Chapter Fifty-Eight

Regeneration and Replacement of the Intervertebral Disc

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I. Introduction
II. IVD Structure and Function
III. Biomaterials for Nucleus Pulposus Replacement
IV. Cell-Biomaterial Constructs for IVD Regeneration
V. Cellular Engineering for Intervertebral Disc Regeneration
VI. Growth Factors and Biologics for Intervertebral Disc Regeneration
VII. Gene Therapy for Intervertebral Disc Regeneration
VIII. Concluding Remarks
IX. Acknowledgments
X. References

I. INTRODUCTION

The intervertebral disc (IVD) is the fibrocartilaginous part of a "three-joint complex" that governs motion, flexibility, and weight bearing in the spine (Fig. 58.1). As part of this complex, the disc undergoes a lifetime of "wear and tear" that contributes to multiple IVD disorders of enormous consequence for human disability and suffering. These IVD disorders are poorly understood musculoskeletal pathologies characterized by multiple anatomic features, including internal disc disruption, IVD tears, and herniated disc fragments (Andersson, 1996; Bodguk, 1988; Boos *et al.*, 2002). These anatomic features are believed to associate with nerve root compression or irritation, spinal canal narrowing (stenosis or spondylolisthesis), or loss of disc height, which contribute to symptoms of low-back pain, neurological deficits, and disability that affect between 4% and 33% of the U.S. population annually (Hurri and Karppinen, 2004; Praemer *et al.*, 1999; Woolf and Pfleger, 2003). Like most cartilaginous tissues, the IVD is an avascular and alymphatic structure that exhibits little to no capacity for repair following injury and experiences aging-related cell density losses that may further limit biologically mediated repair (Urban and Roberts, 2003). The extreme mechanical demands on the IVD may also contribute to tissue failure and degeneration, due to the high magnitudes of pressure, compressive, tensile, and shear stresses, and strains that result from joint loading, muscle activation, and spinal flexibility. As a result, strategies to intervene in the progression of IVD disorders are met with significant biological and mechanical challenges that frustrate success.

Numerous surgical procedures have been developed to treat IVD disorders, focused largely on bony fusion across the disc space to restore stability and eliminate symptomatic motions and weight bearing. The majority of these procedures have relied on fixation of devices to inhibit motion during the bony fusion process. In cases where the pathology permits, removal of extruded IVD fragments may be performed in a procedure termed *discectomy*. Together these procedures comprise more than 300,000 inpatient hospitalizations annually in the United States alone (DeFrances and Podgornik, 2006). More recently, intervertebral disc replacements have been approved as an alternative therapy to bony fusion for disc-related pathologies (McAfee, 2004). The concept for these "motion-preservation devices"

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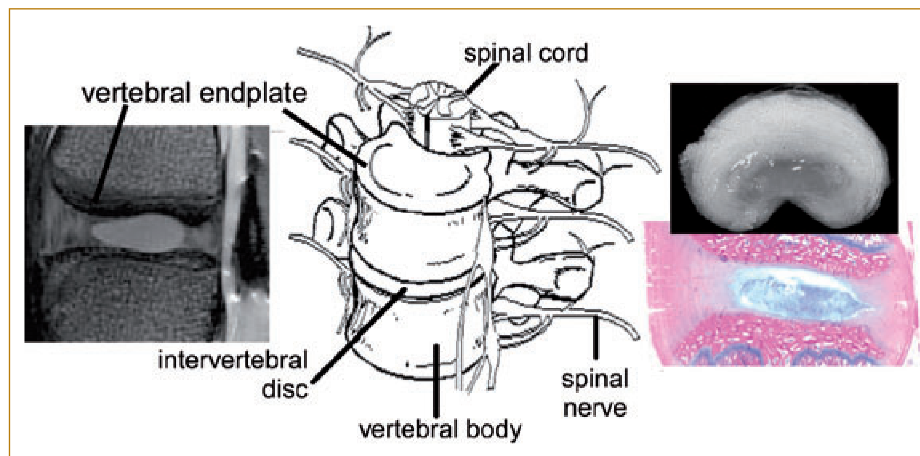

FIG. 58.1. Schema of spinal motion segment illustrating location of intervertebral disc between superior and inferior vertebral bodies. (*Left*) MRI appearance of immature lumbar disc with characteristic intense nucleus pulposus region. (*Right, top*) Gross appearance of nondegenerate lumbar disc and (*right, bottom*) histological appearance of immature disc in a stained section. Modified schema reprinted with permission from Columbia-Presbyterian Neurosurgery at www.cumc.columbia.edu/dept/nsg.

is that maintenance of load sharing across the IVD and a small range of motion are important to provide full range of spinal motion, to maintain IVD health, and to minimize IVD height loss, facet joint degeneration, stenosis, and related symptoms that may occur in progressive IVD pathology. These devices present all the risks associated with conventional joint replacements, such as subsidence, wear, and failure, and are indicated for only a small portion of symptomatic pathologies. There exists a very compelling need to develop alternative strategies not to only treat the consequences of IVD disorders, but also to detect and limit the progression of symptomatic IVD pathology.

Success with cellular therapies for articular-cartilage regeneration, gene therapy, and *in vitro* regeneration of cartilaginous tissue has raised hope for tissue-engineered treatments for IVD disorders. Tissue-engineered approaches to IVD regeneration have been focused on implantation of cell-supplemented or acellular biomaterials that may partially replace the IVD structure as well as on delivery of cells or bioactive factors designed to promote the natural repair process. In this chapter, a review of these tissue-engineering strategies is provided, along with evaluations of their adaptation and implementation for treatment of IVD disorders.

II. IVD STRUCTURE AND FUNCTION

In all structures of the IVD, the extracellular matrix provides physical and biochemical cues that regulate cell-mediated repair or breakdown in mature or aging tissues (Oegema, 1990, 2002). The native matrix organization and interaction with the local IVD cell population will be important considerations in the design of any tissue-engineered regeneration strategy. The IVD is composed of a centrally situated and gelatinous tissue, the nucleus pulposus, which differs substantially from the more fibrocartilaginous annulus fibrosus, on the radial periphery (Fig. 58.2). On both superior and inferior faces is a cartilaginous endplate that provides an intimate mechanical and biophysical connection between the vascularized vertebral bone and the avascular IVD. Both the annulus fibrosus, with a vascularized periphery, and the cartilaginous endplates are believed to be important routes of nutrient transport to all cells of the IVD (Nachemson *et al.*, 1970; Urban *et al.*, 1977). Given the very low cell density of the IVD, maintenance of both cellularity and a generous nutrient supply are often held to be critical to a successful biologically based regenerative strategy.

The immature nucleus pulposus is highly hydrated (>80% water), with extracellular matrix components that include randomly organized type II collagen fibers and multiple forms of negatively charged proteoglycans (Table 58.1, Roughley, 2004). A population of large and highly vacuolated cells is present in the nucleus pulposus during development and growth, with a shift toward a more chondrocyte-like cell population by age 7 (Meachim and Cornah, 1970; Taylor and Twomey, 1988; Trout *et al.*, 1982). Like all IVD regions, the nucleus pulposus contains multiple collagenous and noncollagenous proteins, including types III, V, VI, and IX collagens, elastin, fibronectin, and laminin (Hayes *et al.*, 2001; Oegema, 1993; Roberts *et al.*, 1991; J. Yu *et al.*, 2002). The nucleus pulposus is largely loaded in compression (Fig. 58.3) and experiences high interstitial swelling and fluid pressures, which arise from joint loading and a high density of osmotically active, proteoglycan-associated negative charges (Urban and McMullin, 1985, 1988). Nachemson and coworkers showed, as early as the 1960s, that this interstitial fluid pressure is greater than 0.5 MPa (or approximately five times atmospheric pressure) in the nucleus pulposus region (Andersson *et al.*, 1982; Nachemson, 1960, 1992). An early loss of hydration or tearing in the nucleus pulposus (Boos *et al.*, 2002), often detected as a loss of MR signal (S. Yu *et al.*, 1989), is believed to contribute to a loss of fluid pressurization in the IVD, which may lead to herniation or stenosis with aging (Buckwalter, 1995; McNally and Adams, 1992; Schultz *et al.*, 1982). With loss of fluid pressurization, the load distribution to the annulus fibrosus will shift from a characteristic outward "bulging" of the annulus to one of inward displacements (Adams *et al.*, 1996; Nachemson, 1992; Panjabi *et al.*, 1988; Shirazi-Adl, 1992). Partial or complete removal of the nucleus pulposus, occurring in some

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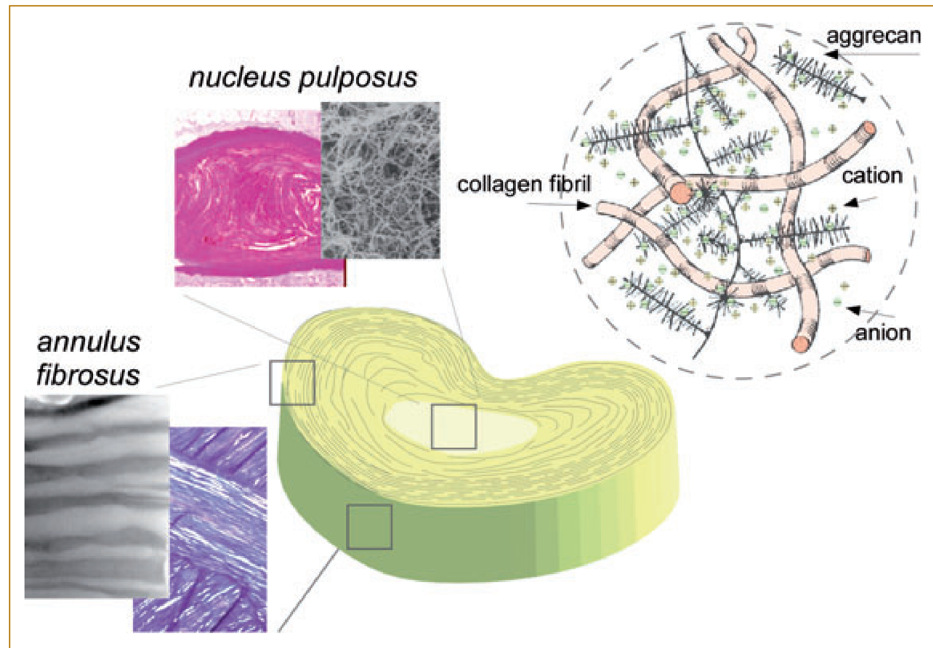

FIG. 58.2. Schema of different zones and microstructures comprising the intervertebral disc. Annulus fibrosus insets of macroscopic appearance and stained section illustrate the lamellar structure of the tissue. The lamellae comprise aligned collagen fiber bundles that are oriented with alternating angles of ±60°. Nucleus pulposus insets of a stained section and scanning electron micrograph illustrate the randomly organized network of fine collagen fibers and gelatinous nature of the tissue. Circular inset contains a schema of building blocks for these cartilaginous tissues that include banded type I and type II collagen fibrils, aggrecan and smaller proteoglycans, water, and multiple ionic species.

Table 58.1. Ranges reported for compositional features and mechanical properties for nucleus pulposus and anulus fibrosus tissue regions of the nondegenerate intervertebral disc[a]

	Water (% wt)	*Collagen (% dry wt)*	*Proteoglycan (% dry wt)*	*Other proteins (% dry wt)*	*Compressive modulus (MPa)*	*Shear modulus (MPa)*	*Tensile modulus (MPa)*	*Interstitial pressure (MPa)[b]*
Nucleus pulposus	70–90	15–35	25–60	20–45	0.5–1.5	0.005–0.01	*NA*	0.5–3.0
Anulus fibrosus	65–80	10–65	10–35	15–40	0.5–1.5	0.08–0.40	20–50 – ∥ circ	0.1–1.0
Notes		Types I, II, VI, IX, XI	Aggrecan, decorin, biglycan, fibromodulin, versican, and more				0.5–5.0	– ⊥ circ

[a]Both composition and mechanical properties of the disc vary substantially with region and with degeneration. Additional mechanical features important to tissue function, such as failure strength, are not shown here.
[b]Reported also as peak hydrostatic pressures, or swelling pressures.

discectomy procedures, may lead to a loss of disc pressurization and disc height that will transfer loads to facet joints of the spine, increase segmental range of motion, and impact overall spinal stability. Restoration of this interstitial swelling pressure in the nucleus pulposus, or restoration of MR signal intensity, is an oft-cited criterion for restoration of a healthy functioning disc.

The annulus fibrosus is a lamellar, fibrocartilaginous structure that is highly organized into distinct lamellae (Coventry *et al.*, 1945a, 1945b) of highly oriented, and largely type I collagen–containing fiber bundles (Cassidy *et al.*, 1989; Hickey and Hukins, 1980). Type II collagen concentration increases toward the innermost region of the annulus fibrosus as the concentration of type I collagen is diminished. As with the nucleus pulposus, the annulus fibrosus contains proteoglycans within the collagenous extracellular matrix, although at lesser concentrations, which vary from outer to inner regions of the tissue. The collagen reinforcement within the annulus fibrosus resists the tensile loads, which arise during physiological joint motions, and the swelling effects, which give rise to significant anular bulging and deformation. Consequently, the annulus fibrosus has a very high stiffness in tension, with moduli that vary with the angle of orientation along the principal collagen fiber direction (Table 58.1, Ebara *et al.*, 1996; Elliott and Setton, 2001; Fujita *et al.*, 1997; Galante, 1967; Holzapfel *et al.*, 2005;

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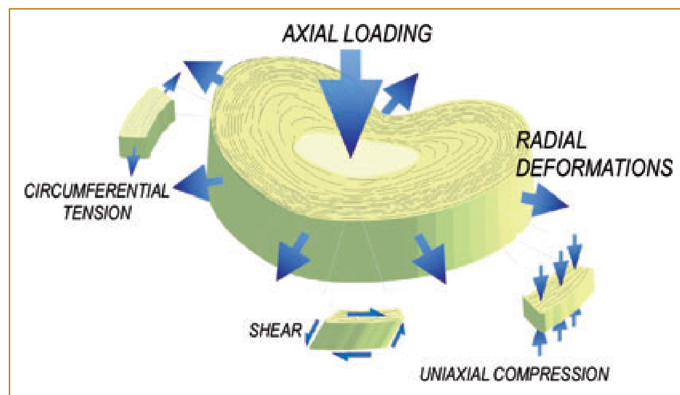

FIG. 58.3. Axial compressive loading of the intervertebral disc gives rise to a radial deformation, or "outward bulge," as the disc deforms in response to the compressive load. The high tensile stiffness of the healthy annulus fibrosus in the circumferential direction acts to restrict this outwardly directed deformation. Tissues of the disc will be variably loaded and experience a combination of compression, tension, and shear, as shown. Pressurization of the central and gelatinous nucleus pulposus is an important mechanism for load support and load transfer to the annulus fibrosus, which contributes to maintenance of disc height.

Skaggs *et al.*, 1994). Cells of the annulus fibrosus originate from the mesenchyme and exhibit many characteristics of fibroblasts and chondrocytes (Bayliss and Johnstone, 1992; Oegema, 2002; Postacchini *et al.*, 1984; Rufai *et al.*, 1995; Urban and Roberts, 1995). These cells are sparsely distributed in the mature IVD and exhibit very little intrinsic ability for self-repair. Disorders of the IVD that involve displacement or herniation of an IVD fragment are believed to arise from tears in the annulus fibrosus region, and discectomy procedures frequently involve removing a portion of this annulus tissue. Some tissue-engineering strategies are being developed around restoration of healthy annulus fibrosus function or composition, although the complexities of annulus structure and composition make this a very challenging goal.

The hyaline cartilage endplates of the IVD are important structures that transmit and distribute loads of the spinal column to the discs. Because of their direct contact with both the annulus fibrosus and the nucleus pulposus, the endplates are believed to be an important route of nutrient transport, particularly to cells of the nucleus pulposus (Antoniou *et al.*, 1996; Benneker *et al.*, 2005; Roberts *et al.*, 1996; Selard *et al.*, 2003; Urban *et al.*, 1977). With aging, the cartilage endplate will thin, as it undergoes mineralization and eventual replacement by bone. This mineralization of the endplate is thought to impede diffusion and nutrient flow to the disc, principally the nucleus pulposus, which is lacking in an alternate short-diffusion pathway. Endplate changes, such as sclerosis, fracture, and modified vascularity, may be detected by MRI changes (Modic *et al.*, 1984) and are believed to contribute to symptomatic IVD degeneration (Bodguk, 1988; Kokkonen *et al.*, 2002; Weishaupt *et al.*, 2001). Thus, tissue-engineering strategies that preserve the health of the endplate without inducing additional damage are believed to be critical to restoring IVD function.

III. BIOMATERIALS FOR NUCLEUS PULPOSUS REPLACEMENT

In Situ Hydrating Polymers

The development of biomaterials and cellular therapies for tissue-engineered IVD replacement has a long history but has not often progressed past preclinical evaluations. The complexity of the IVD, with its three distinct substructures and multiple pathologies, together with very harsh loading conditions and mechanical requirements, has led to challenges for engineering tissue replacements. The concept that nucleus pulposus changes are an important contributor to IVD disorders has led to an initial focus on the use of acellular biomaterials for restoration of the nucleus pulposus tissue or function (Carl *et al.*, 2004; Di Martino *et al.*, 2005; Klara and Ray, 2002). In this section, attention is given to strategies developed around the concept of using "*in situ* hydrating," synthetic polymers to restore nucleus pulposus hydration and, consequently, IVD disc pressure and disc height. The device with the longest clinical history is based on a copolymeric hydrogel encased in a polyethylene-fiber jacket (polyacrylonitrile and polyacrylamide, PDN™, Raymedica Inc., Fig. 58.4). When implanted in a dessicated state, the polymers absorb water while the polyethylene jacket restricts excessive swelling of the polymer. Similar concepts have been developed based on implantation of preformed devices constructed from semihydrated poly(vinyl) alcohol (Aquarelle™, Stryker Spine Inc.), a copolymer of poly(vinyl alcohol) (PVA) and poly(vinyl pyrrolidone) (PVP) (Thomas *et al.*, 2003), or modified poly(acrylonitrile) reinforced by a Dacron mesh (Bertagnoli *et al.*, 2005, NeuDisc™, Replication Medical). As shown for the PVA/PVP copolymeric implant (Fig. 58.5), the design goal is to exploit implant swelling pressure to restore the high compressive stiffness of the IVD, which is lost on dehydration or denucleation of the nucleus pulposus (Joshi *et al.*, 2006). The relevant stiffness is that measured after placement of the implant, with stiffness values reflecting both the material behaviors of the implant as well as the integration with the containing annulus fibrosus and endplates. An additional concept that has promoted development of these devices is an ability to maintain disc height.

As with many tissue replacements, there is a long list of requirements that must be satisfied for biomaterials to be used in this application, including the needs to achieve (1) "favorable mechanical stiffness" or mechanical properties matched to that of the native structure; in particular, the compressive stresses generated must not exceed the failure strength of the adjacent endplate, in order to avoid endplate fracture or subsidence of the device, IVD height loss, and associated problems; (2) integration with adjacent struc-

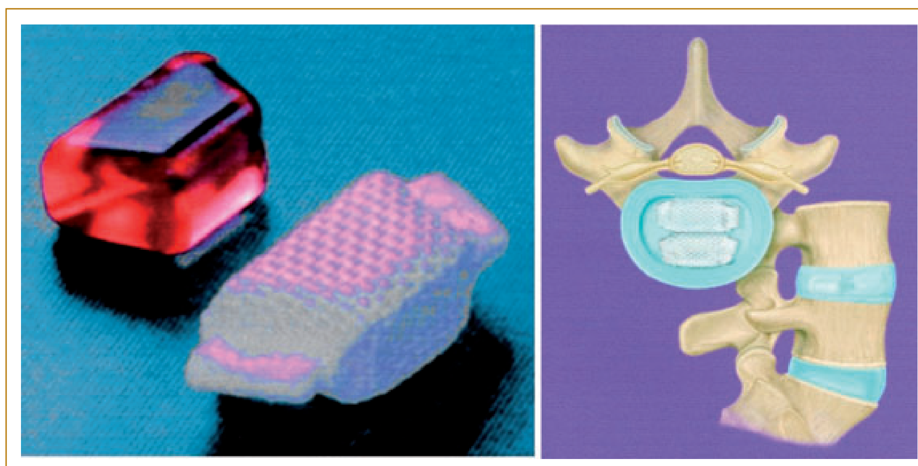

FIG. 58.4. **(left)** Prosthetic disc nucleus (PDN, Raymedica), shown on the left, is composed of an *in situ* swelling synthetic polymer. The pellet is encased in a polyethylene jacket that restricts water imbibition, shown adjacent to the pellet. **(right)** The PDN is designed to fit into either the anterior portion of the vacated disc nucleus or the posterior portion. Shown is a schematic of placement of both anterior and posterior components within the nucleus pulposus disc space. Modified from Klara and Ray (2002) with permission.

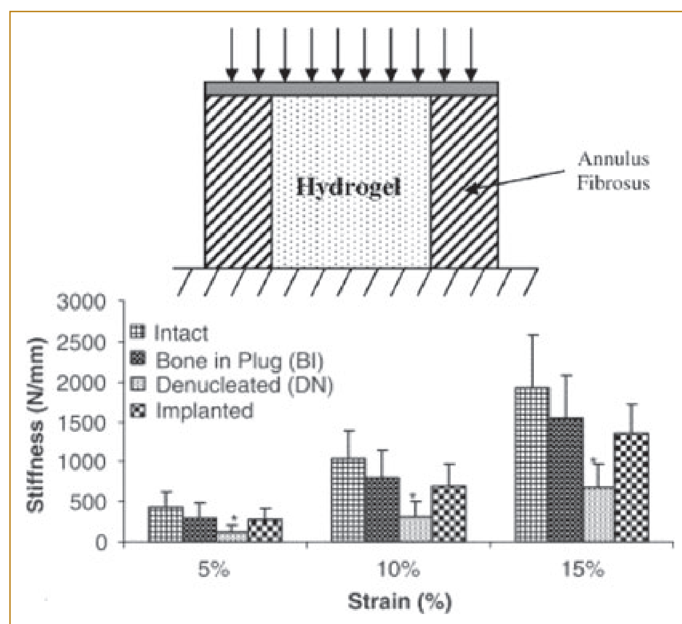

FIG. 58.5. **(top)** Schematic of polymeric hydrogel implants placed within the contained space of the intervertebral disc. The compressive stiffness of the disc implant can be measured as the relationship between axial deformation and applied compressive load, as shown. **(bottom)** Stiffness values were obtained for an intact human motion segment (devoid of posterior elements). When denucleated, the stiffness drops significantly. Implantation of a copolymeric hydrogel of PVA/PVP is capable of restoring stiffness for the motion segment to intact values. Modified from Joshi *et al.* (2006) with permission.

tures in order to promote load transfer, minimize device migration or extrusion, and restore stability for the motion segment; (3) durability, or an ability to maintain physical support over millions of cycles of loading; (4) minimal generation of wear debris, if appropriate; (5) standards of biocompatibility without eliciting systemic, cellular or immunotoxicity. Some of the polymeric devices for nucleus pulposus replacement have experienced device extrusion, endplate failure, and endplate sclerosis after implantation. These observations are thought to relate to a mismatch in mechanical stiffness that leads to excessive endplate loading, and poor integration associated with device migration (Boyd *et al.*, 2004; Huang *et al.*, 2005). Controlling interstitial hydration of the polymer is a desirable feature for this class of polymers in general, because excessive swelling can cause implant stiffness and endplate overloading.

In Situ Forming Polymers

Injectable polymer systems, such as polymers that will undergo a physical transition to a gel-like or solidlike form via cross-linking or thermal or pH-induced transitioning, have been evaluated for placement into a vacant nucleus pulposus space (Bao and Yuan, 2002; Boyd *et al.*, 2004; Temenoff and Mikos, 2000; Thomas *et al.*, 2003). Many of the requirements for success of this strategy are similar to those just described, with the additional requirements that the polymers must exhibit minimal leaching during the *in situ* forming procedure and must provide the benefit of minimally invasive insertion into the disc space. *In situ* curing polyurethane is one widely studied *in situ* curing polymer that has been delivered to the disc space through an inflatable polyurethane "balloon" in order to contain excessive swelling.

Two alternative injectable polymers evaluated for nucleus pulposus replacement have been developed from cross-linkable biopolymers. The BioDisc^{TM} (Cryolife) is composed of bovine serum albumin that is cross-linked via glutaraldehyde at the time of injection to form a mechanically stiff implant. Similarly, Nucore^{TM} (SpineWave Inc.) is a protein hydrogel developed from a silk and elastin peptide–containing sequence that is cross-linked via di-isocyanate at the time of injection (Boyd *et al.*, 2004). The cross-linking confers an extra stiffness to protein polymers, which is necessary to achieve satisfactory stiffness values for a disc implant. Both implant systems have been able to maintain disc stiffness and to restore disc height when implanted,

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providing evidence that successful integration is being achieved on injection. Furthermore, systems composed of native IVD polymers, such as the elastin peptide sequences, may confer some additional benefit as recognized components of the body. These approaches are promising as they move through clinical trials of implant feasibility in the current period.

IV. CELL-BIOMATERIAL CONSTRUCTS FOR IVD REGENERATION

A persistent limitation of materials-based replacements of the IVD is their biologically mediated or mechanically induced failure due to the harsh loading conditions and cellular responses within the disc space. These challenges are rooted in the fact that the materials used for such applications have no capacity for self-renewal or self-repair. This has led to increasing interest in tissue-engineering methods to regenerate new IVD *in situ* or to transplant IVD tissue that has been generated *ex vivo*. Such strategies have been employed to augment repair of other types of cartilage, most notably articular cartilage, and meniscus, which share some features of the harsh biologic and mechanical loading environment within the IVD.

Scaffolds for Cell-Based Tissue Engineering in the IVD

As in other cartilage-tissue-engineering applications, a main strategy for IVD regeneration has been the inclusion of cells with biomaterials to enable production and long-term maintenance of newly generated tissue. Biomaterials that enable appropriate cellular phenotypes and matrix biosynthesis and that sometimes enable polymeric degradation or resorption have been proposed as alternative implantable biomaterials and have been studied largely *in vitro*. The goals for use of these scaffolds are similar to those for other biomaterial implants, with the added requirements that the biomaterial must generate no cytotoxic or immunogenic degradation or breakdown fragments and that new matrix formation is enabled. Studies of cell-biomaterial constructs cultured *in vitro* have demonstrated potential for many materials (Table 58.2), including thermosensitive gels such as chitosan, modified chitosans, and elastin-like polypeptides (Au *et al.*, 2003; Betre *et al.*, 2002; Mwale *et al.*, 2005; Roughley *et al.*, 2006), self-associating gels composed of agarose, collagen, and fibrin (Gruber *et al.*, 2004; Peretti *et al.*, 2006) or modified forms of these same materials, cross-linkable alginates, polyethylene glycol, poly(glycolic acids), and more (Baer *et al.*, 2001; Burkoth and Anseth, 2000; Elisseeff, 2004; Masuda *et al.*, 2003; Mercier *et al.*, 2004; Sontjens *et al.*, 2006). *In vitro* studies with these materials are based on evaluating new matrix formation and sometimes degradation characteristics, through culturing native disc or other cell types within these matrices. Hydrogels, such as alginate and gelatin, have been used most commonly for engineering nucleus pulposus tissue, likely due to the fact that such materials reasonably approximate the gel-like properties of the native tissue. Cells of different origin, including native IVD cells, stem cells, and chondrocytes, are capable of synthesizing and depositing collagen and glycosaminoglycans within these hydrogels, although there is little agreement on the targeted composition necessary to achieve a satisfactory tissue construct. This is a particularly challenging determination for the intervertebral disc because the matrix contains varying amounts of both types I and II collagen, so the exclusive presence of type II collagen does not serve as a phenotypic matrix marker, as is the case for articular cartilage.

Efforts to regenerate annulus fibrosus have also involved gels such as alginate, agarose, gelatin, and collagen as well as fibers or sponges made from materials such as poly(glycolic) acid, collagen, hyaluronic acid, and/or glycosaminoglycans. Often, the same scaffolds evaluated for nucleus pulposus cells are also studied with cells of the annulus fibrosus, with findings that generally illustrate the importance of cell origin in determining the resultant extracellular matrix synthesis. A common observation, however, is that cells of either origin that are maintained in a rounded morphology tend to generate more type II collagen, characteristic of hyaline cartilage, whereas those that are cultured in an elongated morphology generate more type I collagen (Fig. 58.6). A main challenge has been reproducing the intricate lamellar arrangement of collagen fibers that give the annulus fibrosus its unique mechanical properties and the cells their unique morphology. For this reason, the majority of annulus-tissue-engineered materials have been generated from scaffolds that lack any apparent lamellar microstructure. Some investigators have developed polymeric scaffolds with anisotropic features, such as an oriented honeycomb structure, demonstrating production of multiple collagen types as well as proteoglycan (Sato *et al.*, 2003). These results are indeed suggestive of the potential to engineer anisotropic collagenous tissues, although no results for generating new, functional lamellar annulus tissue are known to exist to date.

Assessment of the success of IVD-tissue-engineering efforts is critical to moving this technology toward clinical application. Most studies have focused on generating new IVD *in vitro*, with few documenting tissue formation and integration in preclinical evaluations *in vivo*. To date, the most common tool for assessment of newly generated IVD tissue has been histology as a method to evaluate cell and tissue morphology. Given the structural complexity of the tissue, analysis of gene expression and extracellular matrix composition has been commonly employed to confirm the appropriate phenotypic behavior in engineered IVD. Relatively few studies have documented mechanical analysis of engineered IVD tissue, but this will undoubtedly be critical as efforts to engineer functional tissue continue. Nevertheless, these *in vitro* studies begin to lay the foundation for

Table 58.2. Representative overview of studies involving cell scaffold–based tissue engineering of IVD using cells obtained from native tissues only

Material	*Cell type*	*Cell source*	*Cell density*	In vitro/in vivo	*Assessment*
PCL[a]	AF, NP	Bovine	5×10^3/cm^2	*In vitro*	Histology, SEM, gene expression
Alginate[b]	AF, NP	Porcine	4×10^6/mL	*In vitro*	DNA, ECM analysis
Gelatin/C6S/HA[c]	NP	Human	20×10^6/mL	*In vitro*	Histology, DNA, ECM analysis, gene expression
CPP[d]	NP	Bovine	16×10^6/cm^2	*In vitro*	Histology, mechanical analysis
Agarose, collagen[e]	AF	Human	0.2×10^6/mL	*In vitro*	Histology, ECM analysis
Gelatin, PLA[f]	NP	Porcine	5×10^6/mL	*In vitro*	Histology, ECM analysis, gene expression
Collagen/GAG[g]	AF	Canine	40×10^6/mL	*In vitro*	Histology, ECM analysis
Collagen/HA[h]	AF, NP	Bovine	13×10^6/mL	*In vitro*	Histology DNA, ECM analysis, gene expression
Alginate[i]	AF, NP	Porcine	1–10×10^6/mL	*In vitro*	Histology, gene expression, mechanical analysis
Collagen[j]	AF	Lapine	10×10^6/mL	*In vivo*	Histology
PGA, alginate[k]	AF, NP	Ovine	25–50×10^6/mL	*In vivo*	Histology, DNA, ECM analysis, mechanical analysis

Abbreviations: PCL (polycaprolactone); C6S (chondroitin-6-sulfate); HA (hyaluronan); CPP (calcium polyphosphate); PLA (polylactic acid); GAG (glycosaminoglycan); PGA (polyglycolic acid); AF (annulus fibrosus); NP (nucleus pulposus); SEM (scanning electron microscopy); DNA (deoxyribonucleic acid); ECM (extracellular matrix).
[a]Johnson *et al.* (2006).
[b]Akeda *et al.* (2006).
[c]Yang *et al.* (2005a, 2005b).
[d]••; Hamilton *et al.* (2005).
[e]Seguin *et al.* (2004).
[f]Gruber *et al.* (2006).
[g]Brown *et al.* (2005); Saad and Spector (2004).
[h]Rong *et al.* (2002).
[i]Alini *et al.* (2003); Baer *et al.* (2001); Wang *et al.* (2001).
[j]Sato *et al.* (2003).
[k]Mizuno *et al.* (2004, 2006).

necessary and/or sufficient characteristics of a successful scaffold for nucleus pulposus replacement. From these studies, for example, it is evident that a high starting cell density and a high degree of initial matrix stability are essential for promoting long-term construct stability and matrix accumulation, eventually to restore mechanical function and swelling pressure (Wilson *et al.*, 2002).

Composite Cell-Biomaterial IVD Implants

Cell-based regeneration of the IVD *ex vivo* is complicated by the inherent multicomponent structure of the IVD, which includes two distinct regions, the annulus fibrosus and the nucleus pulposus. Given this added complication, it is not surprising that there are fewer examples of efforts to engineer in integration of the multiple components of the IVD *ex vivo*. This can prove to be a critical limitation because integration is needed to ensure proper load transfer and to limit damaging motions during disc loading. In studies conducted by Bonassar and coworkers, IVD regeneration was attempted with a fully integrated scaffold combining poly(glycolic *co*-lactic acid) as a scaffold for annulus fibrosus and a cross-linked alginate hydrogel as a scaffold for nucleus pulposus tissue (Fig. 58.7) (Mizuno *et al.*, 2004, 2006). Primary cells for culture within each scaffold region were derived from the corresponding native IVD tissues, and the resultant cell-laden scaffolds were implanted subcutaneously into athymic mice for a period of 12 or 16 weeks. Results illustrate spatially directed matrix regeneration with extracellular matrix that exhibited distinct morphologies and contained both collagen and glycosaminoglycans (Fig. 58.8). In biomechanical tests, the composite tissue-engineered disc was found to have a compressive modulus about one order of magnitude lower than that of the native tissue, with a permeability to fluid flow that fell between values for the nucleus pulposus and the annulus fibrosus. Thus, this approach illustrated an ability for cell-laden scaf-

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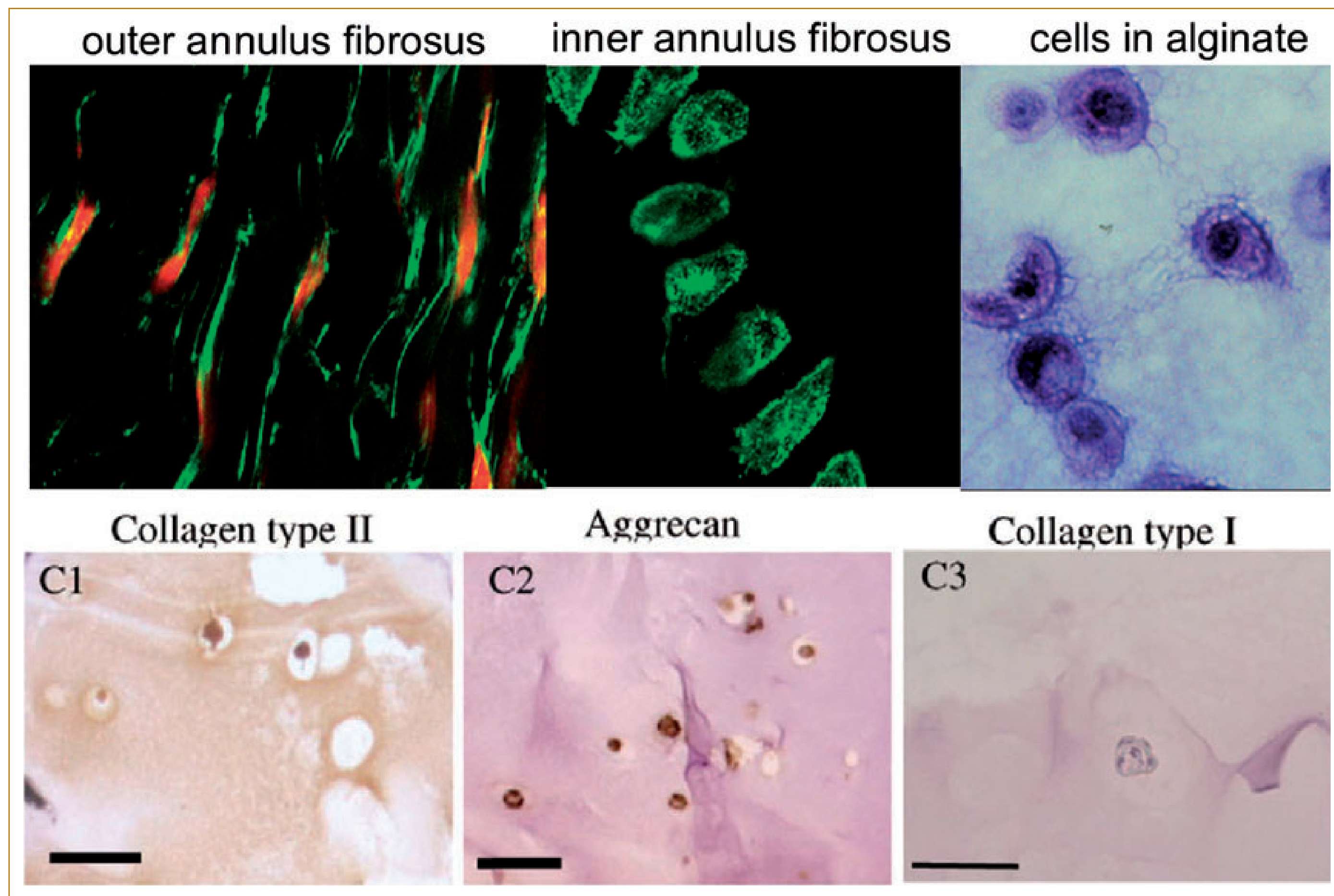

FIG. 58.6. Top: (*left*) Annulus fibrosus cells are highly elongated in the outermost regions of the tissue but adopt a more rounded morphology in the inner tissue regions, as shown (*middle*). (*right*) When annulus fibrosus cells are embedded in an alginate hydrogel, they assume a rounded morphology. **Bottom:** Cells in alginate will express matrix proteins associated with chondrocytes, such as type II collagen and aggrecan. Intervertebral disc cells will also synthesize type II collagen. Bottom figure modified from Le Maitre *et al.* (2005) with permission.

folds to regenerate extracellular matrix with some of the functional and compositional features of the native tissue.

In another integrative tissue-engineering study of note, investigators Kandel and coworkers generated nucleus pulposus tissue *in vitro* by culturing primary bovine nucleus pulposus cells at high density on a calcium polyphosphate substrate, in order to mimic the natural integration of the nucleus pulposus against the vertebral endplate (Fig. 58.9, Seguin *et al.*, 2004). A similar strategy was employed to generate a calcified tissue–cartilaginous endplate–nucleus pulposus construct by first generating a hyaline cartilage tissue layer on the calcium polyphosphate substrate prior to seeding with nucleus pulposus cells (Fig. 58.9 Hamilton *et al.*, 2006). The nucleus pulposus cells formed tissue with a proteoglycan, but not collagen content matched to that of the native nucleus pulposus. Importantly, functional properties in some testing configurations approached that of the native tissue. Additional work will be required in adapting these integrative tissue-engineering approaches to ensure that mechanical integration with adjacent tissues is adequate, but these studies focused on generating integrated nucleus–endplate or nucleus–annulus are an important step in illustrating feasibility for this approach.

V. CELLULAR ENGINEERING FOR INTERVERTEBRAL DISC REGENERATION

Given the relatively small numbers of studies in the area of IVD-tissue engineering, there is a surprising amount of breadth not only to the biomaterials, but also to the cell sources utilized for regeneration. The question of cell source is of particular note for IVD-tissue engineering, given that the availability of autologous disc cells is extremely low in the adult and that the phenotype of cells varies so substantially with both spatial position and with age. In animals studied for IVD-tissue engineering *ex vivo*, the origin of cells in the nucleus pulposus may be partly notochordal or mesenchymal, depending on the age of the animal in question.

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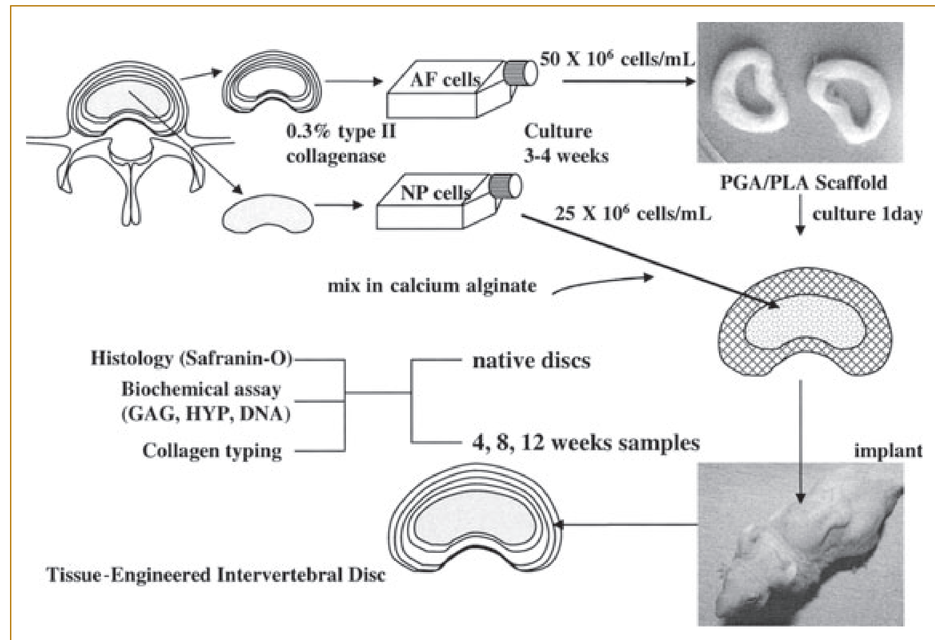

FIG. 58.7. Schema of tissue engineering for intervertebral discs. Annulus fibrosus and nucleus pulposus cells were isolated and cultured separately prior to seeding onto PGA/PLA scaffolds (annulus fibrosus) or suspension in a solution of 2% alginate (nucleus pulposus). The cell–alginate mixture was injected into the empty center of the PGA/PLA construct. The assembled tissue-engineered intervertebral disc constructs were implanted subcutaneously onto the dorsum of athymic mice and harvested at times up to 12 weeks. Reprinted with permission from Mizuno *et al.* (2004).

As such, the choice of species used as a source of cells may be quite important. Due to the ease of availability, porcine and bovine cells are the most commonly used, with other efforts reporting the use of cells of canine, lapine, and ovine origin as well as human. However, cells derived from bovine tissues may be exclusively mesenchymal in origin, while those derived from porcine, lapine, or ovine sources may be largely notochordal. These phenotypic differences add an additional and unique complicating factor for investigators studying preclinical models for IVD-tissue regeneration.

Given the very limited availability of native IVD cells that can be effectively harvested for tissue engineering, there has been interest in using other cells as sources for these efforts. The primary target for other sources has been mesenchymal stem cells (MSCs) derived from sources such as bone marrow (Richardson *et al.*, 2006a) and adipose tissue (Li *et al.*, 2005). A major challenge in this approach has been the development of methods to guide the development of MSCs toward phenotypes found in the IVD (see next section). This has been attempted through manipulation of the culture medium and gas conditions (Risbud *et al.*, 2004) as well as through coculture with primary cells from the IVD (Richardson *et al.*, 2006b). In comparison to the use of adult primary disc cells derived from often-pathological or degenerated IVDs, the use of autologous or other MSCs or progenitor cells may be most promising to the future of *ex vivo* tissue-engineering strategies that rely on cell supplementation.

In addition to origin, cell density is known to have a profound effect on the efficacy of the tissue-engineering process. Here there has been a great deal of variability in protocols, with studies reporting densities of delivered cells ranging from 0.2×10^6 to 50×10^6 cells/mL. While the lower end of this scale is likely more reflective of the actual cell density in nucleus pulposus tissue, the densities at the higher end of the scale are more in line with those known to be effective in generating other types of cartilage (Puelacher *et al.*, 1994). A critical concern for disc-tissue regeneration, particularly in the case of strategies that employ high cell densities, is the issue of nutrient and gas supply necessary to maintain cell viability and health. The IVD is both avascular and alymphatic, meaning that the transport of nutrients and oxygen is driven largely by diffusion from the vascularized periphery and through the vertebral endplates (Holm *et al.*, 1981; Maroudas *et al.*, 1975; Nachemson *et al.*, 1970; Stairmand *et al.*, 1991; Urban *et al.*, 1977). It is noteworthy that calcification and endplate changes in the degenerating IVD can led to impaired nutrient transport, which is presumably linked to decreased cell viability (Benneker *et al.*, 2005; Roberts *et al.*, 1996). Thus, supplementation of scaffolds with very high cell densities may not be optimized for long-term survival in the largely hypoxic and glucose-poor, lactate-rich environment within the IVD. This concern has been expressed for cell-laden IVD scaffolds, but it has not been directly addressed or investigated as an issue in IVD-tissue regeneration.

Cellular Supplementation in the IVD

If the local environment within the IVD is conducive to the survival of cells, direct cell supplementation without biomaterial scaffolds may hold promise for IVD repair. This strategy has been pursued by several groups, using either IVD cells, chondrocyte-like cells, or progenitor cells. In the first reported work, Nishimura and Mochida (1998) inserted nucleus pulposus cells from the rabbit, following removal of nucleus tissue, and showed some beneficial effects in

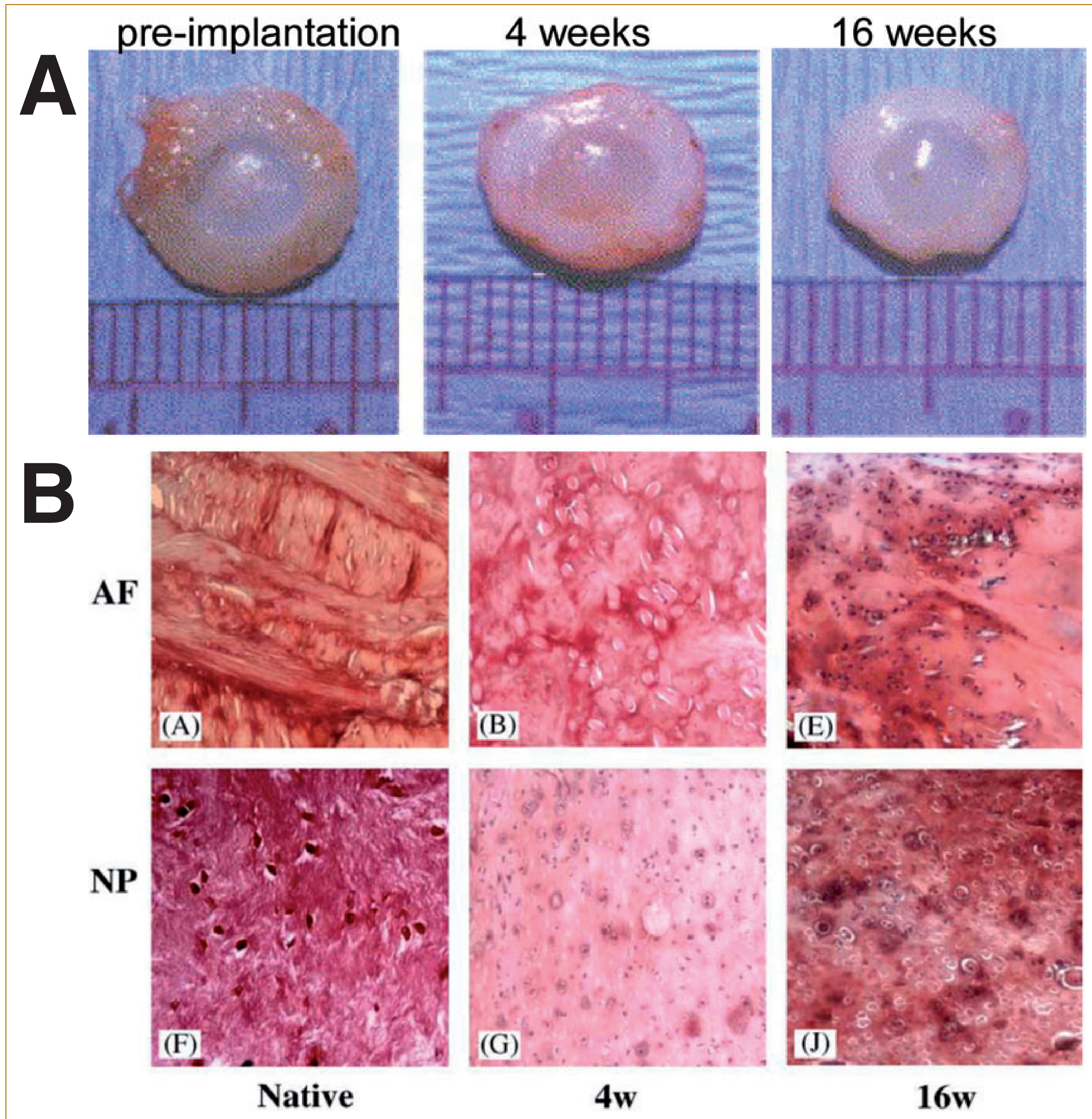

FIG. 58.8. **(A)** Gross morphology of tissue-engineered intervertebral disc constructs before implantation (*left*) and 16 weeks after implantation in the subcutaneous pouch of athymic mice. **(B)** Safranin O–stained sections of annulus fibrosus from (*left*) native ovine lumbar intervertebral disc and (*middle*) tissue-engineered intervertebral disc constructs at 4 weeks and (*right*) 16 weeks after implantation. **(C)** Safranin O–stained sections of nucleus pulposus from (*left*) native ovine lumbar intervertebral disc and (*middle*) tissue-engineered intervertebral disc constructs at 4 weeks and (*right*) 16 weeks after implantation. Modified from Mizuno *et al.* (2006) with permission.

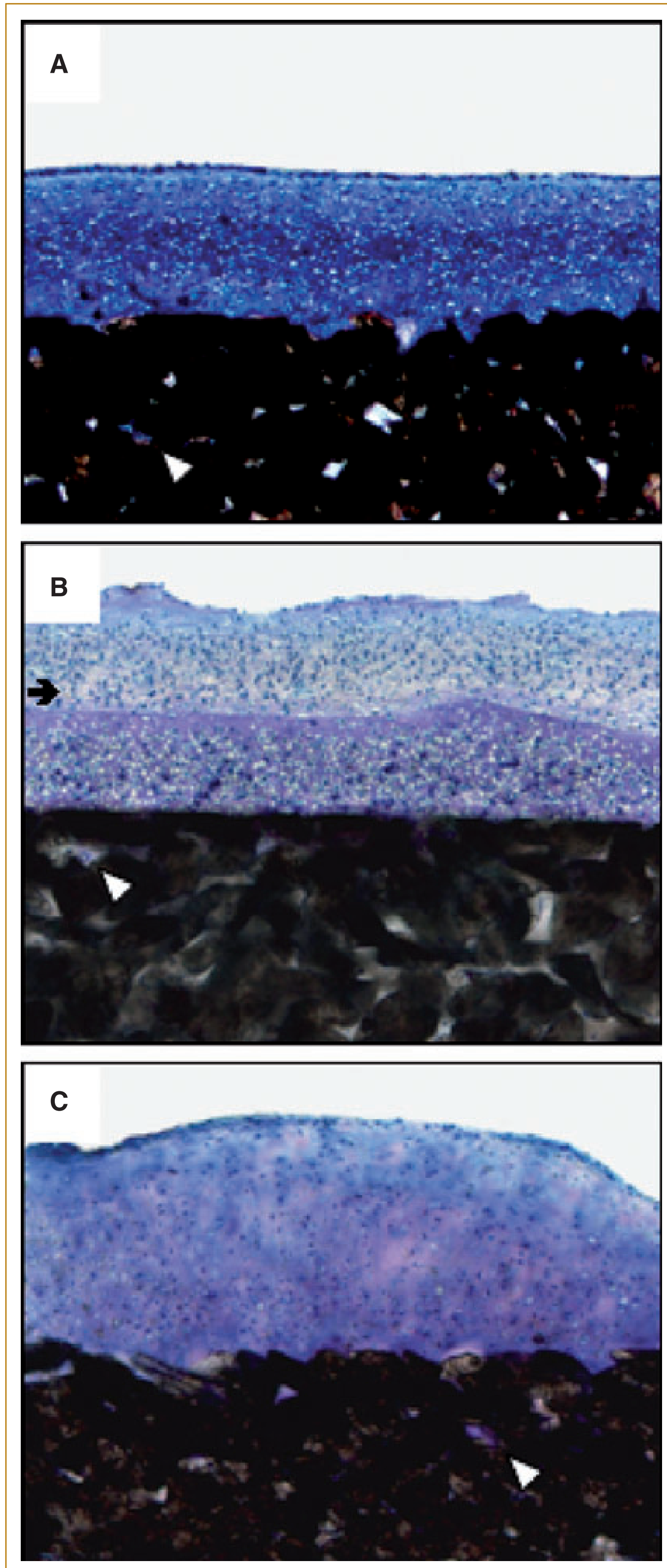

FIG. 58.9. Histological appearance of multicomponent tissue-engineered construct. Bovine articular chondrocytes were placed on the top surface of a porous calcium polyphosphate construct (CPP) and allowed to form cartilage *in vitro*. Nucleus pulposus cells were then placed onto the *in vitro*–formed hyaline cartilage and cultured for periods out to eight weeks. **(A)** *In vitro*–formed cartilage at two weeks (time at which the nucleus pulposus cells would be seeded); **(B)** *in vitro*–formed nucleus pulposus-cartilage–CPP composite (triphasic construct) at eight weeks following seeding of chondrocytes; and **(C)** *in vitro*–formed cartilage tissue alone (no nucleus pulposus cells) at eight weeks. Arrowheads indicate tissue growing within the pores of the CPP; arrow indicates interface between cartilage and nucleus pulposus tissue. (Toluidine blue stain; original magnification ×50). Reprinted from Hamilton and coworkers (2006) with permission.

inhibiting the degenerative IVD changes of nucleotomy. Similar procedures have also shown the effectiveness of autologous disc cell implantation in both a sand rat model of spontaneous disc degeneration (Gruber *et al.*, 2002) as well as a canine model of disc degeneration (Ganey *et al.*, 2003). Furthermore, work by Nomura and coworkers (2001) has shown that supplementation with allogeneic nucleus pulposus cells did not induce any appreciable host-versus-graft rejection response and also retarded disc degeneration in a rabbit nucleotomy model. It is noteworthy that nucleus pulposus cell insertion resulted in a slightly poorer outcome than did insertion of the allograft nucleus pulposus tissue itself, indicating that inclusion of the extracellular matrix present in the allograft may be as important as or more important than the absolute number of cells inserted (Nomura *et al.*, 2001).

Limitations will always exist in obtaining sufficient numbers of autologous or allogeneic disc cells from a single site, as well as concerns about impaired cellular activity for the native cells. Some studies have thus focused on using coculture of nucleus pulposus and annulus fibrosus cells to stimulate cell metabolism prior to reinsertion (Okuma *et al.*, 2000). These approaches were shown to be effective in delaying some degenerative features, such as the loss of disc architecture following reinsertion of the "activated" cells in a rabbit model. Still other studies have focused on delivery of cells through allograft tissues based on the concept that preservation of extracellular matrix is an equally important criteria for regeneration (Matsumoto *et al.*, 2001a; Sato *et al.*, 2003; Seguin *et al.*, 2004; Yung Lee *et al.*, 2001).

Since 2002, a prospective, controlled, multicenter study has been performed to compare autologous disc cell transplantation plus discectomy against discectomy alone (Meisel *et al.*, 2006). The interim analysis of the first 28 patients at two years showed a clinically significant reduction of low-back pain in the transplantation group as compared to the discectomy group, suggesting a potential benefit of the cell transplantation strategies described earlier. Little is understood about the mechanism by which the cell supplementation provides this benefit, although

disc hydration but not disc height was found to be higher in the patients receiving the cell transplantation as compared to the discectomy group. This clinical study underscores the role of autologous cell–mediated biological factors in regulating symptoms with IVD pathology and illustrates a potentially important role for sustaining cell viability of the IVD in inhibiting this pathology.

Clinically, the autologous reinsertion of the nucleus pulposus cells into the degenerative disc remains challenging (Evans, 2006). As a source of cells for transplantation, MSCs that can be harvested from a patient's own bone marrow are a possible candidate (Leung *et al.*, 2006; Risbud *et al.*, 2004; Sakai *et al.*, 2005). *In vitro*, the differentiation of MSCs into nucleus pulposus–like or chondrocyte-like cells has been shown under hypoxic and high-osmotic conditions (Risbud *et al.*, 2004) and also with TGF-β stimulation (Steck *et al.*, 2005). Studies have followed injection of autologous MSCs embedded in atellocollagen gel as well as direct injection of MSCs into rabbit or rat models of IVD degeneration, and these have observed an ability for these cells to differentiate or regenerate matrix (Crevensten *et al.*, 2004; Sakai *et al.*, 2005, 2003). In one set of studies, transplantation of MSCs with an atelocollagen carrier into the rabbit discs effectively maintained disc height, MR signal intensity, and the histological appearance of the nucleus pulposus and annulus fibrosus regions at 24 weeks after transplantation (Fig. 58.10, Sakai *et al.*, 2003). Some of the positive outcomes observed for tissue regeneration in the animal models may arise from factors released from MSCs or direct contact with MSCs, which may enhance metabolism of native disc cells (Yamamoto *et al.*, 2004; Richardson *et al.*, 2006b).

Another approach to supplement cells without donor morbidity is the use of established cell lines, as shown recently following transplantation of a human nucleus pulposus cell line (Sakai *et al.*, 2005, 2004) into degenerated discs of the rabbit nucleotomy model (Iwashina *et al.*, 2006). Despite evidence that the cell supplementation was beneficial in retarding the progression of IVD degeneration, concerns about tumorigenesis and/or carcinogenesis associated with cell lines and the consequences of the use of a recombinant SV40 adenoviral vector need to be clarified before this strategy could be widely adopted. Given the increasing importance of MSCs and other progenitor cell therapies in articular-cartilage-, meniscus-, and other cartilage-regeneration strategies, expanded research on use of MSCs can be expected to comprise a significant effort in the future of IVD regeneration.

VI. GROWTH FACTORS AND BIOLOGICS FOR INTERVERTEBRAL DISC REGENERATION

Disc cells modulate their activity by a variety of substances, including cytokines, growth factors, enzymes, and enzyme inhibitors, in a paracrine and/or autocrine fashion (Masuda and An, 2004). Tissue-engineering approaches to disc regeneration have been based on attempts to up-regulate important matrix proteins (e.g., aggrecan) or to down-regulate proinflammatory cytokines (e.g., interleukin-1 (IL-1), tumor necrosis factor-α (TNF-α) (Ahn *et al.*, 2002; Burke *et al.*, 2003; Igarashi *et al.*, 2000; Kang *et al.*, 1996; Le Maitre *et al.*, 2005; Olmarker and Larsson, 1998; Seguin *et al.*, 2005; Weiler *et al.*, 2005), and matrix-degrading enzymes (e.g., metalloproteinases and aggrecanases) (Evans, 2006; Le Maitre *et al.*, 2004; Roberts *et al.*, 2000; Sztrolovics *et al.*, 1997). Delivery of these modulating biologic agents, with and without scaffolds and/or through cell transplantation, has been the subject of many years of efforts in tissue engineering. *In vitro* studies have shown that the rate of matrix synthesis or gene expression for matrix proteins, principally proteoglycan or collagen, can be increased several-fold in IVD cells in the presence of supplemental transforming growth factor-β (TGF-β), osteogenic protein-1 (OP-1) (Imai *et al.*, 2002; Masuda *et al.*, 2003), bone morphogenetic proteins (e.g., BMP-2) (Kim *et al.*, 2003; Tim Yoon *et al.*, 2003), growth and differentiation factor-5 (GDF-5) (Chujo *et al.*, in print; Li *et al.*, 2004), epidermal growth factor (EGF) (Gruber *et al.*, 1997; Thompson *et al.*, 1991), or insulinlike growth factor-1 (IGF-1) (Osada *et al.*, 1996). Other studies have demonstrated the potential of these growth factors as well as platelet-derived growth factor (PDGF) to reduce cell apoptosis and to promote cell proliferation (Gruber *et al.*, 1997, 2000). Autologous platelet-rich plasma, which contains a variable mixture of growth factors, has also been shown to be an effective stimulator of cell proliferation, proteoglycan and collagen synthesis, as well as proteoglycan accumulation, when added to IVD cell cultures *in vitro* (Akeda *et al.*, 2006). In a different approach, supplementation of IVD cell cultures with a naturally occurring antiinflammatory molecule, interleukin-1 receptor antagonist (rhIL-1Ra) has been shown to inhibit the down-regulation of biosynthesis induced by the proinflammatory cytokine, IL-1 (Akeda *et al.*, 2006, Pichika *et al.*, 2006). This illustrates that stimulatory factors as well as antiinflammatory or anticatabolic factors may be considered for therapeutic purposes in IVD regeneration. Overall, these *in vitro* studies have illustrated the potential for biologics to assist in matrix regeneration through controlling both cell metabolism and cell number, and they have paved the way for more recent studies evaluating these biologics *in vivo*.

Protein injection into the disc space is relatively simple and practical and has been the most widely studied of all approaches for delivery of biologics for IVD regeneration. Walsh and coworkers (2001) reported the *in vivo* effect of a single injection of several growth factors, including basic fibroblast growth factor (bFGF), GDF-5, IGF-1 or TGF-β, in mouse caudal discs with degeneration induced by static compression. An increase in neomatrix was observed following the injection of GDF-5, while increases in the annulus fibrosus cell population were found under the influence of

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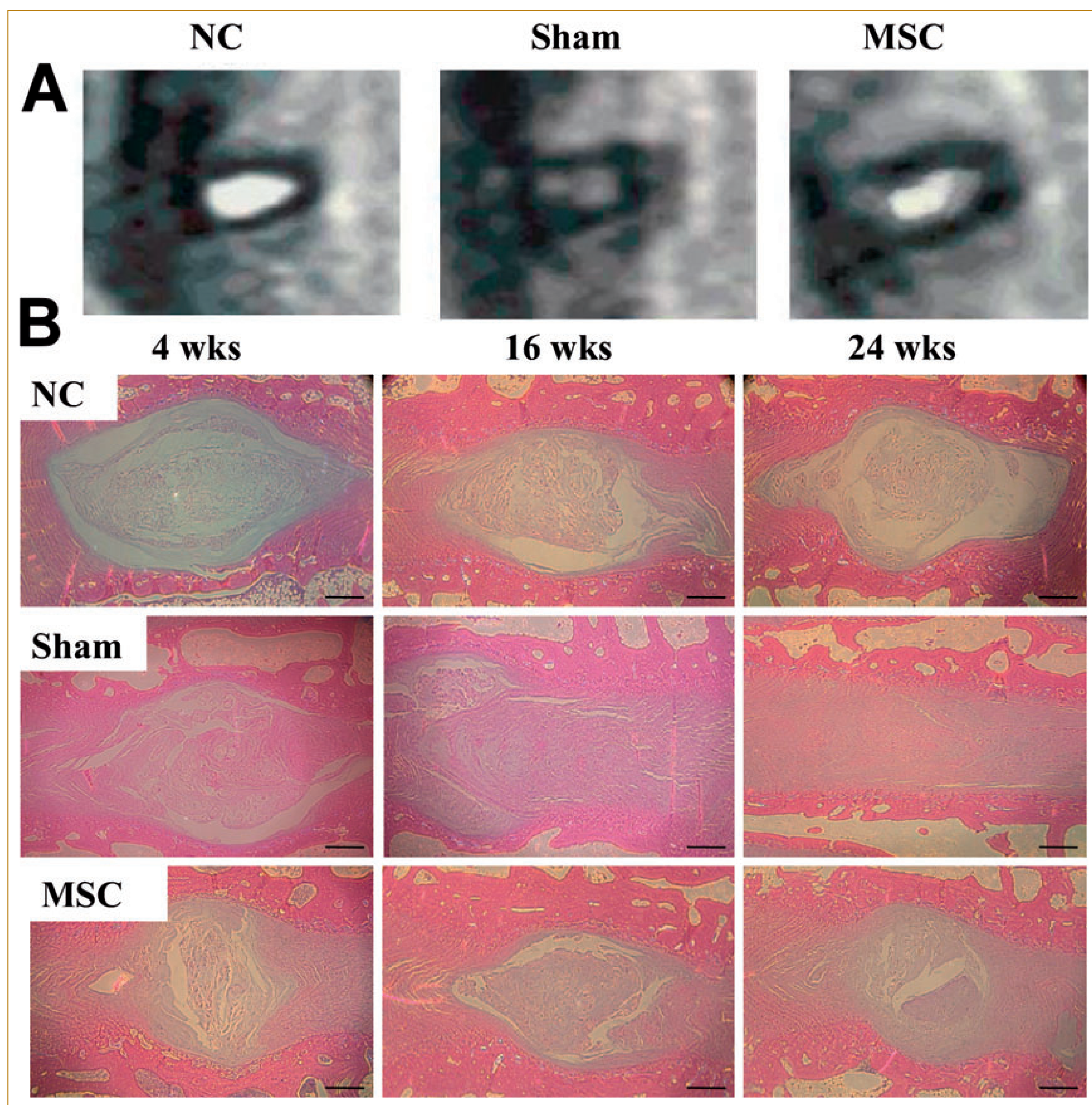

FIG. 58.10. Rabbit IVDs were subjected to annular puncture as a model to induce IVD degeneration in a study by Sakai and coworkers. At two weeks after puncture, subsets of IVDs were transplanted with MSCs or left as sham-operated controls. **(A)** MRI of normal control (NC), sham (disc-degeneration model), and MSC-transplanted rabbits taken 26 weeks postinduction of degeneration (24 weeks after MSC transplantation). Significant recovery of T2-weighted signal intensity is seen in discs of MSC-transplanted discs as compared to very low signal intensity in sham. **(B)** Histological changes seen after MSC transplantation in discs. Control group discs show oval-shaped nucleus, with no collapse of the inner annular structure. Sham operated discs show collapse of the inner annulus morphology from four weeks (six weeks after induction of degeneration) and fibrotic changes in the nucleus pulposus completed by 24 weeks. MSC group discs showed relatively preserved inner annulus structure, with minimal fibrosis in the nucleus region. Bar = 200 μm. Reprinted with permission from Sakai and coworkers (2006).

IGF-1 (Walsh *et al.*, 2002). In separate studies by Masuda and coworkers, a single intradiscal administration of rhOP-1 in normal rabbit discs *in vivo* was shown to result in increased disc height and proteoglycan content in the nucleus pulposus regions, in comparison to a saline injection control group (An *et al.*, 2005). In an animal model of disc degeneration caused by needle puncture of the annulus fibrosus, an injection of rhOP-1 (100 μg/disc) restored disc height, structural change, and mechanical properties (Fig. 58.11) (Masuda *et al.*, 2006; Miyamoto *et al.*, in print). The effectiveness of direct protein delivery was also confirmed in experiments using rhGDF-5, where a single injection of rhGDF-5 resulted in a restoration of disc height and improvements in MRI and histological grading scores in this same animal model of disc degeneration (Chujo *et al.*, in print). These works are important for demonstrating that biologic manipulation of IVD cells *in vitro* may translate to an observed effect *in vivo* and that direct protein delivery may be useful for promoting new matrix formation in the absence of cell delivery. It is noteworthy that the latter studies using rhOP-1 and rhGDF-5 also provide documentation of preclinical measures, such as disc height, disc mechanics, and MRI appearance, that may be important for translating these technologies to noninvasive or minimally invasive clinical outcomes for patient treatment. Indeed, data for the *in vitro* studies have led to an investigational new drug study that has been initiated to test the safety and effect of injections of rhOP-1 into the disc space.

VII. GENE THERAPY FOR INTERVERTEBRAL DISC REGENERATION

While the studies described earlier illustrate a range of proteins considered as possible therapies for IVD regeneration, it is important to consider the unavoidable limitations of protein delivery to the disc space. Issues such as protein half-life and solubility, the need for a proper carrier, the need to preserve mechanical environment or cell numbers, and/or the presence of inhibitors are all factors that can be expected to affect the therapeutic efficacy of protein delivery *in vivo*. A consideration for the use of recombinant protein therapies is also cost, because some disc pathologies and the need to inhibit disc degeneration may be chronic in nature or require multiple treatments. Gene therapy has been advocated as a therapeutic alternative for the delivery of biologics in disc regeneration (Nishida *et al.*, 1999; Wehling *et al.*, 1997; Yoon, 2005; Yoon *et al.*, 2004). DNAs that encode specific proteins may be delivered into the cells by viral or nonviral transfection, with the result that these cells produce proteins to, theoretically, prolong the duration of action. The first successful attempt for *in vitro* gene transfer was reported for chondrocyte-like cells from the endplate of the IVD using a retroviral-mediated technique (Wehling *et al.*, 1997). An *ex vivo* approach was used based on harvesting host cells, infecting these host cells

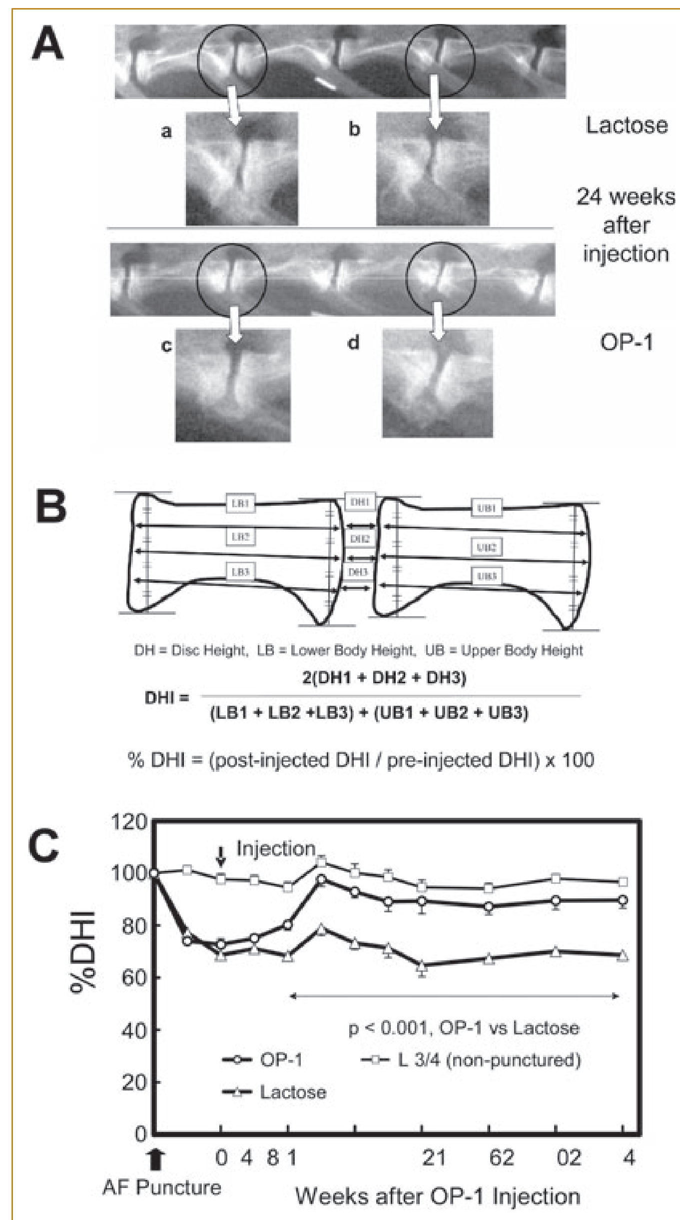

FIG. 58.11. IVDs of rabbit lumbar spines were subjected to annular puncture by an 18-gauge needle to induce disc degeneration. Four weeks after the initial puncture, the vehicle (5% lactose, 10 μL) or OP-1 in 5% lactose (100 μg/10 μL at each level) was injected into the center of the nucleus pulposus. **(A, top)** Radiographs at the 24-week time after injection with lactose. **(A, bottom)** Radiographs of rabbit IVDs following injection with OP-1 in the experimental group, shown at 24 weeks after injection. **(B)** Method for radiographic measurement of disc height index (DHI). The average IVD height (DHI) was calculated by measurements obtained from the anterior, middle, and posterior portions and divided by the average of adjacent vertebral body heights. LBH indicates lower body height; UBH, upper body height. **(C)** Changes in % DHI after the annular puncture and OP-1 injections. As shown, % DHI of injected discs in the OP-1 group was significantly higher than in the lactose-injected control group by four weeks ($p < .001$, repeated ANOVA). This difference in % DHI was maintained out to later periods.

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in vitro, selecting and enriching infected cells, and finally returning these cells to the host. This approach avoids problems associated with low cell numbers and transfection efficiencies, but it is both challenging and costly to perform. Nevertheless, because a decrease of cells by apoptosis or necrosis is considered to be associated with advanced disc degeneration, cell supplementation with genetically manipulated cells will continue to hold promise for disc regeneration.

Adenoviral vectors often possess high titers and infectivity and are able to infect nondividing cells such as IVD cells. Adenoviral-mediated gene transfer to human IVD cells has been shown to be efficient and to produce transcripts across nondegenerative to degenerative cell types, using adenovirus carrying lacZ (Ad/CMV-lacZ) or luciferase "marker" genes (Ad/CMV-luciferase) as well as Sox9 (Ad/Sox9-GFP), GDF-5 and TGF-β1 adenoviral constructs (Moon *et al.*, 2000; Wang *et al.*, 2004; Paul *et al.*, 2003). Yoon and coworkers (2004) also used adenoviral vector to transfer LIM mineralization protein-1 (LMP-1) to rat IVD cells *in vitro* and observed an increase of BMP-2 and BMP-7 gene expression and protein production, and proteoglycan synthesis. The feasibility of using direct *in vivo* adenoviral-mediated gene transfer to disc cells has also been demonstrated using the lacZ, TGF-β, LMP-1, and Sox9 genes (Moon *et al.*, 2000; Nishida *et al.*, 1998; Yoon *et al.*, 2004), finding transgene expression to be present or to exert a biological effect on biosynthesis, often for several weeks.

In addition to up-regulation of anabolic factors, inhibition of catabolic processes has been studied using gene therapy for IVD regeneration. Wallach and coworkers (2003) reported that gene transfer of the tissue inhibitor of metalloproteinase-1 (TIMP-1), an inhibitor of catabolic enzymes, can increase proteoglycan accumulation within pellet cultures of human IVD cells. LeMaitre recently reported that human disc cells infected with Ad-IL-1 receptor antagonist (Ad/IL-1Ra) were resistant to IL-1 (Le Maitre *et al.*, 2006). When *in vitro* infected cells were injected into disc explants *in vitro*, IL-1 receptor antagonist protein expression was also increased and maintained for the two-week time period investigated.

There are significant concerns about adenoviral vector use clinically, however, which may include significant toxicity when used in spinal applications (Driesse *et al.*, 2000; Wallach *et al.*, 2006). These concerns have led investigators to begin consideration of adeno-associated viral vectors (Lattermann *et al.*, 2005) and baculoviral vectors, the latter of which may be nontoxic (Liu *et al.*, 2006). Both approaches may provide safe alternatives for future disc therapies, although much work remains. Also, to avoid safety concerns found with viral gene transfer, several nonviral methods for direct gene transfer to cells have been proposed. Preliminary reports using microbubble-enhanced ultrasound gene therapy (Nishida *et al.*, 2006) and a "gene-gun" method (Chang *et al.*, 2000; Matsumoto *et al.*, 2001b) have shown that introduction of a marker or growth factor gene could be accomplished and provide sustained gene expression without need for viral vectors. Transfection efficacy with nonviral means is lower, however, than that in viral transfection, and further investigation will be needed to apply these in a clinical setting. Nevertheless, both viral and nonviral transfection methods have pros and cons. Safety and immunological reactions as well as the control of expression in viral-mediated gene therapy are potential problems. The comparatively low immunologic exposure of the healthy disc and its low cellularity seem to suggest that safety with gene delivery of therapeutic agents is a lesser concern in the IVD. Cells are needed to transduce the biological effect, however, so transplantation of *ex vivo* transfected cells may be an important part of the future potential for gene therapy in the IVD. During this time, work continues on identification of broader and diverse molecular targets that can be useful for gene delivery in the treatment of IVD regeneration.

VIII. CONCLUDING REMARKS

Efforts to regenerate and replace the tissues of the intervertebral disc have virtually exploded over the last two decades, although the field remains in its infancy. The complexity of the diverse degenerative and pathological processes that affect the IVD as well as the intrinsic complexity of the heterogeneous disc structures demand that multiple strategies be developed for treatment of the IVD. Partial IVD replacements using acellular, preformed, or *in situ*–formed biomaterials have the longest history of development and are important for defining procedural outcomes that will be relevant to long-term functional success. Development of strategies using cells, biologics, or gene therapy is often focused on the restoration of a single tissue source, such as nucleus pulposus or annulus fibrosus, and with or without biomaterial scaffolds. Only a few tissue-engineering solutions have been proposed to integrate two dissimilar tissues in the repair process, and additional work to promote integration among native, neogenerated, and implanted tissues will be critical to restoring IVD function. Many of the identified strategies derive largely from knowledge gained in cartilage-tissue engineering, although the differing cellular, functional, and structural requirements of the IVD suggest that custom approaches are needed. Advances in IVD cell biology are needed to enable the identification of novel therapeutic targets, to select for classes of biomaterials, and to suggest appropriate drug delivery strategies, for disc cell phenotype, cell-biomaterial interactions, and the biology of aging for these cells are still poorly understood. While a diverse array of molecules, cell sources, and materials is suggested as appropriate for IVD regeneration, additional work is needed to reveal some common and unique themes in human IVD cell responses that focus research on IVD specific strategies. Currently underway clinical trials of autologous cell therapies or autologous protein products

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will pave the way for later generations of cellular and biologic-based therapies, because they are expected to illustrate the unique challenges of treating the pathologic and aged human IVD. The next decade promises great advances in the translation of basic and applied sciences to the clinical treatment of IVD regeneration and replacement.

IX. ACKNOWLEDGMENTS

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X. REFERENCES

Adams, M. A., McNally, D. S., and Dolan, P. (1996). "Stress" distributions inside intervertebral discs. The effects of age and degeneration. *J. Bone Joint Surg. Br.* **78**(6), 965–972.

Ahn, S. H., Cho, Y. W., Ahn, M. W., Jang, S. H., Sohn, Y. K., and Kim, H. S. (2002). mRNA expression of cytokines and chemokines in herniated lumbar intervertebral discs. *Spine* **27**(9), 911–917.

Akeda, K., An, H. S., Pichika, R., Attawia, M., Thonar, E., Lenz, M. E., Uchida, A., and Masuda, K. (2006). Platelet-rich plasma (PRP) stimulates the extracellular matrix metabolism of porcine nucleus pulposus and anulus fibrosus cells cultured in alginate beads. *Spine* **31**(9), 959–966.

An, H. S., Takegami, K., Kamada, H., Nguyen, C. M., Thonar, E. J., Singh, K., Andersson, G. B., and Masuda, K. (2005). Intradiscal administration of osteogenic protein-1 increases intervertebral disc height and proteoglycan content in the nucleus pulposus in normal adolescent rabbits. *Spine* **30**(1), 25–31.

Andersson, G. B. J. (1996). Intervertebral disk herniation: epidemiology and natural history. *In* "Low Back Pain: A Scientific and Clinical Overview" (J.N. Weinstein and S.L. Gordon, eds.), pp. 7–21. American Academy of Orthopaedic Surgeons, Rosemont, IL.

Andersson, G. B., Ortengren, R., and Nachemeson, A. (1982). Disc pressure measurements when rising and sitting down on a chair. *Eng. Med.* **11**(4), 189–190.

Antoniou, J., Goudsouzian, N. M., Heathfield, T. F., Winterbottom, N., Steffen, T., Poole, A. R., Aebi, M., and Alini, M. (1996). The human lumbar endplate. Evidence of changes in biosynthesis and denaturation of the extracellular matrix with growth, maturation, aging, and degeneration. *Spine* **21**(10), 1153–1161.

Au, A., Ha, J., Polotsky, A., Krzyminski, K., Gutowska, A., Hungerford, D. S., and Frondoza, C. G. (2003). Thermally reversible polymer gel for chondrocyte culture. *J. Biomed. Mater. Res.* **67**(4), 1310–1319.

Baer, A. E., Wang, J. T., Kraus, V. B., and Setton, L. A. (2001). Collagen gene expression and mechanical properties of intervertebral disc cell-alginate cultures. *J. Orthop. Res.* **19**(1), 2–10.

Bao, Q. B., and Yuan, H. A. (2002). New technologies in spine: nucleus replacement. *Spine* **27**, 1245–1247.

Bayliss, M. T., and Johnstone, B. (1992). Biochemistry of the intervertebral disc. *In* "The Lumbar Spine and Back Pain" (M.I.V. Jayson, ed.), pp. 111–131. Churchill Livingstone, New York.

Benneker, L. M., Heini, P. F., Alini, M., Anderson, S. E., and Ito, K. (2005). 2004 Young investigator award winner: vertebral endplate marrow contact channel occlusions and intervertebral disc degeneration. *Spine* **30**(2), 167–173.

Bertagnoli, R., Sabatino, C. T., Edwards, J. T., Gontarz, G. A., Prewett, A., and Parsons, J. R. (2005). Mechanical testing of a novel hydrogel nucleus replacement implant. *Spine J.* **5**(6), 672–681.

Betre, H., Setton, L. A., Meyer, D., and Chilkoti, A. (2002). Characterization of a genetically engineered elastin-like polypeptide for cartilaginous tissue repair. *Biomacromolecules* **3**, 910–916.

Bodguk, N. (1988). "Clinical Anatomy of the Lumbar Spine and Sacrum." Churchill Livingstone, Edinburgh, UK.

Boos, N., Weissbach, S., Rohrbach, H., Weiler, C., Spratt, K. F., and Nerlich, A. G. (2002). Classification of age-related changes in lumbar intervertebral discs: 2002 Volvo Award in basic science. *Spine* **27**(23), 2631–2644.

Boyd, L. M., Chen, J., Kraus, V. B., and Setton, L. A. (2004). Conditioned medium differentially regulates matrix protein gene expression in cells of the intervertebral disc. *Spine* **29**(20), 2217–2222.

Buckwalter, J. A. (1995). Aging and degeneration of the human intervertebral disc. *Spine* **20**(11), 1307–1314.

Burke, J. G., RW, G. W., Conhyea, D., McCormack, D., Dowling, F. E., Walsh, M. G., and Fitzpatrick, J. M. (2003). Human nucleus pulposus can respond to a pro-inflammatory stimulus. *Spine* **28**(24), 2685–2693.

Burkoth, A. K., and Anseth, K. S. (2000). A review of photocrosslinked polyanhydrides: *in situ* forming degradable networks. *Biomaterials* **21**(23), 2395–2404.

Carl, A., Ledet, E., Yuan, H., and Sharan, A. (2004). New developments in nucleus pulposus replacement technology. *Spine J.* **4**(6 Suppl.), 325S–329S.

Cassidy, J. J., Hiltner, A., and Baer, E. (1989). Hierarchical structure of the intervertebral disc. *Connect. Tissue Res.* **23**, 75–88.

Chang, S., Masuda, K., Takegami, K., Sumner, D., Thonar, E. J.-M. A., Andersson, G., and An, H. (2000). Gene gun–mediated gene transfer to intervertebral disc cells. *Trans. Orthop. Res. Soc.* **25**, 231.

Chujo, T., An, H., Akeda, K., Miyamoto, K., Muehleman, C., Attawia, M., Andersson, G., and Masuda, K. (in print). Effects of growth differentiation factor-5 (GDF-5) on the intervertebral disc — *in vitro* bovine study and *in vivo* rabbit disc degeneration model study. *Spine*.

Coventry, N. B., Ghormley, R. K., and Kernohan, J. W. (1945a). The intervertebral disc — its microscopic anatomy and pathology. Part I: anatomy, development and physiology. *J. Bone Joint Surg.* **27A**, 105–112.

Coventry, N. B., Ghormley, R. K., and Kernohan, J. W. (1945b). The intervertebral disc — its microscopic anatomy and pathology. Part II: changes in the intervertebral disc concomittant with age. *J. Bone Joint Surg.* **27A**, 233–247.

Crevensten, G., Walsh, A. J. L., Ananthakrishnan, D., Page, P., Wahba, G. M., Lotz, J. C., and Berven, S. (2004). Intervertebral disc cell therapy for regeneration: mesenchymal stem cell implantation in rat intervertebral discs. *Ann. Biomed. Eng.* **32**(3), 430–434.

DeFrances, C. J., and Podgornik, M. N. (2006). 2004 national hospital discharge survey. *In* "Advance Data from Vital and Health Statistics," May 4. National Center for Health Statistics, Centers for Disease Control.

Di Martino, A., Vaccaro, A. R., Lee, J. Y., Denaro, V., and Lim, M. R. (2005). Nucleus pulposus replacement: basic science and indications for clinical use. *Spine* **30**(16 Suppl.), S16–S22.

Driesse, M. J., Esandi, M. C., Kros, J. M., Avezaat, C. J., Vecht, C., Zurcher, C., van der Velde, I., Valerio, D., Bout, A., and Sillevis Smitt, P. A. (2000). Intra-CSF administered recombinant adenovirus causes an immune response–mediated toxicity. *Gene Ther.* **7**(16), 1401–1409.

Ebara, S., Iatridis, J. C., Setton, L. A., Mow, V. C., and Weidenbaum, M. (1996). The tensile properties of human lumbar anulus fibrosus and their variations with region in the intervertebral disc. *Spine* **21**, 452–461.

Elisseeff, J. (2004). Injectable cartilage tissue engineering. *Expert Opin. Biol. Ther.* **4**(12), 1849–1859.

Elliott, D. M., and Setton, L. A. (2001). Anisotropic and inhomogeneous tensile behavior of the human anulus fibrosus: experimental measurement and material model predictions. *J. Biomech. Eng.* **123**(3), 256–263.

Evans, C. (2006). Potential biologic therapies for the intervertebral disc. *J. Bone Joint Surg. Am.* **88**(Suppl. 2), 95–98.

Fujita, Y., Duncan, N. A., and Lotz, J. C. (1997). Radial tensile properties of the lumbar annulus fibrosus are site and degeneration dependent. *J. Orthop. Res.* **15**(6), 814–819.

Galante, J. O. (1967). Tensile properties of the human lumbar annulus fibrosus. *Acta Orthop. Scand.* **Suppl. 100**, 1–91.

Ganey, T., Libera, J., Moos, V., Alasevic, O., Fritsch, K. G., Meisel, H. J., and Hutton, W. C. (2003). Disc chondrocyte transplantation in a canine model: a treatment for degenerated or damaged intervertebral disc. *Spine* **28**(23), 2609–2620.

Gruber, H. E., Fisher, E. C., Jr., Desai, B., Stasky, A. A., Hoelscher, G., and Hanley, E. N., Jr. (1997). Human intervertebral disc cells from the annulus: three-dimensional culture in agarose or alginate and responsiveness to TGF-beta1. *Exp. Cell Res.* **235**(1), 13–21.

Gruber, H. E., Norton, H. J., and Hanley, E. N., Jr. (2000). Anti-apoptotic effects of IGF-1 and PDGF on human intervertebral disc cells *in vitro*. *Spine* **25**(17), 2153–2157.

Gruber, H. E., Johnson, T. L., Leslie, K., Ingram, J. A., Martin, D., Hoelscher, G., Banks, D., Phieffer, L., Coldham, G., and Hanley, E. N., Jr. (2002). Autologous intervertebral disc cell implantation: a model using *Psammomys obesus*, the sand rat. *Spine* **27**(15), 1626–1633.

Gruber, H. E., Leslie, K., Ingram, J., Norton, H. J., and Hanley, E. N. (2004). Cell-based tissue engineering for the intervertebral disc: *in vitro* studies of human disc cell gene expression and matrix production within selected cell carriers. *Spine J.* **4**(1), 44–55.

Hamilton, D. J., Seguin, C. A., Wang, J., Pilliar, R. M., and Kandel, R. A. (2006). Formation of a nucleus pulposus-cartilage endplate construct *in vitro*. *Biomaterials* **27**(3), 397–405.

Hayes, A. J., Benjamin, M., and Ralphs, J. R. (2001). Extracellular matrix in development of the intervertebral disc. *Matrix Biol.* **20**(2), 107–121.

Hickey, D. S., and Hukins, D. W. L. (1980). X-ray diffraction studies of the arrangement of collagenous fibers in human fetal intervertebral disc. *J. Anatomy* **131**, 81–90.

Holm, S., Maroudas, A., Urban, J. P., Selstam, G., and Nachemson, A. (1981). Nutrition of the intervertebral disc: solute transport and metabolism. *Connect. Tissue Res.* **8**, 101–119.

Holzapfel, G. A., Schulze-Bauer, C. A., Feigl, G., and Regitnig, P. (2005). Single lamellar mechanics of the human lumbar anulus fibrosus. *Biomech. Model Mechanobiol.* **3**(3), 125–140.

Huang, R. C., Wright, T. M., Panjabi, M. M., and Lipman, J. D. (2005). Biomechanics of nonfusion implants. *Orthop. Clin. North Am.* **36**(3), 271–280.

Hurri, H., and Karppinen, J. (2004). Discogenic pain. *Pain* **112**, 225–228.

Igarashi, T., Kikuchi, S., Shubayev, V., and Myers, R. R. (2000). 2000 Volvo Award winner in basic science studies: exogenous tumor necrosis factor-alpha mimics nucleus pulposus-induced neuropathology. Molecular, histologic, and behavioral comparisons in rats. *Spine* **25**(23), 2975–2980.

Imai, Y., An, H., Thonar, E., Andersson, G., and Masuda , K. (2002). Recombinant human osteogenic protein-1 (rhOP-1) up-regulates extracellular matrix metabolism by human annulus fibrosus and nucleus pulposus cells. *Spine J.* **2**, 101S.

Iwashina, T., Mochida, J., Sakai, D., Yamamoto, Y., Miyazaki, T., Ando, K., and Hotta, T. (2006). Feasibility of using a human nucleus pulposus cell line as a cell source in cell transplantation therapy for intervertebral disc degeneration. *Spine* **31**(11), 1177–1186.

Kang, J. D., Georgescu, H. I., McIntyre-Larkin, L., Stefanovic-Racic, M., Donaldson, W. F., 3rd, and Evans, C. H. (1996). Herniated lumbar intervertebral discs spontaneously produce matrix metalloproteinases, nitric oxide, interleukin-6, and prostaglandin E2. *Spine* **21**(3), 271–277.

Kim, D. J., Moon, S. H., Kim, H., Kwon, U. H., Park, M. S., Han, K. J., Hahn, S. B., and Lee, H. M. (2003). Bone morphogenetic protein-2 facilitates expression of chondrogenic, not osteogenic, phenotype of human intervertebral disc cells. *Spine* **28**(24), 2679–2684.

Klara, P. M., and Ray, C. D. (2002). Artificial nucleus replacement: clinical experience. *Spine* **27**(12), 1374–1377.

Kokkonen, S.-M., Kurunlahti, M., Tervonen, O., Iikko, E., and Vanharanta, H. (2002). Endplate degeneration observed on magnetic resonance imaging of the lumbar spine: correlation with pain provocation and disc changes observed on computed tomography diskography. *Spine* **27**(20), 2274–2278.

Lattermann, C., Oxner, W. M., Xiao, X., Li, J., Gilbertson, L. G., Robbins, P. D., and Kang, J. D. (2005). The adeno-associated viral vector as a strategy for intradiscal gene transfer in immune competent and pre-exposed rabbits. *Spine* **30**(5), 497–504.

Le Maitre, C. L., Freemont, A. J., and Hoyland, J. A. (2004). Localization of degradative enzymes and their inhibitors in the degenerate human intervertebral disc. *J. Pathol.* **204**(1), 47–54.

Le Maitre, C. L., Freemont, A. J., and Hoyland, J. A. (2005). The role of interleukin-1 in the pathogenesis of human intervertebral disc degeneration. *Arthritis Res. Ther.* **7**(4), R732–R745.

Le Maitre, C. L., Freemont, A. J., and Hoyland, J. A. (2006). A preliminary *in vitro* study into the use of IL-1Ra gene therapy for the inhibition of intervertebral disc degeneration. *Int. J. Exp. Pathol.* **87**(1), 17–28.

Leung, V. Y., Chan, D., and Cheung, K. M. (2006). Regeneration of intervertebral disc by mesenchymal stem cells: potentials, limitations, and future direction. *Eur. Spine J.* (in print).

Li, X., Leo, B. M., Beck, G., Balian, G., and Anderson, G. D. (2004). Collagen and proteoglycan abnormalities in the GDF-5-deficient mice and

E1

molecular changes when treating disk cells with recombinant growth factor. *Spine* **29**(20), 2229–2234.

Li, X. D., Lee, J. P., Balian, G., and Anderson, D. G. (2005). Modulation of chondrocytic properties of fat-derived mesenchymal cells in cocultures with nucleus pulposus. *Connect. Tissue Res.* **46**(2), 75–82.

Liu, X., Li, K., Song, J., Liang, C., Wang, X., and Chen, X. (2006). Efficient and stable gene expression in rabbit intervertebral disc cells transduced with a recombinant baculovirus vector. *Spine* **31**(7), 732–735.

Lotz, J. C., and Kim, A. J. (2005). Disc regeneration: why, when, and how. *Neurosurg. Clin. N. Am.* **16**(4), 657–663, vii.

Maroudas, A., Stockwell, R. A., Nachemson, A., and Urban, J. (1975). Factors involved in the nutrition of the human lumbar intervertebral disc: cellularity and diffusion of glucose *in vitro*. *J. Anat.* **120**, 113–130.

Masuda, K., and An, H. S. (2004). Growth factors and the intervertebral disc. *Spine J.* **4**(6 Suppl.), 330S–340S.

Masuda, K., Takegami, K., An, H., Kumano, F., Chiba, K., Andersson, G. B., Schmid, T., and Thonar, E. (2003). Recombinant osteogenic protein-1 upregulates extracellular matrix metabolism by rabbit annulus fibrosus and nucleus pulposus cells cultured in alginate beads. *J. Orthop. Res.* **21**(5), 922–930.

Masuda, K., Imai, Y., Okuma, M., Muehleman, C., Nakagawa, K., Akeda, K., Thonar, E., Andersson, G., and An, H. S. (2006). Osteogenic protein-1 injection into a degenerated disc induces the restoration of disc height and structural changes in the rabbit anular puncture model. *Spine* **31**(7), 742–754.

Matsumoto, T., Masuda, K., An, H., Chen, S., Williamson, A., Sah, R., Andersson, G. B. J., Rueger, D., and Thonar, E. J.-M. A. (2001a). Formation of transplantable disc-shaped tissues by nucleus pulposus and annulus fibrosus cells: biochemical and biomechanical properties. *Trans. Orthop. Res. Soc.* **26**, 897.

Matsumoto, T., Masuda, K., Chen, S., An, H., Andersson, G. B. J., Aota, Y., Horvath, E., and Thonar, E. J.-M. A. (2001b). Transfer of osteogenic protein-1 gene by gene gun system promotes matrix synthesis in bovine intervertebral disc and articular cartilage cells. *Trans. Orthop. Res. Soc.* **26**, 30.

McAfee, P. C. (2004). The indications for lumbar and cervical disc replacement. *Spine J.* **4**, 177S–181S.

McNally, D. S., and Adams, M. A. (1992). Internal intervertebral disc mechanics as revealed by stress profilometry. *Spine* **17**, 66–73.

Meachim, G., and Cornah, M. S. (1970). Fine structure of juvenile human nucleus pulposus. *J. Anat.* **107**(2), 337–350.

Meisel, H. J., Ganey, T., Hutton, W. C., Libera, J., Minkus, Y., and Alasevic, O. (2006). Clinical experience in cell-based therapeutics: intervention and outcome. *Eur. Spine J.* (in print).

Mercier, N. R., Costantino, H. R., Tracy, M. A., and Bonassar, L. J. (2004). A novel injectable approach for cartilage formation *in vivo* using PLG microspheres. *Ann. Biomed. Eng.* **32**(3), 418–429.

Miyamoto, K., Masuda, K., Kim, J., Inoue, N., Akeda, K., Andersson, G., and An, H. (in print). Intradiscal injections of osteogenic protein-1 restore the viscoelastic properties of degenerated intervertebral discs. *Spine J.*

Mizuno, H., Roy, A. K., Vacanti, C. A., Kojima, K., Ueda, M., and Bonassar, L. J. (2004). Tissue-engineered composites of anulus fibrosus and nucleus pulposus for intervertebral disc replacement. *Spine* **29**(12), 1290–1297.

Mizuno, H., Roy, A. K., Zaporojan, V., Vacanti, C. A., Ueda, M., and Bonassar, L. J. (2006). Biomechanical and biochemical characterization of composite tissue-engineered intervertebral discs. *Biomaterials* **27**(3), 362–370.

Modic, M. T., Weinstein, M. A., Pavlicek, W., Dengel, F., Paushter, D., Boumphrey, F., Duchesneau, P. M., Ngo, F. Q. H., and Meaney, T. F. (1984). Magnetic resonance of intervertebral-disk disease. *Magn. Reson. Med.* **1**(2), 207–208.

Moon, S. H., Gilbertson, L. G., Nishida, K., Knaub, M., Muzzonigro, T., Robbins, P. D., Evans, C. H., and Kang, J. D. (2000). Human intervertebral disc cells are genetically modifiable by adenovirus-mediated gene transfer: implications for the clinical management of intervertebral disc disorders. *Spine* **25**(20), 2573–2579.

Mwale, F., Iordanova, M., Demers, C. N., Steffen, T., Roughley, P., and Antoniou, J. (2005). Biological evaluation of chitosan salts cross-linked to genipin as a cell scaffold for disk tissue engineering. *Tissue Eng.* **11**(1–2), 130–140.

Nachemson, A. (1960). Lumbar intradiscal pressure. Experimental studies on postmortem material. *Acta Orthop. Scand. Suppl.* **43**, 1–103.

Nachemson, A. (1992). Lumbar mechanics as revealed by lumbar intradiscal pressure measurements. *In* "The Lumbar Spine and Back Pain," 4th ed. (M. I. V. Jayson, ed.), pp. 157–171. Churchill Livingstone, New York.

Nachemson, A., Lewin, T., Maroudas, A., and Freeman, M. A. (1970). *In vitro* diffusion of dye through the endplates and the annulus fibrosus of human lumbar intervertebral discs. *Acta Orthop. Scand.* **41**(6), 589–607.

Nishida, K., Kang, J. D., Suh, J. K., Robbins, P. D., Evans, C. H., and Gilbertson, L. G. (1998). Adenovirus-mediated gene transfer to nucleus pulposus cells. Implications for the treatment of intervertebral disc degeneration. *Spine* **23**(22), 2437–2442.

Nishida, K., Kang, J. D., Gilbertson, L. G., Moon, S. H., Suh, J. K., Vogt, M. T., Robbins, P. D., and Evans, C. H. (1999). Modulation of the biologic activity of the rabbit intervertebral disc by gene therapy: an *in vivo* study of adenovirus-mediated transfer of the human transforming growth factor-beta 1 encoding gene. *Spine* **24**(23), 2419–2425.

Nishida, K., Doita, M., Takada, T., Kakutani, K., Miyamoto, H., Shimomura, T., Maeno, K., and Kurosaka, M. (2006). Sustained transgene expression in intervertebral disc cells *in vivo* mediated by microbubble-enhanced ultrasound gene therapy. *Spine* **31**(13), 1415–1419.

Nishimura, K., and Mochida, J. (1998). Percutaneous reinsertion of the nucleus pulposus. An experimental study. *Spine* **23**, 1531–1538.

Nomura, T., Mochida, J., Okuma, M., Nishimura, K., and Sakabe, K. (2001). Nucleus pulposus allograft retards intervertebral disc degeneration. *Clin. Orthop.* **389**, 94–101.

Oegema, T. R. (1990). Recent advances in understanding the biochemistry of the IVD. *In* "Disorders of the Lumbar Spine (Y. Floman, ed.), pp. 53–71. Aspen, Rockville, MD.

Oegema, T. R. J. (1993). Biochemistry of the intervertebral disc. *Clin. Sports Med.* **12**(3), 419–439.

Oegema, T. R. J. (2002). The role of disc cell heterogeneity in determining disc biochemistry: a speculation. *Biochem. Soc. Trans.* **30**(6), 839–844.

Okuma, M., Mochida, J., Nishimura, K., Sakabe, K., and Seiki, K. (2000). Reinsertion of stimulated nucleus pulposus cells retards intervertebral disc degeneration: an *in vitro* and *in vivo* experimental study. *J. Orthop. Res.* **18**(6), 988–997.

Olmarker, K., and Larsson, K. (1998). Tumor necrosis factor alpha and nucleus-pulposus-induced nerve root injury. *Spine* **23**(23), 2538–2544.

Osada, R., Ohshima, H., Ishihara, H., Yudoh, K., Sakai, K., Matsui, H., and Tsuji, H. (1996). Autocrine/paracrine mechanism of insulin-like growth factor-1 secretion, and the effect of insulin-like growth factor-1 on proteoglycan synthesis in bovine intervertebral discs. *J. Orthop. Res.* **14**(5), 690–699.

Panjabi, M., Brown, M., Lindahl, S., Irstam, L., and Hermens, M. (1988). Intrinsic disc pressure as a measure of integrity of the lumbar spine. *Spine* **13**(8), 913–917.

Paul, R., Haydon, R. C., Cheng, H., Ishikawa, A., Nenadovich, N., Jiang, W., Zhou, L., Breyer, B., Feng, T., Gupta, P., *et al.* (2003). Potential use of sox9 gene therapy for intervertebral degenerative disc disease. *Spine* **28**, 755–763.

Peretti, G. M., Xu, J. W., Bonassar, L. J., Kirchhoff, C. H., Yaremchuk, M. J., and Randolph, M. A. (2006). Review of injectable cartilage engineering using fibrin gel in mice and swine models. *Tissue Eng.* **12**(5), 1151–1168.

Pichika, R., Betre, H., Setton, L.A., An, H. S., and Masuda, K. (2006). Bioactivity of IL–1 receptor antagonist (IL1Ra) and a related fusion protein in inhibiting IL-1–mediated effects in human intervertebral disc cells. *Trans. Orthop. Res. Soc.* **52**,

Postacchini, F., Bellocci, M., and Massobrio, M. (1984). Morphologic changes in anulus fibrosus during aging: an ultrastructural study in rats. *Spine* **9**(6), 596–603.

Praemer, A., Furner, S., and Rice, D. P. (1999). Back and other joint pain. *In* "Musculoskeletal Conditions in the United States," pp. 27–33. American Academy of Orthopaedic Surgeons, Rosemont, IL.

Puelacher, W. C., Kim, S. W., Vacanti, J. P., Schloo, B., Mooney, D., and Vacanti, C. A. (1994). Tissue-engineered growth of cartilage — the effect of varying the concentration of chondrocytes seeded onto synthetic-polymer matrices. *Int. J. Oral Maxillofac. Surg.* **23**(1), 49–53.

Richardson, S. M., Curran, J. M., Chen, R., Vaughan-Thomas, A., Hunt, J. A., Freemont, A. J., and Hoyland, J. A. (2006a). The differentiation of bone marrow mesenchymal stem cells into chondrocyte-like cells on poly-L-lactic acid (PLLA) scaffolds. *Biomaterials* **27**(22), 4069–4078.

Richardson, S. M., Walker, R. V., Parker, S., Rhodes, N. P., Hunt, J. A., Freemont, A. J., and Hoyland, J. A. (2006b). Intervertebral disc cell-mediated mesenchymal stem cell differentiation. *Stem Cells* **24**(3), 707–716.

Risbud, M. V., Albert, T. J., Guttapalli, A., Vresilovic, E. J., Hillibrand, A. S., Vaccaro, A. R., and Shapiro, I. M. (2004). Differentiation of mesenchymal stem cells towards a nucleus pulposus-like phenotype *in vitro*: implications for cell-based transplantation therapy. *Spine* **29**(23), 2627–2632.

Roberts, S., Menage, J., Duance, V., Wotton, S., and Ayad, S. (1991). 1991 Volvo Award in basic sciences. Collagen types around the cells of the intervertebral disc and cartilage end plate: an immunolocalization study. *Spine* **16**(9), 1030–1038.

Roberts, S., Urban, J. P., Evans, H., and Eisenstein, S. M. (1996). Transport properties of the human cartilage endplate in relation to its composition and calcification. *Spine* **21**(4), 415–420.

Roberts, S., Caterson, B., Menage, J., Evans, E. H., Jaffray, D. C., and Eisenstein, S. M. (2000). Matrix metalloproteinases and aggrecanase: their role in disorders of the human intervertebral disc. *Spine* **25**(23), 3005–3013.

Roughley, P. J. (2004). Biology of intervertebral disc aging and degeneration: involvement of the extracellular matrix. *Spine* **29**(23), 2691–2699.

Roughley, P., Hoemann, C., DesRosiers, E., Mwale, F., Antoniou, J., and Alini, M. (2006). The potential of chitosan-based gels containing intervertebral disc cells for nucleus pulposus supplementation. *Biomaterials* **27**(3), 388–396.

Rufai, A., Benjamin, M., and Ralphs, J. R. (1995). The development of fibrocartilage in the rat intervertebral disc. *Anat. Embryol.* **192**, 53–62.

Sakai, D., Mochida, J., Yamamoto, Y., Nomura, T., Okuma, M., Nishimura, K., Nakai, T., Ando, K., and Hotta, T. (2003). Transplantation of mesenchymal stem cells embedded in Atelocollagen gel to the intervertebral disc: a potential therapeutic model for disc degeneration. *Biomaterials* **24**(20), 3531–3541.

Sakai, D., Mochida, J., Yamamoto, Y., Toh, E., Iwashina, T., Miyazaki, T., Inokuchi, S., Ando, K., and Hotta, T. (2004). Immortalization of human nucleus pulposus cells by a recombinant SV40 adenovirus vector: establishment of a novel cell line for the study of human nucleus pulposus cells. *Spine* **29**(14), 1515–1523.

Sakai, D., Mochida, J., Iwashina, T., Watanabe, T., Nakai, T., Ando, K., and Hotta, T. (2005). Differentiation of mesenchymal stem cells transplanted to a rabbit degenerative disc model: potential and limitations for stem cell therapy in disc regeneration. *Spine* **30**(21), 2379–2387.

Sato, M., Asazuma, T., Ishihara, M., Ishihara, M., Kikuchi, T., Kikuchi, M., and Fujikawa, K. (2003). An experimental study of the regeneration of the intervertebral disc with an allograft of cultured annulus fibrosus cells using a tissue-engineering method. *Spine* **28**(6), 548–553.

Schultz, A., Andersson, G., Ortengren, R., Haderspeck, K., and Nachemson, A. (1982). Loads on the lumbar spine. Validation of a biomechanical analysis by measurements of intradiscal pressures and myoelectric signals. *J. Bone Joint Surg. Am.* **64**(5), 713–720.

Seguin, C. A., Grynpas, M. D., Pilliar, R. M., Waldman, S. D., and Kandel, R. A. (2004). Tissue–engineered nucleus pulposus tissue formed on a porous calcium polyphosphate substrate. *Spine* **29**(12), 1299–1306.

Seguin, C. A., Pilliar, R. M., Roughley, P. J., and Kandel, R. A. (2005). Tumor necrosis factor-alpha modulates matrix production and catabolism in nucleus pulposus tissue. *Spine* **30**(17), 1940–1948.

Selard, E., Shirazi-Adl, A., and Urban, J. P. (2003). Finite-element study of nutrient diffusion in the human intervertebral disc. *Spine* **28**(17), 1945–1953.

Shirazi-Adl, A. (1992). Finite-element simulation of changes in the fluid content of human lumbar discs. Mechanical and clinical implications. *Spine* **17**, 206–212.

Skaggs, D. L., Weidenbaum, M., Iatridis, J. C., Ratcliffe, A., and Mow, V. C. (1994). Regional variation in tensile properties and biochemical composition of the human lumbar anulus fibrosus. *Spine* **19**, 1310–1319.

Sontjens, S. H., Nettles, D. L., Carnahan, M. A., Setton, L. A., and Grinstaff, M. W. (2006). Biodendrimer-based hydrogel scaffolds for cartilage tissue repair. *Biomacromolecules* **7**(1), 310–316.

Stairmand, J. W., Holm, S., and Urban, J. P. (1991). Factors influencing oxygen concentration gradients in the intervertebral disc. A theoretical analysis. *Spine* **16**, 444–449.

Steck, E., Bertram, H., Abel, R., Chen, B., Winter, A., and Richter, W. (2005). Induction of intervertebral disc–like cells from adult mesenchymal stem cells. *Stem Cells* **23**(3), 403–411.

Sztrolovics, R., Alini, M., Roughley, P. J., and Mort, J. S. (1997). Aggrecan degradation in human intervertebral disc and articular cartilage. *Biochem. J.* **326**, 235–241.

Taylor, J. R., and Twomey, L. T. (1988). The development of the human intervertebral disc. *In* "The Biology of the Intervertebral Disc" (P. Ghosh, ed.), pp. 39–82. CRC Press, Boca Raton, FL.

Temenoff, J. S., and Mikos, A. G. (2000). Injectable biodegradable materials in orthopaedic tissue engineering. *Biomaterials* **21**, 2405–2412.

Thomas, J., Lowman, A., and Marcolongo, M. (2003). Novel associated hydrogels for nucleus pulposus replacement. *J. Biomed. Mater. Res.* **67**(4), 1329–1337.

Thompson, J. P., Oegema, T. J., and Bradford, D. S. (1991). Stimulation of mature canine intervertebral disc by growth factors. *Spine* **16**(3), 253–260.

Tim Yoon, S., Su Kim, K., Li, J., Soo Park, J., Akamaru, T., Elmer, W. A., and Hutton, W. C. (2003). The effect of bone morphogenetic protein-2 on rat intervertebral disc cells *in vitro*. *Spine* **28**(16), 1773–1780.

Trout, J. J., Buckwalter, J. A., Moore, K. C., and Landas, S. K. (1982). Ultrastructure of the human intervertebral disc: I. Changes in notochordal cells with age. *Tissue Cell* **14**, 359–369.

Urban, J. P., and McMullin, J. F. (1985). Swelling pressure of the intervertebral disc: influence of proteoglycan and collagen contents. *Biorheology* **22**(2), 145–157.

Urban, J. P., and McMullin, J. F. (1988). Swelling pressure of the lumbar intervertebral discs: influence of age, spinal level, composition, and degeneration. *Spine* **13**(2), 179–187.

Urban, J. P., and Roberts, S. (1995). Development and degeneration of the intervertebral discs. *Mol. Med. Today* **1**(7), 329–335.

Urban, J. P., and Roberts, S. (2003). Degeneration of the intervertebral disc. *Arthritis Res. Ther.* **5**(3), 120–130.

Urban, J. P., Holm, S., Maroudas, A., and Nachemson, A. (1977). Nutrition of the intervertebral disk. An *in vivo* study of solute transport. *Clin. Orthop.* **129**, 101–114.

Wallach, C. J., Sobajima, S., Watanabe, Y., Kim, J. S., Georgescu, H. I., Robbins, P., Gilbertson, L. G., and Kang, J. D. (2003). Gene transfer of the catabolic inhibitor TIMP-1 increases measured proteoglycans in cells from degenerated human intervertebral discs. *Spine* **28**(20), 2331–2337.

Wallach, C. J., Kim, J. S., Sobajima, S., Lattermann, C., Oxner, W. M., McFadden, K., Robbins, P. D., Gilbertson, L. G., and Kang, J. D. (2006). Safety assessment of intradiscal gene transfer: a pilot study. *Spine J.* **6**(2), 107–112.

Walsh, A. L., Kleinstueek, F., Lotz, J., and Bradford, D. (2001). Growth factor treatment in degenerated intervertebral discs. *Trans. Orthop. Res. Soc.* **26**, 0892.

Walsh, A. L., Lotz, J., and Bradford, D. (2002). Single and multiple injections of GDF-5, IGF-1, or TGF-beta into degenerated intervertebral discs. *Trans. Orthop. Res. Soc.* **27**, 0820.

Wang, H., Kroeber, M., Hanke, M., Ries, R., Schmid, C., Poller, W., and Richter, W. (2004). Release of active and depot GDF-5 after adenovirus-mediated overexpression stimulates rabbit and human intervertebral disc cells. *J. Mol. Med.* **82**(2), 126–134.

Wehling, P., Schulitz, K. P., Robbins, P. D., Evans, C. H., and Reinecke, J. A. (1997). Transfer of genes to chondrocytic cells of the lumbar spine. Proposal for a treatment strategy of spinal disorders by local gene therapy. *Spine* **22**(10), 1092–1097.

Weiler, C., Nerlich, A. G., Bachmeier, B. E., and Boos, N. (2005). Expression and distribution of tumor necrosis factor alpha in human lumbar intervertebral discs: a study in surgical specimen and autopsy controls. *Spine* **30**(1), 44–53.

Weishaupt, D., Zanetti, M., Hodler, J., Min, K., Fuchs, B., Pfirrmann, C. W. A., and Boos, N. (2001). Painful lumbar disc derangement: relevance of endplate abnormalities at MR imaging. *Radiology* **218**(2), 420–427.

Wilson, C. G., Bonassar, L. J., and Kohles, S. S. (2002). Modeling the dynamic composition of engineererd cartilage. *Arch. Biochem. Biophys.* **408**, 246–254.

Woolf, A. D., and Pfleger, B. (2003). Burden of major musculoskeletal conditions. *Bull. World Health Organ.* **81**, 646–656.

Yamamoto, Y., Mochida, J., Sakai, D., Nakai, T., Nishimura, K., Kawada, H., and Hotta, T. (2004). Up-regulation of the viability of nucleus pulposus cells by bone marrow–derived stromal cells: significance of direct cell-to-cell contact in coculture system. *Spine* **29**(14), 1508–1514.

Yoon, S. T. (2005). Molecular therapy of the intervertebral disc. *Spine J.* **5**(6 Suppl.), 280S–286S.

Yoon, S. T., Park, J. S., Kim, K. S., Li, J., Attallah-Wasif, E. S., Hutton, W. C., and Boden, S. D. (2004). ISSLS prize winner: LMP-1 upregulates intervertebral disc cell production of proteoglycans and BMPs *in vitro* and *in vivo*. *Spine* **29**(23), 2603–2611.

Yu, J., Winlove, P. C., Roberts, S., and Urban, J. P. (2002). Elastic fiber organization in the intervertebral discs of the bovine tail. *J. Anat.* **201**(6), 465–475.

Yu, S., Haughton, V. M., Sether, L. A., Ho, K. C., and Wagner, M. (1989). Criteria for classifying normal and degenerated lumbar intervertebral disks. *Radiology* **170**, 523–526.

Yung Lee, J., Hall, R., Pelinkovic, D., Cassinelli, E., Usas, A., Gilbertson, L., Huard, J., and Kang, J. (2001). New use of a three-dimensional pellet culture system for human intervertebral disc cells: initial characterization and potential use for tissue engineering. *Spine* **26**(21), 2316–2322.

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