SPINE Volume 29, Number 23, pp 2757–2769
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Growth Factors and Treatment of Intervertebral Disc Degeneration

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Study Design. Literature review.

Objective. To review the most recent findings of the effects of growth factors on the intervertebral disc and, further, to discuss trends in the biologic repair of the degenerated intervertebral disc.

Summary of Background Data. Since early in 1990, advancements in molecular biology and cell culture technology have enabled researchers to accumulate knowledge about the *in vitro* actions of growth factors on intervertebral disc cells. More recently, the use of growth factors for the biologic regeneration of the intervertebral disc is of increasing interest to the orthopedic field, and indeed, some preliminary *in vivo* studies have proven their efficacy.

Methods. Based on a literature search conducted using available databases, such as the National Library of Medicine, as well as data presented at scientific conferences held in the past 2 years, primarily in the United States, the current status of biologic therapy for disc degeneration using growth factors was summarized.

Results. With increasing evidence to support the feasibility of biologically regenerating intervertebral disc tissues, the clinical application of growth factors has become more plausible. The effects of growth factors on the metabolism of intervertebral disc cells or tissues have been extensively studied using *in vitro* approaches. More recently, the efficacy of an injection of growth factor protein to reverse disc regeneration has been shown *in vivo* using a small animal disc degeneration model. The confirmation of those effects and a detailed dose-response study, as well as a long-term safety study, in a large animal model is highly anticipated. Hopefully, the expansion of the clinical use of improved imaging techniques for the early detection of disc degeneration and promising results about the effects of growth factors on intervertebral disc regeneration will benefit the human population in the near future.

Conclusions. The results from these *in vitro* and *in vivo* studies reviewed here clearly suggest the potential usefulness of growth factor injections as a new approach to restore intervertebral disc degeneration at an early stage.

Key words: growth factor, intervertebral disc, anulus fibrosus, nucleus pulposus, regeneration, bone morphogenetic protein. **Spine 2004;29:2757–2769**

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Supported in part by NIH grants 2-P50-AR39239 (K.M.) and 1-P01-AR48152 (K.M., H.S.A.).
The manuscript submitted does not contain information about medical device(s)/drug(s).
No funds were received in support of this work. No benefits in any form have been or will be received from a commercial party related directly or indirectly to the subject of this manuscript.
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Growth Factors and Their Functions

Growth factors, originally discovered in body fluids, including blood and synovial fluid, and in tissue extracts, are polypeptides that are involved in the modulation of cell growth and differentiation.[1] Growth factors, which bind to cell membranes *via* specific transmembrane receptors resulting in the activation of an intercellular signaling cascade, exert biologic effects, such as stimulation of cell proliferation, differentiation, migration, and apoptosis. They also regulate matrix production and repair by various types of cells (*e.g.*, chondrocytes, skin fibroblasts, endothelial cells).[2] Because the amount of those substances in body fluids and tissue extracts is small, experimental approaches to reveal the mechanism of action of growth factors were difficult in the past. With recent advancements in biochemical techniques, these molecules were isolated and identified. New techniques in molecular biology have enabled researchers to clone and characterize growth factors, and eventually, more detailed biologic functions were revealed using those recombinant molecules *in vitro* and *in vivo*. Advancements in monoclonal antibody production also enabled researchers to study detailed *in vivo* functions of growth factors during development and pathologic stages.

Endocrine, Paracrine, and Autocrine Mechanisms

Growth factors exert their biologic functions through endocrine, paracrine, and autocrine mechanisms. In the endocrine mechanism, some growth factors are first produced in the liver, kidney, or other organs, and are subsequently secreted into the blood circulation. For example, insulin-like growth factor-1 (IGF-1) is principally synthesized by the liver and is present in serum. These "endocrine" growth factors are eventually carried to cell surface receptors by the tissue fluid. In the paracrine mechanism, cells that are the source of the growth factor target neighboring cells that have specific receptors for the initiation of a biologic effect. Most connective tissue cells produce growth factors that are involved in repair mechanisms. In the autocrine mechanism, the cell self-regulates by first synthesizing a growth factor that attaches to the appropriate receptor on the surface of the same cell, thus resulting in a response to the signal.

In the intervertebral disc (IVD), there is little vascularity, but tissue fluids can deliver growth factors through the endplate by an endocrine mechanism. In the outer layer of the anulus, neovascularization after injury or degeneration can induce an influx of growth factors. In addition, the autocrine and paracrine production of growth factors is considered to be major regulatory mechanisms in IVD tissues.[3]

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Pathogenesis of Degenerative Disc Disease

IVD degeneration is one of the major causes of low back pain and lumbar disc herniation. In the United States, approximately 5.7 million people are diagnosed with IVD disorders each year, reaching a maximum rate of incidence in the 45- to 64-year-old age group.[4] In addition to direct costs for treatment of IVD disorders, indirect costs, such as loss of productivity, are enormous.

Although the exact pathogenesis is unknown, degenerative disc disease is considered to be a pathologic condition, induced mechanically and mediated biologically, often concurrent with aging changes. Structurally, the IVD consists of an outer anulus fibrosus (AF), which is rich in collagens that account for its tensile strength, an inner nucleus pulposus (NP), which contains large proteoglycans (PGs) that retain water for resisting loading by compression, and an intermediate zone between the AF and NP, the transitional zone (TZ).[5,6] Biologically, disc cells residing in both the AF and NP actively regulate the homeostasis of IVD tissues by maintaining a balance between anabolism and catabolism. Disc cells modulate their metabolic activities by a variety of substances including cytokines, enzymes, enzyme inhibitors, and growth factors in a paracrine and/or autocrine fashion. The degeneration of an IVD may result from an imbalance between the anabolic and catabolic processes or the loss of steady state metabolism that is maintained in the normal disc. Alterations in both anabolic and catabolic processes are thought to play key roles in the onset and progression of IVD degeneration, but the biochemical processes that regulate these changes are poorly understood.

The anabolic regulators include polypeptide growth factors, such as IGF-1, transforming growth factor-β (TGF-β), and the bone morphogenetic proteins (BMPs).[3,7] The present review mainly concentrates on this type of regulator.

Furthermore, in the IVD, the extracellular matrix (ECM) content of PGs and the synthesis of PGs by chondrocytes embedded in the ECM decrease markedly with age and degeneration, as also shown in articular cartilage.[8–16]

Nutrition and Cells in the Intervertebral Disc

Nutrition is another important factor in the pathogenesis of disc disease.[17–24] The IVD, which lacks proper nutrition and receives its main supply of nutrients by diffusion from the vertebral bodies through endplates, might undergo a significant degeneration by the loss of the steady-state metabolism of its cells.[17] For example, trauma, cigarette smoking, and other factors that affect the integrity of the endplates may affect diffusion and disturb the nutrition of the disc cells. A recent paper suggested that an increase in cellular demand or a fall in supply can lead to cell death.[23] The effect of growth factors under conditions where levels of nutrition are compromised is an area requiring further investigation.

Catabolic Regulators: An Antagonistic Role to Growth Factors

Several cytokines (*i.e.*, interleukin-1 [IL-1]) and proteinases (*i.e.*, stromelysin) have been detected in the degenerated or herniated IVD.[25–29] While macrophages that infiltrate into herniated tissue or granulation tissue seem to be the main source of those cytokines,[30–32] there is a possibility that IVD cells themselves may also be the source.[33] After cytokine stimulation, the catabolic process is mediated by various enzymes, such as the matrix metalloproteinases (MMPs)[25–29] or the aggrecanases.[34]

IL-1, a proinflammatory cytokine, is thought to contribute significantly to the loss of matrix homeostasis during joint disease in articular cartilage.[35–37] Although IL-1 affects both synthetic and degradative pathways, it is worth noting that, at lower concentrations, IL-1 is much more effective in inhibiting the synthesis of aggrecan than in stimulating its degradation in articular cartilage.[36,38,39]

Shinmei *et al* showed, for the first time, the possible involvement of IL-1 in the degradation of PGs in IVD cells.[40] This early *in vitro* study of the effect of IL-1α on rabbit AF cells showed that IL-1 enhances caseinase activity, a neutral proteinase, and PG degradation, but also inhibits PG synthesis. These findings were recently confirmed by other investigators.[8,41] IL-1α also stimulates the production of some of the MMPs, nitric oxide, and prostaglandin E_2 by normal IVD cells.[42] These *in vitro* data suggested that IL-1α is a likely candidate to be one of the key mediators in IVD degeneration.

Clinical Application and the Basic Science of Growth Factors

The effect of growth factors on the regulation of matrix metabolism and cell proliferation has been extensively studied in articular cartilage, whose cells are phenotypically closely related to some cells in the IVD.[43,44] The clinical application of growth factors has been proposed as a method of enhancing cartilage repair in osteoarthritis and after traumatic cartilage injury.[45] These proposals include injection of growth factors and the transplantation of a scaffold containing growth factors into the joint cartilage.[45]

The IVD, which is composed of different tissues, such as those of the NP and the AF (inner and outer), shows a gradual transition in cell population and phenotype.[6] The differences in cell phenotype within the various IVD tissues and the difficulties in maintaining those phenotypes *in vitro* have made a detailed investigation of growth factors difficult. In addition, as also found in cartilage, the cell number in IVD tissue is extremely low per volume compared with skin or other connective tissue; this makes it difficult to perform *in vitro* experiments in a reproducible manner.

In the last decade, new tissue culture techniques, especially three-dimensional culture methods, including agarose,[46] alginate,[47–49] and pellet cultures,[50] were developed or adapted to study the effect of growth factors on the metabolism of IVD cells. The difficulties in cultur-

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ing IVD tissues in organ culture were partially overcome by new organ culture techniques using gels[51] or by culturing with intact endplates.[52,53]

For *in vivo* studies, the availability of recombinant protein and the use of gene transfer techniques accelerated progress in this field of research. The development of a reproducible animal model for quantitative studies in a reasonably sized animal, such as the rabbit, makes it possible to analyze the effects of growth factors *in vivo*.[54,55] The results from these *in vitro* and *in vivo* studies clearly suggest the potential usefulness of recombinant growth factors as therapeutic drugs or as medical devices.

In a landmark paper in which they studied the effect of various growth factors on PG synthesis and cell proliferation, Thomson *et al* described the important concept of using growth factors to treat degenerative discs.[56] They pointed out the structural uniqueness of the IVD as an isolated, confined space where a single injection can be effective, by writing that, "... the avascular, alymphatic, and aneural structure of the IVD makes it an ideal structure for therapeutic injection." Now, more than a decade later, we agree with their concept that early clinically significant disc degeneration, detectable by magnetic resonance imaging, can be treated by the injection of a growth factor. Recently, we have shown in our laboratory, using the rabbit anular puncture model, that a single injection of growth factor, osteogenic protein-1 (OP-1, also designated BMP-7), induced a significant restoration of disc height and an improvement in the histologic and biochemical parameters of disc degeneration.[57]

In this review, we will summarize the most recent findings of the effects of growth factors in the IVD, including the available databases and the recent presentation of data at scientific conferences held in the past 2 years, mainly in the United States. Finally, we will discuss the possibility of using growth factors as a treatment tool and the limitations for the biologic repair of the degenerated IVD.

■ Autocrine Expression of Growth Factors

As the name implies, growth factors play an important role in the development of the spine during growth and, interestingly, several growth factors have been found in normal and degenerated IVD tissues. The following section presents the most recent evidence that shows that IVD cells are capable of expressing and producing growth factors.

IGF-1. IGF-1 is a single chain polypeptide (70 amino acids; 7.6 kDa) that shares structural similarity with insulin, the first effecter found to enhance PG synthesis in articular cartilage.[58]
In 1996, Osada *et al* demonstrated both the expression of IGF-1 mRNA and IGF-1 production at the protein level in cultured bovine IVD cells and proposed the presence of an autocrine/paracrine mechanism for the action of IGF-1 in the IVD.[3] The expression of IGF-1 mRNA and the number of IGF-1 receptors were found to be decreased in cells from adult tissues compared with those from fetal tissues, suggesting that the autocrine/paracrine action is more active in young tissues.[3]

In NP cells isolated from disc tissues of rats of different ages (8, 40, and 120 weeks), Okuda *et al* reported an age-related decline in the responsiveness of PG synthesis to IGF-1.[59] They also examined levels of IGF-1 and IGF-binding protein (IGFBP) production in these NP cells. The authors found no significant difference in the amount of IGF-1 synthesized by the NP cells, except a marginal decrease in the IGF-1 production by cells derived from 120-week-old rat IVDs. The expression of the IGF-1 receptor was also decreased in cells from 120-week-old rat IVDs. Interestingly, the production of IGFBP by NP cells was significantly elevated with increasing age. The authors suggested that, at early stages of aging, the increased level of IGFBP is responsible for the decline in PG synthesis, whereas at the later stages, the downregulation of the IGF-1 receptor is a major factor.

Specchia *et al* immunohistochemically analyzed protruded disc samples from 30 patients and 7 age-matched autopsy controls for immunoreactivity to anti-IGF-1 antibody.[60] They reported that chondrocytes in the herniated discs expressed stronger staining for IGF-1 than in the control discs. The authors suggested that the presence of IGF-1 in the human herniated discs might represent an attempt to repair the matrix.[60]

Basic Fibroblast Growth Factor (bFGF). The fibroblast growth factor (FGF) family includes acidic FGF (aFGF, FGF-1: 115 amino acids, 16 kDa) and basic FGF (bFGF, FGF-2: 155 amino acids, 16–18 kDa) as well as other FGFs (FGF-3 to FGF-23). Basic FGF is an important regulator of PG metabolism in growth plate and articular cartilage.[61–63] On the other hand, bFGF also stimulates the production of matrix degrading enzymes, such as collagenase and stromelysin.[64] In addition, synergistic effects of bFGF on IL-1-mediated collagenase have been reported.[65] Using surgical specimens, Tolonen *et al* showed, for the first time, the presence of bFGF in small vessels and scattered disc cells in human AF tissues.[66] Based on the biologic activities of bFGF, the authors suggested that bFGF contributed to the absorption of herniated disc tissue by regulating matrix degrading enzymes such as collagenase, stromelysin, and plasminogen activator.[66]

In a spondylosis model, Nagano *et al* showed that the expression of bFGF in degenerated rat discs accompanies the proliferation of anulus cells with a chondrocytic appearance and an increased amount of surrounding ECM; this suggests that bFGF is one of the autocrine modulators of IVD cell metabolism.[67] Doita *et al* also showed the presence of bFGF in the chondrocytic cells of a degenerating fibrocartilage fragment of human extruded disc specimens.[30] These authors suggested that the release of bFGF might stimulate the proliferation of endothelial and fibroblastic cells.

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In an ovine anular injury model, Melrose *et al* showed the presence of bFGF and TGF-β in the outer third of the AF, which reached a maximum level 12 months after the injury.[68] Cell proliferation at the site of matrix reorganization was also observed, and the authors speculated that those cytokines play an important role in the repair process after an injury.

Platelet-derived Growth Factor (PDGF). Platelet-derived growth factor is a dimer composed of an A chain (17 kDa) and a B chain (16 kDa); A-A or B-B homodimers or A-B heterodimer form.[1] It is mitogenic for connective tissue cells such as endothelial cells and fibroblasts and is involved in wound healing. In cartilage, it stimulates PG synthesis and cell proliferation.[69,70] Platelet-derived growth factor also stimulates collagenase production in fibroblasts.[71]

In human disc herniation tissue, positive staining for PDGF in disc cells was observed in 38% of the samples.[72] The staining is stronger in the herniated tissue than the control tissues; the author speculated that PDGF might stimulate ECM component production and cell proliferation as was seen in cartilage tissue.[61,73] It might play a role in absorption of herniated tissue[72] through the stimulation of matrix degrading enzyme production.[74]

BMP Family. BMPs are a subfamily of the TGF-β superfamily, and more than 47 members of the BMP family have been identified. Those molecules play a critical role in modulating mesenchymal differentiation, bone formation during embryogenesis, postnatal growth, and remodeling and/or regeneration of the skeleton.[44,75]

TGF-β

TGF-β is a member of a family of multifunctional growth factors (112 amino acids, 25 kDa, TGF-β_{1-3}) that regulates cell growth and matrix formation.[1] *In vitro* studies have implicated TGF-β in the regulation of PG metabolism in articular cartilage.[76–78] Because articular chondrocytes are able to produce TGF-β that can bind to various matrix molecules, such as decorin, fibronectin, and thrombospondin, cartilage thus acts as a reservoir of TGF-β.[79] In 1986, Roberts *et al* reported that TGF-β also participates in angiogenesis and inflammation.[80] In IVD tissue, the presence of an endogenous production of TGF-β is reported, but its role remains controversial as described below.

Using immunohistochemistry and the reverse transcriptase polymerase chain reaction, Konttinen *et al* studied the presence of TGF-β and TGF-β Type II receptor (TGF-βRII) in human herniated IVDs from 10 patients and found low levels, or the lack, of immunostaining and expression for TGF-β and TGF-βRII.[81] This suggests that the low level of expression of anabolic growth factors and their receptors may be a risk factor predisposing to IVD degeneration.[81] Okuda *et al* reported an age-related decrease of expression of TGF-β and its receptors in rat IVDs.[82] They also found an age-related decline of TGF-β-dependent PG synthesis.[82]

Using senescence-accelerated mice, Matsunaga *et al* showed the presence of TGF-β_{1-3} and TGF-βR-I and II in AF and NP cells in the IVDs of young mice, which decreased with aging.[83] These authors suggested that the reduction in TGF-β levels might signify the loss of the functions of TGF-β, such as matrix synthesis, which maintains tissue integrity.[83]

Tolonen *et al* first identified the presence of TGF-β in the cells of herniated human disc tissues.[84] Significantly more cells in herniated discs demonstrated immunoreactivity for TGF-β-I, TGF-β-II, and TGF-βR-II compared with control discs. These authors speculated that TGF-β may participate in the regulation of extracellular proteolysis in disc herniation tissue, which results in the disappearance of prolapsed disc materials with time.[85]

In human degenerated IVD tissues, TGF-β has been found in chondrocytes and in endothelial cells, where there was no staining observed in the normal control tissues.[60] Specchia *et al* hypothesized that in the herniated disc TGF-β is produced in response to tissue damage, leading to the formation of granulation tissue.[60]

Taken together, the above findings suggest that TGF-β might play a role in the repair response of IVD tissues, as well as in the induction of neovascularity into herniated disc tissues and in the proteolytic process of absorbing the herniated disc.

BMP-2 and BMP-4

In 1999, Takae *et al* reported the immunolocalization of BMP-2 and BMP-4 and their respective receptors in the cervical spine of senescence-accelerated mice.[86] In young mice, the staining for BMP-2 and -4 and their receptors was located in hyaline cartilaginous cells within the endplate of the vertebrae. In older mice, the staining for these molecules was mainly observed in fibrous cartilaginous cells within the AF. The authors suggested that the expression of BMP-2 and -4 and their receptors at the site of enthesis might be related to the formation of osteophytes during degeneration.

BMP-4, BMP-6, and Growth Differentiation Factor-5 (GDF-5)

Using a murine spondylosis model, Nakase *et al* reported the expression of BMP-4 and its receptors (BMP-receptor IB [ALK6], BMP-receptor II [BMP-RII]) in the outer layer of the anterior anulus at an early stage of experimental cervical spondylosis.[87] At a later stage, the expression of BMP-6 and GDF-5 became predominant, along with their receptors (BMP-receptor IA [ALK3], ALK6, and BMP-RII). The authors concluded that BMP-4 and -6 and GDF-5 are localized in cells undergoing chondrogenesis and that the differential expression of those molecules reflects the possible involvement of molecular signaling by BMPs in the chondrogenic progression in spondylosis.

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Table 1. *In Vitro* Studies of the Effects of Growth Factors

Factor	Cell Type	Effect	Culture Type	Condition	Dose	Reference
IGF-1	Canine NP, AF, TZ	PG synthesis ↑ (NP); cell proliferation ↑ (NP)	Organ culture	Serum (−)	20 ng/mL	7
IGF-1	Bovine NP	PG synthesis ↑ (NP, AF, TZ); cell proliferation ↑ (NP, TZ)	Monolayer	Serum (−)	1–1000 ng/mL	3
IGF-1	Bovine NP, AF	PG accumulation ↑		ITS	100 ng/mL	115
IGF-1	Human IVD (mix)	Apoptosis ↓	Monolayer	1% FCS	50 ng/mL	93
EGF	Canine NP, AF, TZ	PG synthesis ↑ (NP, AF, TZ); cell proliferation ↑ (NP, AF, TZ)	Organ culture	Serum (−)	1 ng/mL	7
FGF	Canine NP, AF, TZ	PG synthesis ↑ (NP, AF, TZ); cell proliferation ↑ (NP, AF, TZ)	Organ culture	Serum (−)	300 ng/mL	7
PDGF	Canine NP, AF, TZ	No effect	Organ culture	Serum (−)	NA	7
PDGF	Human IVD (mix)	Apoptosis ↓	Monolayer	1% FCS	100 ng/mL	93
TGF-β1	Rat IVD	Cell viability ↑	Organ culture with endplate	20% FCS	5 ng/mL	53
TGF-β	Canine NP, AF, TZ	PG synthesis ↑ (NP, AF, TZ); cell proliferation ↑ (NP, TZ)	Organ culture	Serum (−)	1 ng/mL	7
TGF-β1	Bovine NP, AF	PG accumulation ↑	Collagen/Hyaluronan Scaffold	ITS	10 ng/mL	115
TGF-β1	Human IVD (mix)	Cell proliferation ↑	Monolayer, Alginate, agarose	1% FCS	0.25–5 ng/mL	46
TGF-β1	Human IVD (mix)	PG synthesis ↑; cell proliferation ↑	Monolayer	Serum (−)	10 ng/mL	126
OP-1	Rabbit NP, AF	PG synthesis ↑ (NP, AF); cell proliferation (NP, AF) ↑ Collagen synthesis ↑ (NP, AF); Col II, aggrecan mRNA ↑	Alginate bead	10% FCS	50–200 ng/mL	100
OP-1	Rabbit NP, AF	After IL-1 treatment, PG accumulation ↑ (NP, AF)	Alginate bead	10% FCS	200 ng/mL	110
OP-1	Rabbit NP, AF	After C-ABC treatment, PG accumulation ↑ (NP, AF)	Alginate bead	10% FCS	200 ng/mL	111
OP-1	Bovine NP, AF	PG synthesis ↑ (NP, AF); PG accumulation ↑ (NP, AF)	Alginate bead	10% FCS	200 ng/mL	106
OP-1	Rabbit IVD	PG content ↑	Organ culture with endplate	10% FCS	200 μg/disc	52,112
OP-1	Human NP, AF	PG synthesis ↑ (NP, AF); PG accumulation ↑ (NP, AF)	Alginate bead	10% FCS	200 ng/mL	101
BMP-2	Rat AF + TZ	GAG, collagen release in media ↑	Monolayer	1% FCS	100–1000 ng/mL	95
BMP-2	Rabbit AF	GAG, collagen release in media ↑ BMP-6, 7, TGF-β1 mRNA ↑	Monolayer	NA	200 ng/mL	97,98
BMP-2	Human IVD (NP + TZ)	PG synthesis ↑; cell proliferation ↑ (NP, TZ) Type I, II, collagen, aggrecan mRNA ↑	Monolayer		>300 ng/mL	99
BMP-2	Human degenerative IVD	PG synthesis ↑; collagen synthesis ↑	Monolayer	Serum (-)	50–100 ng/mL	108
BMP-12	Human NP	PG synthesis ↑; collagen synthesis ↑	Monolayer	Serum (-)	25–50 ng/mL	108

BMP-2 = bone morphogenetic protein-2; BMP-6 = bone morphogenetic protein-6; BMP-12 = bone morphogenetic protein-12; EGF = epidermal growth factor; FGF = fibroblast growth factor; IGF-1 = insulin-like growth factor-1; OP-1 = osteogenic protein-1; PDGF = platelet-derived growth factor; TGF-β1 = transforming growth factor-β1; AF = anulus fibrosus; FCS = fetal calf serum; GAG = glycosaminoglycan; IVD = intervertebral disc; ITS = media containing insulin-transferrin-selenium; NA = not applicable; NP = nucleus pulposus; PG = proteoglycan; TZ = transitional zone.

Effects of Growth Factors *In Vitro*

Effects of Growth Factors on Matrix Metabolism and Cell Proliferation

In 1991, Thompson *et al* published the first experiment on the responsiveness of the IVD to growth factors.[7] They used the dog IVD organ culture system and investigated the effects of various growth factors on PG synthesis and cell proliferation. The sources of the growth factors or the growth factors studied included fetal calf serum (FCS), plasma-derived equine serum (PDES, as a source of a serum lower in PDGF), IGF-1, epidermal growth factor (EGF), FGF, and TGF-β. Since that initial paper, a large number of studies reporting the effects of growth factors have been published as summarized in this section. There are several factors that should be considered when comparing the effects of each growth factor in those studies. First, growth factors obtained from commercial sources or in collaboration with a manufacturer have different relative activities and levels of purity. Also, each growth factor has a unique optimal concentration and duration of activity. Some growth factors can induce a significant enhancement of biologic activity by being exposed to the cells for a longer period of time. The simple comparison of activity based on concentration might not be adequate to address these issues (Table 1).

Second, different culture conditions (*e.g.*, organ culture with native ECM, monolayer culture, or three-dimensional cultures such as agarose or alginate) and different sources of cells or explants (species, age, and location) affect the results. Different results can also be obtained depending on the presence or absence of serum or other supplements in the culture medium. The results should be properly interpreted based on the scope of the study.

The section below summarizes recent findings on the effect of growth factors on IVD cells in culture.

IGF-1. In the study by Thompson *et al*[7] on dog IVD tissues, IGF-1, at 20 ng/mL, showed a marginally significant stimulatory effect in NP tissues, but not in the TZ or AF tissues. Studies of articular cartilage suggest that any differences in the enhancement of PG synthesis by IGF-1 reflect the maturity of the tissue studied or a species difference.[58,63,88–90]
Osada *et al* showed that IGF-1, in doses ranging from 1 to 1,000 ng/mL, stimulated PG synthesis by bovine NP cells cultured in monolayer in the absence of serum.[3]

A decrease in cell number is one characteristic of aging IVD tissue.[91] Gruber and Hanley reported a high incidence of apoptosis in AF tissue.[92] Furthermore, they found that the surviving AF cells were not synthetically

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inactive but were producing inappropriate matrix molecules during aging and degeneration. Based on these initial findings, they studied the effect of IGF-1 on apoptosis in human AF cells grown in monolayer culture.[93] When apoptosis was induced by a decreased level of FCS (1%) in the media, a reduction from the standard level of 20%, the addition of IGF-1 (50–500 ng/mL) significantly reduced the percentage of apoptotic disc cells.

EGF. EGF is a polypeptide (53 amino acid, 6 kDa) that stimulates the proliferation of various types of cells and is involved in wound healing.[1] EGF and the EGF receptor are expressed by chondrocytes; the former is known to stimulate cell proliferation in rabbit articular cartilage.[94]

In the organ culture of dog IVD tissues, EGF (1 ng/mL) stimulated matrix synthesis (up to 300%) and cell proliferation (up to 180%).[7] The response to EGF was greater in the NP and TZ compared with that in the AF. The rate of PG synthesis in the presence of EGF was nearly 50% higher than that of 20% FCS.[7]

bFGF. bFGF (300 ng/mL) increased PG synthesis by NP and AF cells (up to 300%), reaching the level achieved by 20% FCS.[7] Cell proliferation was also enhanced by bFGF at 300 ng/mL, more so in the AF and TZ compared with the NP. bFGF preferentially stimulated the outer tissue lesions (the AF and the TZ), whereas EGF stimulated the inner tissue lesions (the NP and the TZ).[7]

PDGF. Thompson *et al* demonstrated that PDES, which is a low-PDGF serum source, and FCS showed similar biosynthetic and proliferative activities. They therefore concluded that PDGF was not a major factor in modulating the matrix metabolism of IVD cells.[7]
The effect of PDGF on apoptosis in human AF cells grown in monolayer culture has been reported.[93] After the induction of apoptosis by a 1% serum condition, the addition of PDGF (100 ng/mL) significantly reduced the percentage of apoptotic disc cells.

BMP Family of Growth Factors.

TGF-β (TGF-β)

In the experiment by Thompson *et al*, among the different factors studied, TGF-β (1 ng/mL) induced the greatest response in PG synthesis by NP cells (500%); this response was even higher than that observed with 20% FCS. In addition, TGF-β stimulated the proliferation of cells in the NP and the TZ by 200%. However, AF cells did not respond to TGF-β either with respect to PG synthesis or cell proliferation.[7]

Gruber *et al* reported that human AF cells in three-dimensional cultures, such as agarose and alginate, produced and accumulated more abundant ECM molecules, such as PGs, than those cultured in monolayer.[46] In that study, TGF-β induced a significant enhancement of cell proliferation.

Risbud *et al* recently reported a new organ culture system for rat IVDs using a microdissection technique.[53] The IVD disc with endplates was maintained in a phenotypically stable condition under high osmolarity (410 mOsm/kg) in the presence of TGF-β (5 ng/mL) in an insulin-transferrin-selenium containing media with 20% FCS. The inclusion of TGF-β resulted in the best results for the maintenance of PGs.

BMP-2

Using rat AF cells cultured in monolayer in the presence of 1% FCS, Yoon *et al* showed that recombinant human BMP-2 (rhBMP-2, 10–1,000 ng/mL) increased cell proliferation and PG synthesis.[95] Under these same conditions, BMP-2 also increased mRNA expression of Type II collagen, aggrecan, SOX9, and osteocalcin. Recently, using rat NP cells in monolayer culture, this group demonstrated that the stimulatory effect of BMP-2 (at 1–100 ng/mL) was inhibited by the addition of nicotine (10–100 μg/mL) in the culture media.[96] This suggests that nicotine may contribute to the process of disc degeneration by a direct effect on NP cells, possibly by antagonizing the effect of BMP-2.

Yoon *et al* also showed that in rabbit AF cell monolayer culture in the absence of FCS, BMP-2 (at 200 ng/mL) significantly increased the release of PG and collagen Type II into the media, as well as the gene expression of those molecules.[97,98] In addition, BMP-2 upregulated the gene expression of BMP-6, BMP-7, and TGF-β.[97,98]

Kim *et al* reported that BMP-2 facilitated the expression of the chondrogenic phenotype of human IVD cells.[99] Recombinant human BMP-2 stimulated PG synthesis (67% at 300 ng/mL and 200% at 1,500 ng/mL rhBMP-2) and upregulated expressions of aggrecan, collagen Type I, and collagen Type II mRNA compared with the untreated control levels. On the other hand, under the same conditions, rhBMP-2 did not increase the level of expression of osteocalcin mRNA.

BMP-7/OP-1

Masuda *et al* reported the stimulatory effect of OP-1, otherwise known as BMP-7, on PG and collagen metabolism in rabbit NP and AF cells cultured in alginate beads in the presence of 10% FCS.[100] In both cell types, recombinant human OP-1 (rhOP-1) stimulated the synthesis of PGs and collagens in a dose-dependent manner (50–200 ng/mL) with an associated increase in the expression of mRNA for aggrecan and collagen Type II. Importantly, continuous treatment with rhOP-1 (100 ng/mL) stimulated the accumulation of PGs and collagens as well as an increase in cell number,[100] which suggests that the stimulation of matrix synthesis is associated with the retention of newly synthesized matrix molecules. It is noteworthy that at this time OP-1 is the only growth factor that can stimulate matrix synthesis in the presence of 10% FCS. A comparison of the effects of growth factors (*i.e.*, OP-1, BMP-4, IGF-1) on rabbit IVD cells cultured in the presence of 10% FCS showed that rhOP-1 has more potent effects on PG synthesis than similar concentrations of the other growth factors, although a direct comparison might be difficult.

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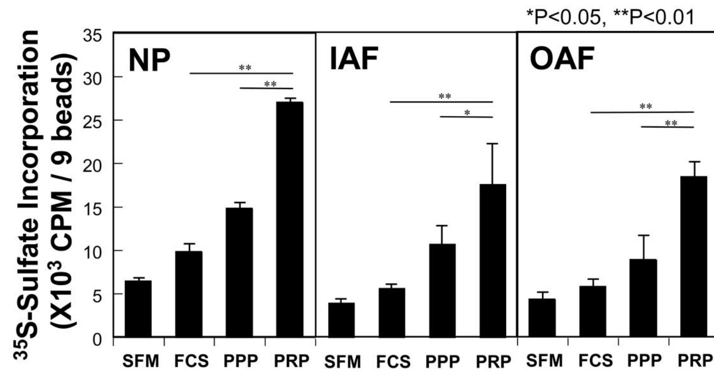

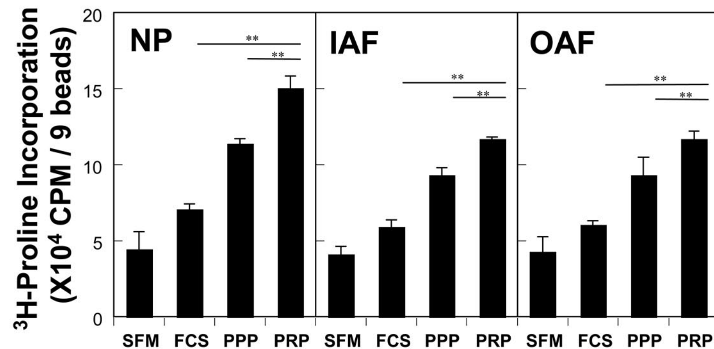

Figure 1. **A,** proteoglycan (PG) synthesis: Bovine nucleus pulposus (NP), inner anulus fibrosus (IAF), and outer anulus fibrosus (OAF) cells were cultured in alginate beads for 7 days in the presence of 10% fetal calf serum (FCS) and, after depletion of serum, cells were cultured with serum-free media (SFM), 10% FCS, 10% porcine platelet-poor plasma (PPP) or 10% porcine platelet-rich plasma (PRP) for an additional 3 days. ^{35}S-sulfate incorporation in each condition was assessed during the last 4 hours of treatment. The PRP treatment resulted in a significant increase in PG synthesis by the bovine NP, IAF, and OAF cells ($*P < 0.01$), when compared with SFM, FCS, or PPP. The rate of PG synthesis remained significantly elevated in NP, IAF, and OAF cultures when expressed per μg DNA. **B,** Collagen synthesis: The cells were treated as described for the PG synthesis experiment. ^{3}H-proline incorporation into a pepsin-resistant protein in each condition was assessed during the last 16 hours of treatment as a measure of collagen synthesis. The PRP plasma stimulated collagen synthesis by all cell types ($*P < 0.01$), but the magnitude of the response was less marked than that found for PG synthesis (**A**).

Imai *et al* recently reported that OP-1 (100–200 ng/mL) enhanced the *in vitro* production of PGs by human NP and AF cells cultured in alginate beads in the presence of 10% FCS.[101] Similar to rabbit cells, OP-1 also enhanced the accumulation of PGs in the matrix. Interestingly, AF cells, which are more fibrochondrocytic, strongly responded to OP-1, suggesting that OP-1 might be beneficial not only for nucleus repair but for anulus repair as well.

Furthermore, to study the effects of growth factors in a more biologically relevant system, the effect of OP-1 on the maintenance of PGs in NP and AF tissues was studied in a newly established rabbit IVD organ culture system, which includes endplates.[52] Intact discs recovered with endplates from young rabbits were cultured in the presence of 10% FCS as a "whole organ" culture system. Osteogenic protein-1 (2 μg in saline) or saline alone was injected into the NP and then cultured for up to 14 days. The discs that received OP-1 injections showed a higher PG content on day 7 and day 14 than those that received the saline injection. These results strongly suggest that OP-1 contributed positively to the maintenance of steady state metabolism. These data led to the various animal studies that are described below in the *in vivo* experimental section.[52]

Platelet-rich Plasma (PRP). Platelet-rich plasma (PRP), which has been used clinically for enhancing bone and wound repair, is a fraction of plasma in which TGF-β and PDGF are concentrated at high levels. Masuda *et al* have studied the effects of porcine PRP on ECM synthesis and accumulation by bovine inner and outer AF and NP cells using the alginate bead cell culture system.[102] PRP (10%) significantly stimulated the synthesis of PG (NP, 183%; inner AF, 164%; outer AF, 209%; Figure 1A) and collagen (NP, 117%; inner AF, 125%; outer AF, 125%; Figure 1B) when compared with platelet-poor plasma; the response to PRP was greater in outer AF cells than in NP cells. Further investigations on the possibility of applying PRP locally, such as by an injection into the NP and/or AF, are worth pursuing. In addition, given the risks of using animal serum for tissue engineering, autologous blood may gain favor as a source of PRP growth factors and serum supplements needed for stimulating cells to engineer IVD tissues.

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Synthetic Peptide of Link Protein (Link-N). Link protein is a glycoprotein that stabilizes the noncovalent binding between aggrecan and hyaluronan. The N-terminal peptide of link protein (link-N [sequence: DHLSDNYTLDHD-RAIH]), which can be generated by proteolysis, has been reported to act as a growth factor.[103,104] Mwale *el al* recently reported that link-N, at concentrations of 10 and 100 ng/mL, stimulated matrix assembly in pellet culture of NP and AF cells by increasing the production and/or accumulation of PGs and collagen.[105] This suggests that a certain level of degradation products of link protein, which can be generated by MMPs, acts as a "growth factor" in a feedback mechanism.

Aging and the Response to Growth Factors

Okuda *et al* reported that the responsiveness of IVD cells to IGF-1 and TGF-β decreases with increasing age in rabbit IVD cells.[59] As described above in the section on the autocrine expression of growth factors, this is associated with either a decrease in the production of IGF-1 and its receptors or an increased level of an inhibitory protein such as IGFBP. Contrary to the effects of IGF-1 and TGF-β, OP-1 stimulated PG synthesis by fetal, adult, and old bovine NP and AF cells.[106] Although the baseline matrix synthesis of cells from older animals is very low, when exposed to OP-1, IVD cells from those older animals produced almost the same amount of PGs as IVD cells from younger animals exposed to OP-1. This is especially noteworthy because increasing consideration is being given to treating disc degeneration by injecting growth factors in the vicinity of the IVD, and those indications are mostly for symptoms or pathologic changes in adults or older individuals.[107]

Effect of Growth Factors on Metabolically Impaired Cells or Metabolically Perturbed Cells

BMP-2 and BMP-12. It remains to be determined whether cells in a degenerated disc, especially metabolically impaired cells, can respond to growth factors and regenerate a damaged IVD. However, Ahn *et al* recently reported that BMP-2 and BMP-12 stimulated PG and collagen synthesis by human NP cells from degenerated discs cultured in monolayer in the absence of serum.[108] They found that a significant and optimal stimulation of PG synthesis by NP cells was achieved at 50 ng/mL of BMP-2 (560%) and BMP-12 (460%). The stimulation of collagen synthesis did not achieve significance.

IL-1 Receptor Antagonist (IL-1ra)/IGF-1/PDGF. Wehling recently reported the effects of a combination of autologous IL-1ra, IGF-1 and PDGF proteins (IL-1ra/IGF-1/PDGF) on apoptosis and degradation of the ECM in monolayer cultures of human degenerative IVD cells.[109] The results indicated that the mixture of proteins reduced the percentage of apoptosis and the production of biochemical markers of disc degeneration, such as IL-1 and IL-6. Based on the results, the author proposed the strategy that the application of an autologous protein mixture, containing IL-1ra/IGF-1/PDGF, could be used therapeutically for the treatment of degenerative disc disease.[109]

BMP-7/OP-1. Our laboratory has conducted two *in vitro* studies that mimic a perturbed matrix metabolism. The first study examined the repair process after matrix depletion in IVD cells. Using the alginate bead culture system, the degeneration of the matrix of rabbit IVD cells was first induced by the pro-inflammatory cytokine, IL-1, and the process of matrix repair was investigated.[110] In this study, OP-1 was effective in promoting, *in vitro*, the repair of the IL-1-damaged PG matrix surrounding the NP and AF cells.

In the second study, alginate beads containing rabbit IVD cells were first briefly exposed to the enzyme, chondroitinase-ABC (C-ABC), which selectively depletes the glycosaminoglycan chains of PGs. The cultures were then maintained in media containing 10% FCS and OP-1 (100 μg/mL).[111] The accumulation of PGs in the C-ABC-damaged matrix around the cells was significantly stimulated by OP-1, actually resulting in a marked increase above the normal level of PG content. These two matrix-damage model studies might support the contention that treatment by a growth factor can be applied to repair the matrix of degenerated human IVDs or to promote matrix repair in conjunction with chemonucleolysis.

An *in vitro* organ culture system was used to study the effects of nutrient concentrations on PG metabolism in the rabbit IVD.[112] The discs with endplates were cultured in the presence of 5%, 10%, and 20% FCS for 21 days. The lowest FCS concentration (5%) induced the loss of PG content during culture. To rescue these discs from their "low-nutrient" status, on day 14 OP-1 (200 μg/disc) was injected intradiscally. This injection resulted in a significantly increased PG content when compared with discs cultured under the same conditions without an OP-1 injection. Thus, the administration of a growth factor may be able to protect NP tissue from undergoing degeneration resulting from poor disc nutrition.

Further studies using aging IVD cells are anticipated to prove the therapeutic concept that growth factors will be useful to treat degenerated disc disease in the aging population. The data described above indicate that the appropriate growth factor, or mixture of growth factors, can be most useful as a therapeutic agent in promoting synthesis and repair of the matrix of both the AF and NP elements of degenerating human IVDs.

Utilization of Growth Factors in IVD Tissue Engineering

BMP-7/OP-1. A new method to produce tissue-engineered cartilage without the aid of a synthetic matrix was recently developed.[113] This two-step culture method, termed the alginate-recovered-chondrocyte method, was also used with the optimal enhancement of cell activity by growth factor treatment (20% FCS + OP-1 200 ng/mL) to tissue-engineer IVD discs.[114] The

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Table 2. *In Vivo* Studies of the Effects of Growth Factors

Factor	Species	Model	Dose	Effect	Reference
OP-1	Rabbit	Normal	2 μg/disc	Increase in disc height, initial PG content in the NP ↑	117
OP-1	Rabbit	C-ABC co-injection	100 μg/disc	Increase in disc height, initial PG content in the NP ↑	121
OP-1	Rabbit	After chemonucleolysis degeneration by C-ABC	100 μg/disc	Increase in disc height	119
OP-1	Rabbit	Needle puncture	100 μg/disc	Increase in disc height, higher MRI score	57
IGF-1	Rat	Compression model	8 ng/disc	Clustering of cells	116
GDF-5	Rat	Compression model	8 ng/disc	Clustering of cells Increase in disc height	116
TGF-β	Rat	Compression model	1.6 ng/disc	Clustering of cells, repeated injection effective	116

C-ABC = chondroitinase-ABC; GDF-5 = growth differentiation factor-5; IGF-1 = insulin-like growth factor-1; NP = nucleus pulposus; OP-1 = osteogenic protein-1; PG = proteoglycan; TGF-β = transforming growth factor-β.

first scaffold-free construct with measurable biomechanical properties was successfully created. A significant increase in biomechanical strength was noted during 4 weeks of culture, and the biochemical properties of the tissue-engineered AF and NP constructs reflected the characteristics of the original tissues.

TGF-β. In another study of tissue-engineered IVD tissues for transplantation, Alini *et al* recently reported the successful construction of tissue-engineered NP and AF tissues using scaffolds of Type I collagen and hyaluronan seeded with bovine NP or AF cells in the presence of FCS or a variety of growth factors.[115] The addition of TGF-β (10 ng/mL) to the FCS stimulated the formation of matrix within the scaffold. The PG content of those scaffolds never exceeded 10% of that present in the mature NP.

■ *In Vivo* Studies on the Effects of Growth Factor Protein Administration

bFGF, GDF-5, IGF-1, and TGF-β

Walsh *et al* reported the *in* vivo effects of a single injection and multiple injections of growth factors, such as bFGF (8 ng/disc), GDF-5, IGF-1, or TGF-β, in the mouse caudal disc with degeneration induced by static compression.[116] Although the effects of a single growth factor injection were not apparent within 1 week, the appearance of clusters of inner anular fibrochondrocytes was observed in the GDF-5 group, although this did not reach a significant level quantitatively. Disc height was significantly increased following a single GDF-5 injection compared with the saline injection ($P < 0.05$). Multiple injections (four injections, once per week) of TGF-β showed a stimulatory effect, an increase of fibrochondrocytes in the anulus, although the other growth factors did not show a significant enhancement of their effect by a single injection. The authors suggested that a sustained delivery system or a combined approach with a mechanical or a cell-based device was required to achieve a beneficial therapeutic effect (Table 2).

BMP-7/OP-1

Our laboratory reported that, in normal rabbits, the *in vivo* intradiscal administration of the growth factor, OP-1, resulted in an increased disc height and PG content of the NP, which was not seen in the saline injection group.[117,118] Two weeks after the OP-1 injections (2 μg in saline), the mean disc height index of the OP-1-injected discs was 15% greater than that of the saline group; the increase was sustained for up to 8 weeks. As determined biochemically, the PG content of the NP in OP-1-injected discs was higher than that in the saline group at the 2-week time point, but not at later time points. This might suggest that continuous or repeated local delivery of OP-1 may be needed to maximize its beneficial effects.

Because of inherent differences in the structure, biochemical composition, cell populations, and metabolism of normal and degenerative IVDs, the application of OP-1 to a normal, healthy disc may not result in the same response as that to a degenerated one. To answer those questions, an *in vivo* animal model of IVD degeneration, using a defined-gauge needle puncture of the AF, was developed.[55]

As the first quantitative study of *in vivo* growth factor treatment, we examined radiographic and MRI changes in the rabbit IVD after injection of OP-1 into the NP in the needle-puncture disc degeneration model.[57] In adolescent rabbits, IVD degeneration was established by puncturing the anulus with an 18-gauge needle. After 4 weeks, OP-1 (100 μg/disc) or vehicle solution was injected into the NP. Our quantitative analysis of disc height showed that a restoration of disc height was induced 6 weeks after a single OP-1 injection and was sustained for the entire experimental period up to 12 weeks ($P < 0.01$). Magnetic resonance imaging grading at each time point showed a significant difference at the 8-week ($P < 0.05$) and 12-week time points ($P < 0.01$), suggesting an increased NP matrix. This data, showing the increase of disc height at a later time point, suggested that metabolic changes induced in cells by a single injection might cause long-term changes. The biochemical and histologic analysis supported those data. The PG content in the NP of the OP-1 injection group, not in the AF, was significantly higher than that of the control

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group ($P < 0.05$), whereas DNA and collagen contents did now show any statistical differences.

Another degeneration model using C-ABC chemonucleolysis was established.[119,120] The C-ABC (10 mU) was first injected into IVDs to induce chemonucleolytic effects. Four weeks following the injection of C-ABC, OP-1 (100 μg/disc) or vehicle was injected and the disc height was measured up to 12 weeks after the OP-1 injection. Four weeks after the OP-1 injection, the disc height began to return toward normal, gradually approaching the control level by 6 weeks; this change was sustained throughout the study period (OP-1 *vs.* vehicle, $P < 0.0001$, OP-1 *vs.* control, not significant). These data suggest that OP-1 has the potential to restore IVD height by stimulating cellular metabolism and might be used in patients who received chemonucleolysis in the past that resulted in some IVD height loss.

In this model, similar results were obtained when C-ABC and OP-1 were coinjected.[121] The results showed that the initial disc narrowing (at 2 weeks) had begun to return toward normal by 4 weeks and was no longer significantly different from the control group at 12 weeks (C-ABC + OP-1 *vs.* C-ABC only, $P < 0.0001$). Biochemical analysis showed that the PG content of the NP of the coinjection group was similar to the noninjection control disc, whereas the PG content of the NP of the C-ABC injected showed a significant decrease (C-ABC + OP-1 *vs.* C-ABC only, $P < 0.0001$). These findings support the contention that OP-1 is very effective in stimulating matrix repair *in vivo* and illustrate the potential of this factor in promoting the regeneration of the NP.

■ Discussion and Conclusion

Based on the *in vitro* and *in vivo* data presented here, the clinical application of growth factors by the direct injection of the protein into the NP or the AF is clearly feasible as a new therapeutic intervention for treatment of IVD degeneration. The stimulation of the biologic repair process will create a new category of therapy, where no active treatment currently exists, between conservative therapies and more aggressive therapies, such as fusion or disc replacement. This approach might be applied together with a pharmaceutical approach that can suppress the influx of inflammatory cytokines, such as antitumor necrosis factor or IL-1ra, *etc.*

On the other hand, if a significant tear of the AF, especially a radial tear, presents, the injection of a growth factor, as a liquid solution, might not be effective because the poor retention of a solution is expected under physiologic loading conditions. The use of a proper biocompatible carrier that provides a sustained release of growth factor might be required in such a situation. The suitable format for protein delivery will be different at each stage of disc degeneration. For the repair of a larger defect, the use of tissue-engineered IVD constructs may prove helpful in treating lumbar disc disease biologically rather than by surgery.

Several important considerations should be taken into account in the development of a growth factor therapy. To obtain the effects of the growth factor, which does not itself change the biochemical and biomechanical properties of the IVD, effecter cells that are able to respond to the applied growth factor are required. Because a reduced cell number is one of the characteristics of IVD degeneration, the transplantation of healthy functional cells, such as, but not limited to, autologous NP cells, might be required.[122–125]

Also, the cells or tissues in the NP and AF stimulated by growth factors may not survive if proper nutrition is not available.[17] In a relatively advanced degenerative condition, the supply of nutrients is disturbed by sclerosis of the endplate. An innovative method to evaluate nutrient supply into the disc space might be required to identify the proper target for growth factor therapy.[21,22] An increased cell number after stimulation by growth factors may create an increased demand for nutrients, which, if not available, could result in cell death. Further investigations of the optimal environment for growth factor stimulation and cell transplantation should be pursued.

The growth factor dose required for the human IVD and the frequency and mode of delivery should be further investigated in order to bring the biologic therapy from the laboratory into the clinic. Because growth factors have pleiotropic functions, such as chondrogenic, osteogenic, and morphogenic activities, a careful safety assessment is necessary. Although many difficulties arise because of remarkable differences in biologic response among different species, the knowledge gained during applications to bone or skin diseases will lead to relatively rapid progress.

Patients with disc degeneration come to our clinics for their symptoms, primarily pain. Attention should always be paid to the short and long therapeutic benefit to patients. Federal Drug Administration guidelines characterize therapeutic drugs/devices into two categories, "structural modified" and "symptom modified." The new biologic therapies for disc degeneration resulting from growth factor application can be considered to be 1) "structural modifying therapies with a delay in structural progression claim"; those that prevent disc height loss or facet degeneration, and/or 2) "symptom modifying therapies with an improvement in symptoms claim"; those that provide relief of symptoms, mainly pain. Preliminary data from *in vivo* rabbit experiments showed the possibility of using growth factors as a "structural modifying therapy," but it is not clear whether it is a "symptom modifying therapy" able to resolve the symptoms associated with pathologic changes. Therefore, further studies are essential to shed light on the mechanism of pain in degenerative disc disease. The best solution for those patients who suffer from low back pain with disc degeneration is the development of a therapy that possesses characteristics of both modified therapies.

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■ Conclusion

These *in vivo* and *in vitro* findings illustrate that the use of growth factors has the potential to promote regeneration of IVD tissues in the human. Further *in vivo* studies in large animals or *in vitro* studies using metabolically impaired cells are needed to show the safety and efficacy of these biologic therapies.

■ Key Points

- The effects of growth factors on the metabolism of intervertebral disc cells or tissues have been extensively studied using *in vitro* approaches.
- More recently, the efficacy of an injection of growth factor protein to reverse disc regeneration has been shown *in vivo* using a small animal disc degeneration model.
- The confirmation of those effects and a detailed dose-response study, as well as a long-term safety study, in a large animal model is highly anticipated.

Acknowledgments

The authors thank Ms. Mary Ellen Lenz and Ms. Lori Otten for their assistance in the preparation of the manuscript.

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