Biologically Based Therapy for the Intervertebral Disk: Who Is the Patient?

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Abstract

The intervertebral disk (IVD) is a fascinating and resilient tissue compartment given the myriad of functions that it performs as well as its unique anatomy. The IVD must tolerate immense loads, protect the spinal cord, and contribute considerable flexibility and strength to the spinal column. In addition, as a consequence of its anatomical and physiological configuration, a unique characteristic of the IVD is that it also provides a barrier to metastatic disease. However, when injured and/or the subject of significant degenerative change, the IVD can be the source of substantial pain and disability. Considerable efforts have been made over the past several decades with respect to regenerating or at least modulating degenerative changes affecting the IVD through the use of many biological agents such as growth factors, hydrogels, and the use of plant sterols and even spices common to Ayurvedic medicine. More recently stem/progenitor and autologous chondrocytes have been used mostly in animal models of disk disease but also a few trials involving humans. At the end of the day if biological therapies are to offer benefit to the patient, the outcomes must be improved function and/or less pain and also must be improvements upon measures that are already in clinical practice. Here some of the challenges posed by the degenerative IVD and a summary of some of the regenerative attempts both in vitro and in vivo are discussed within the context of the vital question: "Who is the patient?"

Keywords
- intervertebral disk
- regenerative medicine
- biological therapy
- growth factors
- stem cells

Over the past 20 years, there has been an explosion in the biotechnology sector concerning the use of recombinant proteins such as growth factors for the treatment of injury/disease, (such as the use of bone morphogenic protein in the management of complex fractures). Furthermore, the recent advances in the use of stem/progenitor and induced pluripotent stem cells have offered the possibility that true regenerative medicine could someday become more than a catchy phrase. Biological therapy has been postulated as a potential "game changer" for the management of disk disease since at least 1991 as presented in the seminal paper by Thompson et al.[1] However, despite over 700 published papers, 22 years after the report by Thompson et al the use of biological agents in the management of disk disease is in, at best, its infancy. There is only one phase 1 clinical trial involving the use of growth differentiation factor-5 (GDF-5) underway for the treatment of disk disease; however, several trials using human stem or porcine stem cells have been undertaken.[2–4] With respect to biological agents and disk disease, the important unanswered (perhaps "elephant in the room") question still remains: Who is the patient?

Intervertebral Disk Compartments

The intervertebral disk (IVD) is a unique organ that modulates complex, enormous applied loads to the spine, protects the spinal cord and exiting nerve roots, functions as a major axial support system for the body, and acts as a barrier to metastatic

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disease. These functions are fulfilled as a consequence of the IVD's central location within the spine and its anatomical configuration and biomechanical properties. The disk is composed of several subcompartments, notably the cartilaginous end plates, the annulus fibrosus, and the nucleus pulposus, with each compartment composed of cells that have differentiated to tolerate the unique requirements of the specific compartment. The cartilaginous end plates are composed of chondrocytic cells embedded within a hyaline-like extracellular matrix (ECM) integrated with the vertebral bodies. The functional linkage of disk and vertebral body creates a permissive though delicate portal whereby the diffusion of nutrients, gases, and waste products subserves IVD homeostasis.[5,6] It has been reported that the vertebral body capillary networks centered over the nucleus pulposus (NP) are much denser than those overlying the annulus, a feature of biological importance with respect to the metabolic demands of the cells and tissues within these compartments.[5–8] The cells of the annulus fibrosus are a combination of fibroblastic and chondrocytic cells embedded within an ECM that results in a structure that acts like a ligament, conferring strong compressive and concentric biomechanical resistance acting in concert with the inner nucleus pulposus and cartilage end plates. The nucleus pulposus represents what may be considered the lynchpin of IVD function due to its central, confined location within the center of the disk and its vital contribution to the biomechanical properties of load dispersion and contribution to neuromuscular reflexive activity.[9,10] Significant degradation of the essential cellular and structural aspects of any of the compartments of the disk contributes to breakdown of the entire organ often leading to pain and disability.

Biology of Disk Degeneration

Degeneration of the ICD is a complex process, and although considerable progress has been made with respect to the mechanisms involved in the degenerative cascade, much remains to be learned. Several catabolic cytokines, such as interleukin (IL) 1-β, act in concert with other inflammatory cytokines, such as IL-6, IL-8, prostaglandin E2, nitric oxide, a variety of matrix metalloproteinases, and ADAMTS (a disintegrin and metalloproteinase with thrombospondin motifs) 4/5 enzymes as well as the death-inducing ligand Fas, and result in degradation of the ECM.[11–24] As the degenerative process continues, the viability of NP cells progressively declines with both IL-1β and Fas ligand figuring prominently in these mechanisms as well as increased degradation of the annulus fibrosus.[25–27] The expression of certain genetic anomalies, in particular ones involved with the ECM, may predispose the disk to accelerated/pathological degeneration, which when coupled with environmental/occupational risk factors may result in clinically significant signs and symptoms of spinal and related pain. Finally, although up to 30% of patients demonstrating signs of degenerative disk disease (DDD) on magnetic resonance imaging (MRI) will be asymptomatic, it has been reported that DDD is associated with back pain and more so with increasing evidence of DDD and older age.[21] Although it is widely accepted that degenerative changes seen on MRI cannot determine the disk as a source of pain and that many people in midlife demonstrate degenerative changes on imaging such as MRI, it should be emphasized that not all degenerative changes are benign. There are several reports demonstrating the capacity for degenerative disks to be potent sources of pain; the problem is that contemporary imaging and diagnostic practices may not be sophisticated enough to determine the pain generators leading to sometimes ill-defined therapeutic goals and methods.[25,28,29]

Challenges and Obstacles to Disk Repair

It has been well characterized that pathological changes affecting the vertebral end plates may compromise the already precarious nutritional status of the IVD leading to degenerative change.[5,6,8,30–32] Impaired diffusion leads to decreased pH and oxygen concentration, progressive cellular death (such as via apoptotic mechanisms), and impaired cell–ECM interaction, resulting in a progressively biochemically and biomechanically impaired IVD.[8,33] The peripheral annulus is vascularized separately and receives barely any diffusible nutrition; therefore, impaired nutrient/gas diffusion may have less impact in this region. However, clefts and fissures occur within the annulus fibrosus, allowing the ingrowth of blood vessels and nociceptive-capable neurons into the NP simultaneously with degeneration of the nucleus.[28] In the case of degenerative disease, this process is gradual and requires years to occur, with symptoms that may or may not declare themselves until the process has become more advanced. However, when symptoms of spinal pain and/or radiculopathy become evident, contemporary diagnostic methods that can define the disk as a significant source of pain lack sufficient precision/sophistication. A common clinical vignette would be that of a patient exhibiting long-standing spinal pain who displays common characteristics of pain emanating from the IVD that has proven refractory to conservative care. Another common presentation is the patient with a history of frequent acute low back pain episodes with antalgia with or without leg pain who develops an acutely herniated disk commonly following relatively trivial or even no trauma. Both of these patients are often considered not to be good surgical candidates, and if/when the patient's condition does not respond to nonoperative measures, what should be done? Epidemiological evidence indicates that many cases of disk herniation resolve with time as the disks' inherent healing properties allow some degree of repair and sciatic pain (if present) and the condition resolves.[34] However, many cases continue to be symptomatic long after the initial symptoms have improved with many of these patients finding themselves labeled as chronic pain patients receiving ill-defined treatments for ill-defined reasons.[34–36] It has been demonstrated that many patients with chronic spinal pain suffer from cognitive-related aspects to their pain and do well with activity and cognitive behavior-based therapy.[37] However there is very likely a subcategory of patients who are inappropriately diagnosed with chronic pain but who actually suffer from biologically mediated pain that may well

emanate from the disk; these patients will not respond to exercise and cognitive behavior interventions if their source of pain is biological at its source.[14,28] Therefore, it may be more accurate to characterize patients suffering from an acute herniated disk whose symptoms settle as having experienced resolution from their acute symptoms rather than healing, as many if not most continue to suffer from relapses of axial pain/sciatic pain with variable intervening asymptomatic episodes for many years. It is a rarity for a patient suffering from such an injury or degenerative condition to ever achieve a long-lasting asymptomatic status.

Biological Attempts at Disk Repair: Results to Date

A recent PubMed search using "biological repair of the intervertebral disk" as search term yielded 52 published manuscripts and using "growth factors and intervertebral disk" as search terms yielded 785 hits. The majority of the articles identified in these searches presented work that was based initially upon in vitro evidence of the anabolic/reparative effects of growth factors upon NP cells that led to subsequent in vivo testing in a variety of animal models. Reviews by Masuda et al and Yoon and Patel have summarized much of the work using growth factors for the treatment of disk disease that developed initially from the cartilage literature and was first published by Thompson et al using a canine disk model in 1991.[1,38,39] Since the early reports of the use of growth factors, several methods of enhancing the delivery of these molecules have been developed, ranging from direct injection of recombinant proteins to viral transfection methods whereby cells of the NP could be modified (such as by the use of viral vectors) to secrete increased amounts of a specific growth factor.[40,41] A more recent attempt to augment the effects of growth factor injection is through the use of platelet-rich plasma (PRP). PRP is a method of using autologously derived growth factors released from highly condensed blood plasma whereby the platelets release a host of (as yet, incompletely characterized) growth factors and cytokines. A recent study has reported that PRP releasate has the capacity to induce a reparative response when injected into rabbit IVDs injured by scalpel stab.[42] This PRP study reported that the PRP releasate-treated disks demonstrated a significant restoration of disk height but no statistically significant change in disk NP hydration as evaluated by MRI, but there were more chondrocyte-like cells within the PRP-treated disks compared with controls.[42] With respect to the use of growth factors in human disk disease, there is at least one ongoing phase 1 clinical trial using a specific growth factor (GDF-5) based primarily upon rabbit IVD models of disk injury, with results pending. There have been several other mediators of disk degeneration proposed as potential therapies in addition to direct injection of recombinant proteins (growth factors) or gene therapy such as caspase inhibitors, IL-1 receptor antagonists, and plant sterols such as resveratrol.[43,44] Even the Indian spice used in Ayurvedic medicine curcumin has been reported to have beneficial activity when evaluated in vitro with some of these interventions tested with in vivo studies.[43,45] Beyond the use of modulators of the degenerative process (growth factors and anti–cell death strategies), there have also been attempts at cellular replacement using autologous chondrocytes and a variety of stem cells.[46–50] Some of these attempts have met with early indications of success such as the apparent restoration of hydration as evidenced by T2-weighted MRI imaging as well as immunohistochemical and RNA evidence of anabolic repair.[47,49,50] These results have by and large been confined to in vitro studies as well as small-animal models of disk disease using rat tail, rabbit disk puncture models, and some limited canine species.[12,50–53] One recent pilot study by Orozco et al using autologously derived human stroma mesenchymal stem cells (MSCs) claim to have demonstrated reduced pain and the restoration of some hydration in the treated disks.[4] It is curious that these investigators cite spinal fusion as the gold standard for the treatment of DDD because there are several high-quality studies citing the controversy surrounding spinal fusion and other studies including a recent systematic review indicating that spinal fusion may not be better than appropriate nonoperative management.[54–57] Nonetheless, the Orozco study is an interesting preliminary step involving the use of MSCs for the treatment of human disk disease. However, several areas remain to be understood involving the use of stem cells. For example, MSCs are known to be growth factor "factories," which may be responsible for some initial benefits so long as the cells survive—a factor not addressed by these authors but noted in the review by English.[58] Prockop et al furthered this discussion by illustrating that some effects seen with stem cell transplants are in the form of immune modulation and anti-inflammatory effects conferred upon the transplant milieu by the transplanted MSCs.[59] Furthermore, transplanted stem cells such as MSCs may modulate repair by virtue of their effects upon the endogenous stem cells at the repair site and as conferred by the secretion of growth factors and antiapoptotic and immune modification effects and in so doing act as a kind of repair "booster" rather than effecting repair themselves.[59] Also, the patients involved with the Orozco study averaged 35 years of age—an age considerably younger than expected for significant degenerative disease as opposed to disk injury. The inclusion criteria in this study do not provide any details to this effect or the history of the patients involved other than "degenerative disk disease" or what failure of conservative management of the disorder entailed. Interestingly these patients all received diskography prior to stem cell transplantation—a technique reported by Carragee et al to result in accelerated degeneration.[60] With respect to biological therapies, it may be that the repair response induced by the intervention (growth factors, anti–cell death strategies, stem cells, or some combination) overwhelms any potentially injurious effects associated with the insertion of a needle into the disk as reported by Carragee et al. Numerous studies that purposely induce disk damage by the use of relatively large needles with respect to the size of the disk (up to 18-gauge needles for rat disk injuries) have demonstrated impressive recovery of T2-weighted signal on MRI, suggesting that biological agents may be able to induce profound healing even after relatively severe injury.[61,62] With this in mind, it should be noted that the Carragee study preceded publications concerning probable genetic

influences on disk degeneration, leading to the open questions concerning the possible genetic influence over the expression of disk disease of the patients reported in that study.

Although perhaps not strictly classified as a biological therapy such as the use of growth factors or cellular replacement strategies such as stem cells, the use of hydrogels as a treatment that could enhance the biological properties of the IVD have been more recently studied with several publications detailing their potential utility.[63,64] The publication by Reitmaier et al compared the reimplantation of a removed nucleus to several hydrogel constructs in an ovine ex vivo study and found that none of the interventions were able to restore biomechanical properties of the intact disk. The conclusions of this study suggest that the use of implantable hydrogels may not be able to restore nucleus functionality in particular without anchorage to the surrounding disk structures such as the inner annulus and end plates.[64] Nonetheless, this area of investigation continues and may yet provide better utility with further research.

What Kind of Therapeutic Intervention?

The complex biochemical and cellular processes that lead to disk degeneration are driven by mitigating factors such as genetic influences, age, metabolic status, history of injury, occupational activity, and lifestyle habits such as smoking.[23,65,66] The net result for persons affected by significant DDD is pain and disability and the need for therapeutic intervention. Therefore if biological therapy is considered to be an option for disk disease, the essential questions are what kind of therapy, for which patient, and how best to deliver the therapy in question?

The degenerative disk that has reached what could be characterized as the point of no return with extensive disk collapse, few remaining viable cells, and extensive annular tears and fissures would likely not be a good candidate for the delivery of anabolic/ECM-protective factors (growth factors, anticatabolic factors) and/or stem/progenitor cells. More likely, the disk that is more upstream in the degenerative process would be an appropriate target for regenerative therapies. As disks degenerate, the cell viability within the nucleus decreases such that advanced degenerative disks have virtually no viable cells remaining. Therefore, it would stand to reason that anabolic/anticatabolic intervention(s) ought to occur before cell viability decreases beyond what may be required to stimulate an anabolic response. As the field of stem cell biology has evolved, such a strategy is probably more complex than originally anticipated. A cellular replacement strategy—perhaps originally thought to provide simply increased numbers of cells that may assume the role of degenerative/dead cells—may actually perform far more complex functions, providing of course that they can survive transplantation. It has been reported that MSCs secrete significant amounts of growth factors, and it may be that at least part of the efficacy that such interventions might offer could be the result of not only cellular replacement, but also the longer-term release of necessary growth and other factors within the degenerative disk.[3] In any event, whatever the actual intervention may be—whether it is a cocktail of proteins, progenitor cells, or some combination of these—the active ingredients must be delivered into the disk; the question remains, what is the best method? Direct injection has obvious advantages but would require a needle puncture.

Models of Disk "Disease"

Several methods of inducing disk "disease" have been reported, all of which require damaging the disk to effect a secondary reparative response, which is interpreted experimentally as DDD.[51,52,67–69] The degree to which the particular interventions (gene therapy, direct injection of growth factors/stem cells) are able to improve upon the natural history of the acute disk injury ostensibly validates the effectiveness of the therapeutic intervention. To this end, a suitable animal model of disk degeneration continues to be elusive in the field of disk biology research, although carefully specific hypothesis testing through the use of animal models has shed light on certain aspects of disk disease and the potential for biological therapies. It is therefore important to consider that most if not all reports concerning biological treatments of disk disease to date are based upon disk injuries that do not mimic the human condition. They are performed on acutely injured (mostly needle puncture or scalpel stab injuries), young and otherwise healthy animals with disks that resemble that of a human of childhood age.[61,62,69] The disks are highly gelatinous and the cellular contents are largely notochordal as well as the ill-classified "nucleus pulposus cell" and a population of stem/progenitor cells reported to be of ~1% of the total cellular volume of the NP.[70] The injurious stimulus either via direct relatively large-bore needle puncture (up to 40% of the disk height), scalpel stab, aspiration/denucleation of the disk, or enzymatic digestion imposes an extremely violent injurious stimulus upon an otherwise healthy, youthful disk to challenge the inherent repair properties of the tissue as compared with the delivered therapeutic vehicle. Nonetheless, it should be recognized that most existing animal models and the conclusions drawn from these studies actually represent repair from "disk injury" as opposed to repair of disk degeneration that is representative of the human condition and results of such studies need to be interpreted with this caveat.

As discussed earlier with respect to stem/progenitor cells, it may be that they confer their regenerative/reparative effects after transplantation in more ways than just a source of cellular replacement. Several studies involving animal models have been performed concerning the potential use of stem cell transplants to the IVD with mixed results.[53,71,72] One study involving xenographic transplantation of human MSCs (MSCs) into minipig IVDs reported that all disks injured and treated in this study demonstrated signs of degeneration on MRI, but the disks treated with a gel carrier + human MSCs had less degeneration.[2] Furthermore, human MSCs were detectable up to 6 months posttransplant with evidence of ECM protein secreted by these cells. The investigators in this study suggested that for most optimal cell-based transplant therapies, it may be better to use differentiated cells rather than MSCs. On

the other hand, another recent large-animal model using pigs reported that 12 months after the transplantation of MSCs, little if any proteoglycan matrix was established, and rather than evidence of implantable cells, there remained only scar tissue composed of a mixture of type I/II collagen.[73] A report using chondrodystrophic canines and stem cell transplants reported that 10^6 transplanted MSCs resulted in an astonishing ~94% cell survival, suggesting that a Pfirrmann grade II to III might represent the best candidate level of pathology for such cellular transplant technology.[53] Very likely these impressive and demanding large-animal studies reach such divergent results with respect to cell survival and transplant effectiveness due a host of technical, procedural, and biological reasons. Nonetheless, the results of these investigations need to be interpreted cautiously and carefully.

Who Is the Patient?

At the end of the day with respect to biological treatments, we are still left with the question of who the patient is. Would growth factors with or without adjuvants delivered via a suitable carrier be the best treatment for a more acute disk injury in a younger person? Should this treatment be delivered percutaneously, or would it be best performed at the time of diskectomy, perhaps within a slow-release formulation? Should this same patient have a combination of cell-based therapy plus growth factors? What would be the best treatment for the longer-term degenerative disk thought to be the source of pain? What kind of cells should be delivered and at what cost? Should the patency of the vertebral end plate be a mitigating factor in the decision to apply biological therapy and if so how would this be assessed? Finally and perhaps most importantly, would such biological treatments make any impact on the pain and disability suffered by the patient?

Although considerable progress has been made with respect to understanding the biology of disk degeneration and the generation of pain, all of the previous questions should be borne in mind as potential biological therapies are developed. Although the effectiveness of many proposed biological therapeutics have met with mixed results, thus far these studies must be interpreted as small steps along a very long road in an as of yet somewhat ill-defined direction. It is likely that if and when biological therapy for the IVD does achieve meaningful clinical standards, one size will not fit all. Biological therapies will need to be optimized and matched for their best possible effectiveness and efficacy that is commensurate with specific pathology. To realize this goal, biology, biomechanics, engineering, and epidemiology—although oftentimes strange bedfellows—will need to achieve an uneasy truce and together determine how, when, if, and most importantly who may be the best candidate for their ministrations. Until then, we are really in our infancy in the pursuit of this fascinating and challenging enterprise.

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