

The challenge and advancement of annulus fibrosus tissue engineering

Li Jin · Adam L. Shimmer · Xudong Li

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Abstract

Background Intervertebral disc degeneration, a main cause of back pain, is an endemic problem and a big economic burden for the health care system. Current treatments are symptom relieving but do not address underlying problems—biological and structural deterioration of the disc. Tissue engineering is an emerging approach for the treatment of intervertebral disc degeneration since it restores the functionality of native tissues. Although numerous studies have focused on the nucleus pulposus tissue engineering and achieved successes in laboratory settings, disc tissue engineering without annulus fibrosus for the end stage of disc degeneration is deemed to fail. The purpose of this article is to review the advancement of annulus fibrosus tissue engineering.

Material and Methods Relevant articles regarding annulus fibrosus tissue engineering were identified in PubMed and Medline databases.

Results The ideal strategy for disc regeneration is to restore the function and integrity of the disc by using biomaterials, native matrices, growth factors, and cells that producing matrices. In the past decades there are tremendous advancement in annulus fibrosus tissue engineering including cell biology, biomaterials, and whole disc replacement. The recent promising results on whole disc tissue engineering—a composite of annulus fibrosus and nucleus pulposus—make the tissue engineering approach more appealing.

Conclusion Despite the promising results in disc tissue engineering, there is still much work to be done regarding the clinical application.

Keywords Tissue engineering · Annulus fibrosus · Scaffolds · Intervertebral disc degeneration

Introduction

Intervertebral disc (IVD) degeneration is a common musculoskeletal disease and progresses to disc herniation, spinal canal stenosis, and degenerative spondylolisthesis. The precise etiology of disc degeneration is still far from delineated. Multiple factors have been found involved in the initiation and progression of the disease including aging, loading changes, poor nutrient supply, and hereditary factors [1–7]. IVD degeneration is characterized by changes of cellular microenvironment, loss of proteoglycan, loss of disc height, tears of annulus fibrosus (AF) tissue, spinal stenosis, herniated discs, neoinnervation, hypermobility, and inflammation [8–11]. At present, all treatments for degenerative disc disease are limited to treating the symptoms of the condition. Due to its avascular and aneural structure, the intervertebral disc has little ability to regenerate. When nucleotomy (a surgical treatment for disc herniation) is performed, only minimal regeneration of the annulus is observed, making degeneration of the disc an inevitable consequence of this procedure. Repair of the herniated or degenerative disc or annulus with appropriate analogs would therefore represent an ideal application for tissue engineering technology. A number of artificial disc prostheses have been designed and used in hospitals [12–17]. While certainly a major advancement in the treatment of this condition, artificial

L. Jin · A. L. Shimmer · X. Li (✉)
Department of Orthopaedic Surgery, University of Virginia,
Hospital Drive, Cobb Hall B039, P.O. Box 800374,
22908 Charlottesville, VA, USA
e-mail: xl2n@virginia.edu

discs composed of metal alloys or plastics are inherently limited in their biocompatibility and do not recapitulate native disc tissue. Therefore, both nucleus pulposus (NP) and AF tissue engineering have been investigated. Herein we will review the current advances in annulus tissue engineering. We will address three major components of AF regeneration: cells, growth factor treatments, and scaffolds.

Current attempts to regenerate the intervertebral disc

One of the hallmarks of the disc degeneration is the loss of matrices. Surgical procedures are attempting to relieve symptoms rather than restore the native structure and function. Thus the ideal strategy for disc regeneration is to restore the function and integrity of the disc by using biomaterials, native matrices, growth factors, and cells that producing matrices. The complex biological and mechanical environment of IVD makes the synthesis of an artificial IVD a difficult task. At the early stage of disc degeneration, cell therapy and biological methods— injection of growth factors and enzymatic inhibitors—can be used to augment anabolic metabolism (Fig. 1). However, the half-life of growth factors is short and limits their use in treating disc degeneration. The hydrated proteoglycans in the NP are essential to maintain the osmotic pressure and therefore have a major effect on the load bearing properties of the disc [18]. Thus, NP tissue is more vulnerable than AF towards the environmental changes [19, 20] that suggesting NP tissue engineering is important for regeneration of the functional disc. Considerable effort has been devoted to restoring NP function using growth factors, biomaterial, and cells. The effectiveness of various hydrogel-based scaffolds including natural hydrophilic biomolecules or synthetic polymers for NP engineering or repair has been reported. A variety of biomaterials have been used for fabricating scaffolds in NP tissue engineering, such as chitosan/hydroxybutyl chitosan, alginate, collagen/atelocollagen, gelatin, hyaluronic acid, calcium polyphosphate, poly-D-L-lactide (PDLA), demineralized bone matrix (DBM), small intestine submucosa (SIS), carboxymethylcellulose, and PGA–hyaluronan [21–27]. In addition to the native or synthetic biomaterials, injectable scaffold has also been used as a carrier to deliver NP cells or mesenchyme stem cells to facilitate restoring disc structure and retarding further disc degeneration [25, 28–30]. However, without a functional AF tissue to resist intra-disc pressure, disc regeneration is deemed to fail, which will be discussed in detail in the current review. The purpose of AF tissue engineering is to mend the injured AF to prevent disc herniation and to retard further AF degeneration. Current approaches for

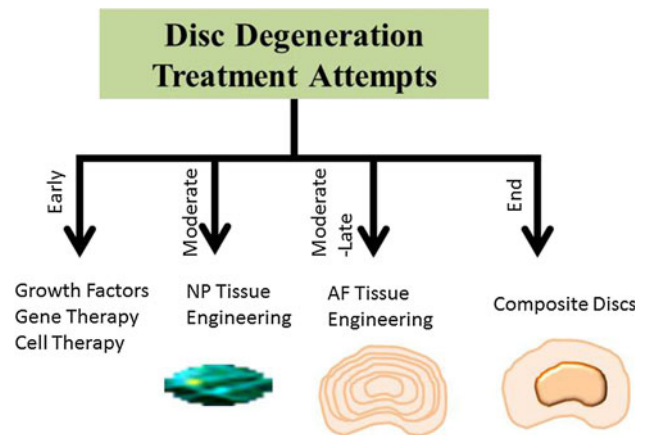


Fig. 1 Scheme showing the current attempts for disc degeneration treatment

AF tissue regeneration combine cells (disc cells or stem cells) and native/synthetic biodegradable scaffolds with different porosities and fiber orientations to resemble the native extracellular matrix component and arrangement. Recently, biological repair of the whole disc with nucleus and annulus composite tissues has become of interest for severe disc degeneration. Bowles et al. [31] constructed a biphasic whole IVD using collagen I for an AF and alginate for the NP. Another study [32] that used electrospinning fabricated a bi-phasic IVD using porcine chondrocytes seeded poly(caprolactone) (PCL) and agarose as the AF and NP tissues, respectively. Recent studies on total disc tissue engineering have attained positive results in small animal models, while the challenges on preclinical and clinical studies remain unknown.

Biology of annulus fibrosus

The AF is originated from mesenchymal cells during embryogenesis, and is composed of highly oriented concentric lamellae sheets that surround the nucleus. It has a much higher collagen and lower water content when compared to the nucleus [33]. These lamella sheets are filled with proteoglycans, interspersed with elastin fibers [34], and provide the tremendous axial load strength. The outer lamellae are majorly type I collagen, as toward the nucleus, type II collagen increases and type I collagen decreases. The inner AF mainly produces type II collagen. Other collagen types such as III, V, VI, IX and XI were also detected in the inner AF. The inner AF consists of greater amounts of glycosaminoglycans as compared with the outer region. Large proteoglycans in AF are aggrecan and versican [35–39], and small proteoglycans are biglycan, decorin, fibromodulin, and lumican [40–42].

What are AF cells

All cells in the AF are derived from the mesenchyme. However, they have different morphologies and compositions of extracellular matrices [23, 43–45]. The outer AF cells are fibrous and ligamentous while the inner AF cells are spherical shaped fibrocartilaginous. Recent studies showed that the expression of type V collagen and tenomodulin was higher in AF cells than in NP and articular chondrocytes, which suggested that they might serve as AF markers [46, 47]. A recent study demonstrated that human AF cells exhibited altered gene profiles among different grades of disc degeneration [48]. Due to interspecies and age variation, however, there is no specific marker to distinguish the AF cells from other cells.

By flow cytometry sorting, we found that some human inner AF cells expressed stem cell surface antigens similar to that of adipose and bone marrow derived stem cells, i.e., CD29, CD49e, CD51, CD73, CD90, CD105, CD166, and CD184 (Fig. 2). In addition, the neuronal stem cell markers, nestin and neuron-specific enolase, were also detected in human inner AF cells. A subpopulation of human inner AF cells was able to differentiate to different cell lineages upon appropriate culture condition: adipocytes, osteoblasts, chondrocytes, neurons, and endothelial cells. These results indicated that a portion of inner AF cells have mesenchymal stem cell (MSC) characterization [49]. In another study, Risbud et al. [50] showed evidence of skeletal progenitor cells in the degenerated human intervertebral disc capable of differentiation into adipose cells, osteoblasts,

and chondrocytes. All these findings suggest that AF tissue contains multipotential stem cells.

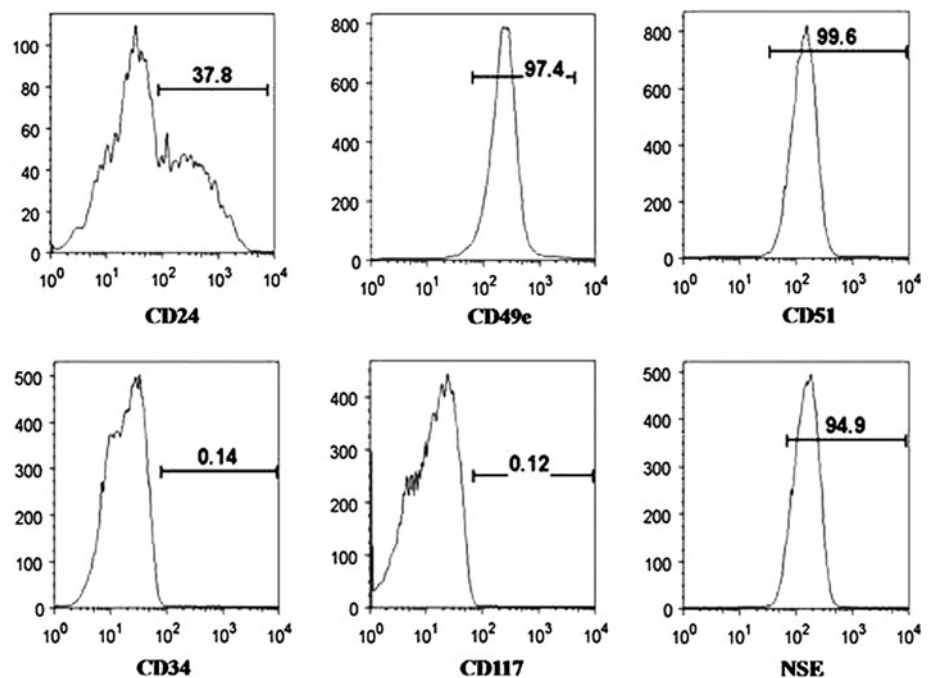
AF cells amplification for tissue regeneration

Studies showed that AF cells cultured in monolayer or three-dimensional matrix can proliferate and produce proteoglycan and collagen [51, 52], which provided the foundation of AF cells in annulus tissue engineering. While compared with a 3D culture, a monolayer culture of AF cells produce less proteoglycan and type II collagen. One of the key elements of cell based therapy or tissue engineering is to amplify a large number of cells. Since the successful cultivation of porcine AF cells in alginate bead [53], many approaches have been used for expanding AF cells in vitro [52, 54, 55]. We have shown that a rotating bioreactor stimulates the anabolic and decreases the catabolic metabolisms of human AF cells. The proliferation of AF cells was greatly enhanced in the rotating bioreactor culture condition [56], which makes the large amplification of AF cells possible in tissue engineering application.

AF cells response to different stimuli

By the immunohistochemistry method, expressions of TGF β RII, BMPRII, FGFR3 and IGFRI growth factor receptors have been detected in human IVDs. There were no significant differences among expressions of four receptors in non-degenerate and degenerate biopsies [57]. This observation suggests that these growth factor

Fig. 2 Flow cytometry showing annulus fibrosus cells express stem cell and neuronal cell markers. The *horizontal axis* represents the parameter's signal value in channel numbers, and the *vertical axis* represents the number of events per channel number. The *number* in each panel shows the percentage of cells expressing the specific cell marker



receptors play a role in normal disc homeostasis and that the administration of growth factors to the degenerate human IVD would stimulate matrix production. Not surprised, TGF β -1 and -2, bFGF and PDGF have been highlighted in herniated intervertebral disc tissue [58]. A study also showed that AF cells produce greater quantities of IL-6, IL-8, PGE2, PGF2 α , and VEGF when co-cultured with macrophages [59]. Both IL-1 and IL-4 are involved in the response of human AF cells derived from non-degenerative tissue to the cyclic tensile strain [60]. Recently Hegewald et al. [61] showed that isolated human AF cells were in response to serum and chemokine migratory effects and expressed chemokine receptors. Of the five tested cytokines (CXCL7, CXCL10, CXCL12, CCL25, and XCL1), CXCL10, a potent attractant for mesenchymal stem cells, and XCL1 recruited the AF cells [61].

Both NP and AF cells from older donors show a decreased production of matrix enriched in aggrecan, but this phenomenon can be overcome by gene therapy or exposure to different stimuli [62]. In *in vitro* culture conditions, a variety of growth factors have been found stimulating matrix production of AF cells (Table 1): TGF- β [52, 54, 63], osteogenic protein-1 [64–66], BMP12 [67], GDF-5 [68–70], IGF-1 [54, 71–73], PDGF, FGF [74, 75], BMP2 [76, 77], BMP13 and the transcription factor Sox9

Table 1 Different growth factors stimulate matrix production of AF cells

Growth factor	Results	References
TGF- β	TGF- β elevated the expression of matrix genes, preserved the expression of TGF-beta receptors, and decreased aggrecan turnover in AF and NP cells.	[52, 54, 63]
Osteogenic Protein - 1	Osteogenic protein-1 increases proteoglycan and collagen contents in both NP and AF cells	[64–66]
BMP-12	Adenovirus mediated BMP-12 significantly increased matrix protein synthesis and DNA content of human AF and NP cells in pellet culture	[67]
BMP-2	BMP-2 up-regulates the expression Col I, type II, and aggrecan in AF cells	[66, 76, 77]
IGF-1	IGF-1 stimulates sGAG, type I and II collagen expression in AF cells	[54, 71–73]
Sox9 and BMP-13	BMPs and Sox9 increase proteoglycan and collagen expression in bovine AF cells	[78]
GDF-5	GDF-5 augments anabolic metabolism of disc cells	[58, 68–70, 80, 81]
FGF and PDGF	Both FGF and PDGF stimulate the proliferation of bovine AF and NP cells	[74, 75]
Link N peptide	Link N peptide increases matrix production in disc cells	[82–84]

[78]. Since GDF5 knockout mice display a degenerated intervertebral disc with disrupted lamellar architecture of the AF and a shrunken and disorganized NP [79], we investigated whether GDF5 gene therapy could reverse the degenerative process [68, 80]. GDF5 protein treatment augmented anabolic metabolism of disc cells from either GDF5 deficient mice or wild-type mice. Intra-disc injection experiments also showed that the administration of GDF5 promotes inner AF cells migrating to injured NP area and presenting chondrogenic phenotype [81].

Growth factors are not the only molecules that stimulate AF cell proliferation, in a serum free culture system, the amino terminal peptide of link protein (DHLSDNYTLHDRAIH) (link N) acts directly on disc cells to stimulate matrix production, which involves increased accumulation of proteoglycan, and type II and IX collagens both *in vitro* and *in vivo* [82–84]. Link N is generated by the cleavage of human link protein by stromelysins 1 and 2, gelatinase A and B, and collagenase between His(16) and Ile(17).

All these studies demonstrated that AF cells reacted to environment cues and were involved in tissue modification in response to injury.

Scaffolds for AF tissue engineering

A number of scaffolds have been used for AF tissue engineering in laboratory settings (Table 2). These scaffolds include porous silks, alginate/chitosan, a demineralized bone matrix, electrospun poly(ϵ -caprolactone) (PCL) fibers, hyaluronic acid/nanofibrous, collagen/glycosaminoglycan, and polyglycolic acid mesh. Scaffolds can be summarized into two categories: single unit (oriented or non-oriented to simulate the organized lamellae) and biphasic to simulate inner and outer layer of AF.

Single unit without fiber orientation

Using an ACHMS scaffold (atelocollagen honeycomb-shaped scaffolds sealed with a membrane), a Japanese group showed that rabbit AF cells grew and maintained phenotype in the scaffold and produced type II collagen and proteoglycan [85]. In a lucana disc model, after laser vaporization of the NP of rabbit intervertebral discs, the honeycomb shaped ACHMS scaffold was implanted. The study showed that AF cells exhibited a proliferation activity, resulting in the production of hyaline-like cartilage similar to the original AF tissue. This prevented the narrowing of the disc space up to 12 postoperative weeks [86].

Adult canine AF cells were able to adhere and proliferate on a collagen–glycosaminoglycan (GAG) scaffold, a

Table 2 Biomaterials used for AF tissue engineering

AF tissue engineering scaffold	Biomaterials	Reference
Single unit without fiber orientation	Atelocollagen honeycomb	[85, 86]
	Collagen-glycosaminoglycan	[87]
	Alginate/Chitosan	[88]
	Poly(1,8-octanediol malate)	[89]
	Poly-D-L-lactide/Bioglass	[90, 91]
	Silk	[92, 93]
	Genipin crosslinked fibrin	[94]
Single unit with fiber orientation	Aligned alginate/chitosan	[88]
	Polycarbonate polyurethane linked with dihydroxyl oligomer	[95, 96]
	Polycaprolactone	[97, 98]
	Poly-L-Lactide	[99]
	Silk fibers/chondroitin sulphate	[100]
Biphasic scaffold	Collagen fibrils	[101]
Composite scaffold	Poly (polycaprolactone triol malate)/DBM	[102]
Composite scaffold	Poly-L-Lactide as AF and hyaluronic acid hydrogel as NP	[24]
	Polyglycolic Acid/poly-l-lactic as AF and alginate hydrogel as NP	[103]
	Silk scaffold as AF and silicon as NP or hyaluronic acid as NP	[104, 105]
	Polycaprolactone fibers as AF and agarose gel as NP	[32]
Composite scaffold	Collagen gel as AF and Polyethylene/alginate as NP	[31, 106]

material that serves as an analog of extracellular matrix [87]. In another study, alginate or alginate/chitosan was fabricated for AF cells culture using a wet-spinning and lyophilization technique. The alginate/chitosan hybrid scaffold exhibited a slower degradation rate, maintained the growth of canine AF cells, and produced specific extracellular matrix molecules [88].

Using a direct one step polycondensation method, we were able to create a malic acid-based polyester poly(1,8-octanediol malate) (POM) film and support the proliferation of rat AF cells [89]. Adjusting post-polymerization time can control the tensile strength of POM: the tensile strength increased from 7.32 to 25.6 MPa at 9 days after

polymerization. In contrast, the elongation decreased over the polymerization time from 14.34 to 3.86 MPa from day 3 to day 9. When rat AF cells were cultured on the POM scaffold, the cells penetrated into the scaffold as visualized by SEM, and expressed higher amount of proteoglycan and collagen II (Fig. 3).

Due to the specific position of intervertebral disc, the deformability is a critical biomechanical property for the AF tissue. The mechanical tests revealed that POM has an excellent deformability: the compressive stress, young's modulus, and tensile strength markedly increased as the polymerization time increased. There was no permanent deformation after 500 press-loading and release cycles with

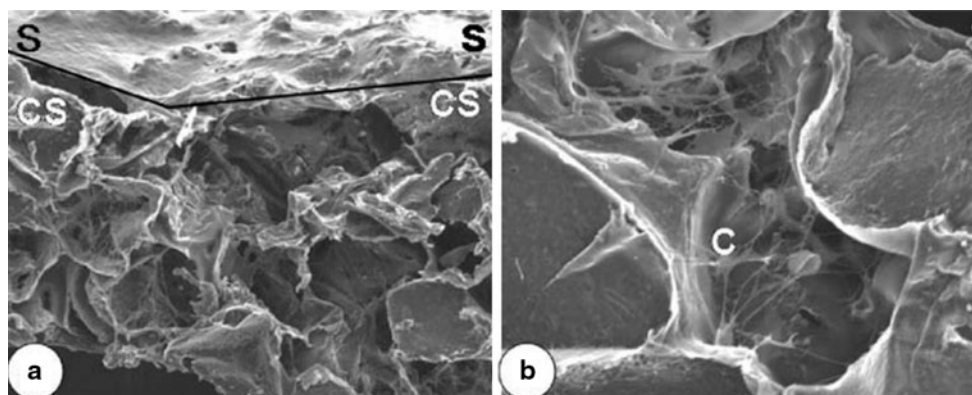


Fig. 3 SEM images of rat AF cells cultured on the POM scaffold **a** 70 \times and **b** 200 \times for 3 weeks. *S* scaffold surface, *CS* scaffold cross-section, *C* cells [89]

30 % maximum strain. When implanted into mice back subcutaneous pocket, there were no inflammation responses were found [89].

Similarly, studies showed that Poly-D-L-Lactide (PDLLA) foams incorporated with different percentages (0, 5 and 30 wt %) of bioglass particles supported bovine AF cell growth and matrix production [90, 91]. The PDLLA and bioglass scaffold, prepared with the thermally induced phase separation process, has a highly porous and foam like structure. The authors claimed that the pore size, mechanical properties, and degradation rates of the PDLLA foams can be controlled by varying the concentration, temperature, and solvent of the PDLLA. Bioactive glasses on the other hand determined mechanics, bioactivity and degradation kinetics of the foams. AF cells were also able to grow on porous silk scaffolds. Modified silk scaffold with arginine-glycineaspartate RGD has been shown to enhance the attachment of other cell types, change the cell morphology and matrix components but did not enhance the cell adhesion [92, 93].

Recently, a genipin crosslinked fibrin gel was used to support the human AF cell growth and adhesion. The authors showed that mechanical modulus of genipin crosslinked fibrin gels can be created similar to native annular tissue. These gels can be used for small AF defects or as an adhesive to augment large annulus repair [94].

Single unit with oriented fiber

The highly organized fibril architecture of AF provides IVD the capacity to resistant tensile and shear stress. Therefore in the design of scaffolds, the fiber alignment is a critical component. Studies have attempted to address the aligned and anisotropic nature of the AF such as collagen gel, aligned PCL fibers, alginate/chitosan, polycarbonate polyurethane (PU), and PPCLM concentric sheets.

With a wet-spinning and lyophilization technique, Shao et al. [88] created an aligned alginate/chitosan scaffold. Collagen gels with varying structure and heterogeneity were used to create circumferentially fibers. AF cells orient themselves along the aligned scaffolds and deposit matrix components that contribute to construct mechanics under loading conditions relevant to the *in vivo* environment.

Polycarbonate polyurethane (PU) was modified by chemically linking with an anionic dihydroxyl oligomer (ADO). The polymeric materials were fabricated into nanoscale fibrous scaffolds using electrospinning. PU nanofibrous scaffolds in the absence or presence of different amounts of ADO were similar in appearance. Increasing the material surface's polar character of the scaffolds resulted in a positive enhancement of AF cell attachment [95]. Both the tensile strength and initial modulus of aligned scaffolds were higher than the random

fiber scaffold. The soluble and non-soluble degradation products were found to be non-toxic to bovine AF cells grown *in vitro* [96].

Other electrospun nanofibrous scaffolds such as polycaprolactone were fabricated in random, aligned, and round-end configurations [97]. A modified electrospinning technique was utilized to generate aligned nanofibrous polymer scaffolds for engineering the basic functional unit of the AF, a single lamella [98]. Uniaxial tension was tested and demonstrated a nonlinear dependence of modulus on fiber angle similar to the nonlinearity and anisotropy of native AF. Culturing bovine AF cells onto a bioactive scaffold with poly-L-lactide incorporated with TGF- β , one study showed that the synthesis of GAG and collagen had markedly increased in the growth factor group compared to the control scaffold alone group [99]. Silk fibers crosslinking with chondroitin sulphate have also been shown to support human chondrocytes re-differentiation [100]. Although not specifically for AF tissue engineering, using molecular crowding and confinement techniques, Saeidi et al. has recently reported producing highly organized arrays of collagen fibrils. In addition, fibrils are organized in multi-layer structures similar to the structure of the collagen fibrils in the extracellular matrices of native tissues, which has potential for AF tissue engineering [101].

Biphasic scaffold

AF is a biphasic structure: an outer layer enriched in collagen I, and an inner layer with more collagen II. Therefore, we constructed a biphasic IVD with a ring-shaped demineralized bone matrix (DBM) as an outer AF and poly (polycaprolactone triol malate) (PPCLM) orientated concentric sheets seeded with chondrocytes as an inner AF [102] (Fig. 4). The DBM was extracted from cortical bone that mimicked the type I collagen structure and fibril property of the outer AF. The resulting PPCLM/DBM biphasic scaffold had excellent elasticity, with no permanent deformation after at least 100 press-loading and release cycles. The compressive stress of the DBM/PPCLM scaffold was significantly higher than that of pure PPCLM, with the incorporation of DBM enhancing the compressive strength of the PPCLM scaffold from 0.21 to 1.26 MPa. The gelatinous pulposus of the IVD absorbs and transmits compressive loads into the tensile stretch at the periphery of the AF. In the strain–stress curve, the stress increased linearly with the strain. The tensile stress of the DBM/PPCLM scaffold was 3.37 MPa, which was much higher than that of the pure PPCLM scaffold at 0.06 MPa for three sheets, and approaching that of rabbit AF at 6.95 MPa.

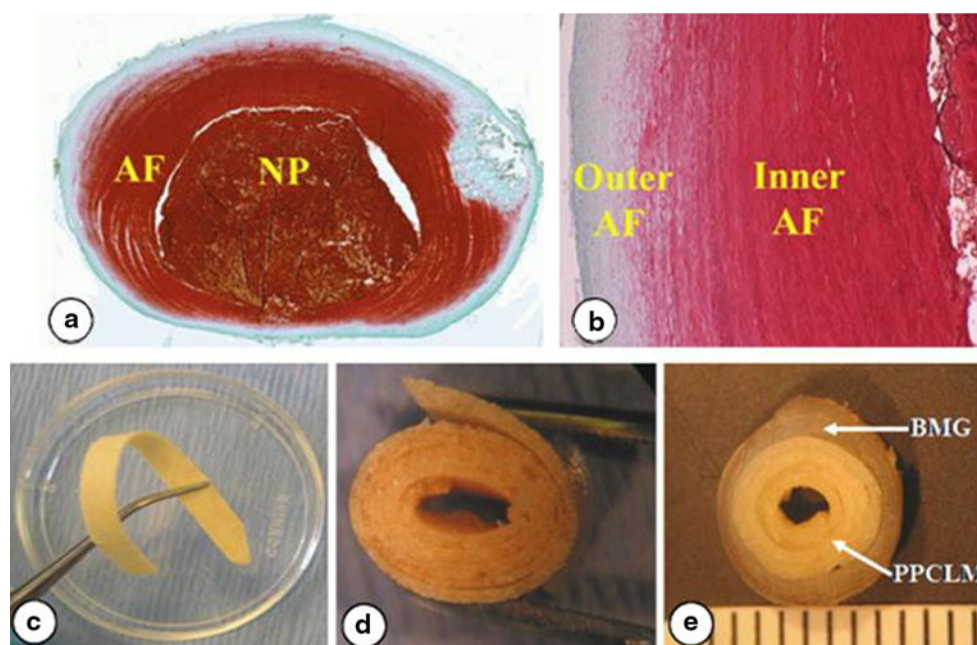


Fig. 4 **a** Horizontal section and **b** vertical section of normal rabbit IVD stained with Safranin-O. The outer layer (*reduced red staining*) and inner layer (*abundant red staining*) of AF can clearly be seen.

c The elastic biomaterial PPCLM orientated in concentric sheets **(d)** and inserted into a BMG ring to mimic the structure of inner and outer AF **(e)**, respectively [102]

Total disc construct

Recently, the tissue engineering field has focused on creating composite tissue engineered total disc replacement, which consists of an inner NP surrounded with AF tissue. The strategy is to resemble the native structure and mechanical property of the intervertebral disc. These works provided advancement in the rational approach to the production of hierarchical and functional scaffolds.

Human mesenchymal stem cells were seeded into a hyaluronic acid hydrogel center and enveloped with a poly(L-lactic acid) nanofibrous scaffold to mimic the native disc structure. Nesti et al. [24] showed that seeded cells exhibited a chondrocyte phenotype. Using a non-woven mesh of polyglycolic acid (PGA) and solvent-cast polylactic acid (PLA) as an outer ‘AF’ ring seeded with AF cells and an alginate hydrogel to serve as the ‘NP’ core, Mizuno et al. created a scaffold in the cylindrical shape of the IVD. The AF and NP cells maintained their phenotypes and integrated over time in culture. When in vitro disc composites were subcutaneously implanted into athymic mice, the implants formed distinct AF and NP tissue as indicated by the expression of extracellular matrix and mechanical properties. By 16 weeks, the biochemical composition and mechanical properties of tissue-engineered intervertebral discs were similar to that of native tissue [103].

By loading rabbit BMSCs (bone marrow stem cells) hyper-confluent cell sheets on a silk scaffold, and a silicon

NP substitute, See et al. showed that the cells are viable, and produced proteoglycan and type I, II collagen after 4 weeks in vitro culture. The ratio of collagen type I to collagen type II within the extracellular matrix of the BMSC sheets also decreased significantly over the period of the culture. The type of collagen found within the BMSC cell sheets were initially predominantly collagen types I. However, following a 4 week culture period within the assembly, collagen type II deposition became more pronounced within the extracellular matrix [104].

In another study, circumferentially orientated polycaprolactone fibers seeding with porcine chondrocytes were used to mimic the AF tissue, and the cell-agarose gel in the center was used to copy NP. They showed that the fibril alignment was formed, the chondrocytes were well distributed around the boundary of NP and AF, and the composite scaffolds had strong mechanical properties than that of agarose gel alone [32]. A different group created the AF/NP composite constructs with silk protein as an AF material and fibrin/hyaluronic acid gel as a NP tissue, and the composites were cultured in vitro up to 6 weeks [105].

Bowless et al. made the composite IVD constructs by using collagen gels seeded with ovine AF cells surrounding with either polyethylene or alginate in the center. By using the contraction feature of cell seeded collagen gel and controlling the boundary conditions, the authors were able to create aligned circumferential fibril structures, and found that more alignment occurred in annular-shaped 1 mg/mL gels compared with 2.5 mg/mL gels [106]. Later, they

implanted the composite constructs into the athymic rat caudal spine, and demonstrated that up to 6 months the engineered IVD maintained similar IVD shapes, disc heights, levels of collagen and aggrecan to the native disc. The scaffold integrated well with the host body. More importantly, the engineered disc had a similar axial load capacity to the native disc. This finding provides the direct evidence that tissue engineering is possible, at least in a small animal model [31].

Conclusions

Despite the promising results in disc tissue engineering, there is still much work to be done regarding the clinical application. Although the combined NP/AF concept seems promising, it might be questionable whether the technique is effective to be used in repairing larger annulus defects. Novel strategies for delivery and fixation may be required. In addition, a suitable cell source for the reengineering of the “whole disc” is still challenging.

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Conflict of interest None.

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