Biological Treatment Approaches for Degenerative Disk Disease: A Literature Review of In Vivo Animal and Clinical Data

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Abstract

Study Design Literature review.

Objective Degenerative disk disease (DDD) has a negative impact on quality of life and is a major cause of morbidity worldwide. There has been a growing interest in the biological repair of DDD by both researchers and clinicians alike. To generate an overview of the recent progress in reparative strategies for the treatment of DDD highlighting their promises and limitations, a comprehensive review of the current literature was performed elucidating data from in vivo animal and clinical studies.

Methods Articles and abstracts available in electronic databases of PubMed, Web of Science, and Google Scholar as of December 2014 were reviewed. Additionally, data from unpublished, ongoing clinical trials was retrieved from clinicaltrials.gov and available abstracts from research forums. Data was extracted from the most recent in vivo animal or clinical studies involving any of the following: (1) treatment with biomolecules, cells, or tissue-engineered constructs and (2) annulus fibrosus repair.

Results Seventy-five articles met the inclusion criteria for review. Among these, 17 studies involved humans; 37, small quadrupeds; and 21, large quadrupeds. Findings from all treatments employed demonstrated improvement either in regenerative capacity or in pain attenuation, with the exception of one clinical study.

Conclusion Published clinical studies on cell therapy have reported encouraging results in the treatment of DDD and resultant back pain. We expect new data to emerge in the near future as treatments for DDD continue to evolve in parallel to our greater understanding of disk health and pathology.

Keywords
- intervertebral disk
- disk regeneration
- back pain
- growth factor
- cell therapy
- platelet-rich plasma
- tissue engineering
- annular repair

Introduction

Occurring in 40% of individuals younger than 30 and in more than 90% of those older than 50 years of age,[1,2] intervertebral disk (IVD) degeneration is a commonly diagnosed disorder that can lead to nerve compression and chronic back pain. Though pharmacologic and physiotherapeutic treatments relieve early symptoms, surgical intervention is eventually required in nearly 4 million patients worldwide.[3] Spinal fusion surgery, most commonly performed in degenerative

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disk cases, presents risks for pseudarthrosis and adjacent segment disease, resulting in higher rates of reoperation in patients.[4,5] Prosthetic total disk replacement (TDR) devices, developed to maintain segmental mobility, are an alternative to fusion surgery. However, recent studies have shown that not only spinal fusion but also TDR alters spine biomechanics leading to adjacent segment disease.[5,6] Hence, it remains controversial whether the theoretical advantage of TDR truly translates to clinical or radiologic superiority over fusion surgery.[5,6] Current treatment options to disk degeneration, both conservative and surgical, fail to treat the underlying etiology; the degenerated disk remains unrepaired.

To overcome the limitations of available treatments, biological repair has emerged as a feasible way to treating pathologic disk segments. Strategies utilized in biological IVD repair are specific to the stage of degeneration and can be classified into three categories: biomolecular therapy, cell therapy, and tissue-engineered IVD construction (►**Fig. 1**).[7–9] Early degenerated disks with sufficient populations of viable cells is treated with biomolecules, such as recombinant genes or proteins (►**Fig. 2**). These agents can enhance selective protein expression to decrease catabolic or increase anabolic cascades, favoring extracellular matrix (ECM) regeneration. Midstage degeneration, characterized by less active and rapidly disappearing viable cells, is treated with cell implantations to meet the increased demand of the disk. When the disk structure and function are severely compromised, reaching terminal stage degeneration, implantation of tissue-engineered disklike constructs is the most potent option for reconstruction of the disk segment. Each treatment modality utilized can target specific structures of the IVD. The IVD is an intricate complex composed of diverse but interrelated tissues: the central gelatinous and highly hydrated nucleus pulposus (NP), the stiffly composed annulus fibrosus (AF) surrounding the NP, and the cartilaginous end plates that connect these tissues to the vertebral bodies provide ample nutrition. Given that degeneration is a multifaceted process that involves the NP, AF, and end plate, one or all components can be targets of potential biological repair.

For many years, NP tissue has been the principal focus of disk repair in early to midstage degeneration, but interest has recently turned to the AF as a more practical target.[10] Strategies targeted at NP, primarily through transannular approaches, compromise the AF tissue.[11] Additionally, an annular defect is the underlying etiology for patients with lumbar disk hernia; diskectomies to remove herniated disks can unveil annular defects, which are often overlooked and left untreated by surgeons due to the limited reparative options. Persistent annular defects are related to reherniation and progressive disk degeneration postdiskectomy.[12,13] Hence, annular repair is an emerging but significant facet in the treatment of degenerative disk disease (DDD). In the present study, we review in vivo animal and clinical studies of biological disk repair. Integrating degenerative stage-specific and IVD component-specific therapy helps elucidate a multifaceted treatment module for disk degeneration. An analysis of the varying research, previous and current, in degenerated disk repair offers greater insight into potential clinical applicability, propelling their transition from bench to bedside.

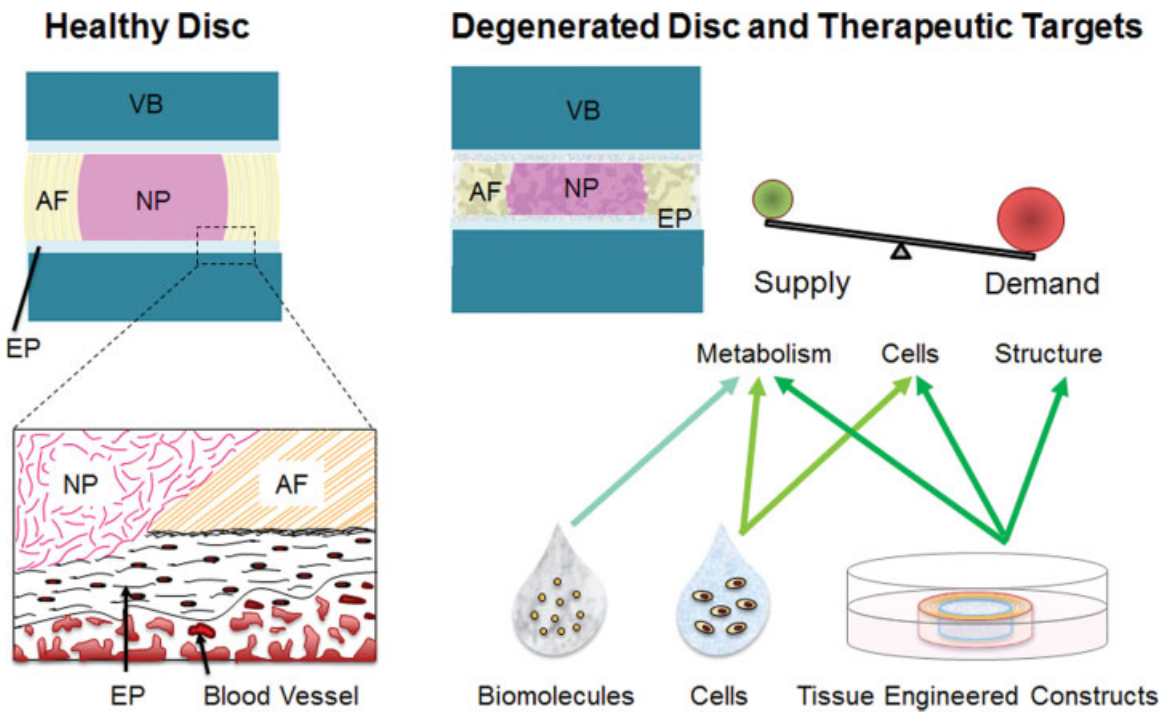

Fig. 1 Schematic picture of intervertebral disk unit, pathologic conditions, and potential treatments. Schematic pictures of the healthy disk show three components of the disk both macro- and microscopically. In degenerated disks, metabolism, cells, and structure encounter imbalance of supply and demand, one, some, or all of which each strategy will redress. Abbreviations: AF, annulus fibrosus; EP, end plate; NP, nucleus pulposus; VB, vertebral body.

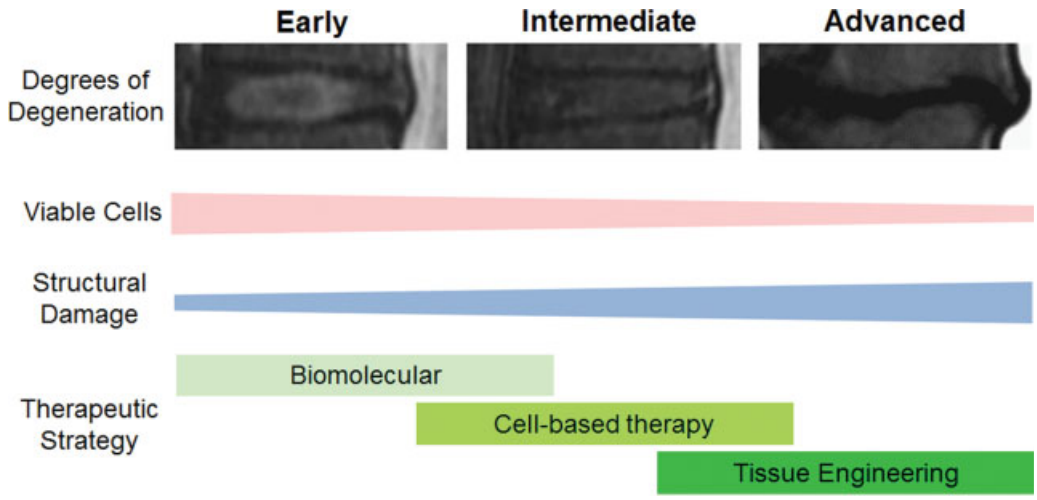

Fig. 2 Strategy for biological intervertebral disk repair. As degeneration progresses, the disk loses viable cells and structure. Biomolecular therapy will repopulate the disk with sufficient responding cells. If those cells are unviable, cell implantation can be necessary. If the structure is completely destroyed, tissue-engineered constructs that mimic the properties of native disks will help with biological reconstruction of the segment.

Methods

A comprehensive search of the literature was performed. Full publications and abstracts available in the electronic databases of PubMed, Web of Science, and Google Scholar as of December 2014 were reviewed. Additionally, data from unpublished, ongoing clinical trials was retrieved from clinicaltrials.gov and available abstracts from research forums. After the initial search, the results were reviewed, duplicates were excluded, and only the relevant studies were included. The primary inclusion criterion was the presence of in vivo and/or clinical results on disk regeneration. The secondary inclusion criterion was the ability to be categorized into one of the following categories: (1) biomolecular therapy, (2) cell-based therapy, (3) tissue-engineered IVD construction, (4) AF repair, (5) published and ongoing clinical studies. After article selection, we extracted the following outcome measures for in vivo animal studies: disk height based on X-ray, signal intensities and degenerative grade based on magnetic resonance imaging (MRI), histologic assessment of reparative tissue, proteoglycan content, and biomechanical functionality. For clinical studies, we extracted questionnaire-based subjective assessment on preexisting pain and physical function in addition to radiologic outcome measures.

In Vivo Animal Studies

Biomolecular Treatment

A defining compositional change in degenerated disks is the gradual decline of NP water content emanating from the loss of proteoglycan and collagen. The loss of swelling pressure in the NP is followed by the loss of mechanical tension in the AF collagen fibers, resulting in abnormal loading of the spine. These alterations often lead to segmental instability with the development of neck or back pain and narrowing of the spinal canal, which may induce neurologic symptoms. In early degeneration, the disk undergoes an imbalance of anabolic and catabolic factors that leads to ECM degradation. Biomolecules such as recombinant proteins and genes can regenerate expression of target molecules through the increase in anabolic or decrease in catabolic factor production, thus facilitating ECM synthesis. The following section will review recent in vivo studies on biomolecules used to treat disk degeneration[14–40] (►**Table 1**).

Protein Injection

Protein solutions directly injected into disks can stimulate cell growth or anabolic responses that may reverse disk degeneration. Since the demonstration of the disk's responsiveness to exogenous growth factors in an ex vivo organ culture system,[41] the various proteins capable of modulating cell growth, differentiation, and ECM synthesis have shown promise in treating degenerative disk disease (DDD). Bone morphogenic proteins (BMPs) such as BMP2, BMP7 also known as osteogenic protein 1 (OP-1), and BMP14 or growth differentiation factor-5 (GDF-5), as well as other members of transforming growth factor (TGF)-β superfamily such as TGF-β1 or TGF-β3 have induced bone and cartilage formation. Their application has been well studied not only in cases of spinal arthrodesis but also disk regeneration.[14–20,22,31] In a single in vivo rabbit study by An et al, intradiscal OP-1 injection induced an increase in proteoglycan (PG) content of NP at 2 weeks and disk height at 8 weeks[14,19]; the treatment has now segued into clinical trials. Protein injection, though promising, is challenged by the short duration of its therapeutic effect, which may be overcome with the development of slow-release carriers or gene-based delivery systems.

Gene Therapy

Gene therapy induces modification of intradiscal gene expression for prolonged effect on degenerated disks. The genes of interest are delivered through either viral (mostly adenovirus) or nonviral vectors, which are then either

Table 1 List of in vivo study of biomolecular treatment

Species	Model	Molecules	Dose	Outcome	Reference
Protein injection					
Rat	Compression	IGF-1, GDF-5, TGF-β, bFGF	IGF-1: 8 ng/8 μL/disk, GDF-5: 8 ng/8 μL/disk, TGF-β: 1.6 ng/8 μL/disk, bFGF: 8 ng/8 μL/disk	GDF-5 and TGF-β aid in expansion of inner annular fibrochondrocytes into the nucleus	16
Rat	Compression	BMP-7 (OP-1)	0.2 μg/μL/disk	OP-1 stimulates anabolic response characterized by the restoration of normal disk morphology	22
Rabbit	Normal	BMP-7 (OP-1)	2 ng/10 μL/disk	Increase in disk height	14
Rabbit	Chemonucleolysis by C-ABC	OP-1	100 μL/10 μL/disk	Increase in disk height and PG content	15
Rabbit	Needle puncture	BMP-7 (OP-1)	100 μg/10 μL/disk	Improvement in disk height and MRI findings	17
Rabbit	Needle puncture	GDF-5	1,100 ng, 1,100 μg/10 μL/disk	Increase in disk height	18
Rabbit	Needle puncture	OP-1	100 μg/10 μL/disk	Increase in disk height and PG content of the NP	19
Rabbit	Annular tear 5 × 7 mm	BMP-2	100 μL/10 μL/disk	Exacerbated degeneration	20
Rabbit	Nucleotomy	PRP	20 μL PRP + microsphere / disk	Less degeneration, more PG	21
Rabbit	Nucleotomy	PRP	20 μL PRP + microsphere/disk	Improvement in disk height and water content	23
Rabbit	Annular puncture	PRP-releasate	20 μL/disk	Better X-ray and MRIs	25
Sheep	Annular incision	BMP 13	300 ug/70 μL saline	BMP 13 prevents loss of hydration	24
Gene therapy					
Rat	Degenerative model induced by unbalanced dynamic and static force	Lentivral CHOP shRNA	1×10^6 PFU/2 μL/disk	Significant decrease of apoptotic incidence in cells treated with CHOP shRNA at 7 wk	30
Rat	Normal	Plasmid DNA mixed with microbubbles	2 ug/2 μL/disk	Reported genes were expressed up to 24 wk	28
Rabbit	Normal	Ad/CMV-hTGFβ1	6×10^6 PFU/15 μL/disk	Leads to double proteoglycan synthesis	26
Rabbit	Normal	Ad-LMP1	1×10^7 PFU/10 μL/disk	LMP1 overexpression increases PG, BMP-2, and BMP-7	27
Rabbit	Annular puncture	ADAMTS5 siRNA oligonucleotide	10 ug/10 μL/disk	Improvement in MRI and histological scores	29
Rabbit	Annulotomy	AAV2-BMP2 or-TIMP1	6×10^6 virus particles/15 μL/disk	AAV-BMP2 and -TIMP1 delayed degeneration	31
Rabbit	Postannulotomy	Ad-Sox9	1×10^9 PFU/10 μL/disk	AdSox9 helped retain chondrocytic appearance, cellular morphology, and ECM at 5 wk	32

Abbreviations: AAV2-BMP2, adeno-associated virus serotype 2 vectors expressing bone morphogenetic protein 2; Ad/CMV-hTGF β1, adenovirus/cytomegalovirus vector expressing human transforming growth factor-beta 1; ADAMTS5, disintegrin and metalloproteinase with thrombospondin motifs 5; Ad-LMP1, adenoviral vectors expressing Lim Mineralization Protein-1; Ad-Sox9, adenoviral vectors expressing Sox9; bFGF, basic fibroblastic growth factor; BMP, bone morphogenic protein; C-ABC, chondroitinase-ABC; CHOP, C/EBP homologous protein; ECM, extracellular matrix; siRNA, small interfering RNA; GDF-5, growth differentiation factor-5; IGF-1, insulin-like growth factor-1; OP-1, osteogenic protein 1; PFU, plaque forming units; PRP, platelet-rich plasma; shRNA, small hairpin RNA; TGF-β, transforming growth factor- β; TIMP1, tissue inhibitor of metalloprotainase-1.
Note: Animal species and model of disk degeneration are shown. "Normal" means normal healthy disks were treated without any induction of degeneration. Biomolecules employed and doses are also shown. Outcomes are briefly summarized with reference information.

directly injected into live tissue (in vivo gene therapy) or transduced/transfected into cells in vitro prior to in vivo transplantation (ex vivo gene therapy or cell-based gene therapy).[42] In one of the earliest in vivo studies in a rabbit model, when the NP cells were transduced with TGF-β1-expressing adenovirus vector, proteoglycan synthesis increased by 100% in the treated tissue.[26] Since then, a variety of proteins have surfaced as promising targets for gene therapy including upstream proteins such as LIM mineralization protein-1 (LMP-1), which regulates BMP-2 and BMP-7, ECM degrading enzymes (disintegrin and metalloproteinase with thrombospondin motifs 5), their inhibitors (tissue inhibitor of metalloprotainase-1), chondrocyte-specific transcription factors (SRY-box 9, Sox9), and apoptosis inducers (C/EBP homologous protein).[26–32] Though gene therapy is advantageous in its sustained effect, the inherent risk of viral gene delivery systems becoming infectious or immunogenic has relayed interest toward nonviral gene delivery systems. Microbubble-enhanced ultrasound gene therapy and injection of small interfering RNA have been shown to achieve long-standing transgene expression in IVD cells in vivo.[28,29] However, nonviral gene delivery systems are curtailed by low transfection efficiency, which must be addressed to enhance their clinical applicability. The feasibility of ex vivo gene therapy, which reduces the risks of infection and immunogenicity and plays an important role in the future of tissue-engineering technology, has been explored in a few studies.[43,44]

Platelet-Rich Plasma

Platelet-rich plasma (PRP), an autologous blood product produced by the centrifugation of whole blood, offers an attractive cocktail of proteins for the treatment of degenerative disks due to its high concentration of platelets. Upon activation, these platelets release a variety of multifunctional growth factors such as platelet-derived growth factor, insulin-like growth factor, TGF-β1, vascular endothelial growth factor, and basic fibroblastic growth factor. When used in early onset disk degeneration, PRP can better enhance disk hydration.[45] Various PRP technologies have emerged to retard the degenerative cascade, including a gelatinous hydrogel scaffold impregnated with PRP and soluble releasate derived from activated PRP.[21,23,25] The in vivo efficacy of PRP in yielding better disk height and hydration has facilitated its transition to ongoing clinical trials.

Cell Therapy

Biomolecules are of limited efficacy in disks with higher grades of degeneration, as the number of cells responsive to injected genes and proteins declines with the progression of degeneration.[46] Cell therapy is the optimal treatment strategy in midstage degeneration, characterized by a decreased number of cells in the tissue; several in vivo studies reported the efficacy of using a vast array of cell sources (►**Table 2**).[46–75]

Differentiated Cells Such as Disk-relevant Cells and Articular Chondrocytes

Implanted differentiated disk chondrocytes, which can produce demanded ECM components such as proteoglycan and collagen type II and I under hypoxia and nutrient stress, can meet the increased cellular and metabolic demands of the disk.[76] Accumulating evidences in an array of animal models demonstrate that autologous or allogeneic disk cells, when transplanted, survive and engraft in the disk, yielding reduction of matrix degradation and recovery of disk height and MRI intensities (►**Table 2**). In fact, the pioneering preclinical study in an injured canine model established that NP disk chondrocyte implantation contributes to ECM regeneration, retarding further disk degeneration.[54] However favorable, disk cell transplantation poses several drawbacks: (1) donor site morbidity; (2) difficulty expanding cells in vitro while maintaining cell phenotype; (3) paucity of allograft donor tissue; and (4) issues of immunocompatibility and disease transmission. Similar to differentiated disk cells, cultured articular chondrocytes are a well-established nondisk cell source in regenerative medicine.[77] Their easy extraction from non-weight-bearing parts of the knee and capacity to produce NP-like ECM when transplanted in vivo makes autologous or allogenic articular chondrocytes a safe and feasible cell source in IVD regeneration.[48,67] Furthermore, potential immune evasion by juvenile articular chondrocytes supports their applicability in allogenic cell transplantation.

Stem Cells

The multipotent mesenchymal stem cells (MSCs) present in adult bone marrow or adipose tissue can replicate as undifferentiated cells, then differentiate into lineages of mesenchymal tissue: bone, cartilage, fat, tendon, muscle, and marrow stroma.[78] These somatic stem cells are potentially an ideal option for disk repair due to their accessibility and ability to differentiate along a chondrogenic lineage and produce the required proteoglycan and collagen for the disk ECM. The feasibility of MSCs to facilitate disk repair has been substantiated. Yet, it remains controversial whether differentiated cells or stem cells are superior in their regenerative capacity of disk morphology. A porcine study comparing the utility of different cell sources found that committed articular chondrocytes are better suited for use in disk repair than MSCs due to their aptness for survival in the ischemic disk microenvironment.[67] Interestingly, a comparative rabbit study found that MSC transplantation can serve as an ideal substitute for differentiated chondrocytes of disk NP owing to better accessibility with equivalent regenerative potential.[68] Studies assessing the combination of both cells demonstrated that in vitro coculture or coimplantation yields better in vivo performance of the implanted cells.[47,69] Nonetheless, pluripotent embryonic and induced pluripotent stem cells,[79–81] unlike the lower potent MSCs, have unlimited proliferative and differentiative capacities, which can be strategically exploited in cell-based disk repair. Sheikh et al extracted murine embryonic stem cells (ESCs) and differentiated them into chondroprogenitor cells; upon implantation into rabbit injured disks, these cells induced notochordal cell formation at the site of injury sans xenograft-associated immune responses.[59] Unstable in vitro differentiation into desired cell lineages and in vivo risks of tumor formation are still major obstacles in the use of ESCs and induced pluripotent stem

Table 2 List of in vivo study of cell therapy

Species	Model	Cell type	Dose	Outcome	Reference
Mouse	Postannular injury	Allogenic bone marrow MSCs	BMSCs 1.0×10^3	ECM augmented in NP via autonomous differentiation and stimulation of endogenous cells at 12 wk	61
Mouse	Annular puncture	Multipotent stem cells derived from human umbilical cord blood	1.0×10^3 cells intradiscally, 1.0×10^6 cells intravenously	Unlike intradiscal injection, intravenous injection did not preserve the IVD architecture nor disk height at 14 wk	71
Sand rat	Diskectomy	Autologous disk cells	1.0×10^4 cells/5 μL/2-mm^3 Gelfoam (Pfizer, New York, NY, United States)	Implanted disk engrafted with the host disk for up to 8 mo	46
Rat	Normal	Bone marrow MSCs	5.0×10^5/50 μL hyaluronan gels	MSCs maintained viability and proliferated over 28 d	49
Rat	Postannular puncture	Human bone marrow MSCs	1.0×10^6/15 μL	Human MSCs survived for 2 wk posttransplantation, increasing disk height and MRI intensity	62
Rat	Postannular puncture	Adipose-derived MSCs	1.0×10^6/50 μL	Disks maintained disk height and restored MRI signal intensity	63
Rat	Nucleotomy	Coculture of NP cells and MSCs	2.5×10^5 cells (25% NP cells and 75% MSCs)	Bilaminar coculture pellet of NP cells and MSCs outperformed solely NP cells or MSCs at 5 wk	69,70
Rabbit	Nucleotomy	Allogeneic NP cells	5.0×10^4 cells/20 μL	Histology indicated delayed degeneration at 16 wk	47
Rabbit	Nucleotomy	Autologous articular chondrocytes	2.0×10^6/150 μL	Chondrocytes survived and produced hyaline-like cartilage at 6 mo	48
Rabbit	Normal	Allogeneic bone marrow MSCs	1.0×10^5 cells	MSCs survived and enhanced PG synthesis	50
Rabbit	Postnucleotomy	Autologous MSCs	4.0×10^4/40 μL Atelocollagen (CELLGEN, Koken, Tokyo, Japan))	Improved disk height, MRIs, and histology at 48 wk	51–53
Rabbit	Postannular injury	Autologous bone marrow MSCs	1.0×10^5/25 μL	Injection of MSCs significantly increased PG synthesis in severely degenerated disks at 16 wk	56
Rabbit	Normal	Allogeneic MSCs	1.0×10^5/15 μL	Injected cells engrafted into inner annulus fibrous at 24 wk	57
Rabbit	Postpuncture	Xenogeneic derivatives of embryonic stem cells	1.0×10^6 cells/20 μL	New notochordal cells observed; no immune response elicited	59
Rabbit	Nucleotomy	Allogenic synovial MSCs	1.0×10^7 cells/100μL PBS	Implanted cells labeled with DiI or GFP detected at 24 wk; disk height and MRI signal intensity were maintained	64

Table 2 (*Continued*)

Species	Model	Cell type	Dose	Outcome	Reference
Rabbit	Compression	Allogenic bone marrow MSCs	0.08 mL of 1.0×10^6 cells/mL	Combination of MSC injection and distraction led to better disk height and histology at 8 wk	66
Rabbit	Postnucleotomy	Autologous NP cells and allogeneic MSCs	1.0×10^6/20 μL	Both NP cells and MSCSs better maintained disk height and GAG content at 16 wk	68
Canine	Postnucleotomy	Disk cells	6.0×10^6 cells/1 mL/disk	Disk remained viable, produced ECM, better maintained disk height	54,55
Canine	Postnucleotomy	Autologous MSCs	1.0×10^6/mL stem cells	MSCs led to better disk height, MRI, and histology grading at 12 wk	58
Canine	Postnucleotomy	Bone marrow MSCs	10^5, 10^6, 10^7 cells	The disk treated with 106 MSCs had more viable cells than 105 and less apoptotic cells than 105 cells at 12 wk	65
Porcine	Postnucleotomy	Human MSCs	0.5×10^6/hydrogel carrier	Implanted cells survived and differentiated into disklike cells at 6 mo	60
Porcine	Nucleotomy	Allogeneic juvenile chondrocytes and MSCs	$7–10 \times 10^6$/0.5–75 mL fibrin carrier	JC outperformed MSCs in proteoglycan synthesis at 12 mo	67

Abbreviations: BMSC, bone marrow stem cell; ECM, extracellular matrix; GAG, glycosaminoglycan; IVD, intervertebral disk; JC, juvenile chondrocyte, MRI, magnetic resonance imaging; MSC, mesenchymal stem cell; NP, nucleus pulposus; PBS, phosphate-buffered saline; PG, proteoglycan.
Note: Animal species and model of disk degeneration are shown. "Normal" means normal healthy disks were treated without any induction of degeneration. Cells employed and dose are also shown. Outcomes are briefly summarized with reference information.

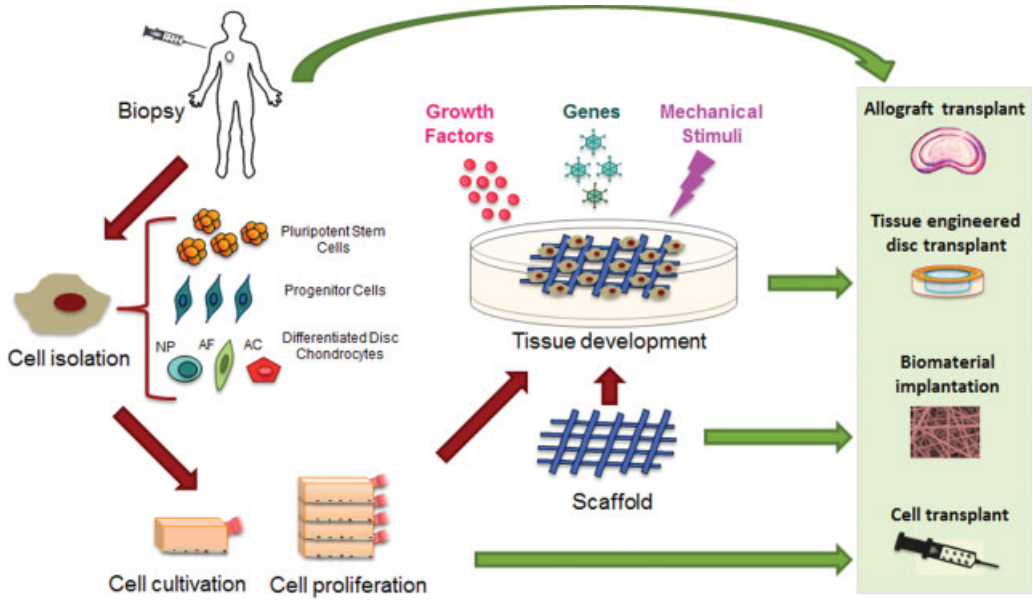

Fig. 3 Cell therapy and tissue engineering. Allograft transplant is the simplest biological disk repair; however, it has critical drawbacks such as limited availability of healthy donor disks and potential risk of disease transmission. Cells harvested from different sources can be expanded in vitro and transplanted in vivo in cell transplant for disk regeneration. Scaffolds can be combined with cells, and, if they have biomimicking properties, these treatments can be regarded as a part of tissue-engineering strategy, which traditionally included cells, scaffolds, growth, and factors, but recently included gene treatment and mechanical conditioning. Abbreviations: AC, articular chondrocytes; AF, annulus fibrosus cells; NP, nucleus pulposus cells.

cells. However, if these issues are overcome, both cells may offer unparalleled promise in degenerative disk repair.

Tissue-Engineering Strategy

Since Langer and Vacanti pioneered the multidisciplinary field of tissue engineering in 1993, much effort has been directed toward the construction of functional substitutes for damaged disk tissues. Tissue engineering originally consisted of three and more recently four components[82]: scaffolds, cells, growth factors, and physical conditioning using electrical or mechanical stimuli (►**Fig. 3**). Because extensive loss of matrix and structural damages are exhibited in advanced stages of disk degeneration, the development of biocompatible and biomimetic scaffolding materials based on engineering innovation can facilitate the recovery of native biological and biomechanical functionality. Numerous studies have assessed tissue-engineered components as well as whole-disk constructs of the disk in vivo (►**Table 3**).[43,83–103]

Scaffold Development

Numerous scaffold materials that mimic the mechanical and biochemical properties of the native NP have been studied including alginate, silk-fibrin/hyaluronic acid composites, Atelocollagen, synthetic polymers, and collagen 2/hyaluronan/chondroitin-6-sulfate composites. Hyaluronic acid, a native NP ECM component, has been extensively studied in vivo.[85–88,94] In a rabbit study, resorbable cell-free implants made of a polyglycolic acid felt, hyaluronic acid, and serum induced disk regeneration after diskectomy, resulting in improved disk hydration and disk height 6 months after microdiskectomy.[86] Cells are frequently used in conjunction with biomimicking materials to encourage de novo ECM production. Ganey et al found that adipose-derived stem cells contributed to recovery of T2 intensity and disk height in a canine disk injury model.[87] Synthetic polymers such as polyglycolic acid or poly (L-lactic-co-glycolic acid) have also been used to construct cell-laden tissue-engineered composites either solely or in combination with hydrogels.[86,89]

Whole Disk Transplantation with Tissue-Engineered Construct

Advanced tissue engineering enables whole IVD construction in vitro, which can be implanted in vivo through TDR. The current standard in whole IVD implantation involves NP and AF composites that replace the structurally damaged tissues of a severely degenerated disk. The first tissue-engineered whole IVD, implanted in vitro within the subcutaneous dorsum of athymic mice, comprised an NP cell-laden polyglycolic and polylactic acid (polyglycolic acid) and AF cell-laden alginate.[84,104] Our group was the first to perform an in vivo implantation of anatomically correct whole tissue-engineered IVDs (TE-IVDs) in the athymic rat tail model; the implanted disks engrafted into the disk space for up to 6 months and exhibited analogous biological, biochemical, and biomechanical properties as the native disks (►**Fig. 4**).[93,105–107] To bring this innovation closer to clinical application, we recently performed TDR using TE-IVDs in the canine cervical spine, characterized by axial loading due to their upright neck, which most closely resembles that in humans. Our preliminary data with 1-month follow-up revealed that TE-IVDs when implanted successfully remained viable in the disk segment, engrafted to the host tissue, and partially maintained disk height.[108] Nonetheless, the addition of growth factors or bioactive molecules can encourage de

Table 3 List of in vivo study of tissue engineering-based treatment

Species	Model	Construct	Outcome	Reference
Rat	Subcutaneous implantation	TE-IVD composed of a NP cell-laden alginate surrounded by an AF cell-laden PGA/PLA	Biochemical markers of matrix synthesis, increasing over time, were similar to native tissue at 12 wk	84
Rat	Subcutaneous Implantation	Porous CII/HyA/CS	CII/HyA/CS scaffolds had satisfactory cytocompatibility and histocompatibility, as well as low immunogenicity	88
Rat	Subcutaneous implantation	Composite IVD consisting of demineralized bone matrix gelatin and CII/HyA/CS scaffolds seeded AF and NP cells	Implant, similar to native disk in morphology and histology, increased proteoglycan synthesis over 12 wk	92
Rat	Total diskectomy	TE-IVD composed of a NP cell-laden alginate surrounded by an AF cell-laden collagen layer	TE-IVD maintained disk space height, produced de novo ECM, and integrated into the spine—yielding intact motion segment with dynamic mechanical properties similar to that of native IVD	93
Rat	Subcutaneous implantation	5.0×10^6 cells/mL in PPS containing polyethylene glycol/HA	MPC/hydrogel composites formed cartilage-like tissue, well tolerated by the host	99
Rabbit	Laser diskectomy	2.0×10^6 cells/Atelocollagen honeycomb-shaped scaffold	AF cells survived and produced hyaline-like cartilage in the disk at 12 wk	83
Rabbit	Microdiskectomy	Cell-free implant composed of a PGA felt, HA, and allogenic serum	Implantation of a cell-free PGA-HA implant immersed in serum after diskectomy improved disk hydration and preserved disk height 6 mo after surgery	86
Rabbit	Postnucleotomy	2.0×10^6 bone marrow MSCs in 0.04 mL PFG-TGF-β1	MSCs-in-PFG-TGF- ß 1 group had less degeneration and a slower decrease in disk height compared with both degenerative and acellular PFG-TGF-ß1 group	90
Rabbit	Nucleotomy	Allogenic NP cell-seeded CII/HyA/CS tri-copolymer construct	Viability of allografted NP cells, extracellular matrix deposition, and disk height maintenance; restoration of T2 MRI signal intensity observed at 24 wk	94
Rabbit	Postpuncture	5.0×10^3 allogenic bone marrow MSCs/10 µL hydrogel	MSCs suppressed collagen I in NP, reduced collagen aggregation, and maintained proper fibrillary properties and function	101
Rabbit	Postnucleotomy	1.0×10^6 human NP cell line infected with HNPSV-5 in Atelocollagen	Deceleration of disk degeneration was evident after HNPSV-5 transplantation as shown by disk height and histologic examination at 24 wk	102
Canine	Total diskectomy	Cell-allograft IVD composites made of allograft and NP cells, with in vitro transduced with r(AAV)-hTERT	The hTERT-loaded NP cells intervention could effectively resist the degeneration of the allogenic transplanted IVD at 12 wk	43

(*Continued*)

Table 3 (*Continued*)

Species	Model	Construct	Outcome	Reference
Canine	Postnucleotomy	ADRC in HA carrier	Disk that received ADRC produced matrix and resembled native disk in morphology at 12 mo	87
Canine	Nucleotomy	Cell-scaffold composite made of three-dimensional porous PLGA scaffolds and NP cells	Disk height, segmental stability, and T2-weighted MRI signal intensity were well preserved at 12 wk	89
Porcine	Nucleotomy	Cell-scaffold composite made of NP cells and injectable hyaluronan-derived polymeric substitute material HYADD (1.0×10^5 cells/mL)	Injected disks had a central NP-like region similar to the normal disk biconvex structure and viable chondrocytes forming matrix like that of normal disk at 6 wk	85
Porcine	Post annular injury	1.25×10^5 autologous MSCs/mL in either Hydrogel PhotoFix (Zimmer Biologics, Inc., Austin, TX, United States) or HA (Zimmer Biologics, Inc.)	Stem cells in hydrogel treatment had significantly higher T2 MRI intensities and lower degeneration grade at 24 wk than hydrogel alone treatment	95
Porcine	Partial nucleotomy	5.0×10^5 autologous bone marrow MSCs transduced with retrovirus encoding luciferase in 1 mL hyaluronan-enhanced albumin hydrogel	In vivo 3-d analysis showed persistent metabolically active implanted cells in the disk	100
Goat	Postdisk injury	2.5×10^5 allogenic bone marrow stromal cells/10 μL PBS + 30 μL chondroitin sulfate-based hydrogel	Significant increase in NP proteoglycan accumulation at 6 mo	96
Sheep	Total diskectomy	Noncrystalline polylactide copolymer interbody cages filled with1.0×10^6 allogenic MPC-laden Gelfoam sponge (Pfizer, New York, NY, United States) formulated with the chondrogenic agent PPS	Biodegradable cage-contained MPCs in combination with PPS produced cartilaginous tissue at 3 mo	91
Sheep	Post-chondroitinase-ABC injection	4.0×10^6 or 0.5×10^6 human MPCs suspended in HA	High-dose injection improved histopathology scores at 3 mo, while low dose at 6 mo	97
Sheep	Nucleotomy	Allogenic or autologous disk cells ($0.4–2.0 \times 10^6$ cells/ 0.5–1 mL hydrogel) in hydrogel containing HA and maleolyl-albumin	Biological repair of traumatic damage occurs in sheep disks at 6 mo; hydrogel-supported disk cells may be beneficial	98

Abbreviations: ADRC, autologous adipose tissue derived stem and regenerative cells; AF, annulus fibrosus; CII, type II collagen; CS, chondroitin-6-sulfate; HA, hyaluronic acid; HNPSV-5, recombinant SV40 adenovirus vector; hTERT, human telomerase reverse transcriptase; HyA, hyaluronate; HYADD, an amide of hyaluronan-derived polymeric substitute materials; IVD, intervertebral disk; MPC, mesenchymal precursor cell; MRI, magnetic resonance imaging; MSCs, mesenchymal stem cells; NP, nucleus pulposus; PBS, phosphate-buffered saline; PFG-TGF-β1, fibrin glue containing 10 μg/L TGF-β1; PGA, polyglycolic acid; PLA, polyglycolic acid; PLGA, poly (L-lactic-co-glycolic acid); PPS, pentosan polysulfate; (rAAV)-hTERT, recombinant adenoassociated virus vector-mediated hTERT gene; TE-IVD, tissue-engineered IVD; TGF-β1, transforming growth factor-β1.
Note: Animal species and model of disk degeneration are shown. "Normal" means normal healthy disks were treated without any induction of degeneration. Constructs employed and dose are also shown. Outcomes are briefly summarized with reference information.

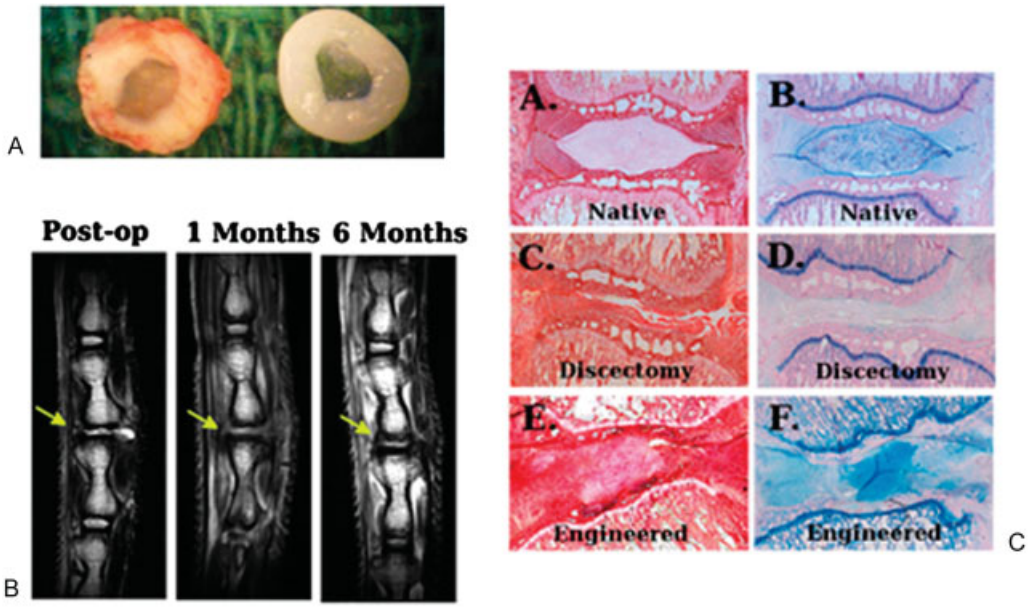

Fig. 4 In vivo implantation of TE-IVD under total disk replacement within rat-tail disks. En bloc total diskectomy was performed in the rat-tail spine. Half of the animals were left untreated and the rest received TE-IVD implants. Resected native disk (right image in A) and TE-IVD (left image in A) are shown. (B) Postoperative T2-weighed magnetic resonance imaging indicating sustained intensity in the disk segment implanted with TE-IVD (yellow arrows in B). (C) Six-month postoperative histology with Alcian blue staining showed implanted TE-IVD integrated with host tissues and production of proteoglycan-rich ECM. The diskectomized segment presented no disklike tissue. This image originates from a previously published study by our group.[93] (Reprinted with permission from Bowles RD, Gebhard HH, Härtl R, Bonassar LJ. Tissue-engineered intervertebral discs produce new matrix, maintain disc height, and restore biomechanical function to the rodent spine. Proc Natl Acad Sci U S A 2011;108(32):13106–13111.) Abbreviations: ECM, extracellular matrix; IVD, intervertebral disk; TE-IVD, tissue-engineered intervertebral disk.

novo ECM deposition. Goldschlager et al demonstrated that adult allogeneic mesenchymal progenitor cells formulated with a chondrogenic agent such as pentosan polysulfate could synthesize a cartilaginous matrix when implanted into a biodegradable carrier and cage and over time might serve as a bioactive interbody spacer following anterior cervical diskectomy.[91] Furthermore, the integration of tissue engineering and gene therapy has been attempted by a group from China that developed a tissue-engineered IVD using an allogenic disk transduced with human telomerase reverse transcripase (hTERT) gene within its NP cells. When implanted in a canine model, the hTERT-loaded NP cells manifested enhanced antidegenerative effect compared with the unloaded NP cell.[43] Such constructions of whole disk implants, the most ambitious therapeutic strategy yet, are met with extensive biological and functional challenges in vivo. Yet, the progressing field of tissue engineering continues to yield promising modifications to meet the higher demands of implanted disks.

Annular Repair

Previous studies of intervertebral disk repair, which aim to halt, delay, or reverse intervertebral disk degeneration, were primarily focused on NP regeneration.[33–38] However, a majority of these strategies are delivered through a punctured AF, which even upon modest injury can generate a degenerative cascade within the disk affecting IVD biomechanics, cellularity, and biosynthesis.[109–112] Annular defects can emerge not only from needle punctures through the AF to reach the NP but also from the early process of intervertebral disk degeneration. Given the sensitivity of the AF, lesions from the NP treatment can provoke further degeneration, inducing leakage of the delivered material and eventual failure of the regenerative treatment. In fact, one retrospective study with 10 years' follow-up found that diskography performed with a small needle puncture accelerated disk degeneration, rate of same-side disk herniation, and changes to the end plate.[12] Another study demonstrated that injecting MSCs through the AF into the NP led to cell leakage and augmented osteophyte formation.[11] Combining an injectable NP regenerative strategy with a sealant that repairs annular defects is the optimal strategy to circumvent leakage of implanted cells or material while enhancing therapeutic outcome. Previous approaches to annular repair have involved mechanical treatments such as suturing and annuloplasty devices, which failed to improve annular healing strength in long-term clinical trials.[113–115] Although several NP regenerative studies and a few in vitro AF studies provide critical insight on the reparative process within the AF tissue,[24,53,83,96,116] there is a very limited number of in vivo studies focusing primarily on annular repair.[107,117–122] Current efforts in the biological treatment for in vivo AF repair include either development of injectable material in conjunction with biologics such as biomolecules/cells or construction of rigid implants derived from synthetic polymer or biological tissue (►**Table 4**). In fact, our group has successfully demonstrated the in vivo efficacy of riboflavin cross-linked high-density collagen gels to facilitate annular repair in a needle-punctured rat-tail model through preservation of NP size, hydration, and prevention of further degeneration (►**Fig. 5**).[107] In another large animal study

Table 4 List of published annular repair studies

Species	Model	Treatment	Outcome	Reference
Rat	Degradation tests with subcutaneous implantation	Fibrin-genipin adehesive hydrogel (Fib-Gen)	60% of Fib-Gen remained at 8 wk and nearly all resorbed at 16 wk; kinetics show better in vivo longevity compared with fibrin	121
Rat	Needle puncture	Injection of cross-linked HDC gels	Cross-linked HDC capable of repairing annular defects most likely due to enhanced stiffness of HDC at 5 wk	107
Porcine	Needle puncture	Injection of Gelfoam (Pfizer, New York, NY, United States), platinum coil, bone cement, and tissue glue	Injection of Gelfoam better improved integrity of punctured disk than the other three to potentially prevent recurrent disk herniation at 2 mo	118
Sheep	Box annulotomy	Patch and plug with SIS and titanium bone screw	SIS-based treatment led to better maintenance of hydration and intradiscal pressure at 26 wk after annulotomy	119
Sheep	Box annulotomy	Triphase AF implant composing two outer phases of absorbable polyglycolic acid and a centric phase of a nonabsorbable PVDF mesh	Implant-treated disks had more reparative tissue, but contrast media leakage tests under provocative pressure did not show the difference between groups	122
Sheep	Microdiskectomy	Allogenic MPCs + PPS embedded in a gelatin/fibrin scaffold	Disks treated with MPC + PPS showed higher PG content than the untreated or ones treated with solely scaffold at 6 mo	120

Abbreviations: AF, annulus fibrosus; HDC, high-density collagen; MPC, mesenchymal progenitor cell; PPS, pentosan polysulphate; PVDF, polyvinylidene fluoride; SIS, small intestinal submucosa.
Note: Animal species and model of disk degeneration are shown. "Normal" means normal healthy disks treated without any induction of degeneration. Treatments employed are also shown. Outcomes are briefly summarized with reference information.

conducted by Oehme et al, injected mesenchymal progenitor cells combined with the chondrogenic agent pentosan polysulfate maintained disk height, disk morphology, and NP proteoglycan content post-microdiskectomy in a sheep model.[120] Despite the few studies dedicated to annular repair, more attention is now being paid to this field given its enhancement of even NP-targeted therapy.

Clinical Studies

Published Clinical Trials

To date, only few clinical trials targeting IVD repair or regeneration have been published (►**Table 5**).[123–130] In 2002, a multicenter prospective, randomized, controlled, nonblinded EuroDISC study comparing the safety and efficacy of autologous disk chondrocyte transplant implanted 12 weeks after diskectomy concluded that the implant attenuated low back pain and preserved disk height over a 2-year span.[55,125,131] The commercially available chondrotransplant DISC is now available for autologous disk chondrocyte transplant. More recently, a variety of stem cells entered the spotlight due to their differentiative and proliferative capacities. The first stem cell–based clinical study published in the field of disk regeneration was focused on hematopoietic stem cells, although patients' discogenic back pain did not improve after 1 year.[123]

MSCs derived from bone marrow are the most well-studied cells in regenerative medicine due to their accessibility through minimally invasive procedures and expandability in ex vivo conditions. Two studies demonstrated the feasibility of autologous bone marrow MSCs to facilitate disk hydration based on follow-up MRIs over 1 year.[127,128] Orozco et al demonstrated rapid improvement of pain and disability despite unrecovered disk height.[128] In another prospective case series using noncultured bone marrow concentrate as a form of autologous point-of-care biologics, patients with either cervical or lumbar DDD receiving a single injection experienced attenuated discogenic pain and improved function for up to 12 months.

Due to their similar biological profile to disk chondrocytes and potential immunoprivileged property, allogeneic juvenile articular chondrocytes are another promising cell source.

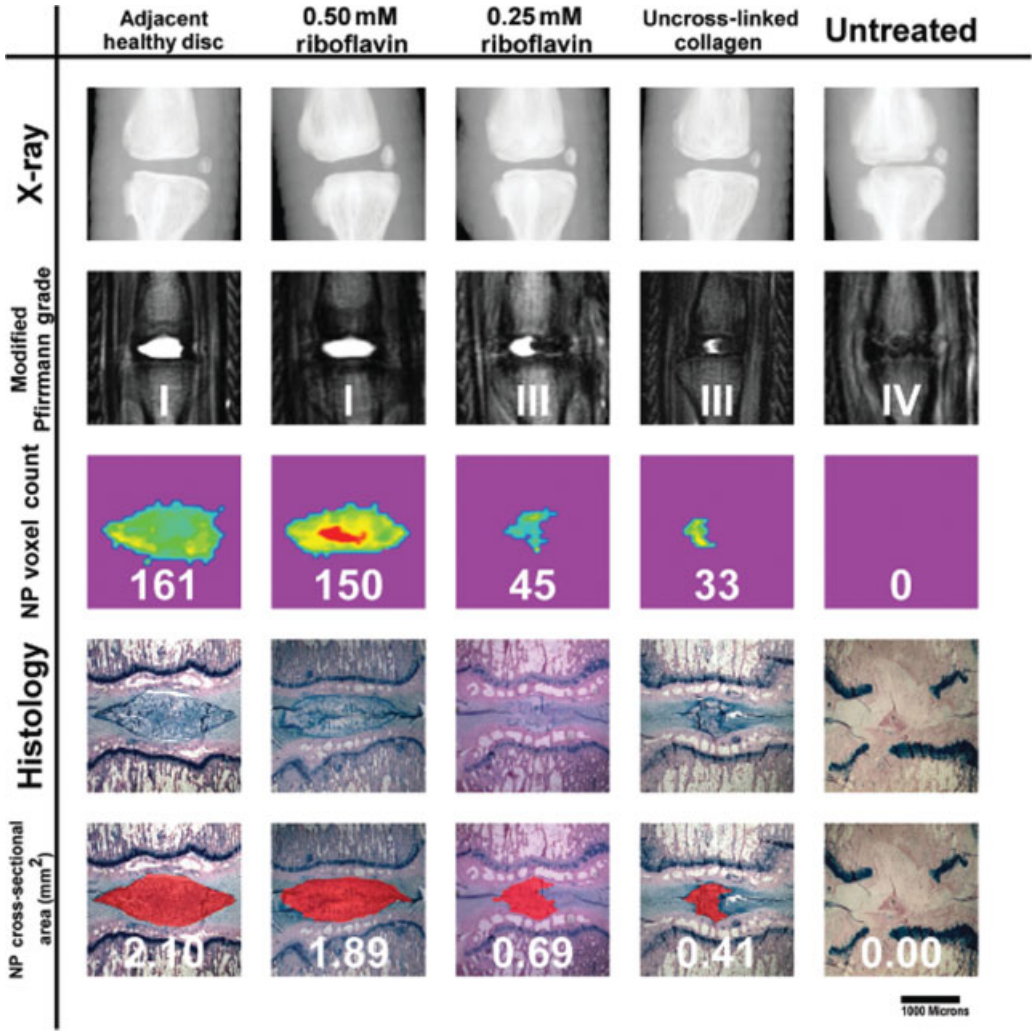

Fig. 5 In vivo annular repair using cross-linked high-density collagen gels. Needle puncture-induced annular injury model in the rat-tail spine was employed to assess feasibility of high-density collagen (HDC) gels to facilitate annular repair. HDC gels were injected into the defect immediately after puncture with an 18-gauge needle. Few HDC gels were supplemented with riboflavin to induce a cross-linking reaction that enhances the stiffness of the gel. In the present study, two distinct concentrations (0.5 and 0.25 mM) were tested along with un-cross-linked HDC without riboflavin (RF). The adjacent segment without puncture and punctured segment without HDC injection served as healthy and model controls, respectively. Five-week outcome examples of all punctured rat-tail disks are shown. The displayed specimen from the 0.5-mM RF group significantly prevented degenerative changes. Disk height, nucleus pulposus (NP) size and hydration, and degeneration grading based on magnetic resonance imaging and histology were all assessed. Both 0.25 RF and un-cross-linked groups had more aggressive degeneration. The untreated group reached terminal degeneration. This image originates from a previously published study by our group.[107]

In a prospective cohort study, Coric et al demonstrated that NuQu (ISTO Techonologies, Inc., St. Louis, Missouri, United States), an injectable percutaneous fibrin-based delivery of juvenile chondrocytes, attenuated otherwise medically refractory low back pain (or pain that does not respond to conservative therapy).[129] A class II study has recently been completed. Despite these studies' promising results, further investigation with a prospective, randomized, double-blinded, placebo-controlled study is necessary to make cell transplantation a valid therapeutic option for DDD.

Although there is no clinical study of tissue-engineering material, efforts have been made to create functional substitutes for NP. Among many clinical studies focusing on NP replacement, a single-center, nonrandomized, prospective feasibility study was undertaken to investigate whether the use of NuCore Injectable Nucleus hydrogel (Spine Wave, Inc., Shelton, Connecticut, United States) post-microdiskectomy prevented early disk collapse to potentially slow the degenerative cascade of the spinal segment over time.[126,132] A group in China was the first to bring a whole allogeneic IVD implantation, inclusive of all three-disk components, to a clinical setting. Though the implant successfully integrated into the host tissue, over the course of 5 years, degeneration was eventually observed. Despite preserving motion in the treated segment,[124] the clinical advantages of allogeneic IVDs are undermined by the limited availability of healthy donor disks and potential disease transmission. De novo tissue-engineered constructs utilizing terminally differentiated cells, yet to reach clinical trials, can potentially overcome limitations of allogeneic implants and yield favorable outcomes.

Table 5 List of published clinical studies

Trial treatment	No. of patients	Study design	Follow-up (mo)	Outcome	Reference
Autologous hematopoietic stem cell injection	10	Case series	12	No patients reported any improvement in their discogenic back pain	123
Total disk replacement with allogeneic IVD	5	Case series	60	Allograft engrafted disk space without apparent immunoreaction; all minus one disk preserved range of motion	124
Autologous disk chondrocyte transplantation (EuroDisc)	28	Control study	24	ADCT with diskectomy shows more pronounced decrease in OPDQ than diskectomy alone	125,131
Injectable biomimetic nucleus hydrogel	14	Case series	24	Significant improvement in leg and back pain after microdiskectomy	126
Autologous bone marrow mesenchymal cell injection	2	Case series	24	Both patients showed improvements in the vacuum phenomenon as well as signal intensity of T2-weighed MRIs	127
Autologous bone marrow mesenchymal cell injection	10	Case series	12	Rapid improvement of pain and disability; disk height was not recovered, but disk hydration was significantly elevated	128
Allogeneic juvenile chondrocytes injection (NuQu; ISTO Technologies, Inc., St. Louis, Missouri, United States)	15	Case series	12	ODI, NRS, SF-36 improved from baseline; 89% of the patients showed improvement on MRI	129
Injection of autologous bone marrow concentrate cells	26	Case series	12	Statistically significant improvement in pain scores and impairment was demonstrated; most dramatic improvement seen in patients with higher CFU-F concentrations; rehydration of the disks observed in 8 of 20 patients	130

Abbreviations: ADCT, autologous disk chondrocyte transplant; CFU-F, colony-forming unit fibroblast; IVD, intervertebral disk; ODI, Oswestry Disability Index; OPDQ, Oswestry Low Back Pain Disability Questionnaire; MRI, magnetic resonance imaging; NRS, Numeric Rating Scale; SF-36, Short Form 36.
Note: Treatments, patient number, study design, follow-up are shown. Outcomes are briefly summarized with reference information.

Nonpublished Ongoing Clinical Trials

Several ongoing clinical trials aiming at disk repair have yet to publish their findings (►**Table 6**).[133–141] The first ever biomolecular treatments with human recombinant growth factors rhBMP-7 (OP-1) and rhGDF-5 (human growth/differentiation factor-5) are currently undergoing clinical trial. PRP or soluble releasate isolated from PRP, both with concentrated growth factors from activated platelets, have been clinically studied in autologous injection models. Akeda et al first demonstrated the feasibility of intradiscal PRP-releasate injection in reducing low back pain in patients with DDD.[140] More recently, a prospective, randomized controlled study assessing the efficacy of intradiscal PRP injections in discogenic mediated low back pain has already shown improvements in pain and function in patients as early as 8 weeks, which was sustained for up to 1 year.139 A 2-year follow-up is currently under process. Mesenchymal lineage adult stem cells (MLCs) (Mesoblast, Melbourne, Australia), the commercially available cell line of in vitro expanded mesenchymal precursor cells (MPCs), is also under clinical trial. The randomized, placebo-controlled phase 2 trial of 100 patients with DDD-induced chronic low back pain already appears promising. Among patients receiving a single injection of either 6 or 18 million mesenchymal progenitor cells, 44 and 42%, respectively, reached target criteria for treatment end point (50% reduction in pain, 15-point improvement in function, and no further treatment needed) at both 6 and 12 months; only 13% of patients administered placebo had the same outcome.[134,142] A few other clinical studies using autologous disk chondrocytes, allogenic juvenile chondrocytes, and autologous and allogenic MSCs derived from bone marrow or adipose tissues are also underway. One such cell-based study is using activated NP cells through in vitro coculture with MSCs to target moderately degenerated disks

Table 6 List of unpublished ongoing clinical trials

Trial	No. of patients	Study design	Follow-up (mo)	Intervention	Assessment	Status	Reference
Safety and efficacy of juvenile chondrocytes injected into the lumbar disk to treat low back pain	44	Double-blind, randomized control, phase 2	24	Single administration NuQu (ISTO Technologies, Inc., St. Louis, Missouri, United States) (allogeneic chondrocyte in fibrin carrier) and a placebo of normal saline	Subject-reported outcomes of pain and disability, health-related quality of life, and subject satisfaction with treatment	Phase II done	133
MPCs (Mesoblast, Melbourne, Australia) in subjects with chronic discogenic lumbar back pain	100	Double-blind, randomized control, phase 2	36	Single dose of either high or low MPCs in a hyaluronic acid carrier and a placebo of either normal saline or hyaluronic acid	MRI, VAS scores, physical exam, and clinical laboratory tests	Phase II done	134
Allogeneic MSCs for DDD	24	Double-blind, randomized, phase 1, 2	12	Single intradiscal transplantation of MSC and sham-treated comparator with drug mepivacaine	VAS, Oswestry Disability Index, and life quality evolution questionnaires; quantitative MRI for disk fluid content	Ongoing	135
Autologous adipose tissue derived mesenchymal stem cells in lumbar IVD degeneration	8	Nonrandomized, open label	6	Injection of autologous adipose-derived MSCs into lumbar IVD at dose 4×10^{e7} cells/1 mL	MRI, VAS scores, physical examination, clinical laboratory tests	Ongoing	136
Adipose tissue-derived stem cells suspended in PRP for DDD	100	Nonrandomized, open label	12	Intradiscal administration of PRP suspended adipose tissue-derived stem cells, obtained from adipose tissue of the abdomen	VAS scores, Oswestry Disability Index, and low back pain questionnaire	Ongoing	137
Intradiscal rhGDF-5 (BMP14) for early stage lumbar DDD	38	Double-blind, randomized, phase 1, 2	36	Single injection of rhGDF-5 into a degenerating lumbar spinal disk	MRI	Ongoing	138
Lumbar intradiscal PRP injections	72; PRP: 48, control: 24	Double-blind, randomized controlled study	6	Single injection of PRP to the patients with concordant discogenic	NRS, modified NASS outcome questionnaire, FRI, SF-36	Ongoing	139

(*Continued*)

Table 6 (*Continued*)

Trial	No. of patients	Study design	Follow-up (mo)	Intervention	Assessment	Status	Reference
				pain and annular disruption			
Intradiscal injection of PRP-releasate for the treatment of lumbar disk degeneration	6	Case series	6	Injection of the soluble releasate isolated form clotted PRP into symptomatic degenerated disks	VAS, RDQ, MRI	Ongoing	[140]
Injection of activated NP cells to moderately degenerated disks adjacent to the fused segment	10	Case series	36	Single injection of autologous NP cells harvested from resected; disk during fusion surgery; activated by in vitro coculture with bone marrow MSCs, and percutaneously injected into adjacent segment	Side effect assessment, blood tests, MRIs	Ongoing	[141]

Abbreviations: BMP14, bone morphogenic protein 14; DDD, degenerative disk disease; FRI, functional rating index; IVD, intervertebral disk; MPC, mesenchymal precursor cell, also termed as mesenchymal lineage adult stem cells (MLCs); MRI, magnetic resonance imaging; MSC, mesenchymal stem cells; NASS, North American Spine Society; NP, nucleus pulposus; NRS, Numeric Rating Scale; PRP, platelet-rich plasma; RDQ, Roland-Morris Disability Questionnaire; rhGDF-5, recombinant human growth/differentiation factor-5; SF-36, Short Form 36; VAS, visual analog scale.
Note: Treatments, patient number, study design, follow-up period, and current status are shown. Intervention and assessment are also briefly summarized.

adjacent to the fused segment. Ultimately, the data emerging from these on-going clinical trials will reinforce findings from published studies and provide new insight for future biological disk repair.

Discussion

The current article is a review study of in vivo experimental and clinical data on treatment approaches for degenerative disk disease, which comprise biomolecular therapy, cell therapy, tissue-engineered construction, and annular repair. A plethora of animal studies have offered critical insight into the regenerative capacity of new IVD therapies due to the similarity in biological and mechanical properties between animals and humans. Yet, the transition from animal to human application is met with challenges. In animal studies, both histologic sections for proteoglycan content and biomechanical testing can be easily evaluated, but these outcome measures are extremely rare in clinical studies. Rather, clinical studies are primarily focused on questionnaire-based subjective assessment on pain and physical function, parameters that are out of scope in animal studies with the exception of two that have developed unique pain assessments in animals.[143,144] Due to these model-based limitations, there is a paucity of studies elucidating direct relation between pain relief and underlying biological repair. Additionally, the animal models may provide mechanistic clues (e.g., genes or signaling factors) to phenotypic manifestations in humans, but complications in comparison arise due to presence of notochordal cells not found in human adults.[9,145] Notochordal cells of NP tissue are present only during the first decade of human life and then are replaced with chondrocyte-like cells, of which the origin has yet to be elucidated. Although cows, horses, and chondrodystrophoid dogs such as beagles demonstrate similar age-related changes to humans, mice, rats, rabbits, pigs, cats, and nonchondrodystrophoid dogs such as mongrels, they are reported to retain notochordal cells past skeletal maturity, which may enhance intrinsic cellular repair.[145] Therefore, results observed in animal models must be carefully translated prior to clinical application.

Within the last decade, there has been significant progress in the field of biological treatments for DDD, despite the few clinical studies published. Injection of biomolecules, such as proteins or genes, and cells can attenuate the degenerative cascade at early to midstages of disease progression. Due to their minimal invasiveness to patients, biomolecular therapy and cellular therapy are easier to maneuver onto clinical application than tissue engineering. In fact, the first clinical trials with rhBMP-7 (OP-1) and BMP-14 (GDF-5) are now underway. Cell therapy, the most exploited therapeutic strategy in both animal and human application, has already been fruitful in treating discogenic pain among patients with nonterminal degeneration. Yet, because all these injectable strategies employ transannular approaches, integration with a treatment for resulting annular defects should be utilized for enhanced clinical outcome.

Annular repair has only recently entered the spotlight due to its potential to spatially retain remnant NP, but merits consideration in its restoration of disk integrity and functionality. TE-IVD, the multicompartment disk analogues using cells and biomaterials, can autonomously regenerate disk morphology and functionality postimplantation. A variety of in vitro studies have indicated the promise of using disk constructs for TDR,[146–149] the most advanced therapeutic strategy for terminally degenerated disks. However, only two translational studies have demonstrated the in vivo efficacy of TE-IVDs for TDR; more are to come in the near future. Though tissue engineering is progressing in its development, TDR using TE-IVDs remains challenging in both implantation and function. Implanting biological constructs as functional disk substitutes requires invasive procedures with increased patient risks; hence, there are greater surgical hurdles to overcome to reach clinical trial with TE-IVDs than with alternative strategies. One preferable option to deliver the implant into the designated site is the use of minimally invasive spine surgery, which can significantly curb surgical damage to surrounding healthy tissue and provide the implant with additional mechanical support based on the premise that minimally invasive surgery causes a significantly lesser increase in range of motion compared with traditional open procedures.[150] As evidenced by prosthetic TDR,[151] displacement of the implant is a complication when positioned in a stand-alone fashion, predominantly due to the weight-bearing human spine yielding severe axial loading. Therefore, the experimental results obtained from quadrupedal animal studies, even when promising, must be interpreted with caution before transitioning the interventions onto humans. Furthermore, the construction of implants that remain viable within a mechanically and trophically inhospitable environment remains technically difficult. However, the combined use of implant with a fixator inducing distraction may enhance the in vivo performance by reducing the risk of implant displacement, stabilizing the implanted segment, and improving the nutrition supply.[66]

In addition to the severe mechanical loading, all therapeutic approaches must combat the disk's poor nutritional supply, diffused from the blood vessels of the vertebral body through the cartilaginous end plate. Despite being one of three IVD components and implicated in disk degeneration, the end plate has hardly been factored into regenerative strategies.[149] In fact, the role of nutrition, critical in the long-term durability of implanted biological treatments, has been largely overlooked. A study by Guehring et al demonstrated that distraction of the disk segment improves hydration, enhances ECM gene expression, and increases protein-expressing gel.[152] As demonstrated by Hee et al, distraction facilitates the regeneration of the ECM in both cartilaginous and osseous end plates as well as the recovery of vascular channels, which are vital to the nutritional supply.[153] The authors corroborated this result through their finding that axial distraction using an external fixator enhanced the regenerative capability of cell injection therapy, based on the hypothesis that individually both a distracted segment and cell injection can stimulate disk repair.[66] Hence, although the implantation of therapeutic biologics can independently regenerate the degenerated disk to some extent, a holistic

treatment with host environmental conditioning that combats the severe mechanical loading and poor nutritional supply is required for optimal and durable clinical outcomes.

Research in the treatment of DDD is continually evolving. Yet, there is a limited understanding of healthy IVD cell phenotype and adaptation in development and maturity, which is paralleled in the limited understanding of degenerative disease pathogenesis and progression. Although the treatment interventions currently being studied in the laboratory and those already applied to patients offer some remedial outcome, there has yet to be an established intervention that offers sustained disk protection, repair, and regeneration. The future of research in the field of disk degeneration will therefore rely on elucidating the differences in the biological features between a healthy and pathologic disk, which may offer insight into optimizing current treatments or the development of more novel treatments. Most innovations in animal models have shown significant promise, although only a few have transitioned onto clinical settings demonstrating their efficacy. Biomolecular and cellular treatments, the only therapeutics undergoing clinical trials, have shown remarkable progress already as determined by the available published and nonpublished data. Tissue engineering and annular repair, on the other hand, are still in the preclinical stage but offer great potential to close the gap between the current treatment attempts and successful disk repair and replacement. The future of human applications with biomolecular and cell-based interventions, combined with tissue engineering, offers exciting prospects in the treatment of DDD.

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