MolecularMedicine

Biologic Treatment of Mild and Moderate Intervertebral Disc Degeneration

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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.

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Submitted July 22, 2014; Accepted for publication August 25, 2014; Epub (www.molmed.org) ahead of print August 25, 2014.

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). Nucleous 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 pulposous 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 nucleous 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 anulous 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 polylactic 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-β1 and rhBMP-2 treatment increased the matrix-related mRNA expression in human cervical and lumbar nucleous 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 intervertebral 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 nucleous pulposus and anulous 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 nucleous 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 nucleous 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 nucleous 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 anulous 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 nucleous 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	*In vitro* culture of rat NP cells	Increase of collagen type II and proteoglycans	20
BMP-2, BMP-12, and adenoviral BMP-12	*In vitro* 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	*In vitro* 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	*In vitro* 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	*In vitro* cultured rabbit NP cells	Upregulated mRNA expression of aggrecan and type I and II collagen	29
TGF-β and BMP-12	*In vitro* cultured human NP cells	Increased matrix-related mRNA expression	31
PRP	*In vitro* cultured human NP cells	Upregulation of Sox9, collagen type II and aggrecan genes	35
PRP	*In vitro* 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, *in vivo* rabbit IVD	Increased proteoglycan synthesis	38
IGF-1 and BMP-2, and TGF-β and BMP-12	Gene therapy, *in vitro* 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, *in vivo* in rabbits	Increased proteoglycan, BMP-2 and BMP-7 synthesis	42
LMP-1	*In vitro* 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, *in vitro* cultured human IVD cells	Increased collagen type II synthesis	44
Sox-9	Gene therapy, *in vivo* in rabbit IVD	Chondrocytic phenotype of IVD, restored architecture of NP	44
Sox-9 and OP-1	Dual gene therapy, *in vivo* 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 anulous 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 nucleous pulposus cells (54,55). Human annulous fibrosus cells stimulated with TGF-β3 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 anulous fibrosus and nucleous

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, *in vivo* in rabbit IVD	Improved MRI and histologic grade scores	47
OP-1	*In vitro* 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	*In vitro* cultured human IVD cells	Attenuated the secretion of NO and PGE in a dose-dependent manner	50
IGF and PDGF	*In vitro* human AF cells	Reduction of the percentage of apoptotic cells	51
IGF-1	Gene therapy, *in vitro* cultured human NP cells	Reversed the apoptotic rate of NP cells	52
PDGF	*In vitro* 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	*In vitro* cultured bovine IVD cells	Stimulated proteoglycan synthesis in NP cells and proliferation of IVD cells	54,55
TGF-β and FGF-2	*In vitro* 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	*In vitro* human IVD cells	Promoted proteoglycan synthesis, decreased levels of ADAMTS-4, ADAMTS-5, MMP-3, MMP-13	58
TIMP-1	Gene therapy, *in vivo* rabbit IVD	Less MRI and histologic evidence of degeneration	60
TIMP-1	Gene therapy, *in vitro* cultured human IVD cells	Increased proteoglycan synthesis	69
IL-1Ra	Gene therapy, *ex vivo* in human IVD	Increased IL-1Ra protein expression, elimination of matrix degradation	15,16
IL-1Ra and TNF inhibitors	*In vitro* cultured human IVD	Decreased levels of MMP-1 and MMP-3	66
Resveratol	*In vitro* 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
Resveratol	*In vivo* 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 anulous fibrosus 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-1β 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-1β 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 nucleous 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 condrocytes 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 anulous fibrosus and chondrocyte phenotype in nucleous 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	*In vivo*, in rabbit IVD	Increased production of hyaline-like cartilage	71
Articular chondrocytes	*In vivo*, in porcine IVD	Increased proteoglycan and collagen type II synthesis	72
Articular chondrocytes	*In vitro*, 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	*In vivo*, 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	*In vivo*, intradiscal injection in dog IVD	Cells remained viable, produced matrix components, disc height retained	76
Autologous chondrocyte-like cells harvested from herniated discs	*In vivo*, 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	*In vivo*, 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	*In vivo*, in human IVD	Increased proteoglycan synthesis	97
MSCs	*In vivo*, 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 nucleous 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 nucleous 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 nucleous 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 nucleous 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 nucleous pulposus cells were observed, a finding supporting the hypothesis that MSCs are ideal substitutes for nucleous pulposus cells (93). Moreover, MSCs induce nucleous 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 nucleous 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 nucleous 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.

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