J Orthop Sci (2010) 15:707–719
DOI 10.1007/s00776-010-1536-3

JOURNAL OF
ORTHOPAEDIC SCIENCE


*Review article*

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

Yun-Tao Wang, Xiao-Tao Wu, and Feng Wang

Department of Orthopaedics, Zhongda Hospital, Southeast University, 87 Ding Jia Qiao, Nanjing, Jiangsu 210009, P. R. China

**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,

*Offprint requests to*: Y.-T. Wang
Received: March 4, 2010 / Accepted: July 6, 2010

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

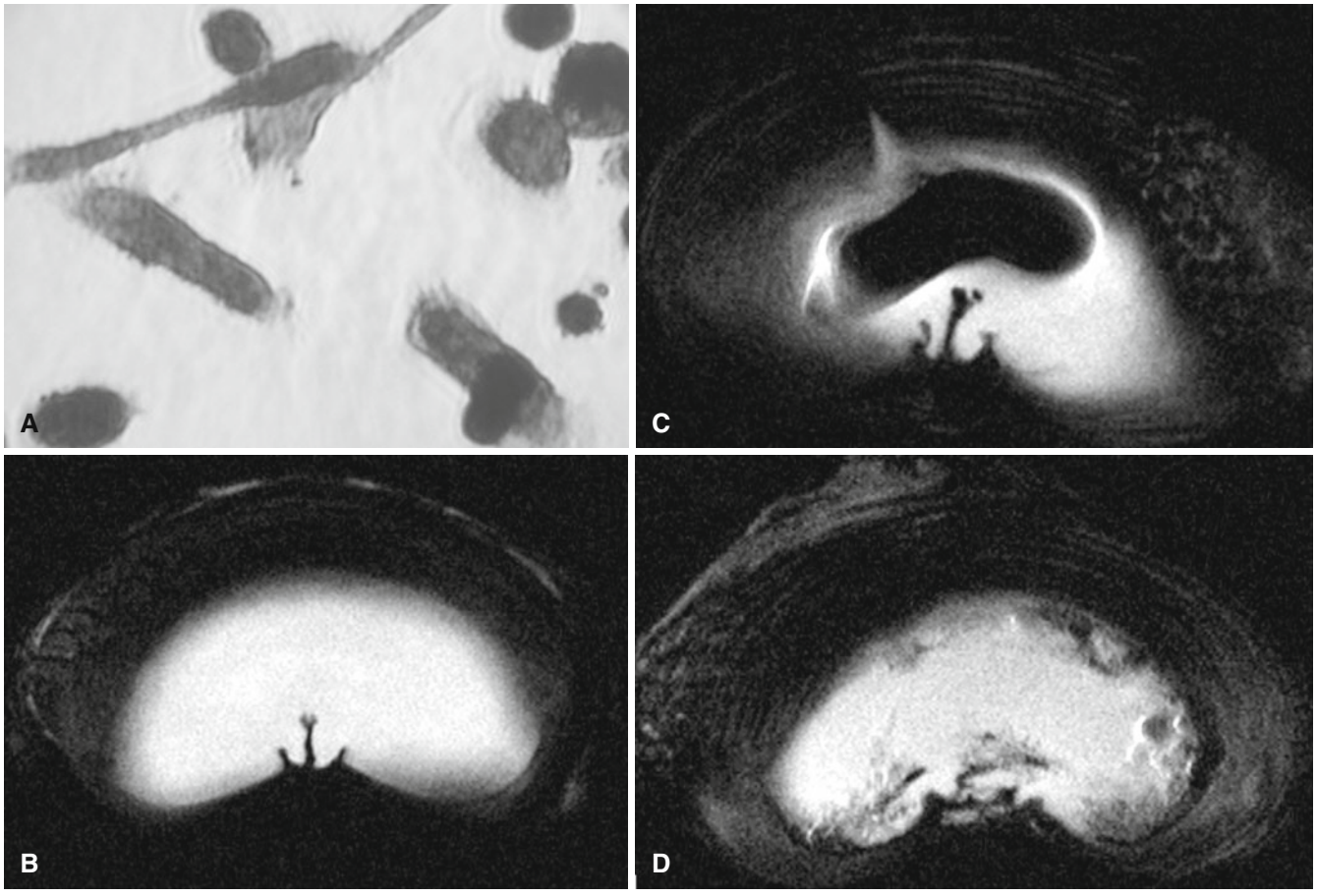


**Fig. 1.** Magnetic resonance imaging (MRI) monitoring of implanted bone marrow-derived mesenchymal stem cells (BMSCs) labeled with superparamagnetic iron oxide (SPIO). **A** Most of the BMSCs stained positive for SPIO. (Prussian blue stain, ×400) **B–D** T2-weighted MRI scans of rabbit nucleus pulposus (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-

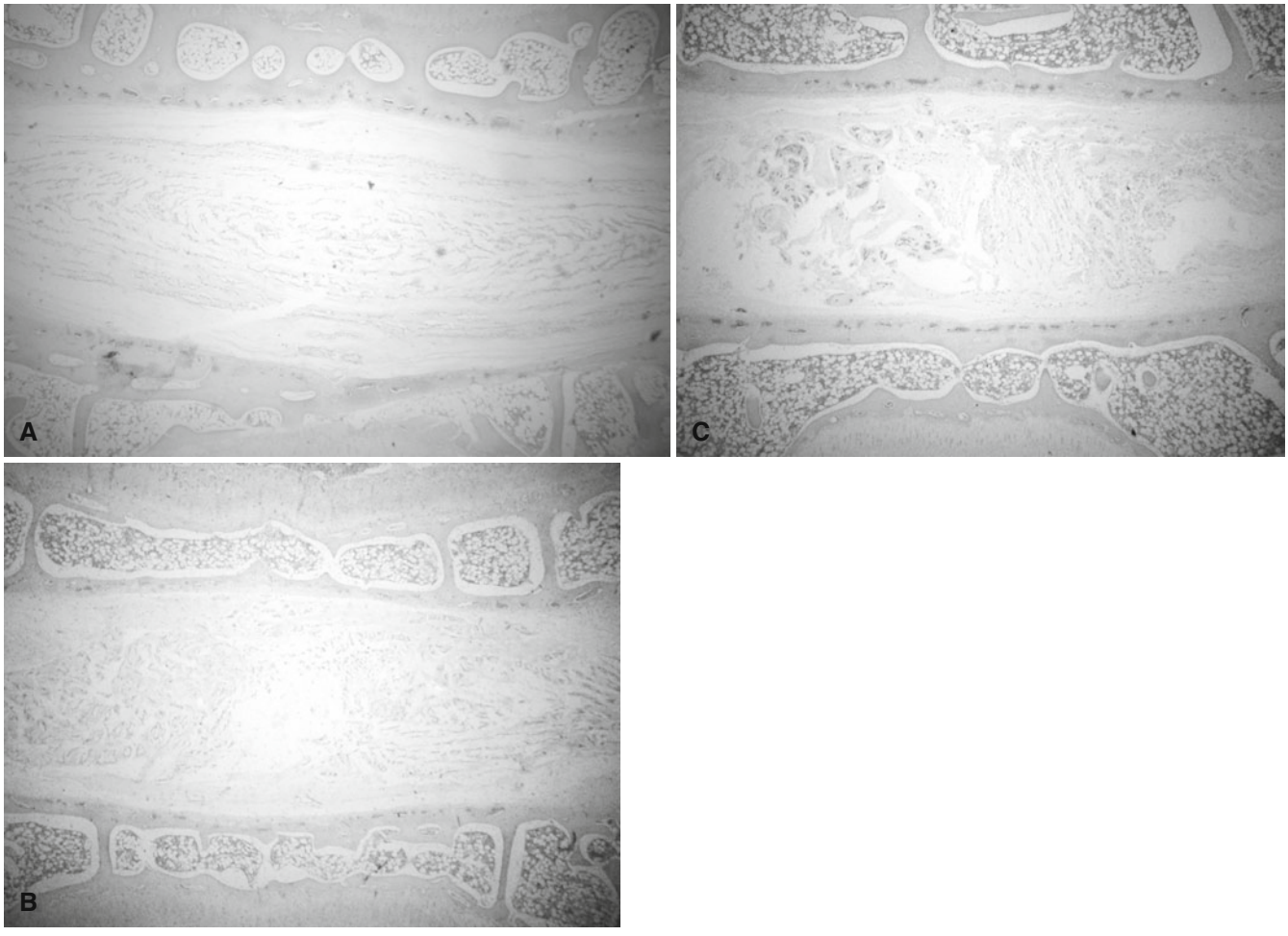


**Fig. 2.** Rabbit intervertebral discs with degeneration induced by nucleotomy and treated with BMSCs for 4 weeks. (safranin-O, ×40) **A** Normal group. **B** Group with transplanted BMSCs. **C** Nucleotomy-only group. Safranin-O staining was minimal in the nucleotomy-only group, whereas there was intense 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.

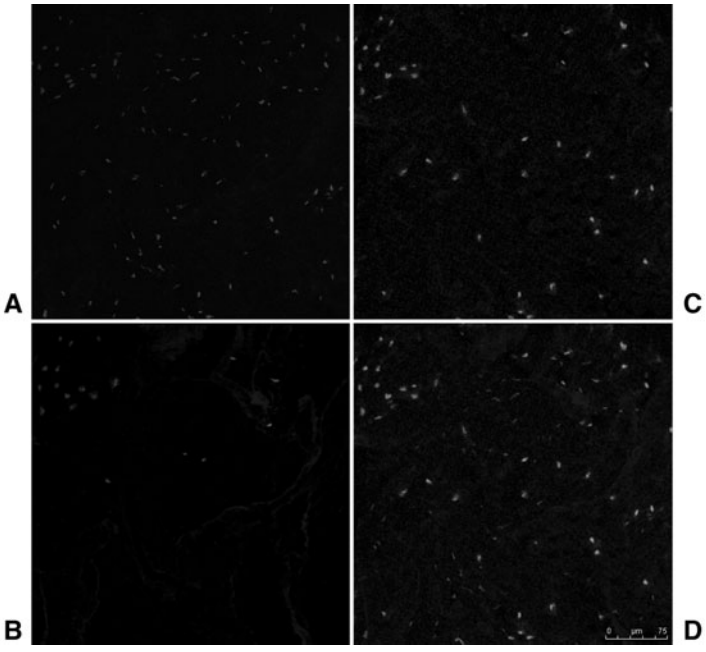


**Fig. 3.** Transforming growth factor β1 (TGFβ1) expression in adenoviral vectors carrying the green fluorescent protein transcript (Ad-GFP)-infected BMSCs in rabbit nucleus pulposus tissue 2 weeks after injection. **A–C** Photomicrographs of 4,6-diamidino-2-phenylindole (DAPI) staining (*blue*), GFP-positive 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 peroxide 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 down-regulating 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.

This work is supported by the National Natural Science Foundation of China (No. 31070876, 81071493)

## References

1. Pye SR, Reid DM, Smith R, Adams JE, Nelson K, Silman AJ, et al. Radiographic features of lumbar disc degeneration and self-reported back pain. J Rheumatol 2004;31:753–8.
2. Hoyland JA, Le Maitre CL, Freemont AJ. Investigation of the role of IL-1 and TNF in matrix degradation in the intervertebral disc. Rheumatology 2008;47:809–14.
3. Miller JA, Schmatz C, Schultz AB. Lumbar disc degeneration: correlation with age, sex, and spine level in 600 autopsy specimens. Spine 1988;13:173–8.
4. Le Maitre CL, Freemont AJ, Hoyland JA. Localization of degradative enzymes and their inhibitors in the degenerate human intervertebral disc. J Pathol 2004;204:47–54.
5. Le Maitre CL, Freemont AJ, Hoyland JA. Accelerated cellular senescence in degenerate intervertebral discs: a possible role in the pathogenesis of intervertebral disc degeneration. Arthritis Res Ther 2007;9:R45.
6. Richardson SM, Knowles R, Tyler J, Mobasheri A, Hoyland JA. Expression of glucose transporters GLUT-1, GLUT-3, GLUT-9 and HIF-1α in normal and degenerate human intervertebral disc. Histochem Cell Biol 2008;129:503–11.
7. Zhao CQ, Wang LM, Jiang LS, Dai LY. The cell biology of intervertebral disc aging and degeneration. Ageing Res Rev 2007;6:247–61.
8. Kim KW, Chung HN, Ha KY, Lee JS, Kim YY. Senescence mechanisms of nucleus pulposus chondrocytes in human intervertebral discs. Spine J 2009;9:658–66.
9. Sobajima S, Vadala G, Shimer A, Kim JS, Gilbertson LG, Kang JD. Feasibility of a stem cell therapy for intervertebral disc degeneration. Spine J 2008;8:888–96.
10. Yasuma T, Koh S, Okamura T, Yamauchi Y. Histological changes in aging lumbar intervertebral discs: their role in protrusions and prolapses. J Bone Joint Surg Am 1990;72:220–9.
11. Thompson RE, Pearcy MJ, Downing KJ, Manthey BA, Parkinson IH, Fazzalari NL. Disc lesions and the mechanics of the intervertebral joint complex. Spine 2000;25:3026–35.
12. Gruber HE, Ingram JA, Norton HJ, Hanley EN. Senescence in cells of the aging and degenerating intervertebral disc: immunolocalization of senescence-associated beta-galactosidase in human and sand rat discs. Spine 2007;32:321–7.
13. Kaneyama S, Nishida K, Takada T, Suzuki T, Shimomura T, Maeno K, et al. Fas ligand expression on human nucleus pulposus cells decreases with disc degeneration processes. J Orthop Sci 2008;13:130–5.
14. Peng B, Hao J, Hou S, Wu W, Jiang D, Fu X, et al. Possible pathogenesis of painful intervertebral disc degeneration. Spine 2006;31:560–6.
15. Richardson SM, Walker RV, Parker S, Rhodes NP, Hunt JA, Freemont AJ, et al. Intervertebral disc cell-mediated mesenchymal stem cell differentiation. Stem Cells 2006;24:707–16.
16. Yoon ST, Patel NM. Molecular therapy of the intervertebral disc. Eur Spine J 2006;15:S379–88.
17. Zhang Y, Markova D, Im HJ, Hu W, Thonar EJ, He TC, et al. Primary bovine intervertebral disc cells transduced with adenovirus overexpressing 12 BMPs and Sox9 maintain appropriate phenotype. Am J Phys Med Rehabil 2009;88:455–63.
18. Masuda K. Biological repair of the degenerated intervertebral disc by the injection of growth factors. Eur Spine J 2008; 17:441–51.

19. Masuda K, An HS. Prevention of disc degeneration with growth factors. Eur Spine J 2006;15:S422–32.
20. Sakai D, Mochida J, Iwashina T, Watanabe T, Nakai T, Ando K, et al. Differentiation of mesenchymal stem cells transplanted to a rabbit degenerative disc model: potential and limitations for stem cell therapy in disc regeneration. Spine 2005;30:2379–87.
21. Kuh SU, Zhu Y, Li J, Tsai KJ, Fei Q, Hutton WC, et al. A comparison of three cell types as potential candidates for intervertebral disc therapy: annulus fibrosus cells, chondrocytes, and bone marrow derived cells. Joint Bone Spine 2009;76:70–4.
22. Gorensek M, Jaksimovic C, Kregar-Velikonja N, Gorensek M, Knezevic M, Jeras M, et al. Nucleus pulposus repair with cultured autologous elastic cartilage derived chondrocytes. Cell Mol Biol Lett 2004;9:363–73.
23. Nishimura K, Mochida J. Percutaneous reinsertion of the nucleus pulposus: an experimental study. Spine 1998;23:1531–9.
24. Nomura T, Mochida J, Okuma M, Nishimura K, Sakabe K. Nucleus pulposus allograft retards intervertebral disc degeneration. Clin Orthop 2001;389:94–101.
25. Iwashina T, Mochida J, Sakai D, Yamamoto Y, Miyazaki T, Ando K, et al. Feasibility of using a human nucleus pulposus cell line as a cell source in cell transplantation therapy for intervertebral disc degeneration. Spine 2006;31:1177–86.
26. Sakai D, Mochida J, Yamamoto Y, Toh E, Iwashina T, Miyazaki T, et al. Immortalization of human nucleus pulposus cells by a recombinant SV40 adenovirus vector: establishment of a novel cell line for the study of human nucleus pulposus cells. Spine 2004;29:1515–23.
27. Sheikh H, Zakharian K, De La Torre RP, Facek C, Vasquez A, Chaudhry GR, et al. In vivo intervertebral disc regeneration using stem cell-derived chondroprogenitors. J Neurosurg Spine 2009;10:265–72.
28. Meisel HJ, Siodla V, Ganey T, Minkus Y, Hutton WC, Alasevic OJ. Clinical experience in cell-based therapeutics: disc chondrocyte transplantation A treatment for degenerated or damaged intervertebral disc. Biomol Eng 2007;24:5–21.
29. Meisel HJ, Ganey T, Hutton WC, Libera J, Minkus Y, Alasevic O. Clinical experience in cell-based therapeutics: intervention and outcome. Eur Spine J 2006;15:S397–405.
30. Hohaus C, Ganey TM, Minkus Y, Meisel HJ. Cell transplantation in lumbar spine disc degeneration disease. Eur Spine J 2008;17:492–503.
31. Anderson DG, Risbud MV, Shapiro IM, Vaccaro AR, Albert TJ. Cell-based therapy for disc repair. Spine J 2005;5: 297S–303S.
32. Bartholomew A, Sturgeon C, Siatskas M, Ferrer K, Mclntosh K, Patil S, et al. Mesenchymal stem cells suppress lymphocyte proliferation in vitro and prolong skin graft survival in vivo. Exp Hematol 2002;30:42–8.
33. Sakai D, Mochida J, Yamamoto Y, Nomura T, Okuma M, Nishimura K, et al. Transplantation of mesenchymal stem cells embedded in Atelocollagen gel to the intervertebral disc: a potential therapeutic model for disc degeneration. Biomaterials 2003;24:3531–41.
34. Horwitz EM, Prockop DJ, Fitzpatrick LA, Koo WWK, Gordon PL, Neel M, et al. Transplantability and therapeutic effects of bone marrow-derived mesenchymal cells in children with osteogenesis imperfecta. Nat Med 1999;5:309–13.
35. Le Blanc K, Tammik C, Rosendahl K, Zetterberg E, Ringden O. HLA expression and immunologic properties of differentiated and undifferentiated mesenchymal stem cells. Exp Hematol 2003;31:890–6.
36. Leung VY, Chan D, Cheung KM. Regeneration of intervertebral disc by mesenchymal stem cells: potentials, limitations, and future direction. Eur Spine J 2006;15:S406–13.
37. Hoogendoorn RJW, Lu ZF, Kroeze RJ, Bank RA, Wuisman PI, Helder MN. Adipose stem cells for intervertebral disc regeneration: current status and concepts for the future. J Cell Mol Med 2008;12:2205–16.
38. Jim JJ, Noponen-Hietala N, Cheung KM, Ott J, Karppinen J, Sahraravand A, et al. The TRP2 allele of COL9A2 is an age-dependent risk factor for the development and severity of intervertebral disc degeneration. Spine 2005;30:2735–42.
39. Noponen-Hietala N, Virtanen I, Karttunen R, Schwenke S, Jakkula E, Li H, et al. Genetic variations in IL6 associate with intervertebral disc disease characterized by sciatica. Pain 2005;114:186–94.
40. Semba K, Araki K, Li Z, Matsumoto K, Suzuki M, Nakagata N, et al. A novel murine gene, sickle tail, linked to the Danforth's short tail locus, is required for normal development of the intervertebral disc. Genetics 2006;172:445–56.
41. Sethe S, Scutt A, Stolzing A. Aging of mesenchymal stem cells. Ageing Res Rev 2006;5:91–116.
42. Bellantuono I, Aldahmash A, Kassem M. Aging of marrow stromal (skeletal) stem cells and their contribution to age-related bone loss. Biochim Biophys Acta 2009;1792:364–70.
43. Ryan JM, Barry FP, Murphy JM, Mahon BP. Mesenchymal stem cells avoid allogeneic rejection. J Inflamm (Lond) 2005;2:8.
44. Gaetani P, Torre ML, Klinger M, Faustini M, Crovato F, Bucco M, et al. Adipose-derived stem cell therapy for Intervertebral disc regeneration: an in vitro reconstructed tissue in alginate capsules. Tissue Eng Part A 2008;14:1415–23.
45. Ganey T, Hutton WC, Moseley T, Hedrick M, Meisel H. Intervertebral disc repair using adipose tissue-derived stem and regenerative cells: experiments in a canine model. Spine 2009;34:2297–304.
46. Vadalà, G, Sobajima S, Lee JY, Huard J, Denaro V, Kang J, et al. In vitro interaction between muscle-derived stem cells and nucleus pulposus cells. Spine J 2008;8:804–9.
47. Murrell W, Sanford E, Anderberg L, Cavanagh B, Mackay-Sim A. Olfactory stem cells can be induced to express chondrogenic phenotype in a rat intervertebral disc injury model. Spine J 2009;9:585–94.
48. Gilchrist CL, Chen J, Richardson WJ, Loeser RF, Setton LA. Functional integrin subunits regulating cell–matrix interactions in the intervertebral disc. J Orthop Res 2007;25:829–40.
49. Henriksson HB, Thornemo M, Karlsson C, Hägg O, Junevik K, Lindahl A, et al. Identification of cell proliferation zones, progenitor cells and a potential stem cell niche in the intervertebral disc region: a study in four species. Spine 2009;34:2278–87.
50. Moore KA, Lemischka IR. Stem cells and their niches. Science 2006;311:1880–5.
51. Risbud MV, Guttapalli A, Tsai TT, Lee JY, Danielson KG, Vaccaro AR, et al. Evidence for skeletal progenitor cells in the degenerate human intervertebral disc. Spine 2007;32:2537–44.
52. Zhao J, Zhang N, Prestwich GD, Wen XJ. Recruitment of endogenous stem cells for tissue repair. Macromol Biosci 2008;8:836–42.
53. Chen WH, Liu HY, Lo WC, Wu SC, Chi CH, Chang HY, et al. Intervertebral disc regeneration in an ex vivo culture system using mesenchymal stem cells and platelet-rich plasma. Biomaterials 2009;30:5523–33.
54. Crevensten G, Walsh AJ, Ananthakrishnan D, Page P, Wahba GM, Lotz JC, et al. Intervertebral disc cell therapy for regeneration: mesenchymal stem cell implantation in rat intervertebral discs. Ann Biomed Eng 2004;32:430–4.
55. Zhang YG, Guo X, Xu P, Kang LL, Li J. Bone mesenchymal stem cells transplanted into rabbit intervertebral discs can increase proteoglycans. Clin Orthop 2005;430:219–6.
56. Hiyama A, Mochida J, Iwashina T, Omi H, Watanabe T, Serigano K, et al. Transplantation of mesenchymal stem cells in a canine disc degeneration model. J Orthop Res 2008;26:589–600.
57. Yang F, Leung VY, Luk KD, Chan D, Cheung KM. Mesenchymal stem cells arrest intervertebral disc degeneration through chondrocytic differentiation and stimulation of endogenous cells. Mol Ther 2009;7:1959–66.

58. Henriksson HB, Svanvik T, Jonsson M, Hagman M, Horn M, Lindahl A, et al. Transplantation of human mesenchymal stems cells into intervertebral discs in a xenogeneic porcine model. Spine 2009;34:141–8.
59. Wei A, Tao H, Chung SA, Brisby H, Ma DD, Diwan AD. The fate of transplanted xenogeneic bone marrow-derived stem cells in rat intervertebral discs. J Orthop Res 2009;27:374–9.
60. Bao JP, Wu XT, Wang YT, Zhang FY. In vivo MR imaging tracking of iron oxide labeled autologous bone marrow mesenchymal stem cells following degenerated disc injection. J Southeast Univ Med Sci Ed 2008;27:166–70 (in Chinese).
61. Zhang Y, An HS, Tannoury C, Thonar EJ, Freedman MK, Anderson DG. Biological treatment for degenerative disc disease: implications for the field of physical medicine and rehabilitation. Am J Phys Med Rehabil 2008;87:694–702.
62. Haufe SM, Mork AR. Intradiscal injection of hematopoietic stem cells in an attempt to rejuvenate the intervertebral discs. Stem Cells Dev 2006;15:136–7.
63. Yoshikawa T, Ueda Y, Miyazaki K, Koizumi M, Takakura Y. Disc regeneration therapy using marrow mesenchymal cell transplantation: a report of two case studies. Spine 2010; 35:E475–80.
64. Le Maitre CL, Baird P, Freemont AJ, Hoyland JA. An in vitro study investigating the survival and phenotype of mesenchymal stem cells following injection into nucleus pulposus tissue. Arthritis Res Ther 2009;11:R20.
65. Yamamoto Y, Mochida J, Sakai D, Nakai T, Nishimura K, Kawada H, et al. Upregulation of the viability of nucleus pulposus cells by bone marrow-derived stromal cells: significance of direct cell-to-cell contact in coculture system. Spine 2004;29;1508–14.
66. Le Visage C, Kim SW, Tateno K, Sieber AN, Kostuik JP, Leong KW. Interaction of human mesenchymal stem cells with disc cells: changes in extracellular matrix biosynthesis. Spine 2006;31:2036–42.
67. Yang SH, Wu CC, Shih TF, Sun YH, Lin FH. In vitro study on interaction between human nucleus pulposus cells and mesenchymal stem cells through paracrine stimulation. Spine 2008;33:1951–7.
68. Watanabe T, Sakai D, Yamamoto Y, Iwashina T, Serigano K, Tamura F, et al. Human nucleus pulposus cells significantly enhanced biological properties in a coculture system with direct cell-to-cell contact with autologous mesenchymal stem cells. J Orthop Res 2010;28:623–30.
69. Deans RJ, Moseley AB. Mesenchymal stem cells: biology and potential clinical uses. Exp Hematol 2000;28:875–84.
70. Richardson SM, Hughes N, Hunt JA, Freemont AJ, Hoyland JA. Human mesenchymal stem cell differentiation to NP-like cells in chitosan-glycerophosphate hydrogels. Biomaterials 2008;29:85–93.
71. Wuertz K, Godburn K, Neidlinger-Wilke C, Urban J, Latridis JC. Behavior of mesenchymal stem cells in the chemical microenvironment of the intervertebral disc. Spine 2008;33:1843–9.
72. Vadalà G, Studer RK, Sowa G, Spiezia F, Iucu C, Denaro V, et al. Coculture of bone marrow mesenchymal stem cells and nucleus pulposus cells modulate gene expression profile without cell fusion. Spine 2008;33:870–6.
73. Bron JL, Helder MN, Meisel HJ, Van-Royen BJ, Smit TH. Repair, regenerative and supportive therapies of the annulus fibrosus: achievements and challenges. Eur Spine J 2009; 18:301–13.
74. Hiyama A, Mochida J, Sakai D. Stem cell applications in intervertebral disc repair. Cell Mol Biol 2008;54:24–32.
75. Kimelman N, Oskouian RJ, Zilberman Y, Pelled G, Gazit Z, Gazit D. Genetically engineered adult stem cells and hybrid scaffolds as a platform for intervertebral disc regeneration. Mol Ther 2006;13:S175–6.
76. Ryan JM, Barry F, Murphy JM, Mahon BP. Interferon-gamma does not break, but promotes the immunosuppressive capacity of adult human mesenchymal stem cells. Clin Exp Immunol 2007;149:353–63.
77. Haynesworth SE, Baber MA, Caplan AI. Cytokine expression by human marrow-derived mesenchymal progenitor cells in vitro: effects of dexamethasone and IL-1 alpha. J Cell Physiol 1996;166:585–92.
78. Caplan AI, Dennis JE. Mesenchymal stem cells as trophic mediators. J Cell Biochem 2006;98:1076–84.
79. Masuda K, Oegema TR Jr, An HS. Growth factors and treatment of intervertebral disc degeneration. Spine 2004;29: 2757–69.
80. Pratsinis H, Kletsas D. Growth factors in intervertebral disc homeostasis. Connect Tissue Res 2008;49:273–6.
81. Gruber HE, Fisher EC Jr, Desai B, Stasky AA, Hoelscher G, Hanley EN. Human intervertebral disc cells from the annulus: three-dimensional culture in agarose or alginate and responsiveness to TGF-β1. Exp Cell Res 1997;235:13–21.
82. Thompson JP, Oegema TR Jr, Bradford DS. Stimulation of mature canine intervertebral disc by growth factors. Spine 1991;16:253–60.
83. Osada R, Ohshima H, Ishihara H, Yudoh K, Sakai K, Matsui H, et al. Autocrine/paracrine mechanism of insulin-like growth factor-1 secretion, and the effect of insulin-like growth factor-1 on proteoglycan synthesis in bovine intervertebral discs. J Orthop Res 1996;14:690–9.
84. Gruber HE, Norton HJ, Hanley EN Jr. Anti-apoptotic effects of IGF-1 and PDGF on human intervertebral disc cells in vitro. Spine 2000;25:2153–7.
85. Pratsinis H, Kletsas D. PDGF, bFGF and IGF-I stimulate the proliferation of intervertebral disc cells in vitro via the activation of the ERK and Akt signaling pathways. Eur Spine J 2007;16:1858–66.
86. Li X, Leo BM, Beck G, Balian G, Anderson DG. Collagen and proteoglycan abnormalities in the GDF-5-deficient mice and molecular changes when treating disk cells with recombinant growth factor. Spine 2004;29:2229–34.
87. Masuda K, Takegami K, An H, Kumano F, Chiba K, Andersson GB, et al. Recombinant osteogenic protein-1 upregulates extracellular matrix metabolism by rabbit annulus fibrosus and nucleus pulposus cells cultured in alginate beads. J Orthop Res 2003;21:922–30.
88. Tim Yoon S, Su Kim K, Li J, Soo Park J, Akamaru T, Elmer WA, Hutton WC. The effect of bone morphogenetic protein-2 on rat intervertebral disc cells in vitro. Spine 2003;28:1773–80.
89. Gilbertson L, Ahn SH, Teng PN, Studer RK, Niyibizi C, Kang JD. The effects of recombinant human bone morphogenetic protein-2, recombinant human bone morphogenetic protein-12, and adenoviral bone morphogenetic protein-12 on matrix synthesis in human annulus fibrosis and nucleus pulposus cells. Spine J 2008;8:449–56.
90. Mochida J. New strategies of disc repair; novel preclinical trials. J Orthop Sci 2005;10:112–8.
91. Yan HS, Wu XT, Wang YT, Shao JS, Cao Y, Bao JP, et al. The rise and fall of the trophic and differentiated effects in cell therapy for regeneration of degenerative intervertebral discs. Chin J Exp Surg 2009;26:125–7.
92. Wei A, Brisby H, Chung SA, Diwan AD. Bone morphogenetic protein-7 protects human intervertebral disc cells in vitro from apoptosis. Spine J 2008;8:466–74.
93. Zhao CQ, Wang LM, Jiang LS, Dai LY. Programmed cell death in intervertebral disc degeneration. Apoptosis 2006;11:2079–88.
94. Broker LE, Kruyt FA, Giaccone G. Cell death independent of caspases: a review. Clin Cancer Res 2005;11:3155–62.
95. Park JB, Lee JK, Park SJ, Kim KW, Riew KD. Mitochondrial involvement in fas-mediated apoptosis of human lumbar disc cells. J Bone Joint Surg Am 2005;87:1338–42.
96. Ariga K, Yonenobu K, Nakase T, Hosono N, Okuda S, Meng W, et al. Mechanical stress-induced apoptosis of endplate chon-

drocytes in organ-cultured mouse intervertebral discs: an ex vivo study. Spine 2003;28:1528–33.
97. Tschoeke SK, Hellmuth M, Hostmann A, Robinson Y, Ertel W, Oberholzer A, et al. Apoptosis of human intervertebral discs after trauma compares to degenerated discs involving both receptor-mediated and mitochondrial-dependent pathways. J Orthop Res 2008;26:999–1006.
98. Park JB, Park IC, Park SJ, Jin HO, Lee JK, Riew KD. Anti-apoptotic effects of caspase inhibitors on rat intervertebral disc cells. J Bone Joint Surg Am 2006;88:771–9.
99. Tapp H, Deepe R, Ingram JA, Yarmola EG, Bubb MR, Hanley EN Jr, et al. Exogenous thymosin beta(4) prevents apoptosis in human intervertebral annulus cells in vitro. Biotech Histochem 2009;84:287–94.
100. Kang Y, Liao WM, Sheng PY, Zhang LJ, Yuan XW, Lei L, et al. Construction and identification of human bone morphogenetic protein-7 recombinant adeno-associated virus type 2 vector and its expression in bone mesenchymal stem cells. Zhonghua Wai Ke Za Zhi 2007;45:1279–83 (in Chinese).
101. Risbud MV, Guttapalli A, Stokes DG, Hawkins D, Danielson KG, Schaer TP, et al. Nucleus pulposus cells express HIF-1 alpha under normoxic culture conditions: a metabolic adaptation to the intervertebral disc microenvironment. J Cell Biochem 2006;98:152–9.
102. Zeng Y, Danielson KG, Albert TJ, Shapiro IM, Risbud MV. HIF-1 alpha is a regulator of galectin-3 expression in the intervertebral disc. J Bone Miner Res 2007;22:1851–61.
103. Gruber HE, Ingram JA, Davis DE, Hanley EN Jr. Increased cell senescence is associated with decreased cell proliferation in vivo in the degenerating human annulus. Spine J 2009;9:210–5.
104. Roberts S, Evans EH, Kletsas D, Jaffray DC, Eisenstein SM. Senescence in human intervertebral discs. Eur Spine J 2006;15:S312–6.
105. Zhou HW, Hou SX, Shang WL, Mei F. Effect of P16INK4a vs Fas on function of intervertebral disc. Chin J Exp Surg 2006;23:1505–7.
106. Nakai T, Mochida J, Sakai D. Synergistic role of c-Myc and ERK1/2 in the mitogenic response to TGF beta-1 in cultured rat nucleus pulposus cells. Arthritis Res Ther 2008;10:R140.
107. Gruber HE, Hoelscher GL, Ingram JA, Bethea S, Hanley EN. IGF-1 rescues human intervertebral annulus cells from in vitro stress-induced premature senescence. Growth Factors 2008; 26:220–5.
108. Niu CC, Yuan LJ, Lin SS, Chen LH, Chen WJ. Mesenchymal stem cell and nucleus pulposus cell coculture modulates cell profile. Clin Orthop 2009;467:3263–72.
109. Lepperdinger G, Brunauer R, Jamnig A, Laschober G, Kassem M. Controversial issue: is it safe to employ mesenchymal stem cells in cell-based therapies? Exp Gerontol 2008;43:1018–23.
110. Momin EN, Vela G, Zaidi HA, Quiñones-Hinojosa A. The oncogenic potential of mesenchymal stem cells in the treatment of cancer: directions for future research. Curr Immunol Rev 2010;6:137–48.
111. Sakai D. Future perspectives of cell-based therapy for intervertebral disc disease. Eur Spine J 2008;17:S452–8.