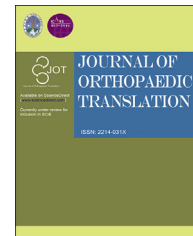




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REVIEW ARTICLE

# Bedside to bench and back to bedside: Translational implications of targeted intervertebral disc therapeutics

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**Summary** Spinal pain and associated disability is a leading cause of morbidity worldwide that has a strong association with degenerative disc disease (DDD). DDD can begin in early–late adolescence and has a variable course. Biologically based therapies to treat DDD face significant challenges posed by the unique milieu of the environment within the intervertebral discs. Many potential promising therapies are still in the early stages of development with a hostile microenvironment within the disc presenting unique challenges.

*The translational potential of this article:* Patient selection, reasonable therapeutic goals, approach, and timing will need to be discerned in order to successfully translate potential therapeutics.

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## Introduction

Low back pain is a leading worldwide cause of disability with degenerative disc disease (DDD) being the most common source of low back pain [1]. In fact, evidence of DDD has

been found in 40% of volunteers aged younger than 30 years, and this rises to more than 90% by age 55 years [2]. A recent systematic review of chronic back pain reported a prevalence of 4.2% between 24 years and 39 years of age, and amongst those between 20 years and 59 years old the prevalence increased to 19.6% [3]. Other studies involving persons aged older than 18 years reported chronic back pain to a similar to a similar degree at between 3.9% and 10.2%, with several others reporting between 13.1% and 20.3% [3]. A cross-sectional study of 876 family health clinic patients

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found that risk factors for chronic back pain included female sex, age 30 years or older, lower education status (4 years or less), anxiety, and an occupation requiring high exertion. Furthermore, quality of life and self-rated health scores were significantly worse among individuals with chronic spinal pain [4]. Furthermore, it has been demonstrated that sexual dimorphism exists with respect to DDD and that postmenopausal women are at an increased risk of disc degeneration perhaps because of the impact of the oestrogen receptor on collagen metabolism [5,6]. Back pain has been reported to be the most common reason for healthcare visits among those with musculoskeletal disorders (more so than hypertension and arthritis) and has the greatest impact and resource use [7]. Back pain is also costly, with 5% of the American workforce missing at least 1 day of work per year, with direct and indirect costs estimated to range between \$19.6 billion and \$118.8 billion in the USA [1,7,8]. Like the lumbar spine, DDD affecting the cervical spine can be painful and disabling; moreover, it is also the main cause of cervical spondylotic myelopathy—the leading cause of spinal neurological impairment in persons aged older than 65 years [9]. DDD may overload segmental muscles, facet joints, and capsules, leading to pain arising from these spinal joints and soft tissues that might otherwise be classified as “muscular” or ill-defined soft tissue pain, perhaps underestimating the impact of DDD and spinal pain [10]. Treatment of spinal pain secondary to DDD is largely afforded by various modes of physical and cognitive behavioural therapy that achieve similar benefits as spinal fusion surgery, leaving the field with no effective disease-modifying therapy [11]. Therefore, new interventions including biologics and/or tissue engineering approaches are currently under intense investigation with a view to being able to influence the course of the disorder [12].

## Intervertebral disc degeneration

### Homeostatic regulation

The intervertebral disc (IVD) complex is composed of specialised cells and extracellular matrix (ECM) that are able to withstand high tensile strength as well as compressive and off-axis loading that affords the spinal column with strength, flexibility and protection of the spinal cord. In youth, the nucleus pulposus (NP) is gelatinous with a proteoglycan (PG) network rich in aggrecan and collagen type 2. PGs within the IVD NP ECM (principally aggrecan) are highly negatively charged; they bind water molecules and are responsible for the high net swelling pressure unique to the IVD NP. The healthy IVD NP is capable of resisting compression and deformation principally owing to the hydrophilic NP rich in highly negatively charged PGs that strongly bind water molecules. Homeostatic regulation of the healthy NP ECM involves a balance between anabolic and catabolic activity. However, in DDD this normally tightly regulated process becomes dysfunctional, such that ECM-degrading enzymes and proinflammatory molecules lead to progressive degeneration, loss of viable cells, and a fibrocartilaginous degenerative phenotype [13–15].

Apart from a limited vascular supply to the periphery of the annulus, the inner annulus and NP is hypoxic,

ischaemic, aneural, and isolated from the immune system, and represents a unique tissue compartment. The cells within the NP have adapted to this harsh environment by relying upon glycolytic metabolism and diffusion of nutrients and waste products into and out of the NP via the vertebral endplates [16]. With maturity and DDD, the cellular and extracellular phenotype within the IVD NP changes from the youthful highly notochordal composition to one where small, chondrocyte-like cells predominate, where there is a gradual replacement of collagen type 1 and relative loss in collagen type 2 leading to the development of a fibrocartilaginous IVD NP [17]. The vertebral endplates form the superior and inferior boundaries of the IVD and act as diffusible barriers between the bone marrow of the vertebral body and the disc itself. With progressive DDD, the small pores within the endplates calcify, thus compromising their diffusion capacity and further compromising the already delicate molecular exchange within the IVD NP [18,19].

### Genetic and epigenetic influences

Some patients develop DDD to a greater degree than others, and it has been demonstrated that certain genes and/or small nucleotide polymorphisms such as collagen IX, the vitamin D receptor, collagen type 1, aggrecan, matrix metalloproteinase-3, and the interleukin (IL)-1 receptor can influence a patient's predisposition to DDD [20,21]. Data concerning the precise mechanisms whereby these genetic anomalies may influence the development and progression are not yet fully understood. However, many of the candidate genes involved with DDD (*collagen 1, IX, XI, aggrecan, matrix metalloproteinase-3, and the vitamin D receptor*) likely result in the deposition of flawed ECM proteins. In the case of IL-1 and its receptor, it is likely that impaired regulation of inflammation and/or even pain could be candidate targets [22]. All the factors listed above plus activities of daily living, trauma, and occupational demands lead to a net decrease in the main PG aggrecan, a decrease in type 2 collagen, an increase in the degradation of collagen type 2, and an increase in type 1 collagen within the NP [23]. The loss of functional aggrecan leads to a progressive inability of the NP ECM to bind water that, in turn, leads to a decrease in intradiscal pressure. The accumulated loss of ECM integrity such as enzymatic cleavage of PG core proteins (such as biglycan, decorin, and fibromodulin) further contribute to DDD, loss of disc height, and a reduced ability of the IVD to resist compressive/shear forces. With respect to epigenetic influences, Matsui et al [24] demonstrated a higher likelihood of DDD in patients with a relative who underwent herniated disc surgery. Furthermore, smokers and patients living with diabetes also have elevated risk of developing DDD [25]. It is therefore likely that certain mutated genes impair cell viability and the deposition and regulation of cellular–ECM interaction that along with multiple processes such as inflammation, leads to progressive DDD [26,27].

For more details with respect to the complexities and changes in cellularity and ECM, the reader is referred to several published reviews such as those by Feng et al [28] and Adams and Roughley [29].

## Targeted IVD therapeutics

The “holy grail” of intradiscal therapy is to mediate the progressive degenerative cascade or even induce repair of the IVD. Necessarily, such putative therapy would need to inhibit inflammatory-driven catabolism of the IVD, stimulate anabolic repair/regeneration of the IVD, and deliver improved biomechanical status to the disc. To this end, there are a number of different therapeutic strategies currently under investigation.

Loss of disc height and swelling pressure within the IVD NP leads to degenerative disease and impaired biomechanical properties. Therefore, interventions that could ameliorate these pathological changes would capture the essence of a minimally invasive therapy. Interestingly, a study published by Klein et al [30] involved the percutaneous injection of a combination of glucosamine, chondroitin sulphate combined with hypertonic dextrose and dimethylsulphoxide. In this study, 30 patients with intractable low back pain of an average of 8.5 years’ duration that demonstrated concordant pain under provocative discography conditions received an injection of the intervention. One year after intervention, 57% of the patients experienced marked improvement in visual analogue pain and functional scores (Roland Morris). No patients experienced undue or serious side effects. Although this study did not propose a mechanism by which the treated patients experienced relief and the study did not have a control group, it remains one of the seminal publications with respect to early attempts to treat the confirmed painful DDD via percutaneous methods.

## Biomolecules

Biomolecules may be able to augment the integrity of the IVD [NP and/or annulus fibrosus (AF)] ECM by directly implanting suitable anabolic/anticatabolic proteins such as bone morphogenetic proteins (BMPs), growth and differentiation factors (GDFs), and various members of the transforming growth factor (TGF)- $\beta$  superfamily and/or catabolic enzyme inhibitors within the IVD NP [12]. Miyamoto et al [31] injected BMP-7, also known as OP-1, into the degenerative discs of rabbits that reportedly led to an increase in disc height with an improved elastic modulus. However, in a canine model of DDD, the injection of recombinant human BMP-7 failed to demonstrate any therapeutic effect on DDD, but did lead to undesired extensive formation of bone outside of the IVD [32]. With respect to therapeutic protein injection, it is vital to deliver the correct molecules in that akin to the experience with BMP-7 injection into canine IVD NPs, injection of BMP-2 into degenerative rabbit discs worsened degeneration with enhanced vascularisation and fibroblast proliferation [33]. An important question therefore with respect to biomolecule delivery is which molecule(s) is/are necessary and sufficient? A possible limitation to the use of therapeutic proteins may be only transient effects of the delivered biomolecules [12]. It may be possible to overcome this potential limitation by a slow release formulation or vector transmission in order to increase the duration of molecular delivery to the target tissue [12]. With respect to increasing

the therapeutic effect of biomolecule delivery, Yan et al [34] investigated GDF-5 packaged in microspheres that reportedly enabled the slow release of GDF-5 for more than 42 days. This approach reportedly demonstrated some improvement in a rat-tail model of DDD in terms of increased disc height, sulphated glycosaminoglycan content and improved histological scores [34]. More recently, Matta et al [35] quantitatively demonstrated robust anti-degenerative/regenerative effects in a rat-tail model of DDD through the use of a single injection of CTGF + TGF $\beta$ 1.

## Gene therapy

The delivery of therapeutic genes into cells within the IVD could potentially lead to the secretion of encoded proteins to enhance IVD homeostasis and possibly suppress degenerative disease. Several nonviral transmission vectors have been created including liposomes and “gene guns.” Although they eliminate the risk of viral vector infection, all of these methods are hampered by transient protein expression because these nonviral vectors do not efficiently incorporate with the host DNA. By contrast, a number of viral vector delivery systems have been evaluated, each with its own benefits and disadvantages. Some viruses can lead to infections, some integrate into the host DNA, and some remain separate. Amongst the most commonly used vectors, adenovirus and adeno-associated virus (AAV) have seen intense investigation. The adenoviral vector does not integrate into the host DNA, which eliminates the risk of mutation because the virus is not replicated with the host DNA. Therefore, the virus only expresses proteins transiently until it is degraded by the immune system that may attack the foreign material. AAV may be advantageous as compared to adenovirus as it requires a helper virus for expression, which limits immune system activation. However, AAV carries only a limited amount of genetic information [36]. Leckie et al [37] investigated AAV2-BMP2 and AAV2-TIMP1 for injection into the degenerated disc. They found there was a slowing of the degenerative cascade in the injected groups [37]. However, these vectors can be directly injected or transplanted *in vivo*. The *ex vivo* injection involves removal of targeted cells, culturing the cells, introducing genes, and replantation. The *ex vivo* introduction is reportedly safer but requires a two-step procedure, and there is difficulty in recreating the *in vivo* environment to expand the cells [36]. However, an element of caution must be introduced to the gene therapy approach, because in a safety study using rabbits, a higher dose of adeno-associated viral vectors expressing TGF $\beta$ 1 and rhBMP-7 induced bilateral lower limb paralysis, clearly indicating the potential risk of this approach [38]. Therefore, although promising, it is clear that further investigation is necessary in order to identify the correct molecules, carriers, transfection method, procedures, and safety profile for gene therapy approaches to treat DDD.

## Platelet-rich plasma

Platelet-rich plasma (PRP) is a method of delivering a small volume of concentrated activated platelets to an area of injury in the hopes that growth factors secreted by the

activated platelets might have a restorative effect upon the tissue. PRP has been shown to stimulate cell proliferation and induce PG and glycosaminoglycan synthesis in tendon and muscle tissue; however, its mechanism of action continues to be under investigation. For example, in tendon-derived fibroblasts, it has been hypothesised that PRP might stimulate reactive oxygen species-based oxidative stress pathways that, in turn, induce a transient proinflammatory event that subsequently triggers inflammation and finally tissue regeneration [39]. With respect to IVD treatment in a short-term, preclinical rat-tail model of injury-induced DDD, Gullung et al [40] suggest that PRP-injected discs prevented degenerative changes compared to a sham injection when injected acutely after injury. Overall, there have been several studies evaluating *in vitro* and *in vivo* injection of PRP as a potential DDD therapy; however, there is considerable inconsistency using *in vivo* preclinical animal models [41]. Tuakli-Wosornu et al [42] performed a double-blind study during discograms with either contrast and PRP or just contrast. The PRP group had significant improvement for at least 1 year in terms of the Functional Rating Index. However, in this study, the control group was limited to only 8 weeks, and although worst pain ratings were reported to be significantly different at 8 weeks after injection and the control group remained relatively constant, the PRP treatment group only dropped by 2 points. Nonetheless, there were reported statistically significant changes in some predictors such as the 36-item Short Form Health Survey and functional rating index, although these were also restricted to only 8 weeks after treatment [42]. Additionally, no imaging studies were performed on any study participants. Potential confounders/limitations to the use of PRP include the lack of standardisation of dose, large donor variability, and method of preparation, and the lack of understanding with respect to purported mechanism of action [41].

## Cell-based therapeutics

Cells within the AF and NP have an intrinsic capacity to effect ongoing homeostasis and repair. To this end, cells expressing stemness genes have been isolated from various components of the IVD including vertebral endplates, NP, and AF tissues, indicating that as with other tissues, the machinery for cell-based repair exists within the IVD [43–45]. The degenerative disc includes senescent and apoptotic cells that do not respond to bioactive molecules such as growth factors. Therefore, biological therapy for more advanced DDD may benefit from the transplantation of healthy cells to repopulate the disc and/or to act as “nurse” cells to improve the NP degenerative milieu.

## Stem cells

In addition to terminally differentiated cells such as donor chondrocytes, stem cells from a variety of sources are another potential choice of cellular replacement. Bone marrow-derived mesenchymal stem cells (BMSCs) possess significant proliferative and differentiation capacity. BMSCs have been transplanted in a rabbit model of DDD, and it has been reported that such cells differentiated into “NP-like”

cells with increases in PG deposition within the IVD NP [46]. Elabd et al [47] injected autologous, hypoxic cultured, bone marrow-derived MSCs into the IVDs of five patients with back pain and reported improvement in a small number of patients 5 years after injection. Furthermore, Cao et al [48] cocultured bone marrow MSCs with NP cells of rabbit IVD origin and demonstrated an upregulation in aggrecan, collagen 2, and SOX-9 expression, suggesting an anabolic effect upon the NP ECM. It is noteworthy with respect to the work by Cao et al that NP cells obtained from the rabbit disc are primarily of notochordal origin and apart from ECM protein expression; there was no specific characterisation of the cellular phenotypic fate. These authors reported an increase in TGF- $\beta$  signalling and decrease in nuclear factor- $\kappa$ B activity, suggesting that these two signalling pathways play a role in preventing degeneration [48]. BMSCs have been intensively studied as potential cellular replacements to treat DDD and in addition to their use in preclinical animal models; BMSCs have been the subject of a number of clinical trials. Despite some early evidence of improvement in some patients, studies have suffered from small sample size and lack of adequate controls [49]. Furthermore, beyond some limited data in preclinical animal studies, the fate of the transplanted cells remains largely unknown. One clinical trial by Red de Terapia Celular (<https://clinicaltrials.gov/ct2/show/record/NCT01860417>) involves the *ex vivo* expansion of bone marrow-derived stem cells and the injection of  $25 \times 10^6$  stem cells/2 mL into the disc. Another trial by Mesoblast Ltd. involves either  $6 \times 10^6$  or  $18 \times 10^6$  cells/disc within a hyaluronic acid carrier (<https://clinicaltrials.gov/ct2/show/NCT01290367?term=mesoblast&rank=3>). The cellular concentration within the cervical IVD NP has been reported to be within the range of  $4200/\text{mm}^3$ . The human IVD NP disc contains cells that are approximately 0.25–1.0%/volume [50]. Therefore, it is important to consider that the transplantation of up to  $25 \times 10^6$  cells is on a scale that is orders-of-magnitude greater than the normal cellular population. Given that DDD is associated with calcification of the endplates and impaired nutrient supply within the IVD NP, it does not appear feasible that such high numbers of transplanted cells could integrate and survive within such a harsh and compromised tissue compartment. Therefore, any beneficial effects conferred by such a massive cellular transplant is, at this point, a matter of conjecture but may be associated with transient growth factor and other secreted factors by the transplanted cells that may confer activity until the cells die. This is an area that requires further, quantitative study.

In addition to BMSCs, other sources of stem cells have been investigated including adipose stem cells, synovial MSCs, olfactory stem cells and neonatal dermal fibroblasts [44,46,51–55]. Colombier et al [51] used lipoaspirates from patients cultured within a TGF- $\beta$ 1 and GDF-containing medium and induced NP cell-like differentiation. In this study, the cells were suspended within a hydrogel and injected subcutaneously into nude mice where it is reported that the cells survived and differentiated into a cellular phenotype similar to NP cells in that they secreted aggrecan and collagen type 2 [51]. Synovial MSCs are other potential stem cell candidates and have been reported to have robust replication rates with *in vitro* cartilaginous ECM production [52,55]. Miyamoto et al [52] implanted synovial MSCs into

rabbit IVD NPs and demonstrated that these cells exhibited anticatabolic and anabolic activity after transplant. Other sources of potential stem cells to be used for IVD repair include olfactory and neonatal dermal fibroblasts; however, donor site morbidity and sufficient tissue sources confound their use [53,54]. However, there have been negative reports with respect to the use of stem cells for IVD therapeutics. Vadalà et al [56] transplanted rabbits MSCs into rabbit IVDs and after transplant detected no signs of the transplanted cells but did detect a significant increase in osteophyte formation. Their conclusions were that cells possibly leaked from the injection site and differentiated into an unwanted osteogenic lineage [56].

Allogeneic and autologous disc and articular chondrocytes have both been explored as potential cells for transplant with Nomura et al [57] reporting that allogeneic rabbit IVD cells slowed degeneration in a needle puncture injury model of rabbit DDD [12,58]. Interestingly, cells transplanted along with their ECM showed greater improvement than cells alone [57]. Another study by Zhang et al [58] used a rabbit degenerative disc model and following injection of articular chondrocytes into the disc. The authors demonstrated cell survival for at least 8 weeks with suppression of associated inflammation; however, the ECM did not resemble the normal NP cell phenotype, and the use of differentiated autologous cells carries with it donor site morbidity [58]. Finally, the choice of cells for transplant is vital in that rabbit IVDs are highly notochordal and contain stem cells. The cellular phenotype of the human IVD is, of course, entirely different; therefore, it is important to exercise caution when drawing conclusions from such animal studies.

Less differentiated stem cells may be advantageous as they have the potential to propagate longer and might better differentiate into the desired lineages. These cells include induced pluripotent stem cells (iPSCs), human umbilical tissue-derived cells, and embryonic stem cells (ESCs) [46,59–63]. iPSCs are somatic cells, such as dermal fibroblasts, that—through genetic reprogramming using a limited number of transcription factors (originally, Oct4, Sox2, c-myc, and Klf4)—assume an embryonic cell-like phenotype and because of their ease of harvest and minimal donor site morbidity offer a virtually limitless tissue source [64]. Since their discovery, it has been reported that considerable variability exists with respect to the transcription factors necessary to reprogramme cells. It has recently become clear that there is considerable tissue and cellular heterogeneity with respect to reprogramming and efficiency such that human papillary dermal fibroblasts and melanocytes can be reprogrammed following transfection with only Oct4 and Klf4 because of their high endogenous expression of Sox2 and c-myc; in these cases, however, there is a lower yield of iPSC clones [65]. Nonetheless, the use of iPSC cells is accompanied by a number of risks including the integration of retroviral DNA into host cells, and the downstream interjection of coding regions that can affect subsequent transcription that heightens the risk of malignant transformation [66]. Takahashi et al [64] found that murine iPSC clones contained retroviral integrations for each transfection factor used, raising a concern that this technology could increase the risk of tumourigenesis [64]. This concern was validated by Okita et al [67], who

reported that close to 20% of mice derived from an iPSC cell line died or were killed because of weakness, wheezing or paralysis, and that approximately 14% of mice developed neck tumours with other tumours in a small number of other mice [67]. Therefore, although iPSCs offer promise in the future, their use must be restricted to research platforms for the present.

ESCs have been intensively investigated for use regenerative medicine. These are cells recovered from the inner cell mass of the developing embryo at the blastocyst stage and have totipotent characteristics. A search of [clinicaltrials.gov](http://clinicaltrials.gov) using ESCs for DDD reveals no studies to date, and ESCs have had limited use as a potential therapy to treat disc disease with studies restricted to preclinical animal models. In a report by Sheikh et al [68], murine ESCs were expanded in a cocktail of growth factors in an attempt to prechondrodifferentiate the cells that were then transplanted in a rabbit model of DDD. This study demonstrated *de novo* notochordal cell-like growth within discs receiving transplants, suggesting that transplanted ESCs, after differentiation, are detectable at least 8 weeks after transplant and that they are capable of differentiation into a notochordal cell-like phenotype. Although ESCs, like iPSCs, offer considerable expansion and differentiation characteristics, ESCs, like iPSCs, have risks of oncogenesis as well as ethical concerns as they are harvested from developing embryos, raising challenges with translation into the clinic.

Another exciting area of cell-based therapeutics involves sourcing endogenous stem cells that reside within the IVD NP itself. Erwin et al [43] reported that stem cells obtained from canine IVD NPs display pluripotential differentiation characteristics and express stemness genes. However, it has been reported that stem cells obtained from degenerative human discs undergo “exhaustion,” possibly limiting their use in a cell-based therapy approach [69]. Finally, the process of tissue culture/cell expansion is labourious and costly, and the use of ESCs is fraught with ethical concerns including risks of malignancy/teratoma development.

## Tissue engineering strategies

The goal of a tissue-engineered scaffold is to function as a load-bearing component. While under the proper conditions, transplanted cells suspended within the construct could repopulate the degenerative disc. The scaffold should be biocompatible, biodegradable and allow for sufficient cellular migration and ECM assembly. The scaffold also must have similar mechanical properties as the native NP or AF [70]. High molecular weight hyaluronic acid (hyaluronan) has potential advantages as a drug carrier, but also when cross-linked as a biomaterial. To this end, a study by Moss et al [71] reported that a thiol-modified hyaluronic acid and elastin-like polypeptide conferred an environment that was suitable for human NP cells and demonstrated desirable biomechanical properties both *in vitro* and *in vivo* in a preclinical rabbit model of DDD. This construct also provided a permissible environment for cell viability and ECM production, suggesting that such composite constructs might have a place in tissue engineering approaches to disc repair [71]. Composite constructs involving cross-linked

hyaluronic acid with type II collagen have also been investigated that support cell viability and also offer a desirable high intradiscal swelling pressure [72].

Numerous reports have been published with respect to NP scaffolds though these strategies remain at the pre-clinical level. Gorapalli et al [73] used human NP cells obtained from discectomy patients and demonstrated that NP cells cultured within poly *N*-acetyl glucosamine resulted in significantly increased PGs, cell viability, metabolic activity, collagen type 2, and aggrecan production *in vitro*. Hyaluronic acid is a major component of the IVD NP ECM, and various forms of this macromolecule have been investigated for IVD tissue engineering including a thiol-modified hyaluronan and elastin-like polypeptide composite [71,74].

Each of the above treatment strategies—whether it is biomolecules, gene therapy, cell-based or tissue engineering—may have a role and possibly can be incorporated in treatment based on the extent of the degeneration of the disc. See Figure 1 for a summary of grades of DDD and possible intradiscal therapeutics.

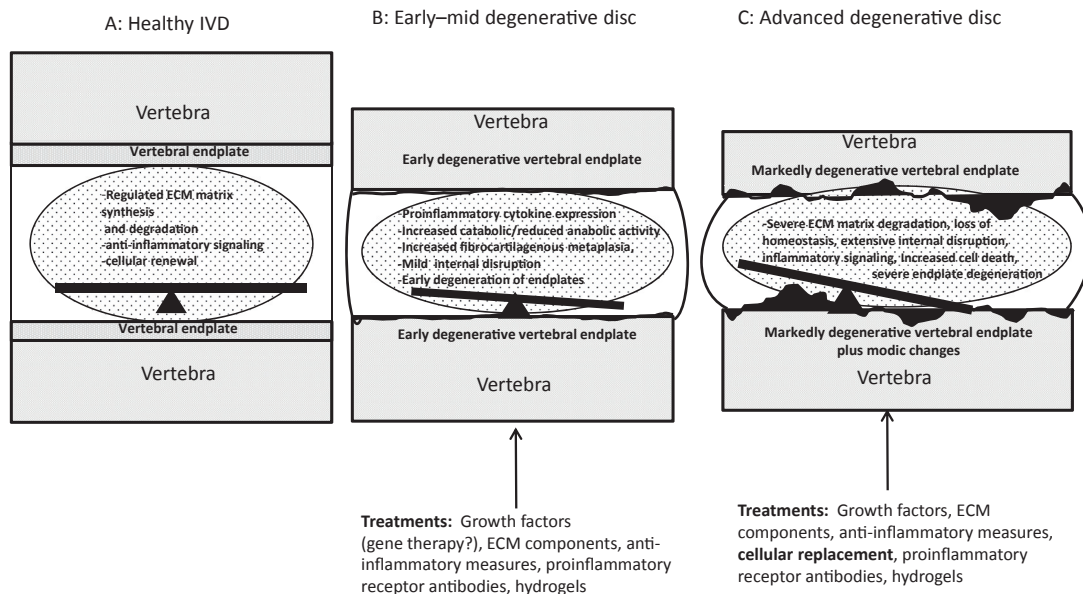
## AF repair or augmentation

Coexistent with degeneration of the IVD NP are similar changes affecting the AF. Such changes involve progressive delamination of the concentric lamellar rings composed primarily of type 1 collagen, fibroblasts, and interposed ECM [75]. AF repair or augmentation could theoretically assist with the mediation of DDD and prevent leakage of

injectable or implantable treatment modalities. Currently, two main therapeutic strategies exist for AF repair: (1) suture or implant repair; and (2) augmentation. Bateman et al [76] have reported the feasibility of a suture application device that was able to close the AF in a porcine model that was maintained for at least 4 weeks. Other approaches include implantable hydrogels designed to mimic the material properties of native AF tissues or the use of cell-based therapy or tissue-engineered scaffolds [77,78]. Additionally, various other strategies have been used in attempts to address AF insufficiency including biodegradable and synthetic materials as well as composites, different forms of bioactive glass, electrospun collagens, and nanofibers [79]. However, vexing problems remain with these tissue engineering strategies including immunogenicity of construct/materials, sufficient biomaterial strength, cellular/tissue milieu, and incorporation and degradation of constructs.

## Modelling IVD degeneration

In order to test potential intradiscal therapies, a number of different animal models of DDD have been investigated. However, differences between species are another potential confounder when one considers the interpretation of scientific literature. O'Connell et al [80] compared the geometry of the human lumbar disc to lumbar discs of baboons, sheep, rabbits, rats, and mice along with bovine, mouse, and rat-tail disc geometry, and concluded that with respect to geometry, the lumbar disc of the mouse, rat, and



**Figure 1** Schematic of (A) healthy versus (B) early degenerative disc and (C) more severe DDD. (A) Balanced homeostatic regulation of ECM synthesis, cell renewal, and apoptosis. (B) For early/mid degenerative disease, there is a loss of ECM regulation, accelerated cell death, increased proinflammatory enzymatic activity, mild loss of disc height, bulging of the annulus, and early degeneration of the vertebral endplate. (C) For more advanced DDD, the changes seen in (B) are more severe with extensive internal disruption of the IVD NP, more severe endplate changes, reactive bone marrow oedema (modic changes), extensive cell death, and marked loss of ECM regulation. Possible therapeutic interventions are highlighted for (B) and (C). DDD = degenerative disc disease; ECM = extracellular matrix; IVD = intervertebral disc; NP = nucleus pulposus.

mouse-tail were most similar to the human lumbar disc. With respect to animal models, the cellular phenotype is of vital importance when attempting to model human DDD. The composition of the human NP at birth and in youth is largely notochordal in nature, but this declines by adolescence/early adulthood into a mostly chondrocyte-like IVD NP. Bovine, ovine, and chondrodystrophic canine IVD NPs resemble the human IVD NP that is characterised by chondrocyte-like cells and a fibrocartilaginous NP. By contrast, the IVD NP in mice, rats, cats, minks, pigs, and rabbits remain rich in notochordal cells largely throughout life—a very different situation than the human condition.

In most animal models, it is necessary to induce DDD through either mechanical or chemical methods. Commonly, DDD is induced by stab injuries using a needle or scalpel that, in turn, leads to degeneration of the tissue compartment to assume the more acellular, fibrocartilaginous morphology typical of DDD [12,37,81–85]. In addition to acute mechanical injury, biomechanically induced DDD has also been performed in the form of compression and distraction through the use of affixed clamps to the rodent tail disc [85]. Furthermore, the introduction of digestive enzymes such as chymopapain and/or chondroitinase ABC also results in degenerative change; however, it must be noted that these methods by definition utilise both needle puncture injury (injection) as well as enzymatic activity. Also, such enzymatically induced DDD occurs very rapidly, unlike DDD that occurs over time or secondary to needle puncture that requires many weeks to achieve a mature DDD phenotype [35,85]. Pathologies induced by these methods include the loss of disc height as well as collagen type 2 and notochordal cells and an increase in the expression of ECM remodelling and proinflammatory enzymes such as MMPs, aggrecanases, and IL-1 $\beta$  and tumour necrosis factor (TNF)- $\alpha$ .

Any experimentally-induced animal model of DDD has its own inherent limitations that must be considered when interpreting experimental results. For example, the *de novo* IVD cellular phenotype, age of the animal, weight bearing (relevant in rodent models), and genetic variations figure prominently in the response of the animal's IVD to experimental manipulations [12]. With respect to genetic variations, recently, the chondrodystrophic dog and the ovine models have been proposed to be the best animal models of disc degeneration because of their similarity to the human condition in terms of cellular and ECM phenotypes [83,86]. Notwithstanding the inherent limitations in animal models of DDD, understanding the key pathologies and mechanisms involved with the progression of DDD provide vital experimental platforms for the controlled investigation of novel therapeutics.

## Clinical challenges

There are significant challenges involved with the development and implementation of biological therapy for the disc that include diagnostic methods, the best choice of therapeutic agent, method of delivery, outcome measures and, of course, patient selection. With respect to patient selection, the ability to specifically isolate and determine pain of discogenic origin remains a vexing challenge.

Cheung et al [2] found that 60% of symptomatic and non-symptomatic people older than 50 years had more than three degenerative intervertebral levels on magnetic resonance imaging (MRI) examination. Another study involving MRI imaging of 98 asymptomatic people reported that only 36% of individuals had nondegenerative discs at all levels [87]. It is widely accepted that many people display imaging changes on MRI but do not experience spinal pain, indicating that contemporary imaging modalities are not capable of specifically detecting symptomatic disc disease. With respect to determining the presence of a painful disc, the gold standard for many years has been provocative discography. Xi et al [88] performed discograms for patients with presumed discogenic back pain for more than 6 months and performed arthrodesis in patients with positive discograms. Fourteen of 18 patients had significant improvement after arthrodesis in terms of the visual analogue scale and Oswestry Disability Index scores over a year postoperatively [88]. However, provocative discography is interventional, painful and is associated with a number of potential morbidities including accelerated disc degeneration [89]. In order to circumvent some of the limitations of discography, new technologies are under development including single-voxel MRI spectroscopy, whereby noninterventional imaging may be able to differentiate painful discs from non-painful ones [90]. Such technology would substantially enhance the clinician's ability to detect the painful disc without invasive means and develop an appropriate therapeutic strategy thereafter.

The translation of a biologic therapy that could influence the course of the disease would revolutionise the treatment of DDD. Such a revolution in therapy has been seen in the treatment of inflammatory arthropathies such as rheumatoid arthritis, ankylosing spondylitis and, lately, for inflammatory bowel disease, all because of a commonality in the inflammatory pathway modulating the response to TNF $\alpha$  activity. In previous years, joint replacement because of profound destruction was common in rheumatoid arthritis; however, the emergence of anti-TNF therapy has markedly reduced the burden of illness such that surgical intervention for joint replacement occurs with far less frequency. The convergence of TNF $\alpha$  signalling pathways has a commonality in all of these diseases such that suppressing the proinflammatory effects of this inflammatory enzyme can significantly slow down/arrest disease progression and even induce remission. The development of a biologic therapy that could improve cellular viability, mitigate ECM degeneration and maintain the biomechanical properties of the IVD would have a profound effect on the treatment of DDD.

## Measuring success

Treatment success can be measured using a number of available metrics. It is important to consider both image-based as well as patient-derived functional outcome measure success. Therapeutic enhancement of disc structure as determined by conventional medical imaging may not necessarily translate into reduced patient pain and reported functional outcome. In considering the use of patient-reported measures, it remains important to record both generic (e.g., 36-item Short Form) and disease-

specific measures of outcome (e.g., Oswestry Disability Index). As previously described, the emergence of new noninvasive imaging techniques to diagnose painful discs also holds promise as another potential way to objectively monitor a patient's response to therapy.

## Conclusion/future perspectives

The development of a biologic therapy that can influence the course of DDD and its associated morbidity will yield a transformative solution in the management of DDD and lead to tremendous cost savings, avoidance of morbid surgical procedures and untoward effects (such as adjacent segment degeneration and further surgery) and drastically reduce pain medication dependence such as is the case with the current opioid crisis.

## Conflict of interest

The authors wish to confirm that there is no conflict of interest with any contents of this manuscript.

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