

Biologic Treatment of Mild and Moderate Intervertebral Disc Degeneration

Elias S Vasiliadis,¹ Spyros G Pneumaticos,¹ Demitrios S Evangelopoulos,¹ and Athanasios G Papavassiliou²

¹Third Department of Orthopaedic Surgery, University of Athens Medical School, KAT Hospital, Athens, Greece; and the

²Department of Biological Chemistry, University of Athens Medical School, Athens, Greece

Disc degeneration is the most common cause of back pain in adults and has enormous socioeconomic implications. Conservative management is ineffective in most cases, and results of surgical treatment have not yet reached desirable standards. Biologic treatment options are an alternative to the above conventional management and have become very attractive in recent years. The present review highlights the currently available biologic treatment options in mild and moderate disc degeneration, where a potential for regeneration still exists. Biologic treatment options include protein-based and cell-based therapies. Protein-based therapies involve administration of biologic factors into the intervertebral disc to enhance matrix synthesis, delay degeneration or impede inflammation. These factors can be delivered by an intradiscal injection, alone or in combination with cells or tissue scaffolds and by gene therapy. Cell-based therapies comprise treatment strategies that aim to either replace necrotic or apoptotic cells, or minimize cell death. Cell-based therapies are more appropriate in moderate stages of degenerated disc disease, when cell population is diminished; therefore, the effect of administration of growth factors would be insufficient. Although clinical application of biologic treatments is far from being an everyday practice, the existing studies demonstrate promising results that will allow the future design of more sophisticated methods of biologic intervention to treat intervertebral disc degeneration.

Online address: <http://www.molmed.org>

doi: 10.2119/molmed.2014.00145

INTRODUCTION

The spinal column sustains continuous movements in different directions to provide the vital flexibility of the human body. Motion is achieved through the elastic intervertebral discs, which lie between the rigid vertebrae. Flexion, extension, lateral bending and rotation exert mechanical forces over the intervertebral discs, which are well tolerated if they are within specific limits. If mechanical loading exceeds a certain magnitude or duration, then structural changes occur, leading to disc degeneration (1). Apart from the mechanical theory, disc degeneration may occur as a consequence of genetic

disorders and environmental factors.

These affect a number of matrix components, which may be deficient or malfunctioning and thereby altering the mechanical properties of the intervertebral disc (2).

Despite the fact that our knowledge regarding disc degeneration has increased over the past years, the exact cause(s) that triggers the degradation cascade remains elusive. Disc degeneration is the result of an imbalance between matrix synthesis and degradation (3). Failures at the molecular level result in changes at the microscopic level and, eventually, disorganization of microstructure of the

intervertebral disc at the macroscopic level, which is clinically evident for the patient. Disc degeneration is the most common cause of back pain in adults, leading to enormous socioeconomic implications. Treatment options include administration of pharmaceutical agents that are clinically indicated (steroids, nonsteroidal antiinflammatory drugs [NSAIDs], analgesics), physiotherapy and a variety of surgical interventions for a selected group of patients. Although conservative measures at some level represent one of the effective methods to treat intervertebral disc degeneration, they are aiming at the symptoms and not the cause of the disease. By contrast, surgical treatment lacks sustainable long-term effects in most cases (4). Biologic therapies approach the condition at a molecular level, in an attempt to alter the process cascade rather than treat the patient symptomatically; therefore, they could be considered an etiologic method of treatment. In this manner, these novel techniques are gaining popularity in recent years.

Address correspondence to Athanasios G Papavassiliou, Department of Biological Chemistry, Medical School, University of Athens, 75, M. Asias Street, 11527 Athens, Greece. Phone: +30-210-746-2508/9; Fax: +30-210-779-1207; E-mail: papavas@med.uoa.gr. Submitted July 22, 2014; Accepted for publication August 25, 2014; Epub (www.molmed.org) ahead of print August 25, 2014.

The Feinstein Institute
for Medical Research 

Empowering Imagination. Pioneering Discovery.®

The understanding of the different mechanisms that cause disc degeneration at the molecular level is crucial when designing biologic treatment strategies. The identification of possible targets for biologic therapies is the first step of this procedure.

There are three different anatomical zones of the intervertebral disc; the inner nucleus pulposus, the outer annulus fibrosus and the cartilaginous end plate (5). Nucleus pulposus lies in the central part of the disc and is composed of chondrocyte-like cells, water and a highly gelatinous extracellular matrix that contains mainly collagen type II and aggrecan. Aggrecan is a large aggregating proteoglycan consisting of a protein core and up to 100 glycosaminoglycans (GAGs) chains (mostly chondroitin and keratan sulfate, which provide the osmotic pressure for attracting water molecules and maintaining disc hydration). Highly hydrated discs absorb compression forces and distribute hydraulic pressure in all directions when loaded. The water content of the disc depends mainly on aggrecan content. In disc degeneration, the amount of water and GAGs decrease, and thus the nucleus pulposus loses its hydrostatical properties, and the annulus fibrosus and the end plate are sustaining cracks and fissures because of the high stresses applied on them (6). The cartilaginous end plates are progressively ossified and eventually prevent nutrient supply to the intervertebral disc, resulting in cell death (7). Treatments that could potentially regenerate the end plates or restore aggrecan content in nucleus pulposus could be ideal concepts for the treatment of intervertebral disc degeneration.

Disc degeneration is characterized by a reduction in the number of disc cells, because of cell necrosis and/or apoptosis (8). Survival of disc cells is vital for synthesizing matrix components and therefore constitute the key target in designing biologic therapies for disc degeneration. Biologic therapies can be protein-based when they refer to biomolecules with anabolic properties and cell-based when

they involve administration of cells. The aim of such therapies is either to stimulate disc cells to upregulate the production of specific proteins during the early stages or to administer active cells to replace the necrotic cells in more advanced stages of disc degeneration.

Here we review the currently available biologic treatment options in mild and moderate disc degeneration, where a potential for regeneration still exists. Advanced degeneration requires different biologic treatment options, with tissue engineering being the most promising strategy.

PROTEIN-BASED THERAPIES

Protein-based therapies include all treatment strategies that involve administration of biologic factors into the intervertebral disc to enhance matrix synthesis, delay degeneration or stop inflammation. These factors can be delivered by an intradiscal injection, alone or in combination with cells or tissue scaffolds as well as by gene therapy.

A direct injection into the intervertebral disc requires repeated doses because disc degeneration is a chronic condition and these factors have short biologic half-lives; therefore, their clinical use might be limited (9). To overcome this problem, gene therapy has been introduced as a method for ensuring prolonged anabolic effect by delivering genetic material expressing growth factors into disc cells (10). Delivery of genetic material is achieved using vectors, either by direct injection (*in vivo*) or after removal of disc cells, application of the vector *in vitro* and return of the modified cells to the disc (*ex vivo*). *Ex vivo* technique is more appropriate in moderate disc disease, where the number of healthy disc cells is insufficient. Vectors can be viral or nonviral. Viral vectors are genetically modified viruses that lack the pathogenic genetic material but maintain the genetic information for insertion into disc cells together with the therapeutic gene that may be transferred into the disc cells. These vectors can be genome incorporating, such as retroviruses (11)

and lentiviruses (12), and non-genome incorporating, such as herpes viruses, adenoviruses and adeno-associated viruses (13).

The major concerns of gene therapy are safety, efficiency and duration of gene expression. Additional concerns include practicality of *ex vivo* methods, patient acceptance of viral-mediated methods and the choice of genes.

Immunogenicity of vectors and long-term delivery of the transduced genes are key issues that have to be resolved before application of gene therapy for the treatment of degenerative disc disease in humans. Retroviral vectors are ideal for *ex vivo* gene transfer, but are of limited value in treating disc degeneration because retroviruses transfer genes that are replicating. Numerous studies investigated the use of adenoviral vectors in gene therapy of disc degeneration (11,14–16). Because adenoviruses are non-genome incorporating, the risk of oncogenesis is reduced and low cell turnover of the intervertebral disc compensates for the fact that their therapeutic genes are not transferred to daughter cells when the disc cells replicate (15). Adenoviruses can infect nondividing cells, but they cause severe immune reaction. An alternative is adeno-associated viruses, with minimal immune response, but with lower efficiency of transduction (17).

Factors Enhancing Matrix Synthesis

Specific biomolecules can induce matrix biosynthesis at the protein or at the gene level. These biomolecules include growth and anabolic factors and require the presence of viable disc cells to be effective. Therefore, treatment of disc degeneration with factors that enhance matrix synthesis is appropriate for patients with early stages of disc degeneration.

Proteins of the bone morphogenetic protein (BMP) family were found to upregulate proteoglycan synthesis in animal models (18,19). BMP-2 (20) and BMP-7 or osteogenic protein-1 (OP-1) (21) increased proteoglycan production in disc cells. The use of recombinant human BMP-2 (rhBMP-2) and BMP-12 in

human disc cells increased collagen and proteoglycan production in nucleous pulposus cells, but not in anulus fibrosus (22). Injection of rhBMP-7 increased disc height of rabbit discs (23) and can prevent degeneration at 24 wks of allogenic intervertebral discs, which were injected with nucleus pulposus cells expressing rhBMP-7; and then they were transplanted into a canine spine (24).

Administration of growth and differentiation factor-5 (GDF-5) into mouse disc cells resulted in increased proteoglycan and collagen type II synthesis (25). rhGDF-5 increased proteoglycan and collagen synthesis in bovine intervertebral discs *in vitro* and restored disc height and improved magnetic resonance imaging (MRI) and histologic grading scores in rabbit intervertebral discs *in vivo* (26). GDF-5 was successfully transferred into rabbit disc cells with *ex vivo* gene therapy and increased the expression of genes for extracellular matrix proteins (27). RhGDF-5 restored disc height, improved GAGs content and increased collagen type II mRNA levels after being inserted into a rat intervertebral disc encapsulated in poly(lactic glycolic acid) (PLGA) microspheres (28). OP-1 and GDF-5 are currently used in phase I clinical trials for human disc degeneration after approval by the U.S. Food and Drug Administration, with results pending (18).

Additionally, administration of transforming growth factor (TGF)- β (29) and epidermal growth factor (EGF) (30) into mouse disc cells upregulated the mRNA expression of aggrecan and type I and II collagen. TGF- β 1 and rhBMP-2 treatment increased the matrix-related mRNA expression in human cervical and lumbar nucleus pulposus cells from degenerative discs *in vitro* (31).

A limitation in the application of TGF and insulinlike growth factor (IGF) for the treatment of degenerative disc disease is that their receptors were found in blood vessels of the intervertebral disc (32); therefore, their injection might stimulate angiogenesis and consequently nerve ingrowth, which are implicated in exacerbation of symptoms in interverte-

bral disc degeneration (33). On the other hand, BMP receptors were not identified in intervertebral disc blood vessels; therefore, growth factors of the BMP family could be more effective in the treatment of intervertebral disc degeneration (32).

Results of several studies regarding the positive effect of solely injected growth factors into the degenerated intervertebral discs generated the idea of administration of platelet-rich plasma (PRP), which contains many growth factors (34). PRP upregulated matrix gene expression and stimulated cell proliferation of porcine nucleus pulposus and anulus fibrosus cells and activated key regulators of the chondrogenic phenotype of disc cells *in vitro* (35,36). Moreover, PRP induced the reparative capacity (restoration of disc height) of rabbit intervertebral disc injured by scalpel stab (37).

Apart from direct injection of growth factors, gene therapy has been used to enhance matrix synthesis in intervertebral disc cells. Transduction of the TGF- β gene by using an adenovirus vector significantly increased proteoglycan synthesis in nucleus pulposus cells *in vivo* (38). Additionally, transduction of a combination of IGF-1, BMP-2, TGF- β and BMP-12 genes produced a significant anabolic effect in nucleus pulposus cells (39). A future gene therapy approach could involve the combination of different growth factors that enhance matrix synthesis.

Recent studies demonstrated that intradiscal injection of the pharmacological agents simvastatin and lovastatin in rabbit intervertebral discs promoted chondrogenesis and upregulated the gene expression of aggrecan and collagen type II (40,41). Another group of biomolecules that potentially could enhance matrix synthesis are transcription factors. Delivery of latent membrane protein-1 (LMP-1) by an adenovirus vector resulted in upregulation of BMP-2 and BMP-7 and consequently increased aggrecan production by nucleus pulposus cells *in vitro* (42). LMP-1 upregulates BMP-2 and BMP-7 and, through them, it increases aggrecan synthesis and collagen type I

and II. Adenovirus vector was used to transfer the LMP-1 gene in anulus fibrosus cells and chondrocytes, where it similarly increased proteoglycan and collagen production (43). Transfection of degenerated human intervertebral disc cells with an adenovirus vector expressing SOX9 resulted in increased synthesis of collagen type II (44), restored the height of the degenerative rabbit intervertebral disc and promoted the expression of proteoglycan and collagen type II when administered with OP-1 as a dual gene therapy (45).

The various factors enhancing matrix synthesis, the way of their administration and the effect on the intervertebral disc metabolism are summarized in Table 1.

Factors Delaying Degeneration

Considering intervertebral disc degeneration as an imbalance between synthesis and degradation of matrix components, cessation of the catabolic cascade could be a realistic therapeutic target. Although most researchers have focused on growth factors, there are a few studies exploring the potential role of anticatabolic factors in the treatment of disc degeneration.

In disc degeneration, there is an overexpression of proteases such as matrix metalloproteinases (MMPs) and a disintegrin and metalloproteinase with thrombospondin motifs (ADAMTS), which degrade various matrix components. These degrading activities are mediated by cytokines, such as interleukin (IL)-1 and tumor necrosis factor (TNF)- α (46). ADAMTS-5 siRNA inhibited matrix degradation after injection in a rabbit degenerated disc and improved histologic grades of nucleus pulposus tissue (47). OP-1 has an additional anticatabolic effect, since it was found to block TNF- α -induced upregulation of ADAMTS-4 and ADAMTS-5, leading to reversion of TNF- α -mediated degradation of aggrecan and collagen type II in human intervertebral discs *in vitro* (48). Etanercept, a TNF- α antagonist, improved the symptoms of patients with persistent discogenic pain (49), and TNF- α receptors

Table 1. Studies with factors enhancing matrix synthesis, the way of administration, and the effect of their application on the intervertebral disc cells.

Factor	Administration	Effect	References
BMP-2	<i>In vitro</i> culture of rat NP cells	Increase of collagen type II and proteoglycans	20
BMP-2, BMP-12, and adenoviral BMP-12	<i>In vitro</i> incubation of human NP and AF cells	Increase of collagen and proteoglycans only in NP cells with BMP-2 and BMP-12, in both NP and AF cells with adenoviral BMP-12	22
BMP-7 (OP-1)	Direct injection into rabbit IVD	Increased disc height, proteoglycan synthesis and decreased degeneration grades	21,23
GDF-5	<i>In vitro</i> cultured bovine IVD cells	Increase of collagen and proteoglycan synthesis	26
GDF-5	Direct injection into rabbit IVD	Restore IVD height, MRI, and histologic grading scores	26
GDF-5	<i>In vitro</i> treatment of mouse IVD cells; gene therapy	Increased expression of genes for matrix synthesis	27
GDF-5	Direct injection into rat IVD after encapsulated in PLGA microspheres	Restored disc height, improved GAGs content and collagen type II content	28
TGF- β and EGF	<i>In vitro</i> cultured rabbit NP cells	Upregulated mRNA expression of aggrecan and type I and II collagen	29
TGF- β and BMP-12	<i>In vitro</i> cultured human NP cells	Increased matrix-related mRNA expression	31
PRP	<i>In vitro</i> cultured human NP cells	Upregulation of Sox9, collagen type II and aggrecan genes	35
PRP	<i>In vitro</i> cultured porcine NP and AF cells	Upregulated proteoglycan and collagen synthesis and cell proliferation	36
PRP	Direct injection into rabbit IVD	Restoration of disc height, increase of chondrocyte-like cells	37
TGF- β	Gene therapy, <i>in vivo</i> rabbit IVD	Increased proteoglycan synthesis	38
IGF-1 and BMP-2, and TGF- β and BMP-12	Gene therapy, <i>in vitro</i> cultured human IVD cells	Increased proteoglycan synthesis	39
Simvastatin	Direct injection into rat IVD	Increased gene expression of aggrecan and collagen type II, improved degeneration grade	40
Lovastatin	Direct injection into rat IVD	Upregulated gene expression of aggrecan, collagen type II, BMP-2, and Sox-9 genes	41
LMP-1	Gene therapy, <i>in vivo</i> in rabbits	Increased proteoglycan, BMP-2 and BMP-7 synthesis	42
LMP-1	<i>In vitro</i> cultured AF cells and chondrocytes	Increased proteoglycan production, upregulation of mRNA expression of aggrecan, collagen type I and type II, BMP-2 and BMP-7	43
Sox-9	Gene therapy, <i>in vitro</i> cultured human IVD cells	Increased collagen type II synthesis	44
Sox-9	Gene therapy, <i>in vivo</i> in rabbit IVD	Chondrocytic phenotype of IVD, restored architecture of NP	44
Sox-9 and OP-1	Dual gene therapy, <i>in vivo</i> rabbit IVD	Restoration of disc height, upregulation of collagen type II and proteoglycan genes	45

IVD, intervertebral disc.

neutralized the effects of TNF- α on human intervertebral cells *in vitro* (50).

IGF-1 and platelet-derived growth factor (PDGF) when applied to serum-depleted anulus fibrosus cells significantly reduced the percentage of apoptotic cells (51). Transfection of nucleus pulposus cells with adenoviral vectors expressing *rhIGF-1* reversed the apoptotic rate of disc cells *in vitro* (52). In

a recent study, rhPDGF treatment significantly inhibited cell apoptosis, increased cell proliferation and matrix production and maintained mRNA expression of extracellular matrix genes of human disc cells *in vitro* (53). Furthermore, PDGF, IGF-1 and basic fibroblast growth factor (bFGF) were found to induce proliferation of bovine nucleus pulposus cells (54,55). Human annulus fibrosus cells

stimulated with TGF- β 3 and FGF-2 *in vitro* increased the expression of matrix molecules and of MMP-13 in an enriched cartilaginous matrix (56).

Injection of the synthetic peptide Link-N (which has growth factor properties) in the degenerated discs of rabbits after an annular puncture downregulated the expression of *MMP-3* and *ADAMTS-4* genes in both the anulus fibrosus and nucleus

Table 2. Studies with factors delaying disc degeneration and factors that inhibit inflammation, the way of administration and the effect of their application on the intervertebral disc cells.

Factor	Administration	Effect	References
Anti-ADAMTS-5	Gene therapy, <i>in vivo</i> in rabbit IVD	Improved MRI and histologic grade scores	47
OP-1	<i>In vitro</i> cultured human IVD cells	Reversed TNF- α -mediated degradation of matrix macromolecules	48
Etanercept	Subcutaneous injection in patients with symptomatic disc degeneration	Improved symptoms of discogenic pain	49
TNF- α antagonist	<i>In vitro</i> cultured human IVD cells	Attenuated the secretion of NO and PGE in a dose-dependent manner	50
IGF and PDGF	<i>In vitro</i> human AF cells	Reduction of the percentage of apoptotic cells	51
IGF-1	Gene therapy, <i>in vitro</i> cultured human NP cells	Reversed the apoptotic rate of NP cells	52
PDGF	<i>In vitro</i> incubated human IVD cells	Inhibited cell apoptosis, increased cell proliferation and matrix production, and maintained mRNA expression of critical extracellular matrix genes	53
PDGF and IGF-1 and bFGF	<i>In vitro</i> cultured bovine IVD cells	Stimulated proteoglycan synthesis in NP cells and proliferation of IVD cells	54,55
TGF- β and FGF-2	<i>In vitro</i> cultured human AF cells	Cartilaginous matrix was formed, enhanced expression of matrix molecules and of MMP-13	56
Link-N	Direct injection into rabbit IVD	Increased aggrecan gene expression and decreased proteinase gene expression in both the NP and AF	57
Link-N	<i>In vitro</i> human IVD cells	Promoted proteoglycan synthesis, decreased levels of ADAMTS-4, ADAMTS-5, MMP-3, MMP-13	58
TIMP-1	Gene therapy, <i>in vivo</i> rabbit IVD	Less MRI and histologic evidence of degeneration	60
TIMP-1	Gene therapy, <i>in vitro</i> cultured human IVD cells	Increased proteoglycan synthesis	69
IL-1Ra	Gene therapy, <i>ex vivo</i> in human IVD	Increased IL-1Ra protein expression, elimination of matrix degradation	15,16
IL-1Ra and TNF inhibitors	<i>In vitro</i> cultured human IVD	Decreased levels of MMP-1 and MMP-3	66
Resveratrol	<i>In vitro</i> cultured human IVD cells	Antiinflammatory and anticatabolic effect on the mRNA and protein level for IL-6, IL-8, MMP-1, MMP-3 and MMP-13	70
Resveratrol	<i>In vivo</i> in rodent IVD	Reduced pain behavior triggered by application of NP tissue on the dorsal root ganglion for up to 14 d	70

IVD, intervertebral disc.

pulposus and the *ADAMTS-5* gene in annular fibrous tissues (57). Link-N increased the expression of transcription factor SOX9 and of aggrecan and collagen type II, while it enhanced the expression of BMP-4 and BMP-7 in *ex vivo* cultured rabbit disc cells (58). In adult human discs, Link-N can promote aggrecan synthesis and reduce MMP-3, MMP-13, ADAMTS-4 and ADAMTS-5 expression in a dose-dependent manner (59).

Administration of adeno-associated vector serotype-2 (AAV2), bearing the

tissue inhibitor of metalloproteinase-1 (*TIMP-1*) gene into punctured rabbit intervertebral discs, delayed degenerative changes (60). Experimental studies with factors that delay intervertebral disc degeneration are summarized in Table 2.

Factors Inhibiting Inflammation

TNF- α and IL-1 are two proinflammatory cytokines that are upregulated in intervertebral disc degeneration. Increased TNF- α and IL-1 β upregulate nerve growth factor (NGF) expression, which

causes proliferation and penetration of nerve fibers in degenerated discs (61). Blockage of IL-1 could be a possible target to prevent the inhibition of matrix synthesis. Stimulation of human intervertebral discs *in vitro* with TNF- α and IL-1 upregulated *MMP-3* and *MMP-9* gene expression, a finding that supports the hypothesis that TNF- α is implicated in the initiation of matrix degradation (62). Application of TNF- α and IL-1 β to normal porcine annulus fibrosus cells leads to a significant increase in tissue levels of

MMP-1 (63). Inhibition of IL-1 displayed superior results when compared with inhibition of TNF- α in reducing matrix degradation in both normal and degenerated discs (64). There is adequate knowledge of administration of interleukin-1 receptor antagonists (IL-1Ra) in rheumatoid arthritis, where it is injected subcutaneously (65). However, in degenerated intervertebral disc disease, injection of IL-1Ra should be performed directly into the disc, because IL-1Ra cannot be delivered through systemic circulation because of the avascular nature of the intervertebral disc (15). The short half-life of IL-1Ra requires repeated injections to achieve the desired clinical outcome. Therefore, gene therapy for the transduction of the *IL-1Ra* gene into degenerated disc cells to inhibit the stimulation of degradative enzymes might be a solution for long-lasting positive clinical outcomes. *Ex vivo* gene therapy of IL-1Ra in human intervertebral disc explants using adenovirus-mediated vectors showed significant inhibition of the activity of degradative enzymes in degenerated discs (15,16). Incubation of human disc cells with IL-1Ra or TNF inhibitors significantly decreased levels of all forms of MMP-3 and less of MMP-1 *in vitro* (66). Mice intervertebral discs exhibited loss of proteoglycan and normal collagen structure and increased expression of matrix-degrading enzymes MMP-3, MMP-7 and ADAMTS-4 after removal of IL-1Ra (67).

Administration of PRP in combination with TNF- α and IL-1 into human nucleus pulposus cells *in vitro* suppressed the cytokine-induced proinflammatory degrading enzymes MMP-3 and cyclooxygenase-2 (COX-2) and restored the downregulated expression of collagen type II and aggrecan (68).

Inhibition of MMPs could be another target for treatment of intervertebral disc degeneration. Gene therapy of *TIMP-1*, although promising, did not address the upregulation of ADAMTS, which are also important biomolecules in the pathogenesis of disc degeneration (69).

Finally, resveratrol had antiinflammatory and anticatabolic effects on the

mRNA and protein level for IL-6, IL-8, MMP-1, MMP-3 and MMP-13 *in vitro*. When administered *in vivo* in a rodent model of radiculopathy, resveratrol significantly reduced pain behavior triggered by application of NP tissue on the dorsal root ganglion for up to 14 d (70). Studies with factors that inhibit inflammation are summarized in Table 2.

CELL-BASED THERAPIES

In intervertebral disc degeneration, a reduction of metabolically active disc cells has been noticed. Cell population should be adequate to maintain disc homeostasis. Therefore, cell-based tissue replacements or genetic modifications of resident cells could be a therapeutic alternative. Cell-based therapies include treatment strategies aiming at either replacing necrotic or apoptotic cells, or minimizing cell death. Cell-based therapies are more appropriate in later stages of degenerated disc disease, when cell population is diminished; therefore, the effect of administration of anabolic or anticatabolic factors would be insufficient, provided that minimum nutritional requirements are met for the cells to survive.

CELL REPLACEMENT

Potential sources of cells suitable for cell replacement in degenerative disc disease include autologous chondrocytes obtained from articular cartilage, disc cells harvested from intervertebral discs and mesenchymal stem cells (MSCs). Articular chondrocytes injection into degenerated discs was used *in vivo* in rabbit (71) and in porcine models (72) and *in vitro* (73) with promising results. Articular chondrocytes obtained from non-weight-bearing areas of the knee produce aggrecan and collagen type II, but the ratio of proteoglycan to collagen is lower compared with NP cells; thus, they might not be a superior source for cell replacement therapy (74).

In autologous disc cell transplantation, chondrocyte-like disc cells harvested from herniated discs are cultured *in vitro* and return to the intervertebral disc through an injection at a second stage.

Unfortunately, harvesting of autologous cells and culture *in vitro* is restricted only to herniated discs, where the number of available cells is diminished. Furthermore, injection of autologous disc cells could injure the already damaged intervertebral disc and eventually accelerate its degeneration (75).

Autologous disc cells from sand rats, which were expanded *in vitro*, demonstrated spindle-shape morphology in the anulus fibrosus and chondrocyte phenotype in nucleus pulposus after transplantation (8). Autologous disc cells, when implanted in degenerative discs of dogs, remained viable, produced matrix components similar to normal discs and retained disc height (76). Following these findings, the investigators injected autologous disc cells in humans after microdiscectomy. The patients demonstrated an increase in fluid content and in pain relief at 2 years compared with the control group (76). In another clinical trial, patients who received injection of autologous disc cell transplantation showed less reduction in fluid content compared with the control group and better pain relief at 24 months (77). Autologous disc cell transplantation is currently the only therapeutic technique that has been clinically tested in intervertebral disc degeneration and shows long-term clinical outcomes (77).

The regenerative capacity of autologous disc cells harvested from herniated discs was questioned in an *in vitro* study, where they lost their differentiation and their ability to synthesize aggrecan and collagen type II (78). Different studies evidenced that degenerated disc cells demonstrate senescence (16,79–82) and that the potential of harvested autologous disc cells that can be explanted for culture is limited (83).

MSCs are undifferentiated somatic cells, which, during embryonic development, can be differentiated into all cell lineages, but adult MSCs can only differentiate into cells of a particular germ layer. Because of ethical issues in harvesting embryonic MSCs, adult MSCs are used in clinical practice. They can be

Table 3. Studies for cell-based treatment of intervertebral disc degeneration, the source of transplanted cells, the way of administration and the effect on the intervertebral disc.

Source of cells	Administration	Effect	References
Articular chondrocytes	<i>In vivo</i> , in rabbit IVD	Increased production of hyaline-like cartilage	71
Articular chondrocytes	<i>In vivo</i> , in porcine IVD	Increased proteoglycan and collagen type II synthesis	72
Articular chondrocytes	<i>In vitro</i> , coculture with bovine NP cells	Increased aggrecan and collagen gene expression and reduced expression of MMP-3, MMP-13 and ADAMTS-5	73
Autologous chondrocyte-like cells harvested from herniated discs	<i>In vivo</i> , intradiscal injection after expansion in monolayer tissue culture	Spindle-shape morphology of cells in AF and chondrocyte phenotype in NP	8
Autologous chondrocyte-like cells harvested from herniated discs	<i>In vivo</i> , intradiscal injection in dog IVD	Cells remained viable, produced matrix components, disc height retained	76
Autologous chondrocyte-like cells harvested from herniated discs	<i>In vivo</i> , intradiscal injection in human IVD	Increase in fluid content, better pain relief at 2-years postinjection	77
Autologous chondrocyte-like cells harvested from herniated discs	Intradiscal injection	Cells lost differentiation and their ability to synthesize aggrecan and collagen type II	78
MSCs	Intradiscal injection in canine, porcine and rabbit	MSCs differentiated into NP cells phenotype, preserved water, restored disc height	92
MSCs	Direct injection into rabbit IVD	Deceleration of disc height loss, increase in T2 weighted signal intensity, GAGs increased, no differences between MSCs and NP cells	93
MSCs	<i>In vivo</i> , in rabbit IVD cells	Induce NP cells to suppress MMPs and inflammatory cytokines, increased collagen type II synthesis, increased disc height, improved MRI	94
MSCs and Link-N	Intradiscal injection in bovine IVD	Restored GAGs content, increased expression of collagen type II	95
MSCs	<i>In vivo</i> , in human IVD	Increased proteoglycan synthesis	97
MSCs	<i>In vivo</i> , in human IVD	No clinical improvement	98
MSCs and gene therapy with human telomerase reverse transcriptase	Intradiscal injection in dog IVD	Significant resistance to disc degeneration	99
MSCs and gene therapy with human TIMP-1 gene	Intradiscal injection in rabbit IVD	Increased TIMP-1 mRNA and protein expression, reduced degenerative changes	100

IVD, intervertebral disc.

obtained from bone marrow, adipose tissue, skeletal muscles, synovial membranes or umbilical cord blood or can be expanded in large numbers *in vitro*. MSCs can be used either as undifferentiated progenitors or cells with a differentiated disc cell phenotype (72) and are able to differentiate into bone, cartilage, fat and fibrous tissues (84). Additionally, recent studies revealed that disc cells themselves contain progenitor cells, which could be optimal candidates for

transplantation into degenerated intervertebral discs (85,86).

MSCs are differentiated into nucleus pulposus chondrocyte-like cells by several methods (83,87,89). *In vitro* exogenous application of growth factors, such as TGF- β and BMPs, to MSCs can stimulate their differentiation into nucleus pulposus cells (88,90). An alternative is co-cultivation of MSCs with intervertebral disc cells *in vitro*, a method that can provide a rapid production of nucleus

pulposus cells without tissue removal (87). In theory, coexistence of nucleus pulposus cells with MSCs after direct administration of MSCs into the disc could induce differentiation of the latter, but further *in vivo* studies are required (75). For this approach to be effective, the stage of degeneration should be moderate (91) and possibly the cessation of disc cells should be addressed simultaneously (84). A limitation of the above methods is that differentiation of MSCs might be in-

conclusive, and, furthermore, it is unclear whether differentiated cells remain intact or capable for replication.

Autologous bone marrow-derived MSCs are proliferating, differentiating into nucleus pulposus cell phenotype, and preserve water content and disc height into canine, porcine and rabbit models (92). Transplantation of MSCs in a rabbit degenerated intervertebral disc resulted in deceleration of disc height loss and increase in T2-weighted signal intensity. GAGs increased at 16 wks compared with controls, and disc regeneration was evidenced by real-time polymerase chain reaction (RT-PCR). No differences between MSCs and nucleus pulposus cells were observed, a finding supporting the hypothesis that MSCs are ideal substitutes for nucleus pulposus cells (93). Moreover, MSCs induce nucleus pulposus cells to suppress MMPs and inflammatory cytokine production *in vitro* (94). MSCs when administered together with Link-N can restore GAGs content and increase the expression of collagen type II in bovine mild degenerated discs (95). In clinical studies, injection of autologous bone marrow MSCs into nucleus pulposus improved pain and disability and water content was increased; albeit, disc height was not restored at 12-months postinjection (96). Similar outcomes were found in a different study where two patients with disc degeneration experienced pain relief and increase in water content of the disc at 2 years after injection of autologous MSCs (97). In another study, 10 patients who underwent intradiscal injection of hematopoietic precursor stem cells obtained from their pelvic bone marrow experienced no clinical improvement of their discogenic low back pain after 1 year (98).

Recently, an alternative treatment option to cell-based therapy was introduced for severe disc degeneration. This result includes cell-based gene delivery, where promoters are genetically incorporated into stem cells and upregulate chondrogenic and suppress osteogenic genes. Transfection of canine nucleus pulposus cells with a viral vector carrying the gene

for human telomerase reverse transcriptase (AAV-*hTERT*) and then transplantation into dog's disc showed significant resistant to disc degeneration (99).

Transfected bone marrow MSCs with a recombinant adenovirus vector carrying the *hTIMP-1* gene increased TIMP-1 mRNA and protein expression and significantly reduced degenerated changes in punctured rabbit intervertebral discs (100). Table 3 summarizes the studies regarding cell-based therapies for intervertebral disc degeneration.

CONCLUSION: OUTLOOK

Clinical application of biologic treatment of intervertebral disc degeneration is far from being an everyday practice. There are promising studies investigating the role of numerous biomolecules and stem cells *in vitro* and *in vivo* in animal models. Further knowledge concerning the molecular mechanisms underpinning disc degeneration is necessary, and a comprehensive understanding of interactions between these biomolecules will allow the design of more sophisticated methods for biologic treatment of intervertebral disc degeneration.

DISCLOSURE

The authors declare that they have no competing interests as defined by *Molecular Medicine*, or other interests that might be perceived to influence the results and discussion reported in this paper.

REFERENCES

- MacLean JJ, et al. (2003) Effects of immobilization and dynamic compression on intervertebral disc cell gene expression *in vivo*. *Spine*. 28:973–81.
- Hadjipavlou AG, Tzermianios MN, Bogduk N, Zindrick MR. (2008) The pathophysiology of disc degeneration: a critical review. *J. Bone Joint Surg. Br*. 90:1261–70.
- Le Maitre CL, Pockert A, Buttle DJ, Freemont AJ, Hoyland JA. (2007) Matrix synthesis and degradation in human intervertebral disc degeneration. *Biochem. Soc. Trans*. 35:652–5.
- Radcliff KE, et al. (2013) Adjacent segment disease in the lumbar spine following different treatment interventions. *Spine J*. 13:1339–49.
- Roberts S, Evans H, Trivedi J, Menage J. (2006) Histology and pathology of the human intervertebral disc. *J. Bone Joint Surg. Am*. 88 Suppl 2:10–14.
- Urban JPG, Roberts S, Ralphs JR. (2000) The nu-

cleus of the intervertebral disc from development to degeneration. *Amer. Zool*. 40:53–61.

- Moore RJ. (2006) The vertebral endplate: disc degeneration, disc regeneration. *Eur. Spine J*. 15 Suppl 3:333–37.
- Gruber HE, et al. (2002) Autologous intervertebral disc cell implantation: a model using *Psammomys obesus*, the sand rat. *Spine*. 27:1626–33.
- Shimer AL, Chadderdon RC, Gilbertson LG, Kang JD. (2004) Gene therapy approaches for intervertebral disc degeneration. *Spine*. 29:2770–8.
- Levicoff EA, Gilbertson LG, Kang JD. (2005) Gene therapy for disc repair. *Spine J*. 5:S287–96.
- Moon SH, et al. (2000) Human intervertebral disc cells are genetically modifiable by adenovirus-mediated gene transfer: implications for the clinical management of intervertebral disc disorders. *Spine*. 25:2573–9.
- Sugiyama O, et al. (2005) Lentivirus-mediated gene transfer induces long-term transgene expression of BMP-2 *in vitro* and new bone formation *in vivo*. *Mol. Ther*. 11:390–8.
- DelloRusso C, et al. (2002) Functional correction of adult mdx mouse muscle using gutted adenoviral vectors expressing full-length dystrophin. *Proc. Natl. Acad. Sci. U. S. A*. 99:12979–84.
- Wallach CJ, et al. (2006) Safety assessment of intradiscal gene transfer: a pilot study. *Spine J*. 6:107–12.
- Le Maitre CL, Freemont AJ, Hoyland JA. (2006) A preliminary *in vitro* study into the use of IL-1Ra gene therapy for the inhibition of intervertebral disc degeneration. *Int. J. Exp. Pathol*. 87:17–28.
- Le Maitre CL, Hoyland JA, Freemont AJ. (2007) Interleukin-1 receptor antagonist delivered directly and by gene therapy inhibits matrix degradation in the intact degenerate human intervertebral disc: an *in situ* zymographic and gene therapy study. *Arthritis Res. Ther*. 9:R83.
- Basner-Tschakarjan E, Bijjiga E, Martino AT. (2014) Pre-clinical assessment of immune responses to adeno-associated virus (AAV) vectors. *Front. Immunol*. 5:28.
- Zhang Y, Chee A, Thomar EJ, An HS. (2011) Intervertebral disc repair by protein, gene, or cell injection: a framework for rehabilitation-focused biologics in the spine. *PM R*. 3(6 Suppl 1):S88–94.
- An HS, et al. (2011) Biologic repair and regeneration of the intervertebral disk. *J. Am. Acad. Orthop. Surg*. 19:450–2.
- Tim YS, et al. (2003) The effect of bone morphogenetic protein-2 on rat intervertebral disc cells *in vitro*. *Spine*. 28:1773–80.
- Masuda K, et al. (2006) Osteogenic protein-1 injection into a degenerated disc induces the restoration of disc height and structural changes in the rabbit annular puncture model. *Spine*. 31:742–54.
- Gilbertson L, et al. (2008) 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 annu-

- lus fibrosis and nucleus pulposus cells. *Spine J.* 8:449–56.
23. Imai Y, et al. (2007) Restoration of disc height loss by recombinant human osteogenic protein-1 injection into intervertebral discs undergoing degeneration induced by an intradiscal injection of chondroitinase ABC. *Spine.* 32:1197–205.
 24. Chaofeng W, et al. (2013) Nucleus pulposus cells expressing hBMP7 can prevent the degeneration of allogenic IVD in a canine transplantation model. *J. Orthop. Res.* 31:1366–73.
 25. Li X, Leo BM, Beck G, Balian G, Anderson GD. (2004) Collagen and proteoglycan abnormalities in the GDF-5-deficient mice and molecular changes when treating disk cells with recombinant growth factor. *Spine.* 29:2229–34.
 26. Chujo T, et al. (2006) Effects of growth differentiation factor-5 on the intervertebral disc: in vitro bovine study and in vivo rabbit disc degeneration model study. *Spine.* 31:2909–17.
 27. Cui M, et al. (2008) Mouse growth and differentiation factor-5 protein and DNA therapy potentiates intervertebral disc cell aggregation and chondrogenic gene expression. *Spine J.* 8:287–95.
 28. Yan J, et al. (2013) Effects of releasing recombinant human growth and differentiation factor-5 from poly(lactic-co-glycolic acid) microspheres for repair of the rat degenerated intervertebral disc. *J. Biomater. Appl.* 29:72–80.
 29. Lee KI, et al. (2012) Tissue engineering of the intervertebral disc with cultured nucleus pulposus cells using atelocollagen scaffold and growth factors. *Spine.* 37:452–8.
 30. Thompson JP, Oegema TJ, Bradford DS. (1991) Stimulation of mature canine intervertebral disc by growth factors. *Spine.* 16:253–60.
 31. Park JY, Yoon YS, Park HS, Kuh SU. (2013) Molecular response of human cervical and lumbar nucleus pulposus cells from degenerated discs following cytokine treatment. *Genet. Mol. Res.* 12:838–51.
 32. Le Maitre CL, Richardson SM, Baird P, Freemont AJ, Hoyland JA. (2005) Expression of receptors for putative anabolic growth factors in human intervertebral disc: implications for repair and regeneration of the disc. *J. Pathol.* 207:445–52.
 33. Freemont AJ, et al. (1997) Nerve ingrowth into diseased intervertebral disc in chronic back pain. *Lancet.* 350:178–81.
 34. Wang SZ, Rui YF, Tan Q, Wang C. (2013) Enhancing intervertebral disc repair and regeneration through biology: platelet-rich plasma as an alternative strategy. *Arthritis Res. Ther.* 15:220.
 35. Chen WH, et al. (2006) Tissue-engineered intervertebral disc and chondrogenesis using human nucleus pulposus regulated through TGF-beta1 in platelet-rich plasma. *J. Cell. Physiol.* 209:744–54.
 36. Akeda K, et al. (2006) Platelet-rich plasma (PRP) stimulates the extracellular matrix metabolism of porcine nucleus pulposus and annulus fibrosus cells cultured in alginate beads. *Spine.* 31:959–66.
 37. Obata S, et al. (2012) Effect of autologous platelet-rich plasma-releasate on intervertebral disc degeneration in the rabbit annular puncture model: a preclinical study. *Arthritis Res. Ther.* 14:R241.
 38. Nishida K, et al. (1999) Modulation of the biologic activity of the rabbit intervertebral disc by gene therapy: an in vivo study of adenovirus-mediated transfer of the human transforming growth factor beta 1 encoding gene. *Spine.* 24:2419–25.
 39. Moon SH, et al. (2008) Biologic response of human intervertebral disc cells to gene therapy cocktail. *Spine.* 33:1850–5.
 40. Than KD, et al. (2014) Intradiscal injection of simvastatin results in radiologic, histologic, and genetic evidence of disc regeneration in a rat model of degenerative disc disease. *Spine J.* 14:1017–28.
 41. Hu MH, Yang KC, Chen YJ, Sum YH, Yang SH. (2014) Lovastatin prevents discography-associated degeneration and maintains the functional morphology of intervertebral discs. *Spine J.* DOI: 10.1016/j.spinee.2014.03.050 [Epub ahead of print].
 42. Yoon ST, et al. (2004) ISSLS prize winner: LMP-1 upregulates intervertebral disc cell production of proteoglycans and BMPs in vitro and in vivo. *Spine.* 29:2603–11.
 43. Kuh SU, et al. (2008) The AdLMP-1 transfection in two different cells; AF cells, chondrocytes as potential cell therapy candidates for disc degeneration. *Acta Neurochir.* 150:803–10.
 44. Paul R, et al. (2003) Potential use of Sox9 gene therapy for intervertebral degenerative disc disease. *Spine.* 28:755–63.
 45. Ren S, et al. (2013) Treatment of rabbit intervertebral disc degeneration with co-transfection by adeno-associated virus-mediated SOX9 and osteogenic protein-1 double genes in vivo. *Int. J. Mol. Med.* 32:1063–8.
 46. Wang J, et al. (2011) TNF-alpha and IL-1beta promote a disintegrin-like and metalloprotease with thrombospondin type I motif-5-mediated aggrecan degradation through syndecan-4 in intervertebral disc. *J. Biol. Chem.* 286:39738–49.
 47. Seki S, et al. (2009) Effect of small interference RNA (siRNA) for ADAMTS5 on intervertebral disc degeneration in the rabbit annular needle-puncture model. *Arthritis Res. Ther.* 11:R166.
 48. Wang Z, Hutton WC, Yoon ST. (2014) Bone morphogenetic protein-7 antagonizes tumor necrosis factor- α -induced activation of nuclear factor κ B and up-regulation of the ADAMTS, leading to decreased degradation of disc matrix macromolecules aggrecan and collagen II. *Spine J.* 14:505–12.
 49. Tobinick E, Davoodifar S. (2004) Efficacy of etanercept delivered by perispinal administration for chronic back and/or neck disc-related pain: a study of clinical observations in 143 patients. *Curr. Med. Res. Opin.* 20:1075–85.
 50. Sinclair SM, et al. (2011) Attenuation of inflammatory events in human intervertebral disc cells with a tumor necrosis factor antagonist. *Spine.* 36:1190–6.
 51. Gruber HE, Norton HJ, Hanley EN Jr. (2000) Anti-apoptotic effects of IGF-1 and PDGF on human intervertebral disc cells in vitro. *Spine.* 25:2153–7.
 52. Zhang CC, et al. (2014) Effects of adenoviral vector expressing hIGF-1 on apoptosis in nucleus pulposus cells in vitro. *Int. J. Mol. Med.* 33:401–5.
 53. Presciutti SM, et al. (2014) PDGF-BB inhibits intervertebral disc cell apoptosis in vitro. *J. Orthop. Res.* 32:1181–8.
 54. Osada R, et al. (1996) 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.* 14:690–9.
 55. Pratsinis H, Kletsas D. (2007) 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.* 16:1858–66.
 56. Hegewald AA, et al. (2013) Towards biological annulus repair: TGF- β 3, FGF-2 and human serum support matrix formation by human annulus fibrosus cells. *Tissue Cell.* 45:68–76.
 57. Mwale F, et al. (2011) The efficacy of Link N as a mediator of repair in a rabbit model of intervertebral disc degeneration. *Arthritis Res. Ther.* 13:R120.
 58. Ma K, et al. (2013) Effect of a synthetic link N peptide nanofiber scaffold on the matrix deposition of aggrecan and type II collagen in rabbit notochordal cells. *J. Mater. Sci. Mater. Med.* 24:405–15.
 59. Gawri R, et al. (2013) Best paper NASS 2013: link-N can stimulate proteoglycan synthesis in the degenerated human intervertebral discs. *Eur. Cell. Mater.* 26:107–19.
 60. Leckie SK, et al. (2012) Injection of AAV2-BMP2 and AAV2-TIMP1 into the nucleus pulposus slows the course of intervertebral disc degeneration in an in vivo rabbit model. *Spine J.* 12:7–20.
 61. Purmessur D, Freemont AJ, Hoyland JA. (2008) Expression and regulation of neurotrophins in the nondegenerate and degenerate human intervertebral disc. *Arthritis Res. Ther.* 10:R99.
 62. Millward-Sadler SJ, Costello PW, Freemont AJ, Hoyland JA. (2009) Regulation of catabolic gene expression in normal and degenerate human intervertebral disc cells: implications for the pathogenesis of intervertebral disc degeneration. *Arthritis Res. Ther.* 11:R65.
 63. Cho H, et al. (2013) Synergistic effect of combined growth factors in porcine intervertebral disc degeneration. *Connect. Tissue Res.* 54:181–6.
 64. Hoyland JA, Le Maitre CL, Freemont AJ. (2008) Investigation of the role of IL-1 and TNF in matrix degradation in the intervertebral disc. *Rheumatology.* 47:809–14.
 65. Kary S, Burmester GR. (2003) Anakinra: the first interleukin-1 inhibitor in the treatment of rheumatoid arthritis. *Int. J. Clin. Pract.* 57:231–4.
 66. Genevay S, Finckh A, Mezin F, Tessitore E, Guerne PA. (2009) Influence of cytokine inhibitors on concentration and activity of MMP-1

- and MMP-3 in disc herniation. *Arthritis Res. Ther.* 11:R169.
67. Phillips KL, Jordan-Mahy N, Nicklin MJ, Le Maitre CL. (2013) Interleukin-1 receptor antagonist deficient mice provide insights into pathogenesis of human intervertebral disc degeneration. *Ann. Rheum. Dis.* 72:1860–7.
 68. Kim HJ, et al. (2014) Anti-inflammatory effect of platelet-rich plasma on nucleus pulposus cells with response of TNF- α and IL-1. *J. Orthop. Res.* 32:551–6
 69. Wallach CJ, et al. (2003) Gene transfer of the catalytic inhibitor TIMP-1 increases measured proteoglycans in cells from degenerated human intervertebral discs. *Spine.* 28:2331–7.
 70. Wuertz K, et al. (2011) The red wine polyphenol resveratrol shows promising potential for the treatment of nucleus pulposus-mediated pain in vitro and in vivo. *Spine.* 36:E1373–84.
 71. Goresek M, et al. (2004) Nucleus pulposus repair with cultured autologous elastic cartilage derived chondrocytes. *Cell. Mol. Biol. Lett.* 9:363–73.
 72. Acosta FL Jr, et al. (2011) Porcine intervertebral disc repair using allogeneic juvenile articular chondrocytes or mesenchymal stem cells. *Tissue Eng. Part A.* 17:3045–55.
 73. Arana CJ, Diamandis EP, Kandel RA. (2010) Cartilage tissue enhances proteoglycan retention by nucleus pulposus cells in vitro. *Arthritis Rheum.* 62:3395–403.
 74. Mwale F, Roughley P, Antoniou J. (2004) Distinction between the extracellular matrix of the nucleus pulposus and hyaline cartilage: a requisite for tissue engineering of intervertebral disc. *Eur. Cell. Mater.* 8:58–63.
 75. Paesold G, Nerlich AG, Boos N. (2007) Biological treatment strategies for disc degeneration: potentials and shortcomings. *Eur. Spine J.* 16:447–68.
 76. Hohaus C, Ganey TM, Minkus Y, Meisel HJ. (2008) Cell transplantation in lumbar spine disc degeneration disease. *Eur. Spine J.* 17 Suppl 4:492–503.
 77. Meisel HJ, et al. (2006) Clinical experience in cell-based therapeutics: intervention and outcome. *Eur. Spine J.* 15:S397–405.
 78. Hegewald AA, et al. (2011) Adequacy of herniated disc tissue as a cell source for nucleus pulposus regeneration. *J. Neurosurg. Spine.* 14:273–80.
 79. Chadderdon RC, Shimer AL, Gilbertson LG, Kang JD. (2004) Advances in gene therapy for intervertebral disc degeneration. *Spine J.* 4:S341–7.
 80. Zhao CQ, Wang LM, Jiang LS, Dai LY. (2007) The cell biology of intervertebral disc aging and degeneration. *Ageing Res. Rev.* 6:247–61.
 81. Gruber HE, Ingram JA, Norton HJ, Hanley EN Jr. (2007) Senescence in cells of the aging and degenerating intervertebral disc: immunolocalization of senescence-associated beta-galactosidase in human and sand rat discs. *Spine.* 32:321–7.
 82. Roberts S, Evans EH, Kleitsas D, Jaffray DC, Einstein SM. (2006) Senescence in human intervertebral discs. *Eur. Spine J.* 15:S312–6.
 83. Richardson SM, Hughes N, Hunt JA, Freemont AJ, Hoyland JA. (2008) Human mesenchymal stem cell differentiation to NP-like cells in chitosan-glycerophosphate hydrogels. *Biomaterials.* 29:85–93.
 84. Leung VY, Chan D, Cheung KM. (2006) Regeneration of intervertebral disc by mesenchymal stem cells: potentials, limitations, and future direction. *Eur. Spine J.* 15:S406–13.
 85. Erwin WM, et al. (2013) Intervertebral disc derived stem cells: implications for regenerative medicine and neural repair. *Spine.* 38:211–6.
 86. Sakai D, et al. (2012) Exhaustion of nucleus pulposus progenitor cells with ageing and degeneration of the intervertebral disc. *Nat. Commun.* 3:1264.
 87. Richardson SM, et al. (2013) Intervertebral disc cell mediated mesenchymal stem cell differentiation. *Stem Cells.* 24:707–16.
 88. Sobajima S, et al. (2008) Feasibility of a stem cell therapy for intervertebral disc degeneration. *Spine J.* 8:888–96.
 89. Sebastine IM, Williams DJ. (2007) Current developments in tissue engineering of nucleus pulposus for the treatment of intervertebral disc degeneration. *Conf. Proc. IEEE Eng. Med. Biol. Soc.* 2007:6400–5.
 90. Risbud MV, et al. (2013) Differentiation of mesenchymal stem cells towards a nucleus pulposus-like phenotype in vitro: implications for cell-based transplantation therapy. *Spine.* 29:2627–32.
 91. Ho G, Leung VY, Cheung KM, Chan D. (2008) Effect of severity of intervertebral disc injury on mesenchymal stem cell-based regeneration. *Connect Tissue Res.* 49:15–21.
 92. Chan SC, Gantenbein-Ritter B. (2012) Intervertebral disc degeneration or repair with biomaterials and stem cell therapy-feasible or fiction? *Swiss Med. Wkly.* 142:w13598.
 93. Feng G, et al. (2011) Transplantation of mesenchymal stem cells and nucleus pulposus cells in a degenerative disc model in rabbits: a comparison of 2 cell types as potential candidates for disc regeneration. *J. Neurosurg. Spine.* 14:322–29.
 94. Miyamoto T, et al. (2010) Intradiscal transplantation of synovial mesenchymal stem cells prevents intervertebral disc degeneration through suppression of matrix metalloproteinase-related genes in nucleus pulposus cells in rabbits. *Arthritis Res. Ther.* 12:R206.
 95. Mwale F, Wang HT, Roughley P, Antoniou J, Haglund L. (2014) Link N and mesenchymal stem cells can induce regeneration of the early degenerate intervertebral disc. *Tissue Eng. Part A.* 2014, Jun 25 [Epub ahead of print].
 96. Orozco L, et al. (2011) Intervertebral disc repair by autologous mesenchymal bone marrow cells: a pilot study. *Transplantation.* 92:822–8.
 97. Yoshikawa T, Ueda Y, Miyazaki K, Koizumi M, Takakura Y. (2010) Disc regeneration therapy using marrow mesenchymal cell transplantation: a report of two case studies. *Spine.* 35:E475–80.
 98. Haufe SM, Mork AR. (2006) Intradiscal injection of hematopoietic stem cells in an attempt to rejuvenate the intervertebral discs. *Stem Cells Dev.* 15:136–7.
 99. Xin H, et al. (2013) Tissue-engineered allograft intervertebral disc transplantation for the treatment of degenerative disc disease: experimental study in a beagle model. *Tissue Eng. Part A.* 19:143–51.
 100. Yi Z, Guan Jun T, Lin C, Zifeng P. (2014) Effects of transplantation of hTIMP1-expressing bone marrow mesenchymal stem cells on the extracellular matrix of degenerative intervertebral discs in an in vivo rabbit model. *Spine.* 39:E669–75.