Intervertebral Disk Degeneration and Repair

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Intervertebral disk (IVD) degeneration is a natural progression of the aging process. Degenerative disk disease (DDD) is a pathologic condition associated with IVD that has been associated with chronic back pain. There are a variety of different mechanisms of DDD (genetic, mechanical, exposure). Each of these pathways leads to a final common result of unbalancing the anabolic and catabolic environment of the extracellular matrix in favor of catabolism. Attempts have been made to gain an understanding of the process of IVD degeneration with in Vitro studies. These models help our understanding of the disease process, but are limited as they do not come close to replicating the complexities that exist with an in Vivo model. Animal models have been developed to help us gain further understanding of the degenerative cascade of IVD degeneration In Vivo and test experimental treatment modalities to either prevent or reverse the process of DDD. Many modalities for treatment of DDD have been developed including therapeutic protein injections, stem cell injections, gene therapy, and tissue engineering. These interventions have had promising outcomes in animal models. Several of these modalities have been attempted in human trials, with early outcomes having promising results. Further, increasing our understanding of the degenerative process is essential to the development of new therapeutic interventions and the optimization of existing treatment protocols. Despite limited data, biological therapies are a promising treatment modality for DDD that could impact our future management of low back pain.

KEY WORDS: Biologics, Disk degeneration, Disk repair, Gene therapy, Injectables, Intervertebral disk, Stem cells, Tissue engineering, Spine

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The intervertebral disk (IVD) is a fibrocartilaginous structure whose main function is to transmit compressible load between the vertebral bodies, while also providing flexibility. The IVD differs from other connective tissues in the body, in that it shows degenerative and aging conditions early in life.[1] Age-associated maturation of IVDs, suggestive of degeneration, has been observed as early as age 11 and histological evidence of diminished blood supply to the vertebral endplates has been reported to start in the second decade of life with increasing prevalence with advancing age.[1,2] IVD degeneration also involves structural failure and is commonly associated with chronic back pain.[1-3] Back pain is one of the most common ailments with a point prevalence of between 12% and 30%, with around 10% of patients proceeding to develop chronic back pain.[3] The direct medical cost of back pain has reached $253 billion dollars annually in the United States.[4] There have also been effects on the psychological well-being of patients with significant increases in stress, depression, and anxiety after the onset of low back pain. Chronic back pain is a major health issue due to the debilitating nature of the symptomatology and the socioeconomic impact. There are a variety of reasons for back pain with disk degeneration only being one possible

ABBREVIATIONS: IVD, intervertebral disk; **DDD,** degenerative disk disease; **NP,** nucleus pulposus; **AF,** anulus fibrosus; **ECM,** extra cellular matrix; **ROS,** reactive oxygen species; **MSCs,** mesenchymal stem cells; **TE-IVD,** tissue engineered-whole implantable IVD; **TDR,** total disk replacement; **hTERT,** human telomerase reverse transcriptase; **ACAN,** aggrecan; **THBS,** thrombospondin; **CILP,** cartilage intermediate layer protein; **ASPN,** aspor; **TIMP,** tissue inhibitor of metalloproteinase; **TGF,** transforming growth factor; **MMPs,** matrix metalloproteinases; **IGF,** insulin-like growth factor; **FGF,** fibroblast growth factor; **PDGF,** platelet derived growth factor; **HSCs,** hematopoetic stem cells

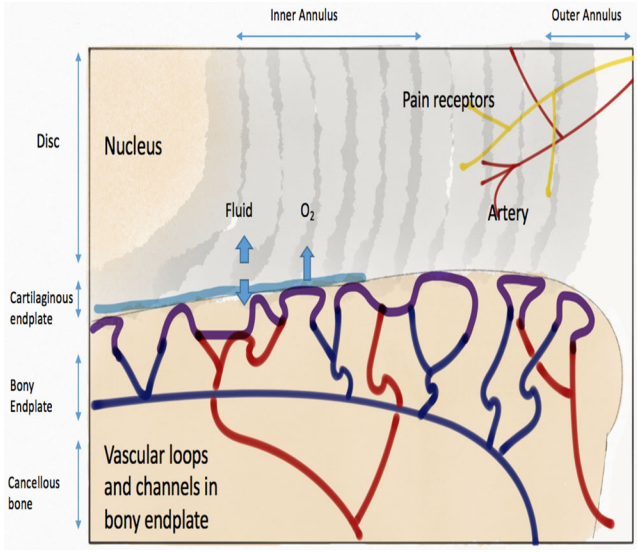


FIGURE 1. *Anatomy of IVD with sectioned view showing blood supply and innervation of IVD. ©Maritza Dowdell, 2016.*

cause. Not every therapy for back pain will focus on pathology at the IVD level. This paper will focus on therapies that are directly related to the IVD. Other conventional treatments for back pain include physical therapy, epidurals injections, surgical decompression, disk replacement, and fusion. These modalities for treatment will be covered in the degenerative thoracic and lumbar section of this focus issue. Significant efforts have been made to further understand the biological basis and clinical significance of IVD degeneration with the goal of preventing or reversing degenerative disk disease (DDD) and improving the clinical outcomes for patients afflicted with low back pain.

ANATOMY AND FUNCTION OF THE IVD

The IVD lies between the vertebral bodies and is composed of 3 main structures: the cartilaginous endplates, the nucleus pulposus (NP), and the anulus fibrosus (AF; Figure 1).[5-8] The NP is composed of collagen II and elastin fibers which are embedded in an aggrecan-containing gel. The fixed charge density of the aggrecan molecules in the NP generates high osmotic pressures, which contributes to the highly hydrated nature of the NP, helps maintain IVD height, and distributes loading across the endplate.[5,6] The cells in the NP have a low density, but are considered notochordal in origin prior to becoming chondrocyte like with aging.[5] In the young individual, prior to the onset of disk degeneration, there is a distinct border between the gel-like nucleus and the concentric rings of the AF.[7] The AF is comprised of 15 to 25 lamellae (concentric rings) with parallel collagen fibers within each lamellae and perpendicular collagen fibers between the adjacent lamellae which give rise to the tensile strength of the AF.[7] The outer AF contains fibroblast-like, thin, elongated cells and the inner AF cells are more of a sphere and appear similar to articular chondrocytes. The AF functions to contain the NP and maintain pressurization of the NP under compressive loads. The cartilaginous endplate is a thin horizontal layer of hyaline cartilage which is avascular in the adult.[7,8]

NUTRITION OF IVD

Early in life, vascular channels transverse the cartilaginous endplates. These disappear by 5 years of age. The IVD blood supply arises from 2 separate capillary plexuses (Figure 1). The first supplies the outer AF and the other arises from the vertebral bodies and terminates in the bone–cartilage junction.[2] The IVD is an avascular structure with some portions of the NP being located 8 mm from the nearest blood supply.[2] Nutrition is driven by a diffusion gradient of glucose, oxygen, and other macromolecules. The cells within the NP are furthest from the blood supply leading to low oxygen tension leading to anaerobic metabolism within the NP. The microenvironment of the NP thus has a higher concentration of lactic acid and lower pH than other portions of the disk, which can negatively affect cell function.

NORMAL IVD AGING

The IVD undergoes age-related changes earlier in life than many other tissues resulting in histomorphologic and functional changes. While age-related changes of the IVD are normal, the process of disk degeneration is a distinct pathological condition that involves structural failure and can occur at an age-accelerated rate.[1,7,9] The cartilaginous endplates have decreased permeability and vascular supply with advanced aging leading to alterations in the microenvironment of the IVD that favor catabolism.[2] The overall proteoglycan content of the IVD declines with aging leading to a less hydrated IVD, which leads to altered biomechanical properties of the IVD. Collagen type II fibers are replaced with collagen type I fibers in the inner AF and NP.[7,8,10] The NP begins to accumulate yellow pigmentation which also makes it less distinguishable from the AF.

MECHANISMS OF IVD DEGENERATION

Etiology of IVD Degeneration

IVD degeneration is attributed to a complex interplay between environmental and genetic factors. DDD is a process that includes a progressive decrease in disk nutrient supply and changes in extracellular matrix (ECM) composition, which weakens the tissue strength and alters the cell metabolism. A decrease in nutrient supply has been shown to negatively impact the IVD in its function to maintain the ECM. Nutrient supply has been found to be altered in the degenerative disk leading to

decreased oxygen concentration and lower pH.[11] Calcification of the endplates has also been shown to lead to a decreased blood supply. Inadequate nutrition inhibits the ability of the IVD to respond to increased load or injury. Structural damage is accrued over time further propagating the degenerative cycle.[12] However, genetics may play a larger role in DDD than both inadequate nutrition and mechanical damage. Twin studies have shown that genetics may in fact contribute as much as 70% of an individual's risk for DDD.[13,14] There are several categories of genes that contribute to DDD and they are grouped based on their function within the IVD. Polymorphisms within the Aggrecan (ACAN), COL1, COL9, COL11, FN, HAPLN1, Thrombospondin, Cartilage intermediate layer protein (CILP), and Asporin (ASPN) genes affect IVD structure.[15] Polymorphisms in the genes for the catabolic MMP1, MMP2, MMP3, PARK2, and PSMB9 and anticatabolic tissue inhibitor of metalloproteinases (TIMPs) favor catabolism and can contribute to the degeneration of the IVD.[15] These polymorphisms affect the delicate balance that exists between the anabolic and catabolic mediators that exists within the IVD. Anything that increases the inflammatory cascade contributes to the degenerative pathway of the IVD, by disrupting the balance between anabolism and catabolism of the IVD. Polymorphisms within the IL-1, IL-6, and COX 2 have been associated with DDD.[16-18] COX-2 specifically has been thought to contribute to the pain cascade within the degenerative disk.[18] Other genetic polymorphisms that have been found to contribute to the onset of DDD are those that code for vitamin D receptor (VDR) and GDF5.[19-20] The polymorphisms in VDR, ACAN, COL9, ASPN, MMP3, IL1, and IL6 have been validated in more than 1 ethnic population and have the broadest association with disk degeneration making them logical targets of any intervention that uses gene therapy.[15]

Environmental factors are also felt to have an impact on the pathogenesis of DDD. It was previously thought that "wear and tear" or repetitive physical loading was a major risk factor for DDD, but twin studies indicated that this only played a minor role in disk degeneration[21]. Obesity has been implicated as a risk factor in the degenerative process. There is mixed epidemiological evidence with regard to the effect of obesity in the degenerative cascade. Recent literature indicates that a BMI > 25 kg/m^2 was an independent risk factor for development of radiographic evidence of DDD and obesity at a young age was a strong risk factor for future increased in the number of degenerated disks.[22] Another hypothesis states that cardiovascular disease and atherosclerosis that are associated with obesity are a homolog for the atherosclerosis of spinal vessels leading to the degenerative cascade. Other studies indicate that obesity is associated with significantly increased levels of IL-6 and proinflammatory cascades throughout the body which could contribute to the inflammatory mediated pathway of disk degeneration.[23]

Cigarette smoking is the only chemical exposure that has been associated with disk degeneration. Twin studies have shown a disconcordance in the amount of disk degeneration on MRI, with those that were exposed to cigarette smoke having higher rates of disk degeneration. Cigarette smoking is presumed to inhibit blood flow at the vertebral endplates. The prevailing theory is that there is an interplay between cigarette smoking and activation of muscarinic receptors located at the endplate.

Biochemical Factors

The most important early change to the IVD is the increased degradation of aggrecan and other aggregating proteoglycans.[8] This biochemical change leads to loss of hydration of the disk which alters the biomechanics of the IVD, leading to accrual of structural damage to the IVD over time. Decreased hydration of the NP forces the AF to resist compression directly. The AF resists tensile forces much better than compressive forces. As the AF acts to resist compressive forces, it becomes stiff and weak and propagates the degenerative pathway. The IVD is characterized by a homeostasis of slow ECM breakdown and synthesis which is regulated by both anabolic (eg, Transforming Growth Factor (TGF), etc) and catabolic (Matrix Metalloproteinases (MMPs), ADAMTS, HRTA1, etc) agents, as well as inhibitors of the previously mentioned factors. Excessive stress can alter this homeostasis and contribute to a degenerative cascade. The imbalance in the anabolic and catabolic pathways often leads to an inflammatory reaction which further propagates the degenerative process. The catabolic environment of the IVD leads to matrix degradations products being formed, which in turn trigger the production of inflammatory mediators leading to further matrix degradation products.[24] Previous literature evaluating DDD showed differences in the expression of catabolic enzymes between nondegenerate and degenerate disks, and this can be modulated by immobilization and mechanical overloading. The production of MMP1 and ADAMTS 4 was seen in low levels in the nondegenerate disks, indicating a role for these molecules in homeostasis, while MMP 3 and MMP 13 were undetectable in the nondegenerate disks. In the degenerative disks, production of MMPs 1, 3, 13 and ADAMTs 4 increased as the severity of the degeneration worsened (Figure 2). However, this was accompanied with an increase in some of the inhibitors of MMPs (TIMPs 1, 2) but not others (TIMPs 3).[25] This study highlighted that while there was an increase in the expression of numerous MMPs with disk degeneration, the most clinically relevant finding was the lack of the increase in specific TIMPs (TIMPs 3). Increasing expressions of TIMPs and restoring the balance between anabolic and catabolic environments could be a possible therapeutic target for inhibition of disk degeneration.

Cell Senescence

There is an association with IVD cell senescence and the development of DDD[26,27]. Cell senescence is defined as irreversible cell cycle arrest caused by a variety of external stimuli or telomere uncapping. Senescent cells show various morphological changes as they will aggregate in clusters, increase in size, and become flat and vacuolized. In addition, these cells will also fail to replicate in response to mitogenic stimuli and aberrantly secrete proinflammatory cytokines and matrix degradation proteases.[28,29] The

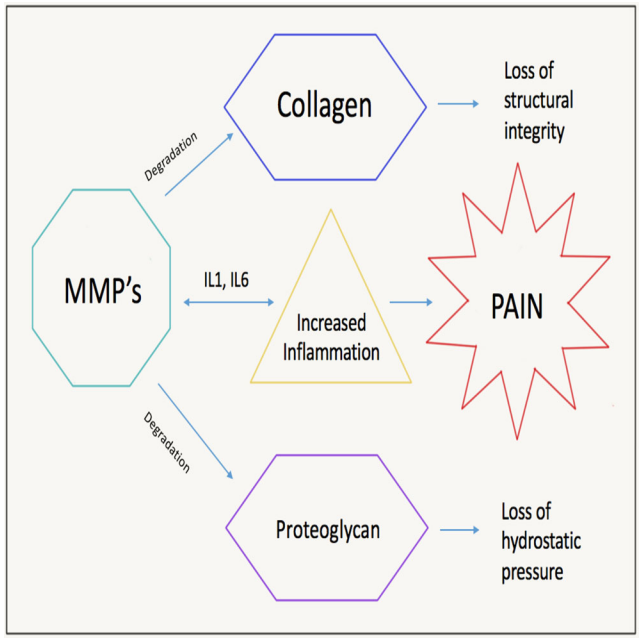


FIGURE 2. *Inflammatory pathways leading to disk degeneration.*

prevailing theory behind how cell senescence contributes to DDD is that senescent cells alter the balance of catabolic and anabolic pathways for ECM production in favor of catabolism.[30] The exact trigger for disk cell senescence is not perfectly understood and is thought to be cause dependent.[31]

Telomere erosion is 1 contributing factor in IVD cell degeneration. During serial replication, the telomere length becomes increasingly shortened leading to incomplete replication of DNA. This activates the p53-p21-Rb signaling pathway that leads to senescence with replication. There is significant correlation between shortening of telomere length and advancing levels of DDD.[32] Oxidative stress in the microenvironment of the IVD also triggers degeneration of the disk. NP cells are a major source of reactive oxygen species (ROS). Increasing levels of ROS have been associated with increasing levels of DDD, inhibition of proliferation of NP cells, and activation of the senescent signal pathways to induce cell cycle arrest of NP cells.[33-35] Reducing nutrient supply and growth factors (Insulin-like Growth Factor (IGF-1), Fibroblast Growth Factor (FGF), Platelet Derived Growth Factor (PDGF)) at the level of the IVD has been shown to increase rates of disk cell senescence. Specifically, IGF-1 has been shown to prevent disk cell senescence induced by oxidative damage.[34] The availability of growth factors to IVD cells is limited by endplate calcification.[36-37] Most importantly, abnormal mechanical loading has also been implicated as the initial event culminating in upregulation of senescence-associated genes (such as p16, p27, RB, PTEN etc).[38,39] In addition to activating the cell senescence pathway through the p53-p21-Rb pathway, mechanical stress has also shown to increase the production of ROS which leads to degeneration and senescence through a different pathway.[40,41]

Programmed Cell Death

Cell senescence is not the only pathway leading to a decrease in IVD cells, with an associated contribution to IVD degeneration. IVD cells also undergo programmed cell death through 1 of 3 apoptosis pathways (mitochondrial, death receptor, and endoplasmic reticulum pathways). The integrity of the ECM plays a vital role in maintaining the IVD. Degenerative processes within the ECM environment lead to increased catabolism causing multiple biological changes in the IVD cells leading to activation of the different apoptosis pathways. The different pathways of IVD cell apoptosis tend to be active at different levels of degeneration of the IVD. The endoplasmic reticulum pathway is most important during mild stages of IVD degeneration, the death receptor pathway is most prominent in mild/moderate stages of IVD degeneration, and the mitochondrial pathway is most prominent in severe stages of degeneration. Potential future therapeutic interventions can be applied toward decreasing cellular apoptosis. It would be important to identify the apoptotic pathway that is most likely to be active prior to implementing any therapeutic interventions. Inhibitors of caspases have been hypothesized to decrease the amount of degeneration and cellular apoptosis of the IVD.[42]

Understanding the pathogenesis of degenerative disk disease can help us target specific pathways in the degenerative cascade with the goal of prevention or reversal of the disease. There are a multitude of therapeutic options that could potentially be harnessed with the goal of preventing DDD including interrupting the inflammatory cascade, increasing anabolism of the ECM, preventing cellular apoptosis or senescence, or gene therapy.

ANIMAL MODELS FOR DISK DEGENERATION

Due to the increasing disease burden of lower back pain, animal models have been developed to further elucidate understanding and improve existing treatment strategies for this condition. In Vitro systems can be helpful in the understanding of specific pathways and components of IVD degeneration. However, an in Vivo animal model more faithfully replicates the inherently complex process of DDD. Several animal models have been developed (including mice, rats, rabbits, dogs, goats, sheep, and primates) using a variety of different mechanisms to simulate disk degeneration (genetic alterations, mechanical loading, or induced structural damage to the IVD).[43,44] Animal models cannot perfectly replicate degeneration of the human IVD for a variety of reasons, but can help us further our understanding of the process beyond what is possible with in Vitro models. Many of the species studied, with the exception of goat and sheep, have notochordal cells that are present into adulthood, which complicates investigations into therapies with cellular regenerative

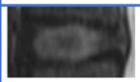
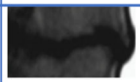
Degrees of Degeneration	Early	Intermediate	Advanced
MRI			
Number of Viable Cells	High	Decreased	Greatly Decreased
Structural Damage	Small	Increased	Greatly Increased
Therapy	Biomolecular	Cell-Based	Tissue Engineering

FIGURE 3. *Therapeutic strategies based on level of disk degeneration.*

therapies.[45,46] Disk size also impacts the degenerative potential seen. The IVD relies on diffusion for the nutritional requirements of the NP. The IVD disk of humans is much larger than of the common animal models that are seen which could hasten the degenerative cascade in humans. Disk geometry has also been studied with the goal of selecting an animal IVD model that most closely replicates the human IVD. The mouse lumbar IVD most closely matches the human IVD.[47] The majority of animal models of DDD are quadrupeds as bipedal models are limited in use due to ethical concerns. The forces seen by the IVD in quadrupeds are hypothesized to be up to 4 times larger due to the complexity of stabilizing a horizontally aligned spine vs a vertically balanced spine.[46] Understanding the differences and similarities between animal models and the human IVD can allow us to implement interventions in the animal model with the goal of translating these therapies a clinical benefit in the future. There are many different avenues for biological intervention at the level of the IVD based on the amount of degeneration present (Figure 3). The amount of degeneration that is present in the IVD provides an insight into the biology of the disk at that time. In earlier stages of degeneration, biomolecular interventions could have an effect to rebalance the anabolic and catabolic pathways in the degenerative cascade. In intermediate stages of degeneration, cell implantation can be used to repopulate the disk. In advanced stages of degeneration, tissue engineering constructs that mimic the native disk would have to be employed for biological reconstruction. Each of these methods has been attempted in small and large animal preclinical trials. More recently, functional behaviors are being assessed in animal models of IVD degeneration to better identify relationships between painful behaviors and DDD.[47]

Therapeutic Protein Injections

Protein solutions can be injected directly into IVD to stimulate cell growth and/or anabolic responses with the goal of reversing the degenerative cascade and preventing further disk degeneration.[48] The IVD has previously been shown to respond to exogenous growth factors.[49,50] Specific growth factors that have been shown to stimulate the growth of both bone and cartilage are the bone morphogenic proteins (OP-1, BMP-14) and members of the transforming growth factor-β. An in Vivo rabbit study showed an injection of intradiscal OP-1 induced an increase in proteoglycan content of the NP and increase of disk height.[51] This study has been repeated and also been shown to improve MRI findings of disk degeneration as well.[52-53] Rat models have shown an anabolic response of the IVD when injections of OP-1 were performed, with a restoration of normal disk morphology.[51,54] Sheep models of DDD have shown that BMP-13 injection prevents loss of hydration of the NP.[55] Negative effects have also been seen with injection of "therapeutic proteins", as injection of BMP-2 in a rabbit model has been shown to exacerbate degeneration of the IVD.[56] The limits to protein injections are the short durations of its therapeutic benefits. This promising potential treatment for disk degeneration could be improved with the development of a slow release carrier vehicle or gene-based delivery to increase to duration of benefit seen.

Gene-based Therapy

Gene therapy is based on inducing changes to intradiscal gene expression. These genes are delivered through a vector and are either injected directly into the cell or transduced into cells via a viral vector. Traditionally used viral vectors are a retroviral vector, adenovirus, adeno-associated virus, and baclovirus.[57] Nonviral vectors are in development, but to this point have not approached viral vectors in terms of their efficacy. The biggest drawback to using a retroviral vector is the potential for insertional mutagenesis leading to potential malignancies. An adenovirus vector has the drawback of the being highly immunogenic leading to major immune response against the foreign transgene-encoded proteins which has the potential to limit the efficacy of this technique. Viral vectors for gene transposition also carry a major expense in their preparation in addition to the still unknown risk to patients. Further development of nonviral transmission agents could mitigate cost and increase the safety of this technique for treating DDD significantly. One of the nonviral transmission agents in development is microbubbles delivered via sonoporation. This technique uses microbubbles to carry plasmid DNA, which encodes for the proteins of interest, and delivers these microbubbles into to the cell with the use of sonoporation (or ultrasound-induced transient holes on the cell surface). Another strategy for intradiscal gene therapy would be to focus less on upregulating the anabolic cascade which carries a significant energy expenditure and focus more on downregulation of gene expression that are harmful to the physiological balance of the disk.[57] Promising targets for gene therapy have included LMP-1 (regulation of BMP-7), disintegrin, MMPs, TIMPs, and chondrocyte-specific transcription factors (Ad-Sox9).[58-63] In rat models, plasmid DNA was mixed with microbubbles as a delivery system and transfected genes were still expressed up to 24 weeks from treatment in the cultured IVD. In a rabbit model, increased LMP-1 led to increased expression of PG, BMP-2, and BMP-7. In a separate rabbit model, increased expression of TIMPs was associated with delayed degeneration and increased expression

of Ad-Sox9 which led to retained chondrocytic appearance and normalization of the ECM.[63]

Cell Therapy

Therapeutic protein injections and gene-based treatment modalities have decreased efficacy with more advanced stages of disk degeneration due to the decreased number of cells available within the IVD to respond to these signals.[64] Cell-based therapy is a good treatment strategy in mid-stage degeneration to increase the number of IVD cells present with the disk. Many studies have proven that both autologous and allogenic disk cells survive within the disk. An injured canine model established that NP chondrocyte implantation contributed to ECM regeneration and prevention of further disk degeneration.[65] Mesenchymal stem cells (MSCs) can differentiate into all lineages of mesenchymal tissue, including the chondrogenic lineage and IVD-cell-specific phenotypes. This makes MSCs a potentially ideal option for repair of IVD as they are easy to obtain and once they differentiate, they would be able to produce proteoglycans and collagen for the disk ECM. Differentiation of MSCs to IVD cells is dependent on growth factors, oxygen tension, as well as a variety of other conditions. In addition, adipose-derived stem cells also show promise for IVD tissue engineering.[66] While there is some conflicting data, MSCs have generally been superior to differentiated disk cells in regeneration of the disk morphology after injection both in Vivo and in Vitro. Porcine studies have shown improved outcomes in disk repair when using articular chondrocytes vs MSCs with the hypothesis that well-differentiated cells are more apt to survive the ischemia of the disk environment.[67] Other studies in rabbit models have shown equivalent outcomes between the 2 cell lineages. MSCs can be an ideal substitute for IVD cells due to their better accessibility and success of this treatment option.[67] Another study shows that a combination of both of these cell lines leads to improved performance and survivability of implanted cells.[68] Both of these cells lines have great potential for repair of the degenerated disk.

Tissue Engineering

Once advanced degeneration and severe loss of cellularity is present within the IVD, there is little potential for reversal of this damage with cell-based implantation or therapeutic protein injections alone. Functional substitutes for damaged disk tissues must be introduced in the form of a scaffold, and physical conditioning of cells using either mechanical or electrical stimuli should also be performed.[69-71] Advancements in tissue engineering has enabled the constructions of tissue engineered-whole implantable IVD (TE-IVD). Both NP and AF composites replace the severely degenerated disk. This has been shown to engraft into the disk space in a rat tail model, where the TE-IVD exhibited similar properties to the native disk in biomechanical and biochemical testing.[72-73] Total disk replacement (TDR) was also performed in the canine cervical spine (axial loading closely matches human cervical spine) and the TE-IVDs engrafted to the host tissue and partially maintained disk height. When the TE-IVDs are used in combination with the protein and gene-based therapies, further clinical improvements are seen. A canine model of TDR with TE-IVDs loaded either with or without human telomerase reverse transcriptase (hTERT) gene in the NP cells showed an antidegenerative effect in the with hTERT group.[74]

Annular Repair

Injury to the AF can generate the catabolic cascade. However, the delivery of many of the therapeutic proteins or cell-based therapies involves puncturing the AF. Injury to the AF with a small needle puncture has been previously established to cause acceleration of disk degeneration in a 10-year follow-up of patients undergoing discography.[75,76] It would not be ideal to iatrogenically cause further degeneration of the IVD while attempting to either halt or reverse the degenerative cascade. AF repair has previously been attempted with suturing and anuloplasty techniques. Both of these techniques failed to improve anular strength in both sheep and porcine models.[77-78] To date no anular repair techniques have proven successful on an in Vivo model, which has led to the development of more robust in Vitro herniation models with aggressive cyclic loading and systematic screening processes. New modalities for anular repair are being studied and developed. Anular repair techniques have great potential to work in conjunction with other treatment modalities in DDD including drug and cell delivery.[79]

Clinical Trials and Treatment of DDD

There have been a limited number of clinical trials for biological treatment of IVD disease. Therapies which have proven benefit in preclinical trials are in varying phases of being attempted in clinical trials to prove their efficacy and safety for human use. There have been several trials of injections of stem cells into degenerated disk. The injections of hematopoietic stem cells for discogenic back pain failed to yield any improvement in the level of low back pain after 1-year follow up.[80] Injection of IVD cells, in the EuroDISC study, 3 months after discectomy, showed reduction in back pain on 2-year follow-up compared to discectomy alone.[81] In addition, disk height was maintained, and there was less adjacent segment disease in the patients who underwent autologous disk chondrocyte transplantation plus discectomy group vs the discectomy alone group.[81] Other clinical trials have also shown some benefit to injections of MSCs into the disk with improvement of pain and MRI findings.[82,83] Injection of allogenic juvenile chondrocytes into the IVD of patients with single-level DD has also been investigated with early results showing improvement in pain in a majority of patients and improved findings in 10/13 patients on MRI at 6 months.[84] However, despite these encouraging preliminary results, there are many obstacles that remain prior to the clinical use of cell-based therapies. The IVD harsh microenvironment had led many to be skeptical that biological and cell-based therapy will make an impact on the process of disk

degeneration. In NP cells, hypoxia inducible factor is stable in an oxygen-independent fashion, which allows these cells to survive the harsh microenvironment.[85] It is unknown if implanted cells acquire the properties of NP cells to adapt to the avascular environment that is present. Future goals of research in cell-based therapies could be to identify the correct cells for implantation (Hematopoetic Stem Cells (HSCs), Mesenchymel Stems Cells (MSCs), chondrocytes, notochordal cells, adult IVD disk cells, etc) and development of new tools to diagnose IVD degeneration needing cell therapy. There have been preliminary results showing a possible benefit of an intradiscal PRP injection, with 47% of patients achieving a 50% improvement in pain via the visual analog scoring system.[86] There are several nonpublished clinical trials examining the effect of GDF-5 and OP-1 on disk repair. Mesenchymal lineage adult stem cells implantation has shown promising results as 42% of patients undergoing treatment had a 50% reduction in pain compared to 13% for placebo, but this is also unpublished data.[87] There are no clinical in Vivo trials that have been performed in humans with regard to gene therapy. In Vitro studies have shown decrease in MMP expression in IVD cells after introductions of IL-1 receptor antagonist via an adenovirus vector.[88] Another in Vitro study transfected human cells with a combination of TGF-β1, IGF-1, BMP-2 and noted proteoglycan synthesis was 4.7 times that of control cells.[89] Prior to any in Vivo or ex vivo (with subsequent cell implantation) studies with viral vectors, there must be questions answered with regard to viral vector safety or whether it would be safer to optimize the less effective but ultimately less or nonvirulent vectors such as the ultrasound microbubble technique previously described.

Summary and Future Therapies

A thorough understanding of the mechanisms for disk degeneration is necessary to design treatment strategies for biological healing and repair of the IVD. The amount of degeneration present within the IVD could dictate the appropriate intervention as different treatment strategies could have differing efficacies at different points along the degenerative cascade. The process of IVD degeneration is complex and multifactorial, and thus the solutions for reversing this process will be equally if not more complex and likely involve multiple solutions depending on the disease phenotype and progression. Future studies should advance the number in Vivo human studies with all of the available treatment modalities. There should also be continued elucidation of intracellular signaling pathways to allow for more targeted regulation of IVD cells. As our ability to understand the process of IVD degeneration grows, new modalities of treatment will be identified and developed, and current modalities of treatment will be optimized. These new treatments will require rigorous testing that can progress to human studies to evaluate their efficacy and impact on reversing IVD degeneration. Biological therapies remain a promising possibility to address the complex processes of painful disk degeneration.

Disclosure

The authors have no personal, financial, or institutional interest in any of the drugs, materials, or devices described in this article.

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