

## Disc Regeneration: A Glimpse of the Future

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Intervertebral disc (IVD) degeneration is a major cause of back pain, disability, and many important diseased states of the spine. The mechanism of disc degeneration is thought to be caused by the inability of the nucleus pulposus (NP) to maintain a fluid pressure in the disc and the consequent loss of ability to resist external loads placed on the disc.<sup>35</sup> The major components of the disc matrix are comprised of proteoglycans and collagen, which are responsible for the trapping of water via osmosis and negative charge.<sup>35</sup> Net disc matrix loss, as well as qualitative changes, are the result of an imbalance between synthesis and degradation of these components. These alterations of the disc and vertebral anatomy are associated with the pathological condition of disc degeneration.<sup>35</sup> At the present time, treatment algorithms for degenerative disc disease are limited to the symptoms of the natural processes and not the underlying biological alterations of the disc. There is currently a growing interest in developing strategies to reverse the underlying biological processes that lead to symptomatic disc degeneration. The focus of disc regeneration is to increase synthesis and decrease degradation of the disc matrix. A variety of approaches have emerged, including the use of anticatabolics, administration of growth factors, cellular transplantation, and gene transfer. However, because many of these techniques have been studied only in animals, their safety must be established before use in humans. In this chapter, we review the current advances and strategies involved in the treatment of disc degeneration.

### DISC STRUCTURE AND BIOCHEMISTRY

The IVD is composed of the NP and annulus fibrosus (AF), which are derived from the embryologically distinct notochord and mesenchymal cells. The annulus encapsulates the nucleus with well-ordered sheets of collagen arranged in different orientations. Although the AF confers excellent resistance to tensile loads across the disc, the NP functions as a shock absorber and provides resistance to compressive loads.

The disc matrix structure and composition directly determines the mechanical properties of the disc. The normal NP has an acidic pH, low oxygen tension, and paucity of

basic nutrients. The gel-like substance of the NP is normally devoid of blood vessels, making anaerobic metabolism the primary means of generating energy. Because the disc must rely on simple diffusion to exchange metabolites and waste products across the barrier of the vertebral endplates, the supply of nutrients is severely limited.<sup>13</sup> Within the disc, the cell concentration is relatively sparse, making up only approximately 1% of the disc volume. Although disc cells can survive in an environment devoid of oxygen, they are highly dependent on the availability of glucose.<sup>4</sup>

The disc is primarily comprised of water, proteoglycans, collagens, and other proteins (*Table 21.1*). Water constitutes 70 to 80% of the weight of the nucleus. Proteoglycans represent a special class of glycoprotein that are heavily glycosylated. They consist of a core protein with one or more covalently attached glycosaminoglycan (GAG) chains. These GAG chains are long, linear carbohydrate polymers that are negatively charged under physiological conditions, because of the occurrence of sulphate and uronic acid groups. Proteoglycans can be categorized depending on the nature of their glycan chains. The most common GAG side chains in discs are chondroitin sulfate and keratan sulfate, with the former predominating in the normal disc and the latter in the degenerated disc.<sup>5</sup> Two classes of proteoglycans are present in the IVD: large aggregating (aggrecan and versican) and small interstitial (biglycan, decorin, fibromodulin, and lumican) proteoglycans.<sup>8</sup> Large proteoglycans are substituted with several negatively charged GAGs, and, through their water-binding capacity, are responsible for providing the tissue with resilience and high hydrostatic pressures.<sup>43</sup> This is especially true in the case of aggrecan, which is the major high molecular weight proteoglycan of IVD and articular cartilage. Small proteoglycans bind to collagens, growth factors, and other matrix components and are thought to play important roles in the regulation of extracellular matrix (ECM) assembly and repair after injury.<sup>8</sup> The family of small proteoglycans is characterized by a leucine-rich core protein substituted by a few GAG side chains.

Several different types of fibrillar and nonfibrillar collagens are also found in the disc, with Type I and Type II collagen constituting the predominant types. Although Type I collagen is found primarily in the AF, Type II collagen is found throughout the disc and is the major fibrillar collagen

TABLE 21.1. Intervertebral disc components<sup>a</sup>

Disc-matrix proteins	Proteoglycans	Collagen	Disc proteinases
Fibronectin	Aggrecan (most abundant)	Fibril-forming collagens	Metalloproteinases (MMPs)
Elastin	Versican	Type I, 0–80%	Collagenases (MMP1, MMP8, and MMP13)
CILP	Decorin	Type II, 0–80%	
Asporin	Biglycan	Type III, <5%	Gelatinases (MMP2 and MMP9)
	Fibromodulin	Type V, 1–2%	
	Lumican	Type XI, 1–2%	Stromelysin (MMP3)
	Perlecan	Short helix collagens	
		Type VI, 5–20%	ADAMS
		Type IX, 1–2%	
		Type XII, <1%	

<sup>a</sup>CILP, cartilage intermediate layer protein; ADAMS, .

in the nucleus. It forms a fibrillar network that serves as a scaffold for the proteoglycans. Type II collagen production decreases and breakdown increases, whereas Type I collagen content increases with aging and degeneration. Consequently, decrease of Type II collagen content is another hallmark of disc degeneration.<sup>2</sup>

### DISC DEGENERATION

Disc degeneration begins when imbalances between catabolism and synthesis of matrix proteins occurs. These changes are characterized by declining disc nutrition, loss of proteoglycan organization and concentration, a decline in cell numerical density and synthetic activity, and increased degradative enzyme activity relative to matrix synthesis.<sup>5</sup> The natural history of disc degeneration has been associated with a genetic predisposition, a tissue response to an insult or altered mechanical environment, diminished blood supply, smoking, and repetitive high mechanical loading.<sup>3,39</sup>

From a molecular standpoint, the most prominent changes in disc degeneration include the progressive loss of proteoglycan, water, and collagen II in the disc matrix of the NP. These alterations are manifest on magnetic resonance imaging (MRI) scans as loss of disc height and loss of signal on T2-weighted images. Grossly, particularly in the NP, the disc becomes less gelatinous and more fibrous, leading to cracks and fissures. More blood vessels begin to grow into the disc from the outer areas of the annulus. Subsequently, an increase in cell proliferation and apoptosis occurs.<sup>13</sup> The cartilage endplate undergoes thinning, altered cell density, formation of fissures, and sclerosis of the subchondral bone.<sup>39</sup>

Although several inflammatory mediators have been identified in degenerated discs, the pathological role played by each of these mediators is not well understood. Nitric oxide (NO), interleukin (IL)-6, prostaglandin (PG)-E<sub>2</sub>, tumor necrosis factor (TNF)- $\alpha$ , fibronectin, and matrix metallopro-

teinases (MMPs) are a few of the many mediators identified.<sup>49</sup>

MMPs are a family of enzymes that, between them, are capable of degrading the major structural components of the IVD, such as collagenases, proteoglycans, gelatins, fibronectins, and elastins. For this reason, elevated MMP levels are found in degenerate discs. MMPs can be inactivated by being bound to natural tissue inhibitors of MMPs (TIMPs), of which, four types are now known.<sup>38</sup>

NO, IL-6, and PGE<sub>2</sub> seem to be the inhibitory factors of proteoglycan synthesis. These factors are recruited into action by IL-1, which also plays a role in the direct breakdown of the proteoglycan matrix. This process of direct breakdown by IL-1 is thought to be mediated by a family of enzymes known as MMPs. IL-1 likely plays a major role in the cascade of inflammatory mediators, but the nature of that role is not well defined.<sup>49</sup>

The expression of endogenous anabolic cytokines in degenerating tissues differs highly depending on tissue, cell type, age, and species. High levels of expressions of basic fibroblast growth factor (bFGF) and transforming growth factor (TGF)- $\beta$ 1 and their receptors were found in the zone of granulation tissue in human degenerate discs.<sup>36</sup> The current consensus is that the early stage degenerative disc is capable of responding anabolically by up-regulating expression of endogenous growth factors.<sup>28</sup> It is uncertain however, whether up-regulation of these anabolic cytokines is part of the degenerative process, or is a cellular reaction to slow or inhibit the degenerative process.

### DISC THERAPY

The balance between synthesis, breakdown, and net accumulation of matrix macromolecules determines the quality and integrity of the matrix, and, thus, the mechanical behavior of the disc itself. The current goals of molecular

therapy involve the prevention or reversal of important changes in the disc ECM by concomitantly stimulating the synthesis and decreasing the degradation of matrix proteins. Therapeutic strategies under investigation for the biological treatment of disc degeneration fall in the realm of tissue engineering and gene therapy. The methods used for disc regeneration include the use of cellular components (mesenchymal stem cells [MSC], chondrocytes, culture expanded disc cells, disc allograft, etc.), matrix derivatives, and molecules influencing disc-cell metabolism, such as growth factors and anticatabolic agents.<sup>49</sup>

### ANTICATABOLICS

The MMP enzyme family of plays an important role in the normal turnover of matrix molecules, and is responsible for the degradation of collagen, aggrecan, versican, and link proteins found in the degenerated disc. Within the matrix, MMP activity is normally inhibited by TIMPs. Anticatabolics such as TIMPs prevent matrix loss by inhibiting degradative enzymes within the disc. Wallach et al.<sup>44</sup> conducted an *in vitro* study on human disc cells transfected with the *TIMP-1* gene using an adenovirus vector. In this study, increased proteoglycan synthesis and content after successful transfection was demonstrated with the adenoviral-induced *TIMP-1* gene. These results suggest that the use of anticatabolics may be useful in slowing the process of disc degeneration.

IL-1 $\beta$  and TNF- $\alpha$  have been shown to mediate the inflammatory cascade in discs and, contrariwise, anticatabolic agents, such as antagonists of IL-1 and TNF- $\alpha$ , have been shown to be chondroprotective.<sup>28</sup> Although much of the *in vitro* data remains promising, further study in higher preclinical models is required before use in humans is considered.

### GROWTH FACTORS

Growth factors, which bind to cell membranes via specific transmembrane receptors, resulting in the activation of an intercellular signaling cascade, exert biological effects, such as stimulation of cell proliferation, differentiation, migration, and apoptosis. Growth factors also regulate matrix production and repair by various types of cells (e.g., chondrocytes, skin fibroblasts, and endothelial cells).<sup>22</sup>

Growth factors have been studied extensively in articular cartilage, and have been shown to regulate matrix metabolism and cell proliferation. Clinical applications of these techniques include the injection of growth factors, scaffolds, and cell transplantation for the repair of articular cartilage defects. Because of the similarity of the phenotype of articular cartilage and disc cells, the success of these studies has led to the evolution of research in disc regeneration.

Many growth factors have been found to be present in the IVD; these include insulin-like growth factor (IGF)-1, bFGF, bone morphogenetic protein (BMP)-2, BMP-4, growth

differentiation factor (GDF)-5, platelet-derived growth factor (PDGF), and TGF $\beta$ .

Growth factors may be delivered directly via *in vivo* injection of the protein into the disc. However, because cytokines are small water-soluble molecules, they rapidly diffuse away from the IVD or become inactivated by other regulatory factors. A single injection strategy may not be optimal for treating and inducing the regenerative changes desired. On the other hand, gene therapy offers an attractive alternative method for delivering the growth factor into the IVD to induce a sustained production of the growth factors. Gene delivery, using a viral vector, may be achieved by either a direct *in vivo* injection of the virus, or alternatively, an *ex vivo* process in which target cells are harvested, transduced *in vitro*, then implanted into the affected disc. Restoration of the disc height *in vivo* typically occurs 6 to 12 weeks after injection in rabbit disc degeneration models.<sup>6,23</sup> *In vitro* and *in vivo* studies support the use of growth factor administration into the disc as a clinically effective new therapeutic intervention for treatment of IVD degeneration. The aim of growth factor treatment is acceleration of the biological repair process by the stimulation of the cellular anabolic capacity. Discs in advanced stages of degeneration do not respond well to growth factor treatment; this may be because of the inherent condition of these discs through the endplate, such as a poor nutrition state and lower cell counts.<sup>13</sup>

### TGF $\beta$

TGF $\beta$  was one of the first morphogenic molecules studied. Thompson et al.<sup>42</sup> not only reported that TGF $\beta$  increased the rate of mitosis, but also showed that TGF $\beta$  was a highly anabolic molecule leading to significantly increased proteoglycan synthesis by the cell compared with endothelial growth factor (EGF), IGF-1, PDGF, and fibroblast growth factor (FGF). TGF $\beta$  has been demonstrated *in vitro* to increase proteoglycan and collagen synthesis rates in degenerate human disc cells.<sup>16</sup> *In vivo* gene therapy studies using an adenoviral vector containing the TGF $\beta$ 1 gene that was directly injected into rabbit discs *in vivo* showed that the injection leads to an increased rate of proteoglycan synthesis. However, the injection of TGF $\beta$  did not produce an increase in disc height.<sup>30</sup>

### BMP-2

BMPs are members of the TGF $\beta$  superfamily and are multipotent proteins that are related to embryogenesis and chondrogenesis. BMPs transmit intracellular signals and affect cell function through binding to heterodimeric receptor complexes essential for cartilage formation and differentiation.<sup>50</sup>

BMP-2 has been shown *in vitro* studies to increase cell proliferation, PG and collagen Type II synthesis, aggrecan, SOX9, TGF $\beta$ 1, BMP-7 messenger ribonucleic acid (mRNA)

expression, and osteocalcin in monolayer cultures of rat AF cells. The increase of aggrecan and Type II collagen mRNA level suggest that BMP-2 stimulates cells to express a more chondrocytic phenotype.<sup>18</sup> These changes reverse the matrix changes characteristic of disc degeneration.

### BMP-7 (Osteogenic Protein-1)

In chondrocytes, BMP-7, also known as osteogenic protein (OP)-1, stimulates the synthesis of proteoglycans and Type II collagen. In vitro studies with human and rabbits IVD cells demonstrated that OP-1 strongly stimulated the production and formation of PG and collagen in a dose-dependent manner. The effect was demonstrated to be present in both AF and NP cells after the depletion of ECM after exposure of IVD cells to IL-1.<sup>21</sup> Disc cells in early stages of degeneration respond better than those in advanced stages when treated with OP-1, with increased deoxyribonucleic acid (DNA) content, PG synthesis and accumulation, and collagen synthesis.<sup>25</sup> In vivo injection of OP-1 induced a restoration of disc height at 6 weeks, which was sustained for the entire experimental period, up to 24 weeks after the injection. The degree of disc degeneration was significantly less in OP-1-treated discs as assessed by proteoglycan content of the NP and AF, MRI scan grading of water content in the NP, and histological grading.<sup>23</sup>

### LIM Mineralization Protein-1

LIM mineralization protein (LMP)-1 is an intracellular regulatory molecule that is known to induce the secretion of multiple different BMPs from leukocytes and osteoblasts. LMP-1 acts to increase the expression of multiple different cytokines, including BMP-2 and BMP-7. In vitro studies with the administration of LMP-1 have shown increased production of aggrecan, BMP-7, and LMP-1 mRNA. The use of noggin, a BMP inhibitor, suggested that the mechanism behind the increased production of aggrecan was the upregulation of production of BMP-2 and BMP-7.<sup>48</sup> Because LMP-1 induces the production of both BMP-2 and BMP-7 (heterodimers), it has the potential to have up to 20 times more activity than homodimers of BMP-2 or BMP-7 in bone formation assays.

### Growth and Differentiation Factor-5

Growth and differentiation factor (GDF)-5 is a member of the BMP family, and is also called cartilage-derived morphogenetic protein (CDMP)-1. GDF-5 was originally found to be a factor responsible for skeletal alterations in mice, stimulating the formation and proliferation of chondrogenic cells,<sup>6</sup> and is necessary for normal synovial joint morphogenesis and limb formation.<sup>46</sup> GDF-5-deficient mice demonstrated disc degeneration characterized by a low T2-weighted MRI scan signal intensity and loss of normal lamellar architecture of the AF, and a shrunken, disorganized NP, with a decreased PG content. In monolayer cultures,

GDF-5 stimulated cell proliferation and aggrecan and collagen Type II expression in IVD cells, with a greater response by NP cells than by AF cells.<sup>6</sup> In vivo injection of GDF-5 protein and virus transfection in a disc degeneration model in rabbits promoted regeneration of the disc, with increased cell proliferation and production of matrix. This was found to be more effective than IGF-1, TGF $\beta$ , or bFGF injection.<sup>45</sup> GDF-5 injection also effected the restoration of disc height and improvements in MRI scans and histological grading scores in rabbit disc degeneration studies.<sup>6</sup>

### GDF-6

BMP-13, also known as GDF-6 or CDMP-2, has been found to only have 50% homology to BMP-2 in amino acid sequence. BMP-13 increases the proteoglycan synthesis rate and chondrocytic phenotype of disc cells, but it is much less potent than BMP-2. It also demonstrated an additive, but not synergistic, effect with BMP-2 on disc cells.<sup>17</sup> BMP-13 has some effect on tendon healing and, therefore, is under investigation for AF repair.<sup>49</sup>

### Link N

The non-covalent bond between aggrecan and hyaluronan is stabilized by the glycoprotein, link protein. LinkN is an amino terminal fragment of link protein.

Initially shown to stimulate production of matrix by cartilage cells in vitro, Link N was shown to stimulate matrix assembly in pellet culture of NP and AF cells by increasing the production and/or accumulation of proteoglycans and collagen, but did not increase cell number.<sup>29</sup> This suggests that degradation products of link protein, which can be generated by MMPs, act as a "growth factor" in a feedback mechanism.

### Sox-9

Sox-9 is an intracellular transcription factor for Type II collagen synthesis and chondrogenesis. The AdSox9 virus efficiently transduced degenerated human disc cells and increased Sox9 and Type 2 collagen productions in vitro. In the rabbit annular puncture model for disc degeneration model, cells infected with AdSox9 maintained a chondrocytic phenotype, and the architecture of the NP was preserved during a 5-week study period.<sup>34</sup>

## NP CELL TRANSPLANTATION

Autologous reinsertion of the NP has been shown to delay degeneration of the disc, including the AF, NP, and endplate.<sup>31</sup> Restoration of disc height has also been demonstrated with autologous disc cell replacement.<sup>12</sup> The reinsertion of activated NP has been shown to retard disc degeneration in vivo, assessed by histological appearance and increased production of collagen 2.<sup>33</sup>

Because the main disadvantage of autogenous transplantation of NP into the degenerated disc is that sufficient

NP cells cannot be harvested from the other IVDs without accelerating degeneration at the donor site, studies have focused on the development of an allograft substitute. In vivo studies on allograft transplantation of NP cells did not induce any appreciable host-versus-graft response.<sup>32</sup> Injection of NP and NP cells retards IVD degeneration, demonstrated by histological grading and staining for Type II collagen. However, injection of intact NP is more effective than injection of NP cells alone, demonstrating that the intercellular matrix plays an important, but poorly understood, role in preserving IVDs.<sup>32</sup>

Moreover, reimplantation of disc cells from discectomy specimens in a nonrandomized series of human patients demonstrated MRI scan improvements consistent with increased proteoglycan matrix within the NP and relief of symptoms.<sup>11</sup>

### STEM CELL THERAPY

With the aim of restoring the normal cellular constituents of the NP, the hunt for a suitable candidate source of autologous cells has led to experiments with MSCs and bone marrow stem cells (BMSC). Stem cells are a readily available source of autologous cells, with minimal donor-associated complications. Apart from bone marrow, adipose tissues have also been shown to be a good source of stem cells capable of differentiating along the chondrogenic lineage.<sup>9</sup> In vitro studies have shown that MSCs that are exposed to hypoxic conditions and TGF $\beta$  in three-dimensional alginate culture can show differentiation toward NP cells, with increased production of matrix components, such as aggrecan, collagen Type II, and fibromodulin.<sup>37</sup>

Autologous MSCs transplanted into degenerate discs of mature rabbits showed increased cell proliferation and increased production of NP cell-associated matrix molecules, such as Type II collagen, keratan sulfate, chondroitin sulfate, aggrecan, and the NP phenotypic markers, hypoxia inducible factor 1 $\alpha$ , glutamine transporter 1, and MMP2. It was also demonstrated that, after MSC transplantation, degenerated discs of rabbits demonstrated improved disc height and MRI scan signal intensity compared with control discs.<sup>41</sup> These data indicate that transplantation of MSCs effectively led to regeneration of IVDs in a rabbit model of disc degeneration. It has been suggested that the transplanted MSCs differentiated into cells expressing a chondrocyte-like phenotype or that the MSCs were induced by the microenvironment of the NP to undergo differentiation toward the NP cell phenotype in vivo.

In vitro coculture of rabbit NP cells and BMSC cells with direct cell-to-cell contact demonstrated increased cell proliferation, production of PG, and increased TGF $\beta$ 1, IGF-1, EGF, and PDGF expression. In vivo studies on rabbits showed that injection of autologous BMSC resulted in restoration of disc height and increased staining for PG, whereas

NP cells cocultured with BMSC injected into the degenerate disc space also resulted in less degeneration assessed by histological appearance and more PG production than untreated degeneration control levels.<sup>26</sup>

In a series of 10 patients who had previous endoscopic discectomies and discogenic back pain confirmed with provocative discograms, an intradiscal injection of BMSC failed to relieve pain in any of the patients at up to 12 months after injection. This study suggests that intradiscal injection of hematopoietic stem cells does not translate to clinical success, despite animal studies showing regeneration of the discs.<sup>15</sup>

### WHOLE DISC TRANSPLANT

Whole disc transplantation is technically feasible but results in mild postimplantation degenerative changes. The preservation of motion it confers makes it a possible alternative to fusion. Autograft transplant including the endplates in the lumbar spine of mature dogs and monkeys resulted in decreased proteoglycan synthesis, degenerative changes, and incomplete restoration of disc height compared with normal discs.<sup>10,19</sup>

Transplantation of cryopreserved dog allograft IVD was performed and x-rays revealed a complete bone union of the vertebral bodies at 5 months and gradual narrowing of the intervertebral space beginning at 6 months.<sup>24</sup> Histologically, the AF was well preserved, and the NP underwent marked degeneration. Transplant of allograft of fresh discs in dogs had an unacceptably high mortality rate, of 46%.<sup>24</sup>

### SCAFFOLDS

The purpose of a cellular scaffold is to provide an optimal microenvironment for cellular migration and proliferation that allows the cells to maintain the appropriate phenotype. It is well known that NP cells will alter their phenotype depending on the culture technique used; therefore, selection of an appropriate scaffold and culture technique is crucial.

Collagen is a physiological biomolecular scaffold compatible with cell attachment, and hyaluronan acts as an anchor for aggrecan retention by promoting proteoglycan aggregate formation. An injectable carrier has the advantage of being dispensed through a small needle, and will conform precisely to the disc defect and, thus, its clinical application is more straightforward, avoiding extensive surgical disruption of the annulus involved with the use of a solid scaffold. In concentrated solution, hyaluronic acid forms a viscous gel that can be a carrier for cells.<sup>7</sup> Chitosan-based polymers have been described that can be maintained as a soluble polymer at room temperature, and induced to gel at body temperature. Such a system might allow disc cells to be injected with the soluble polymer, which can then polymerize and entrap the cells in vivo.<sup>1</sup> Atelocollagen gel is a less antigenic alternative

to conventional collagen, its antigenic telopeptide region having been removed by pepsin digestion and differential salt precipitation during purification. This solution is in liquid form when cooled to temperatures near 4°C but gelatinizes when heated to approximately 37°C.<sup>40</sup>

In vitro and in vivo studies using the above-mentioned scaffolds seeded with cells have shown proliferation of the seeded cells and increased synthesis of proteoglycan and collagen. However, retention of the matrix proved to be difficult because of the porosity of the scaffolds, and normal disc height was not restored.<sup>1,7,40</sup>

### SUMMARY

The normal IVD clinically acts to support and dissipate loads while permitting multiaxial motions of the spine. Its demanding mechanical function is provided by a well-defined microstructural organization and biochemical composition. IVD degeneration is a complex process that disrupts this well-defined organization and biochemical balance. One hallmark of IVD degeneration is the loss of proteoglycan and water in the NP. Because of the central role of proteoglycans in the function of the IVD, restoration of normal proteoglycan production may be critical. Many different biological strategies have been developed, including the use of cells, scaffolds, and molecules. The molecules used to treat disc degeneration include anticatabolics and growth factors, which may influence the cell proliferation rate and phenotypic expression of the cells. Delivery of the molecules may include direct injection into the disc and also in vivo and ex vivo gene therapy using a viral vector. Although many of the in vitro and in vivo studies have exhibited promise in reversing the observed changes of disc degeneration, the unanswered question is whether these efforts will translate to the relief of patients' symptoms, the most common of which is back pain.

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