Published in final edited form as:

Biomaterials. 2017 June ; 129: 54–67. doi:10.1016/j.biomaterials.2017.03.013.

Biomaterials for Intervertebral Disc Regeneration and Repair

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Abstract

The intervertebral disc contributes to motion, weight bearing, and flexibility of the spine, but is susceptible to damage and morphological changes that contribute to pathology with age and injury. Engineering strategies that rely upon synthetic materials or composite implants that do not interface with the biological components of the disc have not met with widespread use or desirable outcomes in the treatment of intervertebral disc pathology. Here we review bioengineering advances to treat disc disorders, using cell-supplemented materials, or acellular, biologically based materials, that provide opportunity for cell-material interactions and remodeling in the treatment of intervertebral disc disorders. While a field still in early development, bioengineering-based strategies employing novel biomaterials are emerging as promising alternatives for clinical treatment of intervertebral disc disorders.

Keywords

intervertebral disc; anulus fibrosus; nucleus pulposus; herniation; disc degeneration; degenerative disc disease; fusion; spine

INTRODUCTION

Intervertebral disc (IVD) disorders can cause pain, neurological deficit, and disability in affected individuals, contributing to low back pain being ranked #1 in disability-adjusted life years (1);(2). In 2012, more than 52 million patients in the US visited a physician with a complaint of back pain with estimated direct medical costs of \$253 billion (3). IVD disorders contribute to back and neck pain in multiple ways with few available treatments other than surgery to this date. The purpose of this review is to introduce the reader to anatomical, molecular and structural changes of IVD disorders, and to discuss how bioengineering advances of material repair and replacement have potential to impact pathology and disability of IVD disorders.

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The IVD is the fibrocartilaginous part of a “three-joint complex” that includes the two posterior articulations of the diarthrodial facet joints, along with the soft tissues of the IVD between the two adjacent vertebral bodies (superior and inferior) (see Figure 1). Together, these joints contribute to motion, weight bearing, and flexibility while protecting the neural anatomy of the entire spine. These structures must tolerate multiple cycles of loading at very high magnitudes of compression, bending, and torsion, that generate IVD pressures of 0.1 to 3 MPa, and loads of similar magnitude within the facet joints (see reviews in (4), (5)). These magnitudes of loading and ranges of motion can separately and together contribute to material failure of the bony endplates and facet joints of the three-joint complex, as well as the soft tissue sub-structures of the IVD – the anulus fibrosus (AF) and nucleus pulposus (NP). Changes to the IVD structure that impact motion, spinal alignment, flexibility, or neural anatomy may contribute to painful impingement of nerve roots exiting the foramen (i.e., herniation, protrusion), narrowing of the canal housing the spinal cord (i.e., stenosis) or an anatomic protrusion of the vertebrae or vertebral bony spurs (osteophytes) known as spondylolisthesis and spondylosis (6),(7). The prevalence of these IVD disorders generally increases with aging and aging-related IVD degeneration (Section 2), and may individually contribute to painful symptoms of neck or back pain.

The intervertebral disc (IVD) has long been associated with back pain and is the target of numerous lines of research to develop novel therapeutics. The IVD undergoes a degenerative process that results in a loss of proteoglycans, disorganization of the extracellular matrix architecture, tears in the IVD, generation of herniation fragments, and a loss of disc height (8–11). As a result of these anatomical changes, nerve root compression, narrowing of the spinal canal, and facet joint impingement can occur and lead to painful symptoms and neurological deficits (7, 12, 13). More recently, the inflammatory environment and neoinnervation found in the degenerated disc has been suggested as a source of discogenic back pain, as well (14). As a result, discogenic back pain is believed to involve complex interactions between the mechanical aspects of the IVD, inflammation, and the nervous system (peripheral and central) (15). While our understanding of the mechanisms of back pain is incomplete, any therapeutic effort to treat pain by targeting the IVD should be considered in this context. As a result, tissue engineering and regenerative medicine strategies that have the potential to provide pain relief and provide functional restoration are of great interest. These strategies are especially promising due to their potential to simultaneously interact with the mechanical, inflammatory, and nervous system components involved in IVD disorders.

IVD disorders that do not spontaneously resolve with conservative therapy (e.g., oral NSAIDs, physical therapy) are candidates for surgery. Surgical treatments may include excision of intrusive IVD fragments (16), fusion surgery to completely limit motions of the three-joint complex (17), (18), or implantation of artificial disc replacements or artificial replacements for the nucleus pulposus designed to restore disc height and antaomic motions (19), (20). These discectomy and fusion procedures are largely palliative in nature, and may fail to restore the baseline motion and mechanical load-bearing characteristics of the IVD, particularly if fused. Artificial replacements for the nucleus pulposus have met an exceptionally high complication rate due to extrusion or migration of the implants that are generally poorly integrated with the surrounding structures (see Section 3). Total disc

replacements of the IVD are not widely used due to the technically challenging nature of the procedure, their generally high cost, and outcomes that are similar to fusion of the entire motion segment (20). One group has reported success with implantation of total disc allografts in lieu of artificial disc replacements for restoration of motion and height in the three-joint complex (21),(22); however, the high risks associated with allograft implantation in this site have limited wide-spread trials with this approach. Finally, procedures such as spinal fusion may be followed by adjacent segment disease (i.e., degeneration of IVDs adjacent to the spinal fusion site) (23), leading to further presentation of herniation, stenosis or spondylosis. There is a clear need for alternate treatments prior to surgery that have the goal to slow or reverse the progression of IVD degeneration that contributes to IVD disorders and associated painful and disabling symptoms.

Despite the common thinking and observation that the IVD exhibits little to no capacity for repair following injury, recent advances in the understanding of IVD development, cell biology, and mechanisms for degeneration point towards the potential for cell-mediated regeneration (11). Bioengineering strategies have potential to guide cell integration with implants and materials, providing great improvements upon the “inert” implants that suffered from poor outcomes in prior decades. We now know that native cell populations within the IVD can respond to physical stimuli such as material stiffness as well as chemokines, cytokines, and numerous soluble mediators of metabolism and extracellular matrix synthesis (11), (24), (25), (26). Furthermore, the IVD becomes increasingly populated by infiltrating macrophages and other monocytes with age, that we know to regulate IVD cell phenotype, biosynthesis and survival (8). For these reasons, interest has expanded in advancing biological and bioengineering approaches that exploit the endogenous cell populations of the IVD for the many and different presentation of IVD disorders.

Here we review strategies to fully replace or partially replace the IVD with cell-supplemented and acellular, biologically based materials that provide opportunity for cell-material interactions and remodeling. There are a very large number of pre-clinical and now clinical studies that reported on outcomes for injection of allogeneic and autologous progenitor and stem cell populations to the IVD (see discussions in (27), (28)), and we provide a brief overview of those clinical studies that make use of biomaterials for cell or drug delivery. The outcomes for cell or drug delivery may have some overlap with the goals of engineered material approaches to restore IVD anatomy (e.g., disc height) to prevent neural impingements and associated pain, or to reverse the radiographic evidence of pathology (e.g., signal intensity upon MRI); however, the challenges to regulatory approval and high demand for cell delivery to the IVD have driven work past a mechanistic or consensus-based understanding of best practice. Below, we will describe strategies and innovations in use of biomaterials being employed or in development to treat IVD disorders, for categories of AF repair, nucleus pulposus replacement or intra-discal material delivery, and combination replacement. While still in early stages of development, we will identify common themes or prior work and discuss outcomes of procedural success for moving forward with biomaterial based strategies to treat IVD disorders that have potential to impact the human patient.

IVD STRUCTURE, FUNCTION & PATHOLOGY

An understanding of the native IVD structure, composition and local IVD cell population is important to guide the design of any therapy for IVD disorders. The IVD is composed of distinct sub-structures (Figure 1): the centrally situated and gelatinous nucleus pulposus (NP), the fibrocartilaginous annulus fibrosus (AF) on the radial periphery, and the cartilaginous end plates on both the superior and inferior faces. The NP and AF of the IVD are considered avascular, alymphatic and largely aneural with substantive nutrient transport occurring between the vascularized vertebral bone and the sub-structures of the IVD (29) (30).

Nucleus pulposus

The NP is a highly hydrated gelatinous tissue that is predominantly water, with a polydisperse population of negatively charged proteoglycans and multiple populations of collagens and noncollagenous proteins (31). Aggrecan is the most abundant proteoglycan found in the NP, but decorin, biglycan, fibromodulin, and versican can be found as well (32) (33). In addition to collagen (principally type II) and proteoglycans, significant amounts of elastin, fibronectin, and laminin are found in the NP (34) (35). The cell population of the NP is derived from the notochord during development and is vacuolated, with marked differences in cellular phenotype with age (32, 36),(37) (38) (39). Throughout, the NP tissue has a very low cell density, $2-5 \times 10^6$ cells/mL, that decreases with age and is similarly avascular and aneural as for the AF (40, 41) (42). There is an increased presence of cell types from the immune system with age (e.g., macrophages, T-cells, lymphocytes), that contribute to an inflammatory milieu that can directly contribute to sensitization of ingrown sensory neurons and growth of new sensory fibers (43), (26) (44),(45). Given the very low cell density for native cells of the NP and AF of the IVD, maintenance of both cellularity and a generous nutrient supply is often held to be critical to successful biologically based regenerative strategies.

Motion and weight bearing of the axial skeleton are transferred as compressive loads to the NP, which is sustained by the high osmotic pressure of the negatively charged proteoglycans contained within. Pressures have been measured to vary from 0.1 MPa when lying prone to upward of 2.3 MPa when lifting a 20-kg weight with a rounded, flexed back (46) (47). IVD degeneration is associated with elevated catabolic processes that promote loss of proteoglycans and consequently osmotic pressure of the NP that reduces support of compressive load and may lead to elevated strains in the tissues of the AF (47). These changes are associated with anatomic evidence of loss of IVD height, reduced hydration measures as reduced T2 signal upon magnetic resonance imaging (“black” disc), and reductions in motion for the affected segment (e.g., reduced segment rotations or anterior-posterior translation with flexion-extension or bending) (48),(49). These anatomic changes in the NP are believed to be a major cause of IVD disorders associated with neural impingement. Indeed, several engineered biomaterial strategies have exploited re-introduction of glycosaminoglycan species to restore IVD hydration and disc height (see Nucleus Pulposus Regeneration and Replacements).

Anulus Fibrosus

The fibrocartilaginous AF is composed largely of highly oriented collagen fibers (principally type I collagen) organized into alternately oriented lamellae (50), that resist the high tensile stresses generated during pressurization of the central NP and during flexion-extension and lateral bending. This tissue is largely avascular and aneural and must rely on a sparsely distributed population of cells ($\sim 5\text{--}10 \times 10^6$ cells/ml) to maintain or repair the AF with material injury or following exposure to factors threatening cell survival. The outer AF has evidence of penetrating sensory nerve fibers and blood vessels that also contribute to nutrient transport to AF cells of the IVD (51). This presence of neo-innervation in the AF of the IVD is one factor that has been suggested to contribute to discogenic back pain directly, although the better studied of IVD disorders (i.e., stenosis, herniation, spondylosis) suggest that pain arises from grossly observable anatomic IVD changes that generate neural impingement or damage (15), (52).

The AF cells are mesenchymal and have both fibroblast and chondrocyte characteristics (53), (54). During IVD degeneration, collagen of the AF loses organization through events that are likely mediated by both proteolytic and mechanical processes (e.g., fatigue, micro-damage). With little reparative capacity for cells of the AF, fissures/tears can develop, causing herniation and displacement of IVD fragments. Surgeons can remove the IVD fragments in a discectomy procedure, but currently the tears remain unrepaired after removal, with no restoration of lost tissue fragments or preservation of IVD height. As a result, techniques to repair the AF are a major focus of bioengineering strategies.

Surrounding bony structures

Vertebral bodies both superior and inferior to each IVD are key contributors to motion of the three-joint complex in the spine. The end plates within each vertebral body are made of hyaline cartilage and are the interfaces between the IVD and highly vascularized vertebral bodies. They are key to maintaining both the mechanical integrity of the IVD and the separation of the vascular and avascular IVD compartments. Much of the nutrition of the disc passes from the capillary buds in the vertebral bodies through the end plate and into the IVD (30, 55–57). During the degenerative process, the end plates thin and mineralize (58) and permeability of the end plates may decrease and hinder nutrient transport to the IVD. Strategies that serve to increase permeability of the endplates, and thus maintain nutrition to the IVD may be helpful to support the long-term survival of the endogenous or delivered cell populations (28–30, 59, 60). An increased presence of nociceptive nerve fibers in the end plates of pathological motion segments is believed to be related to painful conditions with IVD disorders (61) but is very poorly understood. Although engineering strategies to restore end-plate permeability and overcome decreased nutrient transport could be valuable in IVD repair, maintenance of the mechanical integrity of the endplate may be key to the success of an intra-discal biomaterial in order to prevent vertebral fracture and communication with the vertebral marrow. Indeed, the class of in-situ hydrating polymers found subsidence of the biomaterials, or migration through the thinning endplates, to be a major challenging. As such, few regenerative strategies to date have directly targeted the end plates.

On each side of the distal aspect of the vertebral bodies are diarthrodial facet joints that contribute to motion of the motion segment. Under healthy conditions, the facet joint anatomy (planar orientation and surface area) is well-designed to allow smooth motion and to limit excessive torsions, rotations, and translation applied to the IVD (62) (63). With degeneration of the IVD, however, a loss of IVD height and decreased (or sometimes increased) range of motion in vertebrae-IVD complex can contribute to excessive or frequent loading of the facet joints. Pathologies of the facet joints associated with this “mechanical wear and tear” are similar to the osteoarthritis seen in other diarthrodial joints. As a result, techniques to slow or reverse pathology in the facet joints should be the focus of strategies to treat IVD disorders, but have been little studied.

Summary

Bioengineering approaches to treat IVD disorders frequently address the individual sub-structures described above, with their known contributions to pathologies of herniation, stenosis or spondylosis. The degenerative IVD possesses a complex micro-environment that provides design challenges to most biomaterial strategies due to the presence of elevated inflammatory cytokines and active proteases, decreases in pH, glucose/ and oxygen that contribute to cellular stress for implanted cell populations, and altered or difficult-to-control mechanical loading conditions. We describe below a summary of biomaterials approaches that have been developed to treat IVD disorders targeting the individual sub-structures, with a discussion of design challenges and potential design goals.

REPAIR OF THE ANULUS FIBROSUS

The AF tissue exhibits a very limited capacity for self-repair upon injury or tearing (58). With loss of IVD height associated with IVD degeneration, motions of the spine may lead to greater stress transfer to the AF of both compressive and tensile magnitudes. These changes in mechanical loading may contribute to micro-damage observed as fissures or small tears that may progress over time to radiographically observable tears (e.g., via MRI or CT). Left untreated, AF tears may lead to AF fragment displacement and extrusion of the NP known as IVD herniation or a “slipped disc.” While microdiscectomy can be used to surgically remove the displaced fragments that impinge upon neural elements, the persistence of the untreated AF tear may contribute to re-herniation or pathological motions of the IVD (64). AF repair has been proposed as a means to repair the herniation site, in order to restore IVD function and minimize re-herniation of the IVD. AF repair benefits from the opportunity to integrate the repair implant with the adjacent bone, as fibers of the AF directly insert into the endplates of adjacent vertebral bodies and provide opportunities for fixation. Tests of engineering solutions for AF repair have included measuring integration strength between the repaired and native tissue (e.g., suture pull-out strength), and measuring tensile load-bearing properties for the entire motion segment *in vitro* in the presence of the AF repair strategy (Figure 2). How these mechanical measures relate to *in vivo* functional outcomes of IVD re-herniation rates, individual motion segment motion, and presentation of pain is not known; nevertheless, these mechanical tests are pre-requisite to getting an AF repair strategy ready for clinical translation, as described here.

Suture repair methods

The first approaches used to repair torn AF tissue are advanced suturing devices (Table 1). These include several devices with a European Conformity (CE) mark or limited US Food and Drug Administration (FDA) approval that are based on surgical placement of custom-tailored sutures or polymeric meshes over the annular defect site, and fixed with anchors typically attached to bone (e.g., XClose® Surgical Repair, Inclose™ Surgical Mesh, Barricaid™) (Figure 2). These strategies demonstrated success in keeping the NP from extruding and in restoring tensile strength to the torn AF principally *in vitro* (65). Clinical outcomes may be considered successful in the reporting of few adverse events and reduced rates of re-herniation (Table 1). Given surgical complexity in use of these suturing devices that contribute to increased length of procedure along with high device cost, widespread use of these devices has not been achieved (64). Nevertheless, there is broad consensus that less complex techniques that deal with the damaged AF, more broadly than the acellular suture techniques described here, have potential to prevent re-herniation.

Void filling repair methods

Many different materials have been designed for patch or void filling in AF repair, with and without cellular components. These void-filling methods largely include hydrogels, such as alginate, agarose, gelatin, and collagen, as well as sponges made from polyglycolic acid (PGA), polylactic acid (PLA), poly(s-caprolactone), collagen, silk, hyaluronic acid, and/or glycosaminoglycans (Table 1). These hydrogels are often injectable and able to conform to various defect sizes and shapes, and may be prepared for use in combination with biocompatible cross-linkers, such as genipin or riboflavin, to cross-link fibrin or collagen, respectively (66), (67). Alternate approaches, such as the use of a shape-memory alginate to expand into the defect once rehydrated, have also been proposed (68). As an example of pre-clinical tests required for proof-of-concept, the genipin-fibrin gels have been demonstrated to restore compressive mechanical properties to the IVD after repair *in vitro*. More recently, the earlier concepts of crosslinked materials for defect filling were advanced through design of a needle-shaped plug consisting entirely of crosslinked collagen, that was inserted into an AF defect following intra-discal delivery of cells (69). The collagen plug was able to reproduce compressive loading, but also to reduce cell leakage and withstand push-out testing *ex vivo*. Subsequent findings of positive outcomes following implantation in a rabbit AF defect model *in vivo* suggest that these tests of mechanical function have potential to serve as useful surrogates when demonstrating proof-of-concept.

Injectable hydrogels for AF repair also provide an environment that is conducive to AF cell survival and extracellular matrix production, promoting the concept that cell-mediated remodeling of the implanted biomaterial will contribute to device longevity and improved outcomes. With the exception of contracted and aligned collagen gels (70), these materials maintain cells in a rounded morphology that drives infiltrating or seeded cell populations to produce a more hyaline cartilage-like ECM rather than organized fibrocartilage. How critical this feature may be to procedural outcomes in human subjects is not known, particularly over the longer durations of implant survival (> 1 year). And while tests of compression have confirmed utility in restoring IVD motions, the absence of an aligned collagen matrix suggests an inability to transfer tensile stresses across the defect site during loading and

motions of the motion segment. Essential outcomes of an injectable hydrogel have not been defined past pre-clinical in vitro testing, but have gained favor for their ease of use and often high safety profile at other sites in the body.

Tissue engineering for AF repair

Methods to produce engineered AF that mimic the collagen fiber architecture of native tissue may be considered to be the “holy grail” of AF repair. In one approach, decellularized extracellular matrix with a high degree of fiber alignment (small intestinal submucosa) has been used as a patch anchored to bone by titanium bone screws (71). This technique produced an integrated tissue at the defect site that allowed increased pressurization of the IVD in a preclinical model (i.e., ovine) and increased hydration over long periods of time in vivo (24 weeks). The reproduction of aligned fibers *de novo* from synthetic or other materials has been pursued through electrospinning (72–75), collagen contraction (70, 76), silk-fiber winding (77, 78), and the introduction of an oriented honeycomb structure into scaffolds (79). Electrospinning has produced engineered tissues with impressive reconstruction of the multilamella structure, fiber alignment, and anisotropic mechanical properties (80). The electrospinning techniques can produce an engineered AF tissue that appears nearly ideal from an anatomical point; however, use of poly(ϵ -caprolactone) and similar fibers for electrospinning may lack the durability and strength needed to optimize integration with native tissues (81, 82). Emerging thoughts are that an ideal method for AF repair would reproduce the mechanical properties, strength, and oriented microstructure of the AF tissue, but integrate with bone or adjacent AF tissue like that of injectable void-repair methods. A system that can reproduce such design elements falls into the category of “composite” tissue-engineered approaches and is the subject of ongoing research (see Anulus Fibrosus/Nucleus Pulposus Combination Strategies).

NUCLEUS PULPOSUS REGENERATION AND REPLACEMENT

Biomaterials for NP replacement have focused largely on injectable synthetic or biologically based materials that can restore disc height and motion segment stability to an IVD with an intact AF (28, 83, 84). For synthetic materials used in this application, there are no definitive design criteria that must be satisfied to achieve procedural success; instead, engineers have developed materials with material properties matched to those of the native structure, demonstrated integration of implanted materials with adjacent structures through matched displacements (i.e., no migration, slippage), demonstrated integration with tests of integration strength, or demonstrated restoration of motion characteristics and physical properties of the de-nucleated motion segment. Clinical translation of implanted biomaterials can not occur without demonstrations of durability, or an ability to maintain physical support over millions of cycles of loading, and must also generate no or minimal wear debris that could provoke a systemic immune response. Biologically based materials for NP replacement have the potential to be degraded by the endogenous cell population and lead to a remodeling of the NP implant over time. Some of the above-listed tests of implant success may also pertain to this class of materials, but with fewer constraints on durability (due to the remodeling of the biomaterial). Finally, some biomaterials have been proposed to act almost entirely as carriers for cell delivery to the NP, with the goal to provide an

environment supportive of cell-mediated NP tissue regeneration. The development strategy for each approach, documented results and outcomes, are reviewed in the following sections.

In situ hydrating synthetic polymers for nucleus pulposus

The first attempts to augment or replace the NP focused on device classifications, biomaterials that were implanted to restore motion segment function or disc height, with no claims on biologically based outcomes or effects on cell populations. The approaches initially pursued to restore NP height, function, and motion focused on the use of *in situ* hydrating, synthetic polymers to restore NP hydration and, consequently, IVD disc pressure and disc height (Table 2). This concept was intended, in part, to mimic the hydrating properties of the NP glycosaminoglycans that are slowly degraded and modified with aging and degeneration. The approach that has the longest history of clinical use, and that serves as a key precedent in NP replacement, is a copolymeric hydrogel encased in a polyethylene fiber jacket [polyacrylonitrile and polyacrylamide (PDNTM)]. This concept has also been key to the design of multiple implantable devices for NP replacement constructed from either semihydrated poly(vinyl) alcohol (PVA), a copolymer of (PVA) and poly(vinyl pyrrolidone) (PVP) (85), or modified poly(acrylonitrile) reinforced by a Dacron mesh (86). The design concept is that these polymers will absorb water in the otherwise dehydrated, degenerated NP, and the degree of swelling will be restricted by the encasing jacket material. Controlling polymeric swelling proved to be a significant challenge for this class of implants when used *in vivo*, however, as implant swelling was often associated with excessive implant stiffness, end-plate overloading and fracture, device subsidence, and eventual failure. Nevertheless, these polymers have retained some interest for use for NP augmentation and attempts to redesign implant shape have recently been attempted (87). While still in clinical trials, the GelStixTM (Replication Medical, NCT02763956, (88)) consists of a filamentous version of a modified poly-acrylonitrile that will expand in volume upon implantation. As with the earlier uses of in-situ hydrating polymers some complications have already been reported including fragmentation of the gel upon swelling (89). For these reasons, interest in use of this class of polymers for NP implant hydration has recently been approached with caution.

In situ forming synthetic polymers for NP replacement

Another class of biomaterials developed as NP implant devices has developed from injectable polymers that undergo a physical transition to a gel-like or solid-like form when placed into the NP space (60, 85, 90–92). The advantage of this strategy is its ability to cause minimal damage to the AF tissue during implantation of the biomaterials, for example, through a fine-bore needle. Multiple strategies have been explored, including use of chemical cross-linkers and thermal or pH-induced transitioning (Table 2). One example of a chemically cross-linked polymer for use as an NP implant is a silk and elastin copeptide sequence that is mixed with cross-linkers at the time of injection (NuCoreTM) (60). Although it is a biologically based material, the cross-linking inhibits protein degradation and confers an extra stiffness that is necessary to achieve satisfactory stiffness values for a disc implant. The BioDiscTM operates with a similar strategy but with an albumin-based protein material cross-linked by glutaraldehyde. As shown in Table 2, popular materials for *in situ*-forming NP implants include native proteins such as hyaluronan and collagen, as well as glycosaminoglycan-containing components, that provide turgidity to the implant through

osmotic pressure generation. Many of these approaches have been evaluated in human subjects *in vivo*, but few have progressed beyond clinical trials to market approval. What is important about this class of materials is their focus on mechanical design goals upon implantation, which may include restoring disc stiffness, range of motion, neutral axis alignment, or disc height. Despite their presentation of native extracellular matrix protein constituents, they are designed to function as devices, and their interactions with the local cell populations have been poorly studied. Indeed, with one polymerized hyaluronan and collagen implant material designed for NP replacement, deleterious effects were observed for the AF despite achieving implant goals (93). It appears likely that this class of implants will progress toward clinical utility in the near future, but new questions about integration, biological interactions, and mechanical load sharing with other structures may emerge.

Biomaterials as cell carriers for NP regeneration

Reduced cell numbers and phenotypic changes in the resident NP cell population may be the earliest contributors to NP degradation, desiccation, and eventual loss of motion segment function and stability. Thus, cell supplementation to the NP has been widely pursued in preclinical to clinical studies. Cells of different origin, including autologous and allogeneic primary cells as well as multiple types of progenitor cells and chondrocytes, are all capable of synthesizing and depositing some type of collagen and glycosaminoglycan within the NP space, although there is little agreement upon the targeted composition necessary to achieve a satisfactory clinical and long-term outcome. If the local environment within the NP is conducive to the survival of cells, cell supplementation without a supporting biomaterial scaffold may hold promise for NP repair. This strategy has been widely pursued, and excellent reviews of the preclinical and clinical experience with cell supplementation are available (27, 28). No fewer than 50 preclinical studies have evaluated the use of autologous mesenchymal stem cells, or adipose-derived stromal cells; as well as allogeneic or xenogeneic embryonic stem cells; mesenchymal stromal cells from multiple sources; and primary cells of NP, AF, or chondrocytic origin (27). Whereas some studies have reported functional outcomes such as restored disc height or motion segment stability for the acellular biomaterial implants, more have focused on outcomes of synthesis of new extracellular matrix and histological appearance of the new tissue over time. There exists no clear consensus on a set of meaningful biological outcomes from preclinical studies of cell delivery, nor is there a clear mechanism defined according to which delivered cells contribute to improved functional outcomes in NP replacement. Nevertheless, at least 10 clinical trials of cellular products for treatment of NP degeneration or NP regeneration have been performed based on purported mechanisms of promoting matrix biosynthesis or attenuating degradation, and one product has been marketed in Germany for discectomy patients (94).

Of relevance here is the impact that biomaterial design can have on functional outcomes or procedural use of cell delivery for NP treatment. Some early studies compared MSCs embedded in an atelocollagen gel with direct injection of MSCs following delivery into rabbit or rat NP in models of IVD degeneration (95). These studies demonstrated that cells with the collagen carrier maintain disc height, magnetic resonance signal intensity, and the histological appearance of the native tissue for long periods after implantation (95–97).

These findings advanced the concept that preservation of extracellular matrix is important to survival of the delivered cell population. Additional work of bioactive native materials as cell carriers, such as intestinal submucosa, also furthered the idea that native extracellular matrix components were supportive hosts to endogenous cell populations (98). These early studies led to a host of biomaterial developments that made use of biologically based materials and specific components of the extracellular matrix.

Biomaterials as cell carriers for NP regeneration have been more commonly chosen for their handling characteristics and demonstrated safety profile than for their desirable biological, degradation, or bioinductive effects. Preclinical studies have delivered cells in various materials that are commercially available (and safe for human use), including fibrin gels, Gelfoam™, cross-linked or high-molecular weight hyaluronan solutions, hyaluronic acid sponges (e.g., HyStem®, HYAFF), and Puramatrix® (e.g. (27)). Indeed, hyaluronan in liquid or gel forms stands apart for its broader acceptance in clinical trials as a carrier for intradiscal delivery of allogeneic mesenchymal precursor cells, or autologous bone marrow-derived stem cells (NCT01290367, NCT02338271, (99, 100)). Even modifications to hyaluronan, through crosslinking with bis thio-polyethylene glycol solution and albumin, are under investigation in safety trials for intra-discal delivery of ex vivo-expanded autologous IVD cells (NCT01640457, (101)). While it is apparent that intra-discal delivery of hyaluronan as a cell carrier is generally safe in multiple clinical trials, it is not known if the carrier itself confers some required physical or bioinductive features to the cells.

From bench to pre-clinical research, we have begun to consider the features of a biomaterial that might be required to support cell delivery to the NP. Some groups have designed biocompatible scaffolds that may incorporate features supportive of the NP cell phenotype, such as the inclusion of native extracellular matrix components, such as hyaluronan, proteoglycans, or collagens (102, 103) (104). In one example, a polymerizing fibrin containing the growth factor transforming growth factor β (TGF- β) was delivered with allogeneic mesenchymal stem cells and shown to preserve disc height and to inhibit apoptosis beyond that of either the growth factor or cells alone (105). Because TGF- β is naturally present in the healthy IVD, the immobilization of this growth factor in the biomaterial carrier may have conferred some biological effect that contributed to the reported outcomes. This may hold true for injectable gels formed with pentosan polysulfate, a glycosaminoglycan-like factor that supports progenitor cells for IVD regeneration and appropriate matrix accumulation (106).

In our own research, we have exploited the observation that the healthy NP contains several forms of laminin, whereas the AF and cartilage have very little to no laminin protein present (35). We found that both healthy and adult pathological NP cells attach to laminins through specific integrin receptors and that they prefer attachment to laminins over collagens or fibronectin in the healthy young state (107) (108). By designing an injectable, *in situ*-forming hydrogel incorporating full-length laminins (Figure 3), we developed a biomaterial that was able to promote NP cell attachment and appropriate cell signaling and further promote gene expression for NP-specific molecules and higher sulfated glycosaminoglycan synthesis (109, 110). Other groups have evaluated the ability of the arginylglycylaspartic acid (RGD) peptide to promote cell attachment and confer desirable biological outcomes for

AF cells conjugated to solid-formed silk scaffolds, rather than injectable hydrogels as described here for the NP (111). The ability of biological ligands and growth factors to modulate cellular performance when incorporated into biomaterials has been virtually unexplored for application to NP tissue regeneration. Researchers are just beginning to identify the relevant molecules that direct NP cell differentiation (39), promote NP cell synthesis, and promote NP cell survival in the challenging mechanical and physical environment of the degenerative disc. Future design of biomaterials as cell carriers that incorporate these relevant features has the potential to promote NP regeneration through well-understood and disease-specific mechanisms.

ANULUS FIBROSUS/NUCLEUS PULPOSUS COMBINATION STRATEGIES

For over a decade, intervertebral disc replacements (total disc arthroplasty) have been available to replace the integrated anulus fibrosus-nucleus pulposus-endplate units (19) (112). These devices are constructed from well-characterized materials of widespread use in arthroplasty, including metals to integrate with bone and polyethylenes to facilitate the articulation. The concept for these devices has been to preserve motion for individual motion segments back to the 'atural state' when implanted, measured as angles in flexion-extension (typically < 5 degrees) and anterior-posterior translations. They have the advantage of being manufactured as an intact IVD that integrates with bone of the vertebral body providing for rigid fixation. While these devices were developed with the goals to maintain adjacent IVD health, and to minimize facet joint degeneration, stenosis and spondylosis, these devices have not gained widespread use due to surgical complexity, adverse events such as implant migration, and the additional and even greater complexity associated with device removal when needed. As for the NP replacement devices, there has been renewed interest in engineering complete IVD replacements using degradable materials and biological approaches that support cellular survival and remodeling over the lifetime of the implant. All these approaches are at the level of pre-clinical and *in vitro* research, as described here.

In theory, many of the bioengineering strategies described for NP replacement or AF repair could be combined to produce composite IVD replacements, but only a few have been investigated (Table 3). The first study to demonstrate proof-of-concept for a composite tissue-engineered IVD combined a cell-seeded PGA/PLA AF with an alginate NP (113). This study showed promising new matrix production and compressive mechanical properties but did so without introducing any of the collagen organization patterns observed in the AF. Additional efforts to engineer whole IVD without any AF organization include a cell-seeded demineralized bone matrix gelatin AF and a collagen II/hyaluronate/chondroitin-6-sulfate cell-seeded NP (114). It is not clear if the absence of an organized, collagenous AF component will limit the success and survival of the composite IVD, although it would certainly alter the role of the AF in resisting the tensile stresses generated during NP compressive loading and pressurization.

More recent efforts have focused on producing composite IVD that contain varying degrees of the fiber organization observed in the AF in the composite discs (70, 76, 115–117). In an early study, a cell-seeded hyaluronic acid gel core was surrounded by an electrospun, nanofibrous scaffold to represent integrated NP and AF, respectively (116). In another

example, silk scaffolds with oriented lamellar architectures and engineered pores were fabricated and seeded with AF cells to replicate the native AF, with a fibrin-hyaluronan gel serving as the NP (118) (77), (119). While demonstrating an ability to replicate the appearance and key biological features of the composite IVD, only the composite structure with oriented AF was found to reproduce any key mechanical parameters such as integration strength or compressive stiffness. Later studies of composite IVDs with organized AF architecture were produced with contracted collagen gel, silk, and electrospun poly-ε-caprolactone (PCL) (Figure 4). More recently, crosslinked collagen membranes were prepared as AF lamellae with a collagen-glycosaminoglycan co-precipitate to mimic the native composition of the NP (120). In a rigorous evaluation of composite NP-AF function, this study varied the numbers of AF lamellae for a potential impact on ability of the structure to preserve disc height. Their conclusions showed that 10 AF-like lamella was optimal for preserving some features of native IVD biomechanics in compression, giving conclusive evidence of a role for the oriented AF in a composite tissue-engineered structure.

The first whole tissue-engineered IVD to be implanted in the disc space of the rodent tail spine integrated with the native tissue primarily through AF interactions (mechanical integration across AF and composite implant), produced matrix with characteristics similar to that of the native IVD, and showed compressive mechanical properties similar to those of the native IVD in the rodent spine (76). When these whole-IVD constructs were evaluated in the lumbar spine (121), however, disc space was maintained in only 50% of the implants; the disc space collapsed and spinal fusion was observed in the other 50%. This was the same tissue-engineered composite IVD disc that maintained disc space in the tail model without fixation, indicating that the more challenging loading conditions (e.g., torsion, complex bending) of the lumbar spine may limit procedural success. More recently, an electrospun PCL disc was implanted into the tail disc space, which maintained disc height when combined with spinal fixation (81). Unfortunately, these discs did not integrate with the native tissue and displaced from the disc space ~50% of the time without fixation. Although the AF architecture in these discs is promising, future research is needed to promote integration of the electrospun fibers with the native tissue so that they are capable of transmitting tensile load. What is clear from the first *in vivo* investigations of these discs is that additional research in models that better mimic the human disc space will be needed to better understand the integration and design criteria necessary to avoid disc migration out of the disc space after implantation. Additionally, the small disc sizes used for implantation into the rodent disc space may mask possible nutritional deficiencies and challenge functional matrix development in implants sized for use in humans (59).

The integration between the engineered IVD and the native bony end plates may be critical for success of a composite IVD, due the greater possibility for rigid fixation to bone than to AF or NP. In some of the earliest studies of multicomponent IVD tissue engineering, integration between engineered NP tissue and a calcium polyphosphate substrate was investigated to mimic integration with the vertebral end plate (122). This end-plate integration analog demonstrated native-like properties in proteoglycan content and compressive mechanical properties. These results are promising, but it is important to note that integration strength was not tested and will be important for the ultimate success of such strategies. More recent efforts have extended the concept of regenerating the whole disc and

have attempted to produce full-motion segments that include a bony end-plate region, in addition to the AF and NP (123). These investigators used a collagen gel AF, similar to previous whole-IVD work, a collagen-glycosaminoglycan NP, and an osteogenically induced osteochondral subunit. These engineered motion segments may be able to integrate directly with the vertebral body and provide the advantage of eliminating any sensory neuron ingrowth that may be associated with the native IVD. Progress toward increasingly complex regenerative disc replacement techniques continues. Overall, research in this area is promising, but more work is needed to understand how these discs will fare in the human pathological disc space.

SUMMARY OBSERVATIONS

Above, we have reviewed biomaterial based approaches to repair and replacement of tissues of the IVD. In general, we learned that engineering strategies relying upon synthetic materials or composite implants, that do not interface with the biological components of the IVD, were not widely adopted due to complexity of procedural usage or undesirable outcomes. This is important to illustrate that design and proof-of-concept are not the beginning and end of product development for treatment of IVD disorders, that an understanding of biological processes and clinical utility are important parts of the process. This general observation suggests that approaches permissive of cellular interactions, that allow for remodeling of implanted biomaterials over time to accommodate loading or biological interactions, are emerging as promising alternatives for impacting the clinical treatment of IVD disorders. Given the interest in reducing procedural complexity, strategies that support injectable, defect-filling, *in situ* fixation, and minimally invasive implantation, are likely to have the greatest chance of success.

It is important to note that there are a number of critical challenges to regeneration and restoration of the IVD. Significant mechanical loading requirements and difficult integration with cartilaginous and AF structures make IVD treatments challenging. Additionally, the presence of an inflammatory environment, low pH, low oxygen tension, and poor nutritional availability provides a direct challenge to cell based strategies. It will be important to overcome these challenges as the field moves forward and will likely require novel understanding and strategies for interfacing with and regulating IVD physiology in addition to advances in biomaterials development.

After two decades of research, it is tempting to draw conclusions about preferred polymers, extracellular matrix-derived constituents, or protein-based materials as being the chosen solutions for AF repair, NP replacement or combination AF/NP strategies. In the absence of clearly defined outcome criteria, it seems such conclusions are entirely premature. Nevertheless, there are a few unifying themes that emerge from this period of research and development:

1. Reproducing the lamellar structure of the oriented collagenous AF appears to confer essential physical properties to the composite AF/NP regeneration approach; when the NP is pressurized the lamellar AF provides some resistance to deformations of the intact unit.

2. Preservation of the oriented AF lamellar structure does not seem to be essential for AF repair where integrated “plug” and defect-filling strategies appear to be effective.
3. For NP alone replacement, reproducing the hydrating properties of glycosaminoglycans may not be necessary to preserving disc height as many materials have shown utility in this goal. Nevertheless, an ability to recover from mechanical loading through re-hydration or another mechanism may contribute to longevity of an implant in the NP.
4. Integration with the bony vertebra and endplates may be a preferred solution for both AF repair and AF/NP composite regeneration strategies. Bone healing is assisted by a well-perfused vasculature that is lacking in the healthy IVD, yet there is little motive to introduce vascularity or neural innervation into the healing or regenerating IVD to minimize pain and matrix degradation during healing.
5. Finally, we are beginning to see material strategies that incorporate precisely controlled presentation of bioactive elements such as immobilized growth factors, drugs/genes, peptides or matrix proteins, that are a nice complement to strategies that directly modify incorporated progenitor and primary cells (e.g., gene delivery).

Future work in IVD treatment should be guided by carefully defined outcomes and design goals. Most strategies for IVD regeneration and replacement have focused on restoration of key anatomical features observed in the healthy IVD and motion segment, such as “healthy” disc height, MRI signal intensity, decompression of the spinal cord and nerve roots, and restoring range of motion without device slippage or displacement. These criteria come naturally from easily observable measures, such as radiographic imaging, and will likely remain essential primary outcomes for clinical translation of any proposed regenerative strategy. Approaches that merely replicate IVD matrix production, cell survival or preferred tissue alignment, are not likely to make a substantive impact on treating IVD disorders without restoring some key features of the healthy IVD anatomy. Lastly, our lack of a complete understanding of IVD pathology development and its contribution to pain tremendously complicates the task for IVD regeneration and repair. Bioengineering strategies must advance alongside fundamental research advances to better inform the development of design goals, as well as engineered solutions to treat IVD disorders and resultant pathology.

Acknowledgments

This work was performed with support from the NIH (AR047442, AR069588, AR068777).

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Figure 1. Schematic of the intervertebral disc and anatomic sub-structures

Abbreviations: AF, annulus fibrosus; IVD, intervertebral disc; NP, nucleus pulposus. (A) Forces of load bearing and motion contribute to axial loading of the IVD that is balanced by a hydrostatic pressure generated in the gelatinous NP. Pressure in the NP gives rise to a tensile stress in the surrounding AF or a “hoop stress” as depicted. (B) Multiple factors contribute to the onset of degeneration and matrix changes in the IVD, including aging, wear and tear of daily loading, inheritance patterns, and environmental factors such as pH, oxygen, and nutrient supply. (C) Anatomic changes in the substructures of the IVD can give rise to pain or disability due to proximity of the IVD with major sensory and motor nerves. Age- or pathology-related changes can contribute to an increased innervation of the IVD by sensory neurons branching from the dorsal root ganglion that are a purported cause of discogenic pain. (D) Disc degeneration is classified based on anatomic changes that may be distinguished by imaging appearance or gross morphology, as shown here. (i) Healthy young adult disc with a defined NP. (ii) Middle age adult disc that is slightly aged but not yet degenerated. (iii) Moderately degenerated young adult disc. (iv) Severely degenerated young adult disc. [Panel D modified from: Adams M. *Anatomy and Physiology of the Lumbar Intervertebral Disc and Endplates*. In: *The Lumbar Intervertebral Disc*. New York: Thieme Medical Publishers; 2010:14.]

Figure 2.

Intervertebral disc section and schematic image showing the annulus fibrosus insertion into the vertebral body and routes of nutrient transport and gas exchange. (Bottom right) A tear in the AF that falls under the classification of radial tears. (Inset) Suture fixation was among the first strategies explored for AF repair. Suture fixation (inset) was amongst the first strategies explored for AF repair.

Figure 3.

Injectable cell carriers have been used for delivery to the nucleus pulposus (NP). This carrier is mixed with cells and cross-linked in a biocompatible process for *in situ* cross-linking in the NP space. Cell tracking can be accomplished via luciferase, as well as by other means. Delivery by needle puncture to the annulus fibrosus and NP remains the only established method to delivery large volumes of the cells, but may be destructive to the long-term viability of the intervertebral disc. Abbreviations: LM: laminin; NHS: N-hydroxysuccinimide; PEG: polyethylene glycol.

Figure 4.

Engineering of a composite IVD has been performed using collagen contracting gels (A) or electrospun polymers (B) to provide for an aligned AF that is integrated with the NP. (A) Collagen contraction composite IVD fabrication (I.) NP dimensions used to design injection mold in CAD program. (II.) Injection mold 3D printed out of ABS plastic. (III.) Cell-seeded alginate was injected into mold. (IV.) Alginate NP was removed from mold and placed in center of 24 well plate, where cell seeded collagen was poured around alginate NP. (V.) After 2 weeks of culture, cell seeded collagen contracts around alginate NP to form a gel NP encased by a contracted collagen gel. (B) In another approach, electrospinning of polymer solutions is used to create aligned fibrous sheets that are then wound circumferentially to yield an aligned AF to encase the NP. (C) Both approaches for engineering a composite IVD have been evaluated in vivo in small animal models. (D) Histological evaluation of implant performance demonstrates generally good integration with the surrounding, native AF and endplate.

Table 1**Anulus fibrosus repair**

Method	Description	Results	Reference(s)
Suture repair methods			
XClose™ tissue repair	Polymeric suture with soft tissue anchors	Reduced recurrent disc herniation rate up to 2 years; no adverse events	124
Barricaid™	Polymer mesh with titanium anchor to vertebral body rim	At 2-year follow-up, tear closure was associated with no recurrent disc herniation and maintenance of disc height	125
Patch- or void-filling methods			
PTMC scaffold covered with a sutured PU membrane	Human MSCs	Restored disc height in IVD degeneration model and prevented recurrent NP herniation; MSCs seeded onto PTMC had elevated markers of AF phenotype	126
Porous silk fibroin	Bovine AF cells	Cells attached and produced matrix; conjugation with RGD peptide had no effect on cell attachment or morphology	111
Electrospun PCL	Bovine AF cells	Reproduced the anisotropic, angle-ply laminate structure of AF; cells aligned along predominant fiber direction; integration was shown with engineered NP in vivo	80, 81, 115
BMG with PPCLM	Murine AF cells	Supported AF cell survival, alignment, and matrix accumulation; stiffness and degradation was adjusted by postpolymerization time; gelatin component assisted integration and tensile strength	127
POM	Murine AF cells	Supported cell infiltration, elongation, and matrix accumulation; tensile strength and degradation time increased with polymerization time	128
PDLLA/Bioglass	Human AF cells	Foam supported cell cultures and supported cell proliferation and sGAG, collagen type I and collagen type II production	129
Electrospun PU and PCL	Bovine AF cells	Electrospinning increased “yield strain,” promoted AF cell phenotype with retention of collagen and glycosaminoglycan compared with films	130
Fibrin cross-linked with genipin	Human AF cells	Demonstrated suitable mechanical properties that restored compressive properties to IVD; promoted adhesion and elongation; maintained viability of cell population; had a slower in vitro degradation rate	131
Photochemically crosslinked collagen in shape of needle	Placed following intradiscal delivery of MSCs in microsphere carriers	Restored compressive properties to IVD, reduced cell leakage, withstood torsional push-out test. In rabbit model, needle shape revealed placement of the device	69
Collagen cross-linked with riboflavin	No cells	Retained in defect under loading and contributed to restored compressive moduli of IVD	132

Abbreviations: AF, annulus fibrosus; BMG composite bone gelatin; IVD, intervertebral disc; MSC, mesenchymal stem cell; NA, not applicable; NP, nucleus pulposus PCL, poly(ϵ -caprolactone); PDLLA: poly-D,L-lactic acid; POM, poly(1,8-octanediol malate); PPCLM, poly(poly-caprolactone triol malate); PTMC; poly(trimethylene carbonate); PU; poly(ester-urethane); RGD, arginylglycylaspartic acid; sGAG: sulfated glycosaminoglycans.

Table 2

Nucleus pulposus repair or replacement devices

Polymer	Trade name or reference	Clinical trials?	Measurement outcomes/comments
Injectable synthetic polymers			
Copolymeric hydrogel (PAN and polyacrylamide) encased in a polyethylene fiber jacket	PDN	Yes	No longer in use
Semihydrated poly(vinyl) alcohol	Aquarelle	Yes	No longer in use
Copolymer of poly(vinyl alcohol) [and poly(vinyl pyrrolidone) or modified PAN] reinforced by a Dacron mesh	NeuDisc™	Yes	Restored compressive stiffness
Hydrating PAN	NucleoFix™	Yes	Marketed under CE mark
In situ forming (or cross-linked) synthetic and biologically based polymers			
In situ curing polymer using inflatable polyurethane “balloon”	DASCOR™	Yes	Restored multidirectional segment flexibility
In situ curing polymerized water-in-oil emulsion composite	DiscCell™	Yes	From in vitro work: restored motion segment stability ^a
Glutaraldehyde cross-linked albumin	Biodisc™	Yes	Uncertain status
Hydrogel of a chemically cross-linked elastin and silk polypeptide	NuCore™	Yes	Maintenance of disc height; significantly reduced leg and back pain; improved function scores in discectomy patients
Oxidized hyaluronic acid gelatin implant	133	Preclinical	Restored motion segment stability; no changes in stiffness
Thiol-modified hyaluronan and elastin-like polypeptide	134	Preclinical	Restored stiffness
Sulfonate-containing glycosaminoglycan surrogates	135	NA	Restored stiffness; maintained disc height under compression
Silk fibroin/polyurethane composite	136	Preclinical	Mechanical properties stable over time; higher stiffness than native NP but conducive for cell support
PNIPAAm-PEG hydrogels	137	NA	Enhanced recovery from compressive strain, was thermoresponsive, sustained drug release
Chitosan gels	138	NA	Supported matrix accumulation for native cells and retention of proteoglycan
Hyaluronic acid/collagen hydrogel	93	Preclinical	Implant did not improve disc height; increased scarring and inflammation in AF
Hyaluronan polymers	139	Preclinical	Prevented fibrotic changes; supported cell growth; no mechanical outcomes
Hyaluroan / PEG crosslinked copolymer	102	NA	Engineered design that could be targeted to maintain NP or AF cell phenotype in vitro
Collagen type II / hyaluronan-PEG crosslinked hydrogel	104	NA	Supported maintenance of cell phenotype and biosynthesis for native NP cells

Restored stability is equivalent to reduced range of motion in flexion—extension, lateral bending, or restored neutral zone mechanics.

Abbreviations: AF, annulus fibrosus; CE, European Conformity; NP, nucleus pulposus; PAN, polyacrylonitrile; PEG, polyethylene glycol; PNIPAAm, poly(*N*-isopropyl acrylamide).

Table 3

Composite strategies for anulus fibrosus/nucleus pulposus replacement

Anulus fibrosus	Nucleus pulposus	Bone	Outcome	Reference(s)
PGA/PLA, ovine AF cells (isotropic)	Alginate, ovine NP cells	NA	First research to demonstrate tissue-engineered composite IVD, ECM production, and compressive mechanical properties on the order of native ovine IVD	140
PLLA/HA, MSCs (isotropic)	HA, MSCs	NA	First paper to demonstrate a composite IVD structure using electrospun fibers; in vitro study demonstrated histological evidence of an AF and NP region with collagen I, II, and aggrecan production	116
Demineralized bone matrix rabbit AF cells (isotropic)	Collagen (II)/hyaluronate/chondroitin-6-sulfate rabbit NP cells	NA	Composite IVD subcutaneously implanted into athymic mice demonstrated NP and AF region with collagen and proteoglycan deposition	114
Contracted collagen gel, ovine AF cells (anisotropic)	Alginate, ovine NP cells	NA	Composite IVD implanted in a rat-tail and lumbar model showed integration, disc height maintenance, and mechanical properties on the order of the native controls in all implants in the tail model, and disc height maintenance in 50% of the implants in the lumbar model	66, 70, 76, 121
Toroidal scaffolds of porous silk	Fibrin/hyaluron crosslinked hydrogel	NA	In vitro study demonstrated histological evidence of an AF from implanted AF cells, and NP from implanted chondrocytes, with phenotypically appropriate gene expression	77, 141
Multiple lamellae of photocrosslinked collagen membranes	Collagen-glycosaminoglycan co-precipitate	NA	Composite IVD tested mechanically in compression for comparison to native IVD; results showed a role for collagen lamellae in preserving height recovery following loading, and transient behaviors in creep and recovery.	120
Electrospun PCL, MSCs (anisotropic)	Agarose, MSCs	NA	Composite IVD with AF and NP regions showed aligned PCL fiber and lamella AF organization that mimicked those of the native IVD; in vitro study demonstrated	80, 115

Anulus fibrosus	Nucleus pulposus	Bone	Outcome	Reference(s)
			organization and appropriate ECM deposition in the composite IVD constructs	
Electrospun PCL, acellular (anisotropic)	No material acellular	NA	Construct with organized AF architecture was implanted in a rat-tail model showing maintenance of disc height in 50% of samples without fixation and 100% with fixation	81
Contracted collagen gel, MSCs (anisotropic)	Collagen–GAG coprecipitate MSC	Collagen gel, osteogenically differentiated MSCs	Created an engineered IVD with AF/NP/osteochondral subunits with appropriate histological features of each region	123

Abbreviations: AF, anulus fibrosus; ECM, extracellular matrix; GAG, glycosaminoglycan; HA, hyaluronic acid; IVD, intervertebral disc; MSC, mesenchymal stem cell; NA, not applicable; NP, nucleus pulposus; PCL, poly(e-caprolactone); PGA, polyglycolic acid; PLA, polylactic acid.