Intervertebral disc tissue engineering: A brief review

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ABSTRACT

Intervertebral disc (IVD) degeneration (IDD) is associated with low back pain and significantly affects the patient's quality of life. Degeneration of the IVD alters disk height and the mechanics of the spine, leading to chronic segmental spinal instability. The pathophysiology of IVD disease is still not well understood. Current therapies for IDD include conservative and invasive approaches, but none of those treatments are able to restore the disc structure and function. Recently, tissue engineering techniques emerged as a possible approach to treat IDD, by replacing a damaged IVD with scaffolds and appropriate cells. Advances in manufacturing techniques, material processing and development, surface functionalization, drug delivery systems and cell incorporation furthered the development of tissue engineering therapies. In this review, biomaterial scaffolds and cell-based therapies for IVD regeneration are briefly discussed.

KEY WORDS: Intervertebral disc; IVD; cell-based therapies; biomaterials; scaffolds; tissue engineering; IVD degeneration; IDD
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INTRODUCTION

In recent years, our knowledge of human physiology and cell biology has increased exponentially. This significantly impacted the development of tissue engineering in various medical fields, including regenerative techniques for intervertebral disc (IVD) degeneration (IDD). Degenerative discs are among the main causes of chronic segmental spinal instability in both males and females, and significantly affect the quality of life, especially of young patients [1]. IDD may present clinically as axial back pain, spinal stenosis, myelopathy, or radiculopathy [2-4].

Low back pain is associated with IDD [5,6]. The disk height decreases due to degeneration, altering the mechanics of the affected spinal segment. This process accelerates the degeneration of adjacent segments and other spinal structures, such as small joints, ligaments and muscles [5]. In the long term, IDD leads to narrowing of the spinal canal (spinal stenosis) and the subsequent compression of neural tissue. Spinal stenosis is the major cause of pain, especially in the elderly. Considering current demographic trends in Europe [7] such as the increase in the elderly population, the problem of IDD and related conditions becomes more difficult [8]. Current therapies for IDD include conservative and invasive treatments [6], however, none of those approaches can restore the disc structure and function [9,10]. Recently, tissue engineering techniques emerged as a possible approach to treat IDD, by replacing a damaged IVD with scaffolds and appropriate cells [9,11,12].

In this review, biomaterial scaffolds and cell-based therapies for IVD regeneration are briefly discussed.

INTERVERTEBRAL DISC STRUCTURE

IDD and associated back pain are chronic conditions that affect a large number of people worldwide. Thus, prevention and treatment of disk degeneration are the focus of intensive research. Recent findings show the potential of biological methods, such as molecular, cell-based and whole organ tissue engineering therapies, to prevent and manage IDD [13-15]. Because various scaffold materials and cell sources are used in tissue engineering, advances in materials science are particularly important for the development of this field. These include improvements in manufacturing techniques, material processing and development, surface functionalization, drug delivery systems and cell incorporation [16-19].

The IVD is complex in structure and consists of three distinct parts (Figure 1): 1) the fibrocartilaginous annulus fibrosus (AF) with its outer and inner regions, composed of

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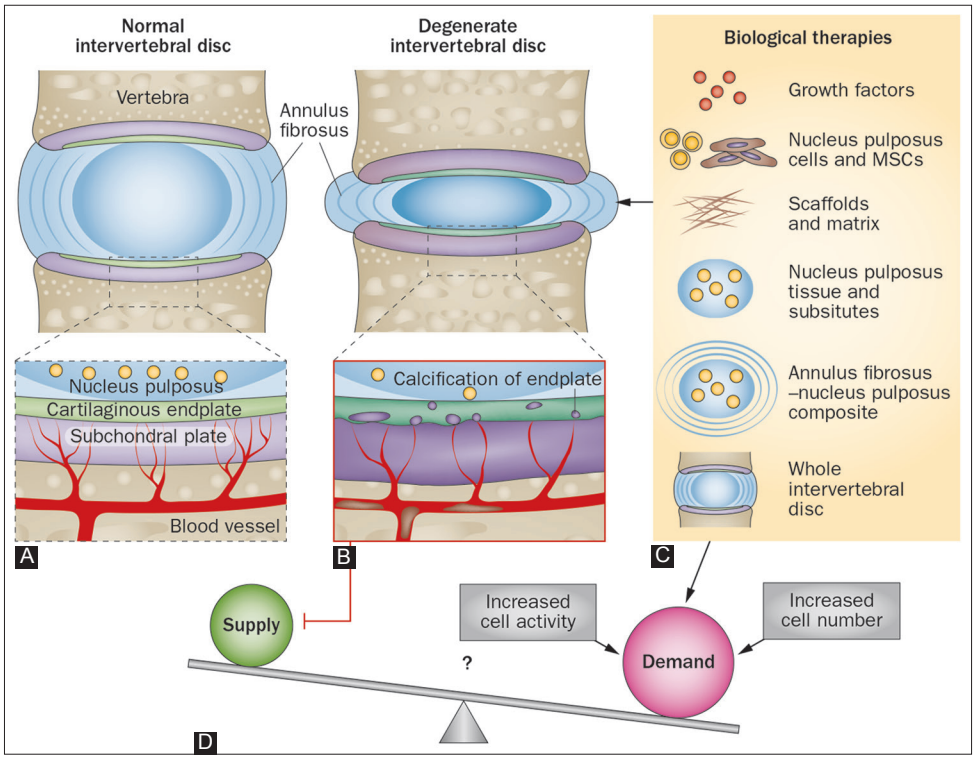

FIGURE 1. Schematic depiction of the normal and degenerate IVD structure with possible therapeutic approaches. A) Nutrient pathways in normal disc. B) Nutrient pathways in a degenerate disc (e.g., calcification of cartilaginous endplate, occlusion of marrow spaces, atherosclerosis of vertebral arteries, reduced capillary density etc.). C) Different forms of biological therapies for disc repair. D) Current therapies increase the cell number and/or cellular activity causing nutrient demand to exceed nutrient supply, which is already diminished in degenerate discs. Reprinted by permission from Macmillan Publishers Ltd: Nature Reviews Rheumatology [20], copyright 2014.

concentrically oriented layers of fibrous tissue; 2) the central nucleus pulposus (NP); and 3) the cartilaginous endplates (EPs) [9,20]. The AF consists of a series of 15 to 25 concentric rings or lamellae, with aligned parallel collagen fibers located within each lamella. Elastic fibers lie between the lamellae and help the IVD to return to its original shape following flexion or extension of the spine. The cells of the AF are elongated and fibroblast-like and are aligned parallel to the collagen fibers. The NP contains a highly hydrated gel-like matrix that is comprised primarily of the proteoglycan aggrecan. A small amount of randomly arranged collagen fibers and radially arranged elastin fibers are embedded in the matrix [20,21,22]. The cells in the NP are spheroidal and chondrocyte-like. The EPs are made up of osseous and hyaline-cartilaginous layers and contain rounded chondrocytes.

The IVD is an avascular structure. Both the cells and extracellular matrix (ECM) are essential for normal IVD function [4]. Regenerative approaches in tissue engineering of IVD aim to restore/preserve the anatomy and function of both AF and NP [23]. Therefore, an ideal scaffold for IVD replacement should have good biocompatibility and moderate porosity, and the shape, structure and mechanical properties similar to the IVD [9,24]. Currently, the research in this area is focused on constructing AF- and NP-scaffolds that have all the properties of the native structures, using various synthetic and natural polymers [12,25,26].

Main challenges in IVD tissue engineering

Mechanical properties of the IVD are important for its proper function. *In vivo,* the IVD transmits the load imposed on the spine, including spinal tension, torsion, compression and bending [27]. Thus, it is essential for tissue engineered scaffolds to have all the (bio)mechanical properties of the IVD so they can replace a damaged disc in the body [9].

Scaffolds represent one of the key components in tissue engineering, as they provide the structural support for cell attachment/proliferation and ECM accumulation [28]. Tissue engineered scaffolds should be able to withstand the physiological load imposed on the IVD *in vivo*, in addition to good biocompatibility, moderate porosity and similarity to the IVD in shape, structure and mechanical properties [29]. Moreover, for cell/whole organ tissue engineering therapy to be successful, the local environment in which transplanted cells or tissue engineered IVDs are introduced must be able to support cell growth and proliferation [9,26].

Scaffolds and appropriate source materials

Tissue engineering provides a promising alternative for the restoration of physiological function of a damaged IVD [30]. Because degeneration of the IVD affects both the AF and NP, composite scaffolds that enable simultaneous repair of the two parts should be used [30]. Studies with small

animal models showed promising results in IVD regeneration and repair. Various techniques are utilized for the preparation of three-dimensional (3D) biomimetic scaffolds, including solvent casting, leaching method, phase separation, freeze drying, and electrospinning [11,31,32]. However, clinical application of these scaffolds is still limited [33]. Collagen, which is the most abundant protein in mammals, is widely used in biomedical applications and may also be appropriate for IVD regeneration [34,35]. In the extraction of collagen from skin tissue, pepsin is added to remove N- and C-terminal telopeptide components from collagen and to solubilize the collagen derivative [36]. This low-immunogenic derivative of collagen is called atelocollagen. Due to its low antigenicity, atelocollagen is regarded as one of the best basic matrices for implantable materials [37]. An atelocollagen scaffold with honeycomb structure is mechanically stable, easily handled, and supports the growth of a large number of cells (high-density cell cultures). Considering these characteristics, atelocollagen may be useful as a 3D scaffold in tissue engineering [38].

Another important material for scaffold construction is silk fibroin, a protein produced by silkworms and some other insects [39]. Silk has a high resistance to compression and is considered to be one of the strongest natural fibers [31,40]. The stability of the silk fibers is the result of the extensive hydrogen bonding, hydrophobic nature of the protein, and high degree of crystallinity due to the specific organization of β-sheets [41]. There are several benefits of using silk fibroin as a scaffold in IVD bioengineering [42]. Mechanical stability is an important property of a scaffold, and in the case of silk scaffolds, it is the result of compressive and tensile strength of silk protein fibers. *In vivo*, IVDs are always to some degree under load [24]. Once implanted, the silk scaffold would degrade at a sufficiently slow rate to allow proper tissue development [39,43]. Another benefit of silk is that other peptides can be covalently attached to it, which could potentially enhance cell attachment during AF and NP development [44].

Other biomaterials that may be used as matrix supporting materials in AF and NP tissue engineering include chitosan and alginate, and both are cheap and easily accessible. Moreover, the two polymers yield superior characteristics when combined into a hybrid scaffold [7,45]. Chitosan is derived from the shells of crustaceans [46]. It is a biodegradable and biocompatible polymer with low toxicity and good antimicrobial properties [47,48]. Chitosan-based scaffolds are soft and spongy, have high porosity and pore interconnectivity, and support cell adhesion and growth [46]. Alginate is a linear polysaccharide composed of α-L-guluronic acid (G block) and β-D-mannuronic acid (M block) residues, which are linked together in different sequences. It represents one of the most abundant natural materials and is derived primarily from brown algae and some bacteria [49]. Due to its outstanding properties in terms of biocompatibility, biodegradability, non-antigenicity and chelating ability, alginate is used in a variety of biomedical applications, including tissue engineering (e.g., as a supporting matrix) and drug delivery [45,49-52].

Gellan gum (GG) is a natural polysaccharide produced by the bacterium *Sphingomonas elodea* [53]. It consists of repeating tetrasaccharide units that are comprised of L-rhamnose, D-glucuronic acid and two D-glucose residues. GG is noncytotoxic and particularly resistant to heat and acid stress [54]. The gelation of this biomaterial leads to the formation of a stable hydrogel structure. At higher temperatures (above ~80 °C), GG exists in a coil conformation. As temperature decreases, a thermoreversible coil to helix transition occurs [55]. Untwined regions of polysaccharide chains link to the oriented bundles of the double helix structures (junction zones) leading to the formation of a 3D gel network, which can be used as a matrix for cell seeding [55,56].

In addition to natural polymers and natural-polymer derived materials, biodegradable synthetic polymers are also utilized as scaffolds in tissue engineering [57,58]. Synthetic polymers have a number of advantages over natural polymers, including highly reproducible synthesis, predictable properties, lack of immunogenicity, and easy processing into desired structures and implants [58]. Synthetic polyesters that are extensively used in AF tissue regeneration include poly(ε-caprolactone) [PCL], polylactide (PLA), polyglycolide (PGA), and copolymers produced from the respective monomers [26,59]. In addition, many other natural and synthetic materials are being investigated as scaffolds for AF, NP and IVD tissue engineering [60].

Tissue engineering of AF and NP

Tissue engineered AF and NP have the potential to repair or replace degenerated tissue in the IVD, thus restoring its functionality. Therefore, considerable research is directed toward developing appropriate scaffolds for AF and NP regeneration [15,42,61]. Many natural and synthetic materials can be used as a supporting matrix in AF and NP scaffolds [25].

Damage to the AF is attributed to numerous factors, such as mechanical stress, biological remodeling, loss of nutrition of cells, and accumulation of cellular waste products [27,42,62]. Silk scaffolds are appropriate for AF engineering due to their mechanical properties and biocompatibility. According to Chang et al. [44], porous silk scaffolds may be used to modulate the phenotype of seeded AF cells, mimicking the native tissue with inner transition and outer zones [44]. AF scaffolds can also be produced from alginate or alginate/chitosan [15,51]. A hybrid alginate/chitosan scaffold has good biocompatibility, promotes AF cell proliferation, supports ECM deposition, and has a slower degradation rate compared to a pure alginate scaffold [51]. The atelocollagen honeycomb-shaped scaffolds,

which allow high-density growth and 3D culture of various cells, may be used for IVD regeneration by transplanting the AF cells [37,51]. In addition, Cabraja et al. showed that a 3D polymer of pure polyglycolic acid (PGA) combined with hyaluronan is an excellent scaffold for AF cell redifferentiation [63].

Similar to AF tissue engineering, different types of materials are used for NP scaffolds, such as collagen, alginate, chitosan, fibrin and hyaluronic acid [24]. Hydrogels for NP engineering should possess good mechanical strength, viscoelasticity, swelling capacity, diffusion properties, biocompatibility, and to be able to support cell growth and ECM accumulation [64]. Generally, the main challenge in NP tissue engineering is to find a biomaterial that can withstand large mechanical loads, imposed on the spine in natural conditions [15]. There are two different cell types in the NP: notochordal cells and mature NP cells. The latter are called chondrocyte-like cells due to their rounded shape and secretory activity, and are found primarily in adults [65]. An example of a scaffold appropriate for NP tissue engineering is a chitosan-based hydrogel seeded with IVD cells [15,66]. This scaffold is suitable for cell-based supplementation, to restore NP function in the early stages of IVD degeneration [66]. Halloran et al. showed that an enzymatically cross-linked atelocollagen type II-based scaffold, containing aggrecan and hyaluronan in varying concentrations, has the potential for developing an injectable scaffold seeded with cells for NP regeneration [67]. Moreover, modification of low viscosity alginate with methacrylate groups produces a photo-crosslinkable alginate hydrogel with tunable material properties and the ability to maintain the viability of the encapsulated NP cells [50]. Similarly, porous silk fibroin (SF)

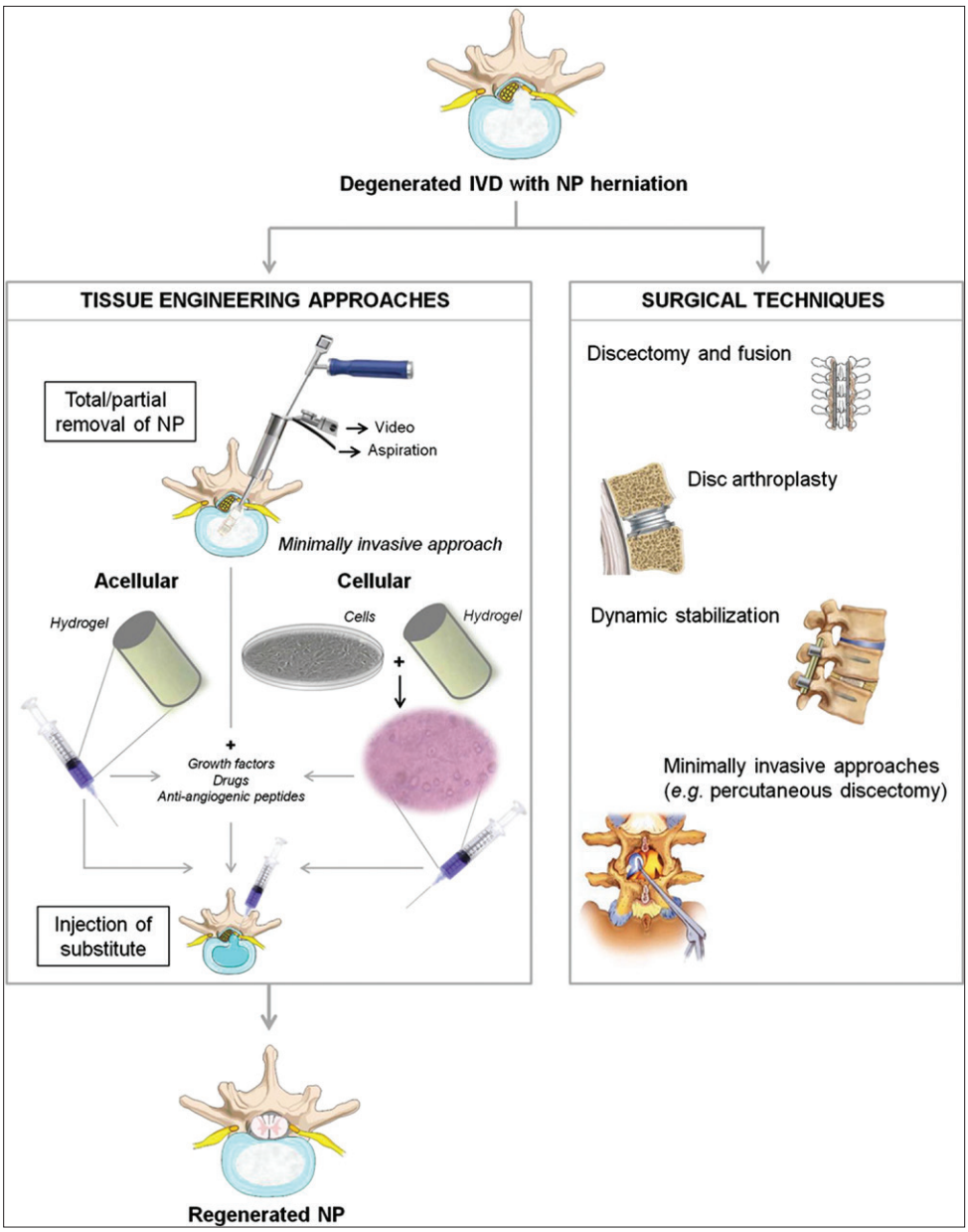

FIGURE 2. Overview of recent strategies related to intervertebral disc regeneration. Reprinted by permission from Elsevier: Biotechnology Advances [94], copyright 2013.

scaffolds represent a plausible candidate for tissue engineered NP as they support NP cell attachment, proliferation and infiltration, and the production of ECM [40]. Other scaffolds for NP regeneration include ionic- and photo-crosslinked methacrylated gellan gum hydrogels, which can be used as acellular or cellular tissue engineering scaffolds; polylactide beads; demineralized bone matrix; gelatin microcarriers; gelatin/chondroitin-6-sulfate/hyaluronan tri-copolymers, which are bioactive scaffolds for culturing human NP cells that preserve cell viability and support cell proliferation and ECM production; and biphasic polyurethane scaffolds, which have good swelling capacity *in vitro*, fast swelling rate after hydration, and dynamic compressive stiffness [15,37,40,50,64,66,67].

Final task: cell integration

For a complete and functional tissue-engineered model of IVD, which includes the cells and ECM, a scaffold needs be able to support the survival and preserve/induce the phenotype of both AF and NP cells. Various cell types are used for regenerative therapy of IDD, and stem cells represent a particularly attractive option [68]. As unspecialized cells capable of long-term self-renewal and lineage-specific differentiation, stem cells can be programmed/induced to differentiate into different types of cells [68-70]. Mesenchymal stem cells (MSCs) are particularly suitable for IVD cell therapy, because they are capable of differentiation into various connective tissue cells and can be obtained relatively easily from a number of sources, including fetal liver, umbilical cord blood, bone marrow, adipose tissue, muscles and dermis [70-81]. MSCs participate in the repair of degenerated disc tissue in several ways, including: 1) directly by differentiation into disc tissue-specific cells to supply lost or damaged cells and promote the formation of the ECM; 2) indirectly by secreting growth factors to enhance tissue regeneration; and 3) by modulating the inflammatory response [82-86]. Two main therapeutic strategies exists for the application of MSCs in tissue repair. In the first approach, undifferentiated MSCs are transplanted, which then undergo differentiation *in vivo* under the stimulation of local factors. In the second strategy, MSC are induced to differentiate *in vitro*, prior to transplantation [86]. A number of studies investigated the ability of MSCs to differentiate into NP or AF cells and promote ECM synthesis, using either co-culture systems with growth factors or animal models in which MSCs are injected directly into the IVD [62,87-92]. When injected directly into the IVD MSCs promote ECM synthesis, resulting in restoration of the disc height. Human cell cultures are also used for IVD regeneration. Transplantation of autologous MSCs from bone marrow into a rabbit model of disc degeneration leads to regeneration of IVDs, providing a new hope for the treatment of degenerative disc disease in humans [93,94]. Figure 2 summarizes the most important recent strategies for IVD regeneration.

CONCLUSION

The pathophysiology of IVD disease is still not well understood and IDD remains a significant health problem worldwide. Although still in the experimental phase, regenerative strategies for IDD such as tissue engineering show great promise. Further research will provide new insights into IVD regeneration mechanisms and, hopefully, enable the integration of regenerative therapies for IDD into clinical practice.

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DECLARATION OF INTERESTS

The authors declare no conflict of interests.

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