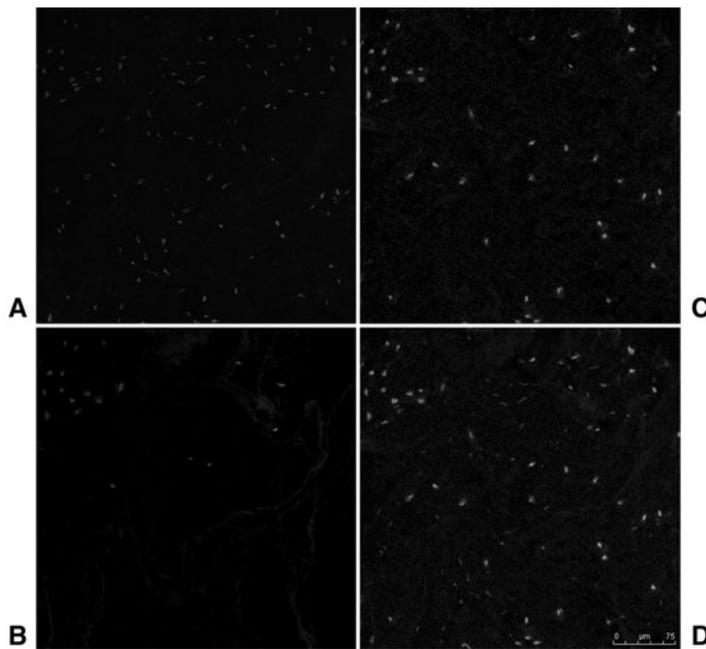
Fig. 3. Transforming growth factor β1 (TGFβ1) expression in adenoviral vectors carrying the green fluorescent protein transcript (Ad-GFP)-infected BMSCs in rabbit nucleus pulposus tissue 2 weeks after injection. **A–C** Photomicrographs of 4,6-diamidino-2-phenylindole (DAPI) staining (*blue*), GFP-positive cells (*green*), and immunofluorescence (*red*) in an identical field of view at the injection sites of intervertebral disc tissue. **D** Merged images show the positive expression of TGFβ1 in transplanted BMSCs. Calculations for the cells were performed using a ×20 image. *Bar* 75 μm

MSCs protect disc cells from apoptosis

Apoptosis of IVD cells has been demonstrated to play a vital role in promoting the degeneration process.[92,93] The apoptotic process[94] can be triggered by either an intrinsic pathway influenced by members of the Bcl family bound to the mitochondrial membrane or an extrinsic pathway stimulated by tumor necrosis factor (TNF), both of which have been detected in IVD degeneration.[95–97] Hence, it is beneficial to retard the progression of apoptosis-associated IVD degeneration or regenerate IVDs by attenuating the apoptosis of IVD cells. Indeed, several previous studies[84,92,98,99] have provided evidence that IGF-1, PDGF, BMP-7, caspase inhibitors, and thymosin-β4 can protect or rescue disc cells in vitro from undergoing apoptosis. Gruber et al.[84] found a significant reduction in the percentage of serum starvation-induced apoptotic disc cells after exposure to IGF-1 50–500 ng/ml or PDGF 100 ng/ml; they showed that selected cytokines can retard or prevent programmed cell death in vitro. Wei et al.[92] cultured human NP cells in monolayer with human recombinant pure BMP-7 (rhBMP-7) and used TNFα or hydrogen perox-

ide to induce an apoptotic environment. They found that the addition of rhBMP-7 resulted in inhibition of the apoptotic effects of both inducers; and further study showed that the antiapoptosis effect originated from the inactivation of caspase-3.

Meantime, IGF-1, PDGF, and BMP-7 were shown to be up-regulated to express and secrete either in the co-culture system or after BMSC transplantation, indicating the potential antiapoptosis effect of BMSCs. Yang et al.[67] reported that the Fas-associated death domain protein of human NP cells was only slightly lowered when co-cultured with human BMSCs, and BMP7 showed no significantly increased expression in either BMSCs or NP cells after co-culture for 3 days, which indicated that the volunteer or naturally occurring antiapoptosis effect from BMSCs was limited without proper intervention. Kang et al.[100] successfully transfected the *BMP7* gene into BMSCs and detected increased expression of BMP7 proteins, suggesting that the antiapoptosis effect would be amplified with the introduction of gene therapy. Risbud et al.[101] reported normoxic stabilization of HIF-1α; and increased HIF-1α transcriptional activity under hypoxia showed metabolic adaptation of the NP cells to the IVD microenvironment, which has a limited vascular supply and generates energy through anaerobic glycolysis. Zeng et al.[102] reported that the inhibition of HIF-1α function in the NP cells down-regulated the promoter of galectin-3 (gal-3), which sequentially enhanced FasL-mediated apoptosis of the IVD cells. HIF-1α can be used as a phenotypic marker of NP cells.[101] Our group transplanted rabbit BMSCs into early degenerated IVDs and detected up-regulation of HIF-1α, which indicated that BMSCs not only had differentiated into the NP-like phenotype but had also increased the antiapoptosis ability by maintaining the HIF-1α.[91] Hiyama et al.[56] used nucleotomy to model IVD degeneration in beagles and found that the proportion of Fas-positive cells in the NP region increased 4 weeks after the induction of disc degeneration. However, after BMSCs were transplanted into the degenerated IVDs the proportion of Fas-positive cells was suppressed compared to that in the normal control group, indicating that BMSC transplantation protected the NP cells from apoptosis by downregulating the Fas proteins.

All the above studies demonstrated the direct or indirect antiapoptosis effect of BMSCs on IVD regeneration.

MSCs inhibit increased disc cells' senescence

Cell senescence occurs when normal cells stop dividing.[12,103] Senescent cells, which remain viable and inhibit alteration of the pattern of gene expression,

become unresponsive to external stimuli and may secrete bioactive factors, such as extracellular matrix degrading proteases, collagenase, and matrix metalloproteinase family members, which can influence neighboring cells and degrade their nearby extracellular matrix.[8,12,103,104]

Direct evidence regarding the close link between cell senescence and disc degeneration — based on the observations that senescence-associated β-galactosidase-positive disc cells increased with the increasing degree of disc degeneration or increased especially in the clustered cells in herniated NP — has been provided by several laboratories all over the world. Recently, Kim et al.[8] confirmed that the telomere-based p53–p21–pRB pathway, rather than the stress-based p16–pRB pathway, played a more important role in the senescence of NP cells in an in vivo condition. These studies indicate that the prevention or reversal of the senescence of disc cells could be an effective therapeutic target for degenerated IVDs. After P16 expression was down-regulated by P16INK4a-specific siRNAs, increased RB phosphorylation and deceased senescent features were detected in cultured NP cells from herniated IVDs.[105] Through reducing expression of the cyclin-dependent kinase inhibitors P21/27 and increasing c-Myc expression to enhance extracellular signal regulated kinase (ERK1/2) signals, TGFβ1, which accelerates proliferation in mesenchymal cells, was reported by Nakai et al.[106] to promote proliferation and cell cycle progression in cultured rat NP cells. Gruber et al.[103] found that there was a significant negative correlation between the percentage of senescent cells and the percentage of proliferating cells in human degenerated annulus in vivo. A further study[107] was performed to investigate in vitro the potential for IGF-1 to prevent or ameliorate stress-induced premature senescence in the human annulus. They found that a significant reduction of senescent annulus cells was present following exposure to IGF-1 500 ng/ml. Therefore, although cell senescence has been long regarded as an irreversible process of G_1/S transition arrest with proliferation ability lost permanently, this programmed process can still be attenuated or even reversed by appropriate modification at certain stages.

At present, the direct evidence regarding BMSCs inhibiting disc cell senescence through either the telomere-based p53–p21–pRB pathway or the stress-based p16–pRB pathway has not been provided. However, the implied information that BMSC-based disc regeneration could be achieved by targeting disc cell senescence can be obtained from the above observations. Indeed, in the co-culture study of BMSCs and NP cells, the cell growth rate and telomerase activity were found to be higher in co-cultured cells than in NP cells cultured alone.[108]

MSCs suppress the local immune system

FasL is expressed on activated T cells, natural killer (NK) cells, and tumor cells and in immune-privileged sites such as the retina, testis, brain, and IVD. In normal IVDs, FasL acts, via induction of apoptosis, on invading Fas-positive activated T cells and thus protects the cells from immune attack. Peng et al.[14] found that abundant macrophages infiltrated the NP and released growth factor or cytokines, which led to the ingrowth of blood vessels forming a zone of vascularized granulation tissue in the painful IVD. Kaneyama et al.[13] compared FasL expression on degenerated discs from spondylolisthesis patients with that on nondegenerated discs from idiopathic scoliosis patients. They found that NP cells in all specimens from the two groups showed strong positive staining for FasL, but the quantitative examination demonstrated a significant decrease in FasL expression in the degenerated disc group compared to that of the nondegenerated disc group. This indicated that FasL and its potential mechanism of immunological privilege could influence the protection of the IVD against degeneration. All these studies demonstrated that as IVDs degenerated the FasL expression was down-regulated with the loss of the immune privilege feature, which subsequently led to infiltration of the activated immune cells and ingrowth of blood vessels into the degenerated IVDs.

Hiyama et al.[56] reported that 4 weeks after nucleotomy-induced IVD degeneration the proportion of FasL-positive cells in the NP region was significantly decreased in the nucleotomy group compared to that in a normal control group. They then transplanted GFP-labeled BMSCs into the degenerated IVDs and found that BMSCs expressed FasL at the genetic level rather than as proteins before transplantation, but the expression was increased in the GFP-positive BMSCs 4 weeks after transplantation. Furthermore, FasL-positive cells increased significantly 8 weeks after BMSC transplantation compared to nucleotomy only — to approximate the level found in the normal control group. Therefore, transplanted BMSCs might contribute directly to the recovery of immune privilege by differentiating into disc cells expressing FasL and indirectly by stimulating the original NP cells to increase FasL expression. Wei et al.[59] reported the same result after transplanting fluorescence-labeled human BMSCs into rat IVDs. They found that the increased FasL-positive cells were mainly distributed around the peripheral regions of the annulus and in the NP. Almost no FasL-positive cells were detected in the cartilage endplate or the inner region of the annulus. More importantly, only a few cells were double-labeled with FasL and fluorescence, indicating that FasL was mainly expressed by the host cells after BMSC transplantation.

All the above studies demonstrated that BMSCs up-regulate the expression of FasL on NP cells, which maintains the FasL-mediated immune privilege state of the IVDs.

Future perspectives

Current in vivo and in vitro studies are encouraging as they reveal the potential and possible mechanisms of autogenic and allogeneic BMSCs to retard or IVD degeneration or encourage regeneration. Nevertheless, BMSC-based therapy for disc degeneration is still in its infancy, and many biotechnological problems related to the biological characteristics of the cells need to be solved.

Human trials will be necessary to determine the safety, feasibility, and ultimate efficacy of BMSC transplantation; a clear understanding of the biological characteristics of BMSCs is of utmost importance. Apart from the regenerative potential mentioned above, it is also worth noting the potential risks of BMSCs.[109–111] The optimal BMSC dose for clinical applications is currently unknown, but it is likely that large amounts of cells would be needed for successful IVD regeneration. However, in vitro expansion possibly poses a risk of accumulating genetic and epigenetic changes to the cells, which in turn may lead to malignant transformation.[109,110] Moreover, in vivo transplantation can likely promote the growth of a latent tumor.[109,110] Future studies are therefore be needed to elucidate the poorly understood potential for oncogenic capacity. In addition, in vitro techniques and reagents used in cell culture may increase the risk of infection from xenogenic compounds.[109] For this reason, a topic of major interest is how to eliminate the contamination of expanding cells with serum-bound pathogenic agents so we can make good use of BMSCs in clinical therapy.

In addition, we need to learn more about native disc cells and their microenvironment, the etiology of IVD degeneration, the cellular and molecular changes in IVDs with aging and degeneration, the exact behavioral and synthetic properties of transplanted BMSCs in degenerated IVDs, the effect of aging on the regeneration capacity of BMSCs, and the interactions between differentiation and trophic effects of BMSCs, among others. These topics should be fully explored before this newly developed technique can be applied to the clinical setting. Furthermore, the best preconditioning and optimal number of BMSCs, culture conditions, implantation strategy, and the window of IVD degeneration remain to be defined for the best efficacy. Despite all these concerns, which seem to indicate that the clinical application of BMSCs to repair IVD degeneration is still far in the future, the developments in this field are increasing rapidly. In the future, when the above issues are addressed, BMSC transplantation combined with minimally invasive treatment for the regeneration of degenerated IVDs will become a clinical reality.

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