Current insights on use of growth factors as therapy for Intervertebral Disc Degeneration

Abstract: Chronic low back pain is a critical health problem and a leading cause of disability in aging populations. A major cause of low back pain is considered to be the degeneration of the intervertebral disc (IVD). Recent advances in therapeutics, particularly cell and tissue engineering, offer potential methods for inhibiting or reversing IVD degeneration, which have previously been impossible. The use of growth factors is under serious consideration as a potential therapy to enhance IVD tissue regeneration. We reviewed the role of chosen prototypical growth factors and growth factor combinations that have the capacity to improve IVD restoration. A number of growth factors have demonstrated potential to modulate the anabolic and antiproliferative effects in both in vitro and animal studies of IVD tissue engineering. Members of the transforming growth factor-β superfamily, IGF-1, GDF-5, BMP-2, BMP-7, and platelet-derived growth factor have all been investigated as possible therapeutic options for IVD regeneration. The role of growth factors in IVD tissue engineering appears promising; however, further extensive research is needed at both basic science and clinical levels before its application is appropriate for clinical use.

Keywords: growth factor; intervertebral disc; tissue engineering; intervertebral disc disease.

Introduction

Chronic low back pain remains a prevalent and widespread complaint among patients seeking medical attention worldwide. Low back pain contends as one of the top chronic debilitating problems for many people, and continues to impose itself as a detriment to the growing healthcare concerns in terms of both direct medical costs and otherwise decreased productivity. While a wealth of variable statistics exists, the lifetime prevalence of low back pain is generally accepted to be in the 70–85% range, suggesting that most people will be afflicted by this ailment at some point in their lives. When considering the annual prevalence, studies estimate a 15–45% annual occurrence rate and an average point prevalence of roughly 30%. Low back pain is associated with huge economic and social burden in the United States. More than $100 billion per year is the estimated total cost of low back pain. 70% of this cost is a result of reduced productivity and lost wages. Disability-adjusted life years burden of low back pain increased from 58.2 million in 1990 to 83 million in 2010.

With annual and lifetime prevalence this high, it stands to reason that the growing interest and research devoted to both the pathophysiology and potential treatment regimens for low back pain are well founded. The overwhelming culprit for the majority of chronic low back pain is degeneration of the intervertebral disc (IVD). Over a 10-year period from 1997–2007, the scientific and medical communities have witnessed a three-fold increase in the number of peer-reviewed journal articles devoted to various aspects of biology and biotherapeutics related to IVD disease. Further, the documented impact on the hampered quality of life and even disability rates are formidable among low back pain sufferers. Among the United States population under 45-years old, back pain is the leading cause of activity limitation, second most common cause for physician visit, third for surgical intervention, and fifth most frequent cause for hospital admission. These are impressive, yet appropriate, numbers that reflect the heightened awareness of low back pain as an area in need of more effective therapeutic treatment.
options. Considering the potential sequelae of the costly and invasive surgical procedures, alternative treatment modalities via biogenic modification is an area meriting investigation. Several biological based therapies have gained attention either as currently practiced options or as potential biologic and genetic manipulation methods. In addition to conventional surgical and pain management options, research communities are investigating disc or stem cell implantation, painful disk denervation, gene therapy, and injection of specific therapeutic proteins. While each of these approaches has its respective promise, for the purpose of this review we will primarily focus on the therapeutic possibilities revolving around prototypical growth factors involved in IVD disease.

With such an impact being made on the quality of life of so many patients, there has been an increase in efforts to find a minimally invasive, safe, and effective treatment through the injection of targeted proteins to delay or reverse the known pathology that comes with IVD disease. Much attention has been paid to prolotherapy as an effective way to provide some improvement to chronic low back pain. Prolotherapy is minimally-invasive injection of factors to promote proliferation of normal cells and tissues. Prolotherapy includes three main types, growth factor injection prolotherapy, growth factor stimulation prolotherapy, and inflammatory prolotherapy. Growth factors injection prolotherapy has been heavily investigated recently to assess its feasibility, safety, and efficacy for IVD degeneration. Preliminary data have been generated over the past few years about the outcomes after the intradiscal administration of the following set of growth factors: platelet-derived growth factor (PDGF), insulin-like growth factor-1 (IGF-1), basic fibroblast growth factor (bFGF), fibroblast growth factor-18 (FGF-18), growth and differentiation factor-5 (GDF-5), transforming growth factor beta-1 (TGF-β1), bone morphogenetic protein-2 (BMP-2), and bone morphogenetic protein-7 (BMP-7). The metabolic activity of IVD cells is modulated and regulated by several enzymes, cytokines, and growth factors via either autocrine or paracrine manner. Any deviation from the homeostatic balance between the anabolic and catabolic factors in the normal, healthy disc will result in degenerative process of IVD. The definite role and mechanism of growth factors within the normal disc are poorly understood. Our aim is to provide a brief evidence-based review about some of the previous and ongoing research concerning the prospect of the growth factors and their role in IVD tissue engineering; we do not aim to comprehensively cover all the existing literature, or all the suspected growth factors involved in degenerative disc disease (DDD).

Pathophysiology Of Intervertebral Disc Disease

Beginning with the anatomical makeup, three key components contribute to the biomechanics and pathophysiology of the IVD: the inner nucleus pulposus (NP), the outer annulus fibrosus (AF), and the adjacent vertebral end plates. The NP is surrounded by the AF in a circumferential manner, while the end plates provide separation from the disc superiorly and inferiorly. These structures are primarily composed of differing ratios of proteoglycans, collagen, and water. While the NP serves as more of a cushion due to its significant fractional content of water, the AF and end plates combine to dissipate the compression forces and shield the vertebral bodies from bony compromise.

At a cellular level, the NP is comprised of a roughly 1:20 ratio of type II collagen to the proteoglycan aggrecan. The aggrecan creates an osmotic gradient that helps draw water into the IVD. The hydrophilic nature of aggrecan and its abundance in the NP are primary contributors to the spongy and cushioning nature of the IVD. Further, these functionally desirable properties are compromised in degenerative disc disease to the extent that much of the type II collagen and aggrecan of the matrix composition is replaced by type I collagen and an associated decrease in aggrecan synthesis. In turn, the IVD loses some of its hydrophilic nature and the accompanying space-retaining aspects of the disc, ultimately leading to abnormal loading, movement, and significant pain which ends up with constricted health-related quality of life.

In contrast to the NP, the AF has a composition heavily weighted with type I collagen yielding a more structurally sound matrix to circumferentially contain the inner NP. Like the NP, the composition of the AF gives insight to its functional properties. Roughly 60% of the AF is collagen, while another 25% is aggrecan, yielding the more stringent properties of this structural component compared to the NP. The many lamellae provide rotational support and counterbalance the loading forces of adjacent vertebrae.

As the natural balance of the forces of the AF and NP is disturbed, shock loading is promoted at the enthesis during ordinary movement. The potential result of this imbalance is painful movement and microtrauma. This microtrauma to the AF and surrounding bone allows angiogenesis to the IVD, where it is typically absent. This angiogenesis has been shown to detrimentally affect patients’ intensity of pain, postoperative pain improvement, mobility, and overall quality of life as a consequence of IVD herniation.
microtraumatic effects in the NP can be seen in the AF and end plates resulting in fracture and facet compromise, resulting in an insult to structural integrity and presumed attenuation of degenerative changes. While a sufficient number of human studies that connect microtrauma with pain are still lacking, the link between in vitro microtrauma with the resulting angio-/neurogenesis and IVD pain yield a convincing juxtaposition that warrants ongoing consideration.

The previously described changes in the motion segment are related to the matrix changes of the IVD; however, the cellular biology of the NP, AF, and end plates serve to mitigate these motion segment variations. It is also worth mentioning that the contentious issue of growth factor activation and the relentless cascade of degeneration continue to create end plate compromise and challenge disc integrity; these issues will surely be the focus of future research. Furthermore, much of the current literature focuses on the composition and alteration of the NP cells; however, manipulating and potentially reversing some of the catabolic changes in the NP cells would also structurally benefit the AF. While the discussion has largely been based on the anatomic and structural aspects of IVD disease, these areas of focus are overshadowed by research in the field of biologic treatment options for degenerative disease. We will discuss several pertinent approaches to this area of study and potential treatment modalities.

The past two decades have seen a surge of research investigating the balance of anabolic and catabolic factors affecting IVD disease. Previously demonstrated in the literature, specific alterations in growth factor and cytokine expression alter matrix synthesis and accordingly adjust IVD homeostasis by shifting cellular metabolism to the anabolic state. In turn, the anabolic state and cell proliferation are likely signs of tissue repair and hallmarks of IVD. We will briefly touch on each factor in the context of reviewing the tissue engineering approaches that are targeted for potential clinical application in the future [Table 1].

**Platelet-derived Growth Factor (PDGF)**

At a fundamental level, PDGF plays a role in angiogenesis and the growth of existing blood vessels. PDGF falls
into the category of anti-catabolic mitogens, which act to retard cellular breakdown and turnover. Paglia et al. (2016) demonstrated that when PDGF-BB is delivered in a thiol-modified hyaluronic acid hydrogel in rabbit preclinical DDD model, it significantly decreases disc degeneration via preventing apoptosis and increasing collagen-3 matrix production maintains the disc structure, and facilitates biomechanical functions. Gruber et al. (2000) demonstrated that PDGF’s influence significantly reduced the percentage of apoptotic AF cells induced by serum depletion in culture. More recently, Pratsinis et al. (2017) reported similar findings that PDGF-BB inhibit IVD cell apoptosis and promote anabolic gene expression. Similar to other proteins we review, PDGF is a focus of research in apoptotic and senescent cells. Gruber et al. (2008) aimed at using IGF-1 exposure on human annulus cells to prevent or ameliorate senescence in vitro; they demonstrated a significant reduction following exposure to 500 ng/mL IGF-1 (control, 56.3% +/- 8.5 (9); mean +/- - SEM, (n) vs. treated, 39.6% +/- - 6.6 (9), p=0.0009). Recently, An et al. (2017) reported that intraperitoneal injection of calcitriol in diabetic rat model protects the degeneration of IVD by upregulating IGF-1 and TGFβ expression. These studies provide a solid foundation for further investigation in the area in hopes of applying these principles in vivo to provide both symptomatic and pathophysiologic therapy.

**Basic Fibroblast Growth Factor (bFGF) & Fibroblast Growth Factor-18 (FGF-18)**

The family of fibroblast growth factors, bFGF and FGF-18 in particular, have been studied and implicated in the regulation of both articular and IVD cartilage homeostasis. These proteins have been shown to bind heparin and heparan sulfate, and serve to modulate cell growth, differentiation, migration, and survival. Along with PDGF and IGF-1, most consider FGF-18 to be anabolic in nature, while bFGF remains more controversial. FGF-18 has yet to be studied and explicitly proven to be anabolic in IVD disease in vivo. However, recent FGF-18 studies indicate a slightly more unified agreement among experts describing anabolic effects in human articular chondrocytes by activating FGFR3, increasing extracellular matrix (ECM) formation and cell differentiation while inhibiting cell proliferation, leading to dispersed cells surrounded by abundant ECM instead of clusters of cells seen after stimulation with bFGF. Furthermore, there are reports that FGF helps mesenchymal stem cell differentiation to chondrogenic lineage.

In discussing the more contentious member of the fibroblast growth factor family, several studies have shown bFGF to be mitogenic and anabolic much like PDGF and IGF-1. Recent studies have analyzed the effects of bFGF on bovine NP cell growth and differentiation cultured in monolayer and alginate, and found that bFGF stimulated increased sulfated proteoglycan (PG) synthesis, lower aggrecan turnover, and differentiation of NP cell phenotype by maintaining responsiveness to TGF-β. However, other studies suggest that the mitogenic effect of bFGF in human articular chondrocytes or spine disc cells signal pathologic degeneration rather than regeneration. While the literature leaves the exact anabolic/catabolic role of bFGF open for debate, more recent research shows bFGF exert catabolic effect on human articular chondrocytes and IVD tissue by activation of FGFR1 and up-regulation catabolic enzymes and inhibition of extracellular matrix synthesis. Hence,
the exact mechanisms and pathophysiology necessitate further research, but it stands to argue that there is likely some utility in the modification of FGF regulation in the therapeutic arena.

**Growth and Differentiation Factor-5 (GDF-5)**

While not technically a member of the BMP family, GDF-5 is closely related to this family of proteins and serves a similar role in cell growth and differentiation in both adult and embryonic tissues; specific to our interest, GDF-5 has been shown to influence joint and skeletal development via matrix production and disc cell proliferation\(^{38-42}\). In a bovine model of NP and AF cells, Chujo et al. (2006)\(^{43}\) showed that recombinant rhGDF-5 increased matrix synthesis and cell proliferation in both sets of cell groups, with a higher response being documented in the NP cells. Recently, Luo et al. (2016)\(^{42}\) demonstrated similar results in human NP cells when cells were infected with adenovirus-mediated GDF-5. Moving toward in vivo responses, Walsh et al. (2006)\(^{44}\) were able to produce a notable increase in disc height after a single GDF-5 injection in a mouse disc compression model. Interestingly, in the same study, the team was unable to elicit a significant disc height increase from IGF-1, bFGF, or TGF-β injection. More recently, Liang et al. (2010)\(^{45}\) developed a murine degenerative disc model via needle puncture, and investigated the effects of adenoviral (Ad) GDF-5 gene therapy. Utilizing bioluminescent imaging, radiographic, and MRI scanning, they showed the percent disc height index at two weeks in the mice injected with Ad-GDF5 increased significantly compared with that of the control mice; the increase was sustained for the rest of the experiment period. The T2-weighted signals were detected in the Ad-GDF-5 at 6 and 8 weeks after injection while none were detected in the control group. On histological evaluation, the GDF-5 group also significantly confirmed the hypothesized disc improvement. They demonstrated notable decreases in glycosaminoglycan levels at two weeks post-insult and decreased DNA levels at four weeks; contrastingly, the discs treated with GDF-5 revealed no decrease in glycosaminoglycan or DNA levels throughout the 8-week trial period\(^{45}\). There are two ongoing multicentric clinical trials investigating the safety, tolerability, effectiveness of intradiscal recombinant human GDF-5 injection for treatment of early stage lumbar disc degeneration [Table 2].

**Transforming Growth Factor Beta-1 (TGF-β1)**

TGF-β1 is secreted polypeptide acting in multiple cellular functions including proliferation, growth, and cell differentiation. In a rabbit model, researchers have shown after one week of in vivo injection of hTGF-b1/adenovirus construct in IVD, there was a 100% increase in PG expression when compared with controls\(^{46}\). Similar to results found with GDF-5, studies have shown increased deposition of PGs compared with basal media or chondrogenic media alone, and further showed abundant aggrecan and type II collagen deposition\(^{47}\). Similar IVD restorative results via increased PG synthesis have been further proven in canine studies\(^{48,49}\). In a study examining the role of TGF-β1 in platelet-rich plasma on IVD regeneration, Chen et al. (2006)\(^{50}\) concluded that growth factors in platelet-rich plasma can effectively react as a growth factor cocktail to induce NP proliferation and differentiation, promote tissue-engineered NP formation, and serve as a possible therapeutic deterrent to IVD disease.

In recent approaches aimed at potential therapeutic treatment, researchers have qualitatively and quantitatively shown the stimulatory effects of a combination treatment with TGF-β1 and IGF-1 on the synthesis of sulphated glycosaminoglycan and type I and II collagen by AF cells; they suggest the role for TGF-β1 in pushing cells towards a

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Table 2: Unpublished Clinical trials investigating Growth factor injection therapy for IVD degeneration.
fibrocartilaginous phenotype. From a signaling cascade perspective, a study recently demonstrated that BMP-2 and TGF-β regulate β1,3-glucuronosyl transferase-1 expression and chondroitin sulfate synthesis in NP cells through a signaling network comprising MAPK, AP1, Sp1, and TonEBP. These researchers draw the conclusion that by controlling glycosaminoglycan and aggrecan synthesis, these growth factors positively influence disk cell function.

Another recent rabbit model, Yang et al. (2010) looked at the effects of the combination therapy of mesenchymal stem cells (MSC) and pure fibrinous gelatin (PFG)-TGF-β1 on disc height. They reported that the MSC-PFG-TGF-β1 group had less degeneration and a slower decrease in disc height compared with both the degenerative model and pure PFG-TGF-β1 groups. Furthermore, they showed up-regulation of type II collagen content in NP cells while demonstrating a decrease in the rate of cell apoptosis in the MSC-PFG-TGF-β1 group. Recently, similar results were reported in in vivo rabbit IVD model using lentivirus and adenoviral mediated transfer of TGFβ1.

Transforming Growth Factor Beta-3 (TGF-β3)

Transforming growth factor (TGF-β3) plays a pivotal role in maintain and induce transformation of IVD structure. TGF-β3 has a synergy with other growth factors to induce discogenic differentiation and reduce the damage caused by degeneration. Risbud et al. showed that TGF-β3 could enhance NP and AF structure and function by elevating the levels of activated ERK1/2, which in turn would regulate the TGF-β-RI and TGF-β-RII. Hegewald et al. confirmed the previous finding and demonstrated that TGF-β3 administration is a possible candidate as a biological treatment of AF degeneration. They presented that stimulation of AF with TGF-β3 was associated with more production of collagen type X.

Bone Morphogenetic Protein-7 (BMP-7)

BMP-7 is also known as osteogenic protein-1 (OP-1). BMP-7 is a known player in bone homeostasis and the transformation of mesenchymal cells into bone and cartilage. Specifically, BMP-7 treatment has been shown to induce all the genetic markers of osteoblast differentiation and extracellular matrix synthesis. Similar to many of the previously addressed growth factors, BMP-7 has been implicated in proteoglycan metabolism and extracellular matrix synthesis in IVD cells exposed to known inflammatory factors such as interleuken-1. Pertinent to possible future clinical trials, this enhanced extracellular matrix synthesis has been demonstrated in both rabbit and human IVD cells in vitro. Regarding in vivo studies, BMP-7 has shown to have a disc height restoration effect similar to GDF-5. Rabbit studies have shown that a single intradiscal administration of BMP-7 yielded both an increase in NP proteoglycan content as well as an overall heightened disc measurement, neither of which was seen in the control groups. In another rabbit model, BMP-7 injection showed a sustained disc height re-establishment at 8-, 12- and 24-week time points, suggesting a sustainable increased water content of the NP of treatment vs. control subjects.

Shifting to in vivo techniques and response, Wang et al. (2011) argued that the recombinant adeno-associated viral vector rAAV2 vector has empirical advantages over adenovirus vector Ad-hBMP-7 to transfer exogenous genes into cells, especially in the clinical use. They found that NP cells transfected by the rAAV-humanBMP-7 vector expressed hBMP-7 for at least two weeks while promoting a remarkable and significant accumulation of both proteoglycans (42% and 77% higher than non-transfected cell, p<0.05) and collagen type II (63% and 94% higher than non-transfected cells, p<0.05). Based on the results of Masuda, Wang, and others, it seems feasible that there is an opportunity to restore degenerative discs by a single NP injection of BMP-7. Recently, Liao et al. performed in vitro and in vivo studies using bone marrow derived mesenchymal stem cells with overexpression of human BMP-7 into cells, especially in the clinical use. They found that BMP-7 transfected stem cells significantly slowed progression of disc degeneration in a rat tail disc model.

Bone Morphogenetic Protein-2 (BMP-2)

Like BMP-7, BMP-2 is a known osteoinductive protein that stimulates osteoblast differentiation and bone formation. Moreover, in vitro and in vivo studies in different animal and human IVDs have shown BMP-2 acting similarly to BMP-7 in upregulation with both aging and induced disc injury. Demonstrating its anabolic nature in DDD, studies have shown that direct administration of BMP-2 to IVD cells results in increased production of components of the extracellular matrix; again, this is a shared commonality between BMP-2 and BMP-7. Furthermore, research has shown upregulation of the BMP pathway via other agents, namely simvastatin and LIM mineralization protein-1, which has resulted in similar outcomes as the direct administration of bone morphogenetic proteins.

To this extent, BMP-2 is currently available in the United States for clinical trials.
States and has shown some clinical efficacy as an adjunct to anterior lumbar interbody fusion procedures for degenerative disc disease. Burkus et al. (2009) combined BMP-2 on an absorbable collagen sponge with the use of dual tapered threaded fusion cages to demonstrate obtained and maintained intervertebral spinal fusion, improved clinical outcomes, and reduced pain after anterior lumbar interbody arthrodesis. Based on the current literature, it stands to argue that by way of either direct or indirect stimulation of BMPs, there is a possible role for these proteins in the treatment of discogenic pain. Use of recombinant growth factors has shown promising results, but contain some risks such as uncontrolled bone formation, immunogenicity, and malignancies. Malignancies has specifically been a concern with the use of recombinant BMP-2. Vavken et al. conducted meta-analysis to assess the risk profile of the use of rhBMP-2 in spine fusion. They reported an increase in general risk of complication such as heterotopic ossification, retrograde ejaculation, and neck swelling in cervical fusion. Moreover, they reported that there is slight increased risk of new tumor, but findings were not statistically significant. Recently, Dettori et al. (2006) performed retrospective cohort study reporting that there was no increase in overall cancer incidence among patients those receiving rhBMP. Use of BMP-2 in spine fusion is controversial, and has been well described in the review article published by Hustedt et al. (2014). It is clear that more basic and clinical research is needed to evaluate the harmful and beneficial effects of BMP-2.

**Summary**

Clearly the key to understanding the pathophysiology of DDD is a stronger understanding of the molecular mechanism underlying IVD disease. Specifically, the prospect of being able to harness the power of the growth factors and cytokines we discussed, and applying these to structural modification in human models is encouraging. The aim is that the injection of growth factors and mitogens could potentially surmount the complex degenerative changes of the IVD disease phenotype. Growth factor protherapy could be considered as a reasonable line of management for intervertebral disc degeneration and associated low back pain. It would provide a true not palliative and long-term cure for the patient. In addition, it might effectively reduce the narcotic consumption and surgical intervention for early stage cases. However, it may not be effective for late stage IVD, and there are still many questionable aspects in regards to alternative sources, numbers of injections, frequency, and safety (malignancies). We believe that numerous growth factors may be administrated. However, administration of the right one based on the level of degeneration is crucial. Unfortunately, the bulk of existing research remains confined to animal and in vitro studies. Certainly, the goal is to apply these principles to growth factor modulation and tissue engineering in human models to attain matrix restoration. A follow up concern to be addressed in the future would be determining the ideal timing of these interventions. Beyond that, little has been discussed regarding the precarious in vivo environment around which the IVD exists, thus opening new questions as to the drawbacks and side effects along with the therapeutic and practical challenges of administration. Currently in the United States, the Food and Drug Administration has only recently allowed the initiation of investigational new drug clinical trials on OP-1/BMP-7 and GDF-5 based on the aforementioned disc height restoration potential seen in multiple animal models. We hope future collective efforts by researchers and clinicians will yield safe and effective therapies that address both functional disc restoration and back pain alleviation.

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