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Review

Advances in biological therapy for nucleus pulposus regeneration

P. Priyadarshani, Y. Li, L. Yao*

Department of Biological Sciences, Wichita State University, Wichita, KS 67260, USA

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SUMMARY

Objective: The intervertebral disc (IVD) is composed of the external annulus fibrosus (AF) and the inner gel-like center, the nucleus pulposus (NP). The elastic NP can function to relieve stress and maintain IVD function by distributing hydraulic pressure evenly to annulus and endplate. Degeneration of the NP, which leads to increased death of NP cells, the loss of proteoglycan (PG), and aberrant gene expression, may result in an overall alteration of the biomechanics of the spinal column and cause low back pain. Recent advances in biological therapy strategies that target therapy at the regeneration of degenerated and damaged NP have been investigated in *in vitro* and *in vivo* studies and demonstrated promising outcomes. In this article, we review recent studies of biological approaches for NP regeneration.
Method: The articles regarding NP regeneration using biomaterials, stem cells, and gene vectors were identified in PubMed databases.
Results: Stem cell-mediated cell therapy demonstrates the potential to restore the function and structure of the NP. The viral or non-viral vectors encoding functional genes may generate a therapeutic effect when they are introduced into grafted cells or native cells in the NP. Biomaterial scaffolds generate an initial permissive environment for cell growth and allow the remodeling of scaffolds in the regeneration process. Biomaterial scaffolds provide structural support for NP regeneration and serve as a carrier for stem cell and gene vector delivery.
Conclusion: Though recent studies advance the body of knowledge needed to treat degenerated discs, many challenges need to be overcome before the application of these approaches can be successful clinically.

Introduction

The intervertebral disc (IVD) is an elastic structure between adjacent vertebrae in the spine. Discs are composed of the external annulus fibrosus (AF) and the inner gel-like center, the nucleus pulposus (NP). The AF of the IVD comprises 15–25 concentric lamellae with aligned collagen fibers within the lamella and elastin fibers between the lamellae[1]. The NP is a jelly-like avascular tissue and the extracellular matrix (ECM) of the gelatinous NP is composed of type II collagen and proteoglycan (PG). The function of the elastic NP is to distribute hydraulic pressure in all directions within each disc under compressive loads. Degeneration of the NP results in an overall alteration of the biomechanics of the spinal column and may cause low back pain[2].

Low back pain and related spine disorders are the leading causes of disability, generating a significant economic burden on the health care system. At some point in their lives, about 80% of the world's population is affected by low back pain, which is the most common problem among individuals in the age group between 20 and 50[3,4]. Surgical treatments of degenerated discs, such as

Abbreviations: BMPs, bone morphogenetic proteins; OP1, osteogenic protein 1; TIMP-1, tissue inhibitor of metalloproteinase; TGF, transforming growth factor; AAV, adeno-associated virus; hUCB-MSCs, human umbilical cord blood-derived mesenchymal stem cells; SDSCs, synovium-derived stem cells; ADSCs, adipose-derived stem cells; CESCs, cartilage endplate-derived stem cells; PGA, polyglycolic acid; hADSCs, human-derived adipose tissue stromal cells; PLGA, poly (L-lactic-co-glycolic acid); C/Gp, chitosan–glycerophosphate; PEG, poly(ethylene glycol); IVD, intervertebral disc; AF, annulus fibrosus; NP, nucleus pulposus; ECM, extracellular matrix; HA, hyaluronic acid; EDC, 1-ethyl-3 (3-dimethyl aminopropyl) carbodiimide; NHS, N-hydroxy succinimide; MSCs, mesenchymal stem cells; LN-NS, link N nanofiber scaffold; CAM, chorioallantoic membrane; GG, gellan gum; iGG-MA, ionic-crosslinked methacrylated GG; MRI, magnetic resonance imaging; GDF_5, growth and differentiation factor 5; (FasL), Fas-ligand; AFSCs, annulus fibrosus-derived stem cells; NPSCs, nucleus pulposus-derived stem cells; hTERT, human telomerase reverse transcriptase; (KRT19), cytoskeletal 19; AAV, adeno-associated virus serotype; AAV2, adeno-associated virus serotype 2.

* Address correspondence and reprint requests to: L. Yao, Department of Biological Sciences, Wichita State University, Wichita, KS 67260, USA. Tel: 1-316-978-6766; Fax: 1-316-978-3772.
E-mail addresses: pxpriyadarshani@wichita.edu (P. Priyadarshani), liyongchao32@yahoo.com (Y. Li), li.yao@wichita.edu (L. Yao).

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discectomy, spinal fusion, lumbar disc herniation decompression surgery, and nucleotomy, are generally performed to relieve patients' symptoms[5–7]. However, these methods normally result in restricted flexibility of the spine and do not allow preservation of the IVD function for a long period of time. Recent advances in tissue engineering approaches try to determine the molecular cascade of disc degeneration and target therapy at the regeneration of the degenerated and damaged NP.

Biological strategies of therapy focus on the regeneration of degenerated tissue, which accounts for long-term cure rather than removing the altered tissue. The development of biocompatible hydrogels that mimic the native ECM of NP has provided the possibility for replacement and repair of the degenerated and damaged NP[3,8–10]. Previous studies that investigated cell–biomaterial interaction showed that a few types of biomaterials were capable of supporting NP cell growth and maintain the cell's biological function[3,8–11]. Biomaterial scaffolds can also serve as a carrier of stem cells and gene vectors to treat the NP. Stem cells have shown an ability to replace NP cells and restore the function and structure of degenerated discs. The viral or non-viral vectors encoding functional genes may generate a therapeutic effect when they are introduced into grafted cells or native cells in the NP. In this article, we will review recent advances of biomaterial scaffold development and transplantation, stem cell therapy, and gene therapy in NP regeneration.

Degeneration of NP

The human spine has 23 IVDs that allow movement of the vertebrae and link adjacent vertebral bodies together. When the spine sustains an impact from body movement, the IVD that keeps two vertebrae separated can absorb shock and keep the bones from rubbing against each other in the spinal column. In an IVD, the NP is a central gelatinous region consisting of a sparse cell population within a complex hydrated ECM of type II collagen fibers (20% of dry weight) with a small amount of other collagen types (VI, IX, and XI) as well as hydrophilic PGs (50% of dry weight)[12]. The ratio between PG and collagen (measured according to the glycosaminoglycan-to-hydroxyproline ratio) in the NP is 27 to 1[13]. Large aggregating PGs that bind to hyaluronic acid (HA) include aggrecan and versican[14,15], while non-aggregating PGs include degradation products of large aggregating PGs and members of the small leucine-rich proteoglycans (SLRPs) such as biglycan, decorin, fibromodulin, keratocan, and lumican[2,16–18]. A high water content is preserved by the PGs[19,20]. Aggrecan is the major PG and is responsible for water-retaining properties of the disc because its net negative charge attracts cations to the ECM[21]. NP cells express significantly higher levels of aggrecan and collagen type II compared to other cells within the disc[22,23]. A previous study investigated the relation of cell density within IVDs and its age-related changes by analyzing the lumbar motion segments of 22 human specimens. This study revealed that the cell density in NP decreased significantly from 0 to 16 years and became stable thereafter[24]. The cell density of the adult human NP is around 1–5 million cells/ml, which is lower than 0.5% tissue volume[24,25]. Though the cell number is extremely low in the NP, these cells are essential for the maintenance of NP tissue by producing PGs and other molecules throughout life[26].

IVD degeneration is characterized by tissue alteration at the cellular and molecular levels. The pathological changes of a degenerative IVD include increased cell death, disc swelling, immune privilege unbalance, and aberrant gene expression[4,27–29]. During the degeneration process, dehydration leads to a decrease in size of the NP and intradisc pressure, resulting in increased stress on the AF with a compensatory increase in functional size. The gelatinous ECM is condensed and replaced by more fibrous tissue[19,20]. In a degenerated disc, the change of its microenvironment, such as reduction of nutrition supplies, and various mechanical loadings, leads to alteration of the ECM and causes stressful impact on the NP cells[20,30]. The expression of matrix degrading enzymes such as aggrecanases and collagenases is up-regulated, while the expression of aggrecan and collagen type II is down-regulated in the NP cells of degenerated discs[31–33].

Recent findings have revealed that genetic factors account for up to 75% of individual susceptibility to IVD degeneration, while only a small part of degeneration is caused by environmental factors[34,35]. Studies of twins and family predisposition as well as genetic polymorphisms have explored the role of genetics in the process of disc degeneration[36–39]. In one epidemiological study, the relative importance of genetics was compared to other risk factors. Here the disc degeneration of 115 pairs of male monozygotic twins was evaluated using observational and digital magnetic resonance imaging (MRI) assessment methods[37]. The study found that genetic influence and unidentified factors including complex, unpredictable interactions may primarily explain the disc degeneration. A number of genes, including vitamin D receptor; growth differentiation factor 5 (GDF5); tissue inhibitor of metalloproteinases (TIMP); collagen types I, IX, and XI; aggrecan; and matrix metalloproteinase (MMP) 1, 2, and 3, have been identified as being associated with the degeneration of IVD. One study revealed that the allelic variation in the vitamin-D receptor gene was associated with multilevel and severe disc degeneration. Here an MRI examination of 205 young adults with or without low-back problems was conducted to evaluate disc degeneration. Results showed that the Tt allele was more frequently associated with multilevel disc disease, severe disc degeneration, and disc herniation than the TT allele[40].

Biomaterials-based NP repair

The development of transplantable biomaterials provides a promising approach to regenerate IVD. Scaffolds of natural and synthetic materials have been investigated for *in vitro* cell–biomaterial interaction and *in vivo* implantation into animal models with degenerative IVDs[3,8,9,41–50] (Supplemental material table 1). Previous studies demonstrated that biomaterial scaffolds can help to retain cells in their desired location and maintain their viability. The microstructure of the scaffolds can facilitate tissue ingrowth. The biodegradable property of biomaterials allows the remodeling of scaffolds in the regeneration process. Some scaffolds can also mechanically support the disc and withstand compressive loads. The function of the implants can be enhanced by introducing biological molecules in the scaffolds or delivering therapeutic cells to the degenerated discs[10,51–54].

A few types of natural materials such as collagen, HA, fibrin, gelatin, alginate, and chitosan were studied for their potential to support NP cell growth *in vitro* and to replace and regenerate NP *in vivo*[3,41,42,56–59]. Natural biopolymers that mimic native ECM biochemistry provide natural cues to cells that may stimulate a healing or regenerative response[60]. Some injectable biomaterials have the ability to self-assemble to a higher-order network[61]. Type II collagen and HA, being major components of IVD, were studied for their ability to repair the NP[3,41]. The biological and material properties of hydrogel generated by collagen and HA are normally enhanced by crosslinking. Studies have been performed to generate hydrogels composed of type II collagen and HA, and crosslink the gels with 1-ethyl-3 (3-dimethyl aminopropyl) carbodiimide (EDC)[41] or poly(ethylene glycol) ether tetrasuccinimidyl glutarate (4S-StarPEG)[3]. It was shown that these hydrogels can support the growth and proliferation of rat mesenchymal stem cells (MSCs)[41] or bovine NP cells[3]. The expression of type II collagen but not type I

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