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REVIEW

Treatment of the degenerated intervertebral disc; closure, repair and regeneration of the annulus fibrosus

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Abstract

Degeneration of the intervertebral disc (IVD) and disc herniation are two causes of low back pain. The aetiology of these disorders is unknown, but tissue weakening, which primarily occurs due to inherited genetic factors, ageing, nutritional compromise and loading history, is the basic factor causing disc degeneration. Symptomatic disc herniation mainly causes radicular pain. Current treatments of intervertebral disc degeneration and low back pain are based on alleviating the symptoms and comprise administration of painkillers or surgical methods such as spinal fusion. None of these methods is completely successful. Current research focuses on regeneration of the IVD and particularly on regeneration of the nucleus pulposus. Less attention has been directed to the repair or regeneration of the annulus fibrosus, although this is the key to successful nucleus pulposus, and therewith IVD, repair. This review focuses on the importance of restoring the function of the annulus fibrosus, as well as on the repair, replacement or regeneration of the annulus fibrosus in combination with restoration of the function of the nucleus pulposus, to treat low back pain. Copyright © 2014 John Wiley & Sons, Ltd.

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1. Introduction

Low back pain is a disorder that affects a considerable proportion of the population. About 60–80% of all people suffer from back pain at some time during their life (Nachemson, 2004). Degeneration of the intervertebral disc (IVD) and disc herniation are two distinct but related causes of low back pain and radicular pain, respectively. Radicular pain is a result of mechanical impingement of the spinal nerves, which usually resolves after surgical or conservative treatment (Thome *et al*., 2005). According to imaging and discographic studies, at least 40% of patients with chronic low back pain showed characteristics of intervertebral disc degeneration (IVDD) (Freemont *et al*., 2002; Kalson *et al*., 2008; Luoma *et al*., 2000). IVDD is an aberrant, cell-mediated response to progressive structural failure (Adams and Roughley, 2006). The aetiology of this disorder is unknown (Mwale *et al*., 2004), but tissue weakening, which primarily occurs due to inherited genetic factors, ageing, nutritional compromise and loading history, is the basic factor causing disc degeneration (Adams and Roughley, 2006).

Current treatments of IVDD and low back pain are based on alleviating the symptoms and comprise administration of painkillers or surgical methods like spinal fusion, with or without discectomy, replacement of the degenerated disc by an IVD or nucleus pulposus (NP) prosthesis, and annuloplasty. None of these methods is completely successful. Furthermore, it is of great clinical importance to prevent reherniation (Hegewald *et al*., 2008).

The problem of IVDD has been analysed from many sides, and the scientific literature on this subject is particularly diverse. Worldwide, groups dealing with IVDD have

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acknowledged the need for therapeutic alternatives that do not remove or replace the IVD but allow it to regenerate (Boyd and Carter, 2006; Bron *et al*., 2009; Kalson *et al*., 2008; Leung *et al*., 2006; O'Halloran and Pandit, 2007; Richardson *et al*., 2007; Sakai, 2008) and the majority of this research has focused on the repair and regeneration of the NP. Limited attention has been paid to the annulus fibrosus (AF), although it is a key determinant in the outcome of these therapies. The purpose of this review is to highlight the importance of restoring the function of the AF by describing its structure and its role in low back pain development. Research focused on the repair, replacement or regeneration of the annulus fibrosus is discussed.

2. Structure of the intervertebral disc (IVD)

Intervertebral discs are fibrocartilaginous tissues that allow motion between the vertebral bodies. They transmit load and absorb the shocks that are experienced by the spine (Kurtz and Edidin, 2006). Each IVD is composed of three distinct but connected structures: the vertebral endplates, the NP and the AF (Bogduk, 2008; Humzah and Soames, 1988) (Figure 1).

The vertebral endplates consist of hyaline cartilage (which resembles articular cartilage) and occupy the inferior and superior interfaces between the intervertebral disc and the adjacent vertebral bodies. As in articular cartilage, the parts of the endplates that are closest to the vertebral bone are calcified. The collagen content is greatest at the periphery of the endplates, while the centre contains most of the proteoglycans and water. The inner third of the AF is directly attached to this cartilage.

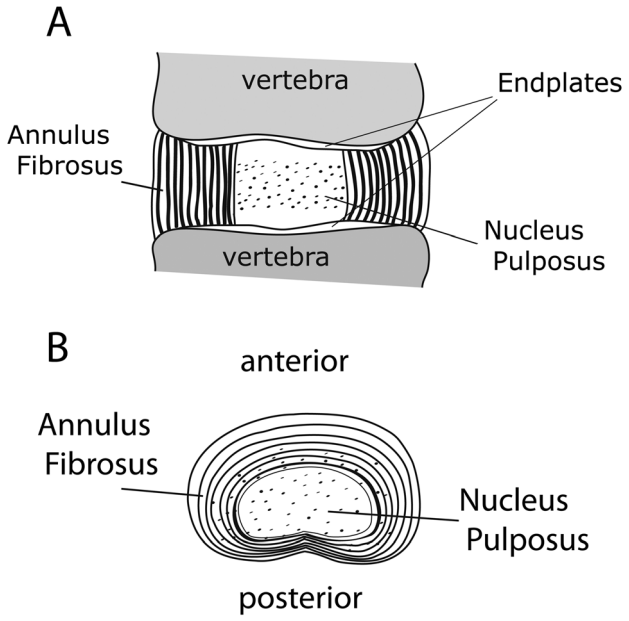

Figure 1. Schematic diagram illustrating the basic structures of the intervertebral disc; this drawing is not to scale. (A) Sagittal view of the intervertebral disc, illustrating the annulus fibrosus, the nucleus pulposus and the endplates. (B) Top-down view, illustrating the different lamellae of the annulus fibrosus: note that the posterior part is much thinner than the anterior part

The NP is the gelatinous structure of the disc that is surrounded by the AF. It is a highly hydrated aggrecan gel, kept together by randomly distributed collagen type II fibrils. The hydrated aggrecans of the NP provide the disc with the ability to absorb and transmit compressive loads acting on the spine. The density of cells in the NP is 4×10^6 cells/cm^3 (Nakagawa *et al*., 2007).

The AF is the tough annular exterior of the IVD, which encases the NP and prevents the NP from herniating (leaking out of the disc). The medial and lateral borders of the AF taper to a thin, free edge (Coventry *et al*., 1945). The AF is composed of water (65–90% of its weight), collagen type I and II fibres (60% of dry weight), and proteoglycans and other proteins (10–20% of dry weight) (Roughley, 2004; Sun and Leong, 2004). The AF has a cell density of 9×10^6/cm^3. The AF is composed of 15–25 loosely connected concentric rings of highly organized collagen fibres (lamellae). These lamellae are thicker towards the centre of the disc (Marchand and Ahmed, 1990). In every lamella, the collagen fibres lie parallel to each other and are orientated at approximately ± 28–43° to the transverse axis. In adjacent lamellae they alternate to the left and to the right of this axis (Cassidy *et al*., 1989; Hickey and Hukins, 1980; Marchand and Ahmed, 1990), resulting in non-linear, anisotropic and viscoelastic properties, which are key to its function (Nerurkar *et al*., 2008). The tensile and compressive moduli of the AF vary in the ranges 0.5–29 MPa and 0.5–1.5 MPa, respectively (Kurtz and Edidin, 2006). Except for the very outer layer of the AF, there is no direct blood supply to the disc. Nutrition of the IVD is based on diffusion of nutrients through the subchondral bone and the endplates of the vertebrae. The outer AF cells receive nutrients from blood vessels of the surrounding vascular plexus (Raj, 2008).

3. Pathophysiology of the intervertebral disc degeneration

Understanding IVD degeneration is an important step towards developing successful therapies for treating low back- and radicular pain. Ageing of the IVD, along with overuse, results in morphological changes, cell transformations and degeneration (Urban and Roberts, 2003). The effects of normal ageing and degenerative disease have striking similarities and are difficult to distinguish. As an integral part of the IVD, the AF is involved in almost all pathological conditions resulting from ageing or degeneration. Previous studies have demonstrated a strong association between annular defects and nuclear degeneration. However, it is not clear which comes first (Osti *et al*., 1990). In humans, ageing and degenerative disease of the NP coincide with an imbalance of the anabolic and catabolic processes, catabolic processes exceeding anabolic ones. This results in loss of proteoglycans

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and a decrease in water content of the NP and in osmotic pressure (Goupille *et al*., 1998).

Degeneration of the endplates, with concomitant endplate calcification, impairs transport of nutrients, amongst which are oxygen and glucose, into the disc and results in the accumulation of waste products such as lactic acid, which reduce the pH (Bernick and Cailliet, 1982; Boos *et al*., 2002). These deficiencies in metabolite transport reduce the number of cells and their metabolic activity, resulting in a reduction of extracellular matrix (ECM) synthesis and a decrease of the water-binding capacity of the NP (Bibby and Urban, 2004). Dehydration of the NP leads to a smaller NP, resulting in a reduced shock absorbance capacity. As a consequence, loads which would ordinarily be taken up by the NP will be transferred to the AF, affecting AF cell metabolism and structure. The lamellae of the AF become thicker, irregular and disorganized and increase in number in the radial direction (Marchand and Ahmed, 1990). Due to reduced turnover of the matrix, the collagen becomes more densely crosslinked and denatured (Hormel and Eyre, 1991), leading to increased stresses on, and delamination and ruptures or cracks of, the AF. Annulus tears are the most common disorders seen by surgeons. These can already start to be formed during the second decade of life. Since the outer part of the annulus is innervated, annular tears (even acute ones) can be painful. In the repair process, neovascularization with concomitant ingrowth of nerve endings and granulation tissue occurs, which leads to discogenic low back pain (Constantinescu *et al*., 2007; Helm Ii *et al*., 2012; Sharma *et al*., 2009a).

Pain radiating along a compressed nerve, radicular pain or leg pain, is caused by expulsion of the NP and herniation of the disc as a result of a weakened AF, allowing the NP to bulge or leak posteriorly towards the spinal cord and nerve roots. The pressure that is induced by a herniated or bulging disc is not the sole cause of pain, since a herniated disc impinging the nerve root is painless in 70% of patients. It is likely that the secretion of products that are involved in the inflammation cascade in a torn AF sensitize the nerve root or increase the number of innervations, thereby causing pain (Freemont *et al*., 1997; Kang *et al*., 1996; Urban and Roberts, 2003).

4. Treatment of low back pain in intervertebral disc degeneration and herniation

4.1. Alleviating low back pain without the use of implants

Patients with low back pain secondary to traumatic or age-related intervertebral disc disease are typically treated conservatively, with rest, cognitive–behavioural treatment, anti-inflammatory medications and physiotherapy (Brox *et al*., 2003, 2006; Frost *et al*., 2004; Helm Ii *et al*., 2012; Lewis, 2012; Mirza *et al*., 2007). For the majority of patients, the back pain will resolve and normal spinal function will be restored.

The natural history of unilateral sciatic pain spontaneously resolves within 1–2 months in 80% of patients, without neurological sequelae. A proportion of patients will suffer recurrent or persistent symptoms; the latter often occurring after several spontaneously resolving episodes. It is for these patients that intervention should be considered.

Epidural injections of steroid and local anaesthetics appear to help some patients and a prospective, randomized, controlled, double-blinded study has shown the efficacy of selective nerve root blocks of patients with lumbar radiculopathy and/or stenosis (Riew *et al*., 2000). Less than 2% of symptomatic patients undergo operative treatment. Surgical intervention is best directed at those with unremitting nerve root symptoms.

Microdiscectomy is the gold standard operative treatment for lumbar disc prolapse (Postacchini and Postacchini, 2011). More recently the use of endoscopes has emerged. The endoscope is inserted through the intervertebral foramen to visualize the herniation and remove it manually (Chiu *et al*., 2004). Often removal of sequesters is sufficient; only incidentally, the AF has to be perforated and is usually sutured at the end of the procedure. No new AF tissue is formed, and the opening remains open or closes with the formation of scar tissue. This not only makes the disc prone to reherniation, one of the major clinical problems, but can also promote degeneration with concomitant irritation of nerve endings in the outer AF. Suturing, which is still an experimental procedure, is difficult, due to restricted access to the AF, and sutures do not hold in degenerated AF tissue. The normal biomechanics of the disc and vertebra are not restored and the degeneration process can continue in ensuing years. In order to restore the biomechanical conditions, there is great need to not only close and seal the AF but also to promote healing by the formation of new AF tissue (Bron *et al*., 2009; Jin *et al*., 2009). More experimental procedures are annuloplasty and nucleoplasty. Anuloplasty is a minimally invasive method, in which heat produced by electricity or radio frequency radiation (RF) can strengthen the collagen fibres and seal fissures in a process similar to tissue soldering (Constantinescu *et al*., 2007). Although annuloplasty offers a therapeutic alternative between conservative therapy and more invasive surgery, there is no strong evidence regarding its efficiency (Helm Ii *et al*., 2012). Nucleoplasty can also be successful. This procedure will release the pressure on the outer AF, allowing the disc to return to normal size, thereby decompressing the nerve (Sharps and Isaac, 2002).

Central canal, lateral recess or foraminal stenosis from facet joint or ligamentum flavum hypertrophy are surgically decompressed by removal of the offending tissue whilst maintaining stability (laminectomy or laminotomy). Although partial discectomy for nucleated NP is a very successful operation, serious instability or degeneration of the vertebral column may finally occur. Lumbar fusion, or spondylodesis, may then be a solution for local symptoms, although also here deterioration of symptoms may occur later on due to degeneration at other spinal levels.

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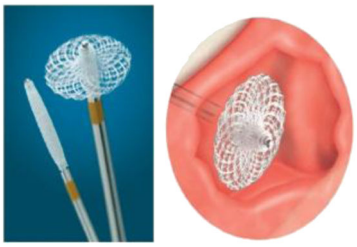
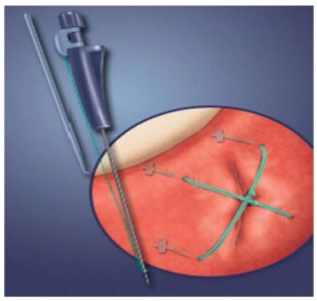
Spondylodesis, fusion of two vertebrae with a bone block after removal of the IVD (Zhang *et al.*, 2007), has a success rate of 32–98%. This technique produces good short-term results, but in the long term there is a significant risk that the altered biomechanics of the treated level may compromise adjacent motion segments, leading to additional pathology (Osti *et al.*, 1990).

4.2. Alleviating low back pain and restoring function using permanent structural implants

The described decompression and fusion techniques yield relatively good short-term clinical results with regard to alleviating pain, but do not restore the biomechanics of the spine. This can lead to further degeneration of the surrounding tissues and neighbouring discs. Artificial discs have been developed that not only relieve the pain but also maintain the function of the spine. Although their implantation is an invasive surgical procedure, total disc replacement may be necessary in case of severe disc degeneration (David, 2007). The entire diseased disc is removed and replaced with a prosthesis prepared from a variety of biomaterials (metals and polymers). Examples of such permanent implant devices are the Prodisk, SB Charité disc, Maverick, Flexicore, Cadisc™-L and Acroflex artificial discs. Preliminary results using these artificial discs were promising; however, recently some of these permanent disc prostheses showed increasing failure rates (Punt *et al.* 2012). For patients with an early diagnosis of intervertebral disc disease, the closure of AF tears is of significant importance, as pathological observations and experimental studies have shown that AF tears occurring in the early stages of disc degeneration are associated with more rapid degenerative changes of the other components of the intervertebral disc (Saad and Spector, 2004; Sharma *et al.*, 2009b). Additionally, successful performance of a NP implant very much depends on its confinement by the AF. If the AF cannot secure the NP tissue or its prosthesis, it will be expulsed and no biomechanical benefit can be expected. Permanent AF closure methods consist of mechanical solutions to reinforce the annulus and simultaneously seal it (Table 1). Several implants have been developed for closing a torn AF. Due to occurring complications, Inclose® and Xclose® (Anulex Technologies), have been withdrawn from the market. Barricaid® is another commercially available implant used in conjunction with discectomies that forms a strong and flexible mechanical barrier that closes the defect. This product is made from PET and a titanium bone anchor (Chan and Gantenbein-Ritter, 2012). Recently, Bron *et al.* (2009) investigated the use of barbed plugs made of polyethylene as AF closure devices. However, the efficacy of these systems to contain a collagenous NP replacement was compromised due to a mismatch of the mechanical properties of the device and the AF tissue (Jin *et al.*, 2009).

Table 1. Commercially available permanent annulus closure devices

Annulus closure device	Material	Form
Inclose® surgical mesh	Polyethylene terephthalate	
Xclose® suturing system	Polyethylene terephthalate	
Barricaid® woven mesh barrier	Polyethylene terephthalate and titanium bone anchor	

Note: Figures were reproduced with permission (Marcolongo et al., 2011) and from http://www.intrinsic-therapeutics.com/barricaid_ard.shtml.

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Despite promising results regarding closure of the AF with the above-mentioned approaches, recurrent disc herniation frequently occurs. This is one of the most common causes of repeated back pain, with the majority of events occurring at the same site and level as the previous herniation (Dewing *et al*., 2008; Jonsson and Stromqvist, 1993; McGirt *et al*., 2009). Furthermore, there are concerns regarding the long-term consequences of implanting artificial materials within the intervertebral disc, as it is subject to temporal biological and biomechanical changes.

5. Regenerative medicine strategies for the treatment of degenerated intervertebral discs

The regenerative medicine approach aims at restoring disc function by reversing the aetiology of disc degeneration and regenerating disc tissues (Gilbertson *et al*., 2008). An increased understanding of the physiology, cell biology and matrix biology of the IVD and the degradation mechanisms involved has led many researchers to investigate the potential of biological treatments for repairing the diseased intervertebral disc. Regenerative medicine strategies address the biological basis of the disease, and can be classified as: therapies based on the use of biological molecules; cell-based therapies; and therapies based on the use of biodegradable scaffolds together with cells and/or biological molecules or gene therapy (An *et al*., 2003; Boyd and Carter, 2006; Bron *et al*., 2009; Hegewald *et al*., 2008; Kalson *et al*., 2008; Zhang *et al*., 2008). The choice of each therapy as a regenerative approach for IVD repair depends on the grade of degeneration of the IVD. These strategies and their suitability for treating discs with different extents of degeneration are discussed below.

5.1. Cell-based therapies to regenerate degenerated IVD tissue

In the degenerated disc, where existing cells do not respond to any biological stimuli, or intrinsic repair capacity has been hampered by inflammatory and catabolic processes, transplantation of healthy or genetically manipulated cells into a degenerated IVD should partially or fully restore the function of the degenerated disc.

Although in animal models the feasibility and the effectiveness of cell transplantation of NP cells is well documented (Hohaus *et al*., 2008; Kuh *et al*., 2009; Nishida *et al*., 2005; Sato *et al*., 2003b; Sobajima *et al*., 2008), an *in vivo* study on cell delivery to the degenerated AF cannot be found in the scientific literature.

Among the different potential cell sources (Table 2) available for cell therapy, mesenchymal stromal cells (MSCs) are a very attractive cell source for use in restoring the normal cellular constitution of the degenerated disc. In contrast to the necessary differentiated autologous or allogeneic cells, which are usually not available in sufficient amounts or in a healthy state, MSCs are readily available. These cells can be differentiated into the required AF cells (Saal and Saal, 2000). Several factors, such as the administration of cytokines, the application of external (e.g. mechanical) forces and co-culture with differentiated cells, can determine the fate of the stem cell (Leung *et al*., 2006; Saal and Saal, 2000). Several studies aiming at increasing the cell population in discus tissues using stem cells have been published (Crevensten *et al*., 2004; Hoogendoorn *et al*., 2008; Risbud *et al*., 2004; Sakai *et al*., 2003). Ho *et al*. (2008a, 2008b) showed that injection of MSCs into a punctured AF significantly reduced the degeneration process (Reza and Nicoll, 2008).

One of the difficulties in cell therapy is the fact that most transplanted cells will not survive the ischaemic conditions immediately after implantation. Of transplanted MSCs, 80–90% died within 5 days after implantation in an osteochondral defect in rabbits (Emans *et al*., 2006). Dying cells at best exert a trophic effect: they excrete a large number of chemokines, cytokines, growth factors, etc., which will stimulate endogenous cells to infiltrate the tissue. These infiltrating cells will promote either the regeneration of IVD tissue or the production of scar tissue (Emans *et al*., 2006; Ma *et al*., 2009; Moioli *et al*., 2008; Potier *et al*., 2007). The current lack of unique molecular markers with which to characterize the different IVD cells, and the uncertainty regarding the final phenotype of the

Table 2. Different cell types and -sources used for direct transplantation into the degenerated IVD

Cell type	Advantages	Disadvantages
Autologous NP or AF cells	No immune response	Not available in sufficient amounts and healthy state, especially for NP (Boos *et al*., 2002; Gruber *et al*., 2009b; Le Maitre *et al*., 2007a, 2007b). Phenotype change upon expansion in monolayer culture (Gruber *et al*., 1997, 2004a). Additional surgery required to obtain NP or AF cells Risk of disc degeneration upon harvesting NP or AF cells (Rousseau *et al*., 2007)
Allogeneic NP or AF cells	No or minimal immune response Healthy cells available	Limited availability of allogeneic human NP or AF cells Risk of disc degeneration upon harvesting NP or AF cells
Autologous stem cells	Available in sufficient amounts No immune response	Lack of definitive phenotype markers for NP or AF cells
Allogeneic stem cells	Off-the-shelf availability No or minimal immune response	Lack of definitive phenotype markers for NP or AF cells
Chondrocytes	Available in sufficient amounts	Difference in phenotype compared to NP or AF cells

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cells, as well as individual variations in the quality of the MSCs and the characteristics of the MSCs in the harsh conditions of the IVD, bring additional complexity (Gilson *et al.*, 2010).

An approach to circumvent the existing limitations associated with protein delivery is gene therapy. Gene therapy strategies offer an opportunity for the sustained expression of synthetic proteins in the disc in order to augment the anabolic functions or decrease the catabolic processes.

Gene therapy can restore the production of a protein that is absent or deficient by introducing a functional gene into the target cell (Kootstra and Verma, 2003; Levicoff *et al.*, 2005). The unique biology of the intervertebral disc may favour gene therapy. The isolated disc environment and its relative avascularity not only may prevent leakage of the contents of the disc but also protect the contents from the immune response that can affect most transgenic expression in other tissues (Yang *et al.*, 1994). Gene therapy can be done *in vitro* by transient or permanent transfection, followed by implantation of the transfected cells into the IVD (*ex vivo* approach) or by *in vivo* injection of vectors for transfection of preferably healthy cells (Cassinelli *et al.*, 2001; Yoon, 2004). For more information about gene therapy for IVD repair, we refer to a recent review (Guterl *et al.*, 2013).

6. Tissue engineering of the intervertebral disc

6.1. Use of biodegradable polymeric biomaterials in regenerating degenerated AF tissue

In NP and AF tissue engineering, the final goal is to achieve biomechanical stability of the disc in the short term and the formation of new tissue in the long term, utilizing scaffolding materials in combination with cells, signalling molecules or both. The scaffolds can also play a significant role as a functional template to guide the cellular remodelling process, supporting the delivery of biological molecules and cells; they can also ensure closure of a defect and immediate restoration of biomechanical function. In the following paragraphs, tissue engineering of the IVD with special focus on the AF is elaborated upon.

6.2. AF tissue engineering

In the absence of a supporting scaffold, repair is limited to the outer layer of the AF, where scar tissue with inferior mechanical properties compared to the native AF tissue develops (Hampton *et al.*, 1989). Replacement of defected or degenerated AF tissue with engineered AF tissue may restore the biomechanics of the disc by confining the NP. The load-bearing nature and the highly organized structure of the AF, which are essential for performing its biomechanical function, make the tissue-engineering strategy challenging.

6.3. Biodegradable polymeric biomaterials used in AF tissue engineering

Several common polymeric biomaterials including synthetic and natural polymers have been used in AF tissue engineering (Table 3). The choice of scaffolding materials used in AF tissue engineering is determined by the physicomechanical properties of the AF. While hydrogels are much used as scaffolding structures in NP tissue engineering, the biomaterials used to fabricate AF tissue engineering scaffolds are much tougher, and stronger. Polyglycolic acid, polylactic acid and glycolide and lactide copolymers have often been used in AF tissue-engineering applications, as these polymers degrade by hydrolysis of the ester bonds. Mizuno *et al.* (2004, 2006) seeded polyglycolic acid and polylactic acid scaffolds with ovine AF cells. After 12 weeks of subcutaneous implantation in athymic mice, the gross morphology and histology of the engineered discs strongly resembled that of native intervertebral discs. However, due to the avascular nature of the AF, the removal of the acidic degradation products of these polymers was not facilitated. An acidic environment is not only favourable for disc proteinase-like aspartate- or cysteine proteinase, which both contribute to matrix degradation, but is also detrimental for AF cells (Roughley, 2004). To control acidity, Helen and Gough (2008) prepared composite scaffolds from poly(D,L-lactic acid) (PDLLA) and Bioglass. In this manner, the acidic PDLLA degradation products were buffered by the ionic dissolution products of the Bioglass. In addition, it was found that bioactivity of the scaffolds was enhanced when compared to controls without Bioglass. Human AF cells cultured on composite PDLLA/Bioglass scaffolds had a greater ability to deposit collagen and proteoglycan after 4 weeks of culture.

Another biodegradable polymer that has been used to prepare AF tissue engineering scaffolds is poly(ε-caprolactone) (PCL). PCL also degrades by hydrolysis of ester linkages, in this case very slowly. It combines good mechanical properties with facile thermoplastic processing. In AF tissue engineering, PCL has been often used to prepare scaffolds by electrospinning methods that mimic the morphological and mechanical features of the AF (Nerurkar *et al.*, 2007, 2008, 2009); these researchers reported the ability to electrospin PCL scaffolds that mimicked the multilamellar architecture of the native AF tissue to direct the deposition of an organized, collagen-rich extracellular matrix by seeded MSCs after 10 weeks of *in vitro* culture.

Most of the synthetic polymers used in AF tissue engineering lack the flexibility and elastic properties that are required for the movement of the disc and the protection of the NP. Wan *et al.* (2007, 2008) therefore developed biodegradable poly(1,8-octanediol malate) and poly(ε-caprolactone triol malate) polymer networks. The deformability of the

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Table 3. Synthetic and natural polymeric biomaterials, matrices and scaffolds used in the engineering of AF tissue

Material	Processing technique	Origin	Injectable (y/n)	Mechanical characteristics	Tissue integration	Cell adhesion	Reference
Alginate	Hydrogel crosslinked with Ca^{2+} ions	Natural	No	Poor	No	No	(Chiba *et al.*, 1997; Drury *et al.*, 2004)
Collagen	Freeze-drying Crosslinking	Natural	No	Dependent on degree of crosslinking	Yes	Yes	(Rong *et al.*, 2002)
Small intestine		Natural	No		Yes	Yes	(Le Visage *et al.*, 2006)
Poly(glycolic acid)	Non-woven mesh	Synthetic	No	High modulus, 5–6 GPa	No	No	(Mizuno *et al.*, 2006)
Chitosan	Thermo-gelling	Natural	Yes		No	No	(Roughley *et al.*, 2006)
Poly(ε-caprolactone)	Electro-spinning	Synthetic	No	Modulus 300 MPa	No	No	(Nerurkar *et al.*, 2007)
Poly(D,L-lactic acid)	Thermally induced phase separation	Synthetic	No	High modulus (2 GPa)	No	No	(Helen and Gough, 2008)
Poly(octane diol malate)	Crosslinking Salt leaching	Synthetic	No	Low modulus (15 MPa)	No	No	(Wan *et al.*, 2007)
Silk	Salt leaching	Natural	No	High modulus	No	Yes	(Chang *et al.*, 2010)
Polycarbonate urethane	Electro-spinning	Synthetic	No	Tunable	No	No	(Yang *et al.*, 2009)
Fibrin	Solvent casting Gelation	Natural	Yes	Poor	Yes	Yes	(Sha'ban *et al.*, 2008)
Polyamide	Electro-spinning	Synthetic	No	Tunable	No	No	(Gruber *et al.*, 2009a)

materials, which is essential for restoration of the biomechanical properties of the spine, could be adjusted by controlling the post-polymerization time.

Biodegradable polyurethanes have been subject of investigation for use as tissue-engineering scaffolds. Whatley *et al.* (2011) reported on the properties of 3D printed AF tissue-engineering scaffolds prepared from biodegradable lysine diisocyanate and poly(ε-caprolactone) polyurethanes. The proliferation rate of chondrocytes on these materials when compared to tissue culture polystyrene was similar or slightly better. Yang *et al.* (2009) investigated the effect of surface energy on AF cell attachment and proliferation. They incorporated various amounts of an anionic dihydroxyl oligomer (ADO) into polycarbonate urethane. Scaffolds containing higher amounts of ADO showed more collagen accumulation, suggesting that surface energy affects AF cell attachment and collagen production.

Many AF tissue-engineering structures have been prepared from natural polymers. These include collagen, chitosan, hyaluronan, fibrin, alginate and silk. As the AF mainly contains collagen, it can be expected that an ideal scaffold should be based on collagen. Neat collagen or chemically modified collagen, alone or combined with glycosaminoglycans or hyaluronan, have been used in AF tissue engineering (Alini *et al.*, 2003; Bowles *et al.*, 2010; Gruber *et al.*, 2004b; Saad and Spector, 2004; Sakai *et al.*, 2006; Sato *et al.*, 2003a). In these studies, collagen has been shown to support AF cell adhesion and proliferation and enhance proteoglycan synthesis. Also, its ability to self-assemble into fibrillar hydrogels make collagen type I an attractive material for AF tissue engineering. Bowles *et al.* (2010) used collagen gels prepared by physical fibrillogenesis to culture sheep AF cells. They observed that the AF cells could be elongated between, and in parallel to, the collagen fibrils. Their alignment and spindle-shaped morphology was similar to that observed in the IVD.

Also chitosan and alginate have been used to prepare AF tissue engineering scaffolds (Mizuno *et al.*, 2004; Shao and Hunter, 2007). However, these materials are usually not strong enough to sustain the high circumferential, longitudinal and torsional stresses occurring in the IVD. Moreover, AF cells were found to lose their phenotype and become like NP cells when cultured in soft hydrogels such as alginate, agarose or chitosan (Alini *et al.*, 2003; Roughley *et al.*, 2006).

Of the natural biopolymers, the use of silk may be advantageous, as it is the strongest known natural fibre. Chang *et al.* (2007) seeded bovine AF cells on porous silk tissue-engineering scaffolds. They observed attachment and proliferation of AF cells on the scaffolds and the synthesis of extracellular matrix.

Currently there is not a single polymer of choice for preparing AF tissue-engineering scaffolds. Morever, the choice for a scaffolding material will likely depend on the extent of degeneration of the AF and the pursued tissue-regeneration strategy.

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6.4. Annulus fibrosus tissue engineering matrices and scaffolds

In the early stages of intervertebral disc degeneration, where loss of extracellular matrix material is minor and the AF is relatively unaffected, injectable matrices that gelate and solidify after injection can be used to fill the disc cavity and to deliver biological molecules or cells. The number of studies on the feasibility of using injectable matrices to deliver relevant cells to the AF is limited. The intervertebral disc and articular cartilage share several features, and lessons can be learnt from the more established field of cartilage tissue engineering (Saal and Saal, 2002). A critical issue in AF tissue regeneration is the ability to be able to reconstruct the highly orientated laminar structure with alternating orientation. Contracting collagen gels have been partly successful in mimicking the layered structure of the AF. Bowles *et al.* (2010) reported on injectable collagen gels seeded with ovine AF cells. Although their preliminary results were successful in aligning the collagen in the circumferential direction, it was not possible to mimic the alternating angles of orientation of the fibres in adjacent lamellae.

At more advanced stages of degeneration of the IVD, when structural changes are more significant, the implantation of fabricated scaffolds seeded with cells and/or loaded with biologically active molecules should be considered. Scaffolds with well-defined pore structure, pore distribution and texture for promoting cell adhesion can be designed and prepared. In AF tissue engineering, a variety of techniques have been employed for the preparation of scaffolding structures with random pore network characteristics, including freeze-drying and salt leaching (Sato *et al.*, 2003b; Schneider *et al.*, 1999; Wan *et al.* 2007, 2008). *In vitro* culture studies of AF cells seeded on silk scaffolds show an average pore size of 600 μm to be optimal for maximum cell attachment and proliferation, resulting in the most uniform AF tissue distribution with the greatest amount of type I collagen formation (Chang *et al.*, 2010).

Although the AF has a complex anisotropic structure that is key to its functional performance, few studies have investigated the effect of the anisotropy of the scaffold pore network in AF tissue engineering (Nerurkar *et al.*, 2007, 2008, 2009). Wan *et al.* (2008) combined ring-shaped demineralized bone matrix gelatin (BMG) with a concentrically orientated sheet made from poly(ε-caprolactone triol malate) to replicate the laminar structure of the AF. This scaffold supported the growth of rabbit chondrocytes, as well as the production of collagen type II (Wan *et al.*, 2008). Nerurkar *et al.* (2009) reported on the performance of electrospun nanofibrous scaffolds that mimicked the layered and angled structure of the AF; although the mechanical properties were similar to that of the AF, tissue integration and supply of nutrients to the cells are yet to be considered. Nonetheless, these scaffolds continue to be optimized (Chan and Leong, 2008; Koepsell *et al.*, 2011a, 2011b).

Despite the interest in AF tissue engineering using scaffolds, strategies for their fixation to and integration in the IVD tissue, and thus for true clinical application, have yet to be developed.

7. Challenges in tissue engineering the annulus fibrosus

Many challenges in engineering the intervertebral disc and AF tissue still remain (Kandel *et al.*, 2008) (Table 4). The AF is isolated from the systemic circulation, transport of nutrients is limited, it has abundant extracellular matrix and a low cell density. Although its complex and anisotropic structure allow it to withstand high dynamic loading, this structure is difficult to mimic.

The low number of cells in the AF and their advanced stage of senescence exclude AF cells as a suitable source of cells for seeding scaffolds. Mesenchymal stromal cells are the most promising alternative. These cells can be isolated from bone marrow and subcutaneous fat and have the ability to differentiate in the required cell lineages, producing matrix components (Gruber *et al.*, 2000; Saal and Saal, 2000).

The healing potential of the AF is low. Most of the intrinsic healing of the AF occurs in its outermost borders (Hampton *et al.*, 1989; Kaapa *et al.*, 1994; Melrose *et al.*, 1992; Smith and Walmsley, 1951). A thin fibrous tissue is then formed that is not as strong as the uninjured disc

Table 4. Difficulties and challenges in tissue engineering the AF

Difficulties and challenges
Sources of cells
Limited sources of human cells due to the unavailability of healthy tissue (Gruber *et al.*, 2007; Roberts *et al.*, 2006)
Biopsies taken from healthy AF do not contain sufficient cells (Roughley, 2004)
Risk of damage to AF during biopsy (Elliott *et al.*, 2008)
Difficulty distinguishing inner and outer AF cells (Bron *et al.*, 2009)
Difficulties in cell culturing. Loss of cell phenotype in 2D cell culture, and the requirement for specific media and culture conditions, e.g. pressure or tension (Chou *et al.*, 2006; Gruber *et al.*, 2002; Johnson *et al.*, 2006; Reza and Nicoll, 2008).
Lack of suitable cell markers for AF cells (Melrose *et al.*, 2008)
Poor survival of transplanted cells (Emans *et al.*, 2006; Potier *et al.*, 2007)
Tissue-engineering scaffolds
Requirement of anisotropic physical and mechanical characteristics mimicking the healthy AF (Guerin and Elliott, 2007)
Requirements change with extent of disc degeneration
Limited integration with native AF tissue
Conformation to the site of implantation, which hinders implantation and restricts implant geometry
Providing an aqueous medium for cell survival, while simultaneously maintaining mechanical properties
Need to promote cell attachment and provide signals for normal cellular activity in the AF ECM
Wish for radio-opacity to allow medical follow-up
Method of surgical implantation or injection
Anatomy and physiology
Avascularity of the tissue
Limited nutrient transport and waste disposal
Low healing potential
Low cell numbers
Lack of an animal model of the human degenerated IVD

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and does not have the regular angle-ply, laminate structure. In the absence of the proper, AF-like structure to provide the critical mechanical properties to the tissue (Guerin and Elliott, 2007), this new tissue is not expected to last very long (Onimus, 2006). Unfortunately, it is not yet possible to give the newly formed tissue the opportunity to mature with only limited loading, which might enable regeneration of the AF, similar as shown for other osteoarthritic joints (Lafeber *et al*., 2006). Finally, the poor healing potential also negatively affects the repair of tears caused by sutures or a surgical incision, making the disc highly susceptible to re-tearing.

7.1. Non-cell-seeded scaffolding and support structures allowing immediate closure of the AF

Although tissue engineering using resorbable biomaterial scaffolds in combination with cells and/or bioactive factors has allowed the generation of AF tissue, no studies have yet demonstrated the ability to restore AF functionality, preventing extrusion of the NP (or NP prosthesis) from the intervertebral disc cavity. As spinal stability is important both for the patient and in the clinical setting, approaches that allow immediate closing of the AF defect at the same time, allowing generation of a functional AF tissue, are much needed.

7.2. Porous scaffolding and support structures

Porous resorbable patches or barriers, used in combination with sutures or adhesives, have been employed to prevent migration of the NP of the disc through an AF tear. These cell-free implants are supposed to recruit relevant differentiated cells or progenitor cells from the surrounding environment, leading to the formation of a repair tissue. The feasibility of such an approach in restoring IVD function has been shown (Abbushi *et al*., 2008). Hegewald *et al*. (2008) introduced a cell-free AF sealing barrier based on a poly(glycolic acid) patch and hyaluronan, which was sutured in a microsurgical procedure to the inner wall of the AF defect, using an inside-out technique. It was reported in these preliminary studies that this approach could restore the biomechanical behaviour in flexion and extension (Singh *et al*., 2002).

We have reported on a biodegradable AF closure device with shape memory properties, prepared from lactide and trimethylene carbonate networks. The implant was introduced into the disc through the annulus defect in a compressed form; upon warming to body temperature, the shape of the implant changed to seal the defect at the inside of the AF, thus preventing extrusion of the NP (prosthesis). Excellent compatibility of these implants with human AF cells was observed. *Ex vivo* studies using canine IVDs, however, showed that optimization of the implant geometry was necessary for optimal performance (Sharifi, 2013).

7.3. Adhesive materials and glues

In closing the AF after a discectomy or herniation with sutures, additional closing force using a glue could be provided to prevent early reherniation. In the longer term, such an approach could reduce disc instability by keeping the NP contained. Such a glue could also be used for the fixation and later integration of AF scaffolding and support structures with native tissue. Heuer *et al*. (2008) reported on the use of biodegradable fibrin and cyanoacrylate glues in combination with sutures to close the opening in the AF after insertion of collagen NP prostheses into bovine lumbar discs. According to their observations in fatigue testing, closing the AF incision with sutures only, or with sutures and fibrin glue, was not successful. The mechanical performance of the cyanoacrylate glue and sutures was significantly better. It should be noted that in other studies cyanoacrylate glue has been shown to be cytotoxic (Chen *et al*., 2007; Thumwanit and Kedjarune, 1999).

At early stages of degeneration, the small fissures and defects in the still relatively unaffected AF can contribute to discogenic pain. As these defects can make the disc prone to further degeneration and biomechanical instability, fissure growth could be suppressed by occluding with a sealant. An injectable fibrin-based sealant has been developed that also serves a tissue repair matrix (Yin *et al*., 2011). The feasibility of using fibrin-based biomaterials for AF repair has been studied by Schek *et al*. (2011), who showed that genipin-crosslinked fibrin gels, with dynamic shear modulus values (measured at 1 Hz) in the range of native annular tissue (80–95 kPa), show promise as gap-filling adhesives for annular fissures.

At later stages of degeneration of the disc, and for closing larger defects of the AF, obtaining biomechanically stable solutions is much more difficult. Strong resorbable glues would then be required to seal the AF. In the liquid state, these materials wet the irregular surface of the annular tissue tears, and upon curing form an interwoven mechanically interlocking structure with the AF tissue. Although there is an obvious need for fixing a temporary patch or barrier, the scientific literature on the use of glues to close the AF is scarce. However, the well-known chemistry of tissue adhesives can be instructive in designing a strong resorbable glue for AF tissue repair.

Wang *et al*. (2007) used methacrylate- and aldehyde-functionalized chondroitin sulphate as an injectable tissue adhesive for cartilage repair. The aldehyde moieties formed covalent bonds with the amine groups of the collagen molecules in the tissue, while polymerization of the methacrylate groups led to solidification of the material. In both in a goat and a rabbit model, the repair of the cartilage tissue was significant. The binding to cartilage was strong, and no cell damage was observed. Using similar chemistry, Murakami *et al*. (2007) synthesized a tissue-adhesive hydrogel that comprised an aldehyde-terminated poly(ethylene glycol)–poly(D,L-lactide) block polymer. The hydrogel rapidly formed when applied *in vivo* to the peritoneum of mice, and adhered well to the tissue surface. Aldehyde-functionalized gelatin and polysaccharides have also been reported (Herget *et al*., 2003; Matsuda *et al*., 1999; Mo *et al*., 2000).

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Sharifi *et al.* (2011) developed an injectable, biodegradable and photocrosslinkable system based on methacrylate-functionalized polyethylene glycol–poly(trimethylene carbonate) block copolymers, which could seal the disc through a minimally invasive surgical procedure. The elastic modulus and water content could easily be varied to match those of the native AF. *Ex vivo* results using canine cadaveric spines showed the potential of the materials to seal an opening in the AF, although the adhesion of the photocrosslinked material to the AF tissue still needs to be improved.

Self-curing acrylate formulations also display potential in this regard. Larraz *et al.* (2005, 2007) developed a system based on a methacrylic acid derivative of Triton X, a non-ionic surfactant, and polymers prepared from acrylic acid, methacrylic acid and hydroxyethyl methacrylate, which was loaded with a biological compound, chondroitin sulphate. These polymers absorbed water, were elastic and demonstrated shape memory properties, making them strong candidates for IVD repair.

Blanquer *et al.* (2012) prepared a biodegradable glue based on isocyanate-terminated low molecular weight poly(trimethylene carbonate)–polyethylene glycol–poly (trimethylene carbonate) triblock copolymers. This glue was used in combination with porous PTMC membranes for the fixation of AF tissue-engineering scaffolds to the surrounding AF tissue. The bond strength to the tissue was of the same order of magnitude as that when using Dermabond® cyanoacrylate glue.

Carbodiimide chemistry has also been used in the synthesis of tissue adhesives. Amine and carboxylic acid groups can be coupled using 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) and *N*-hydroxysuccinimide (NHS) to form amide bonds in aqueous solutions under physiological conditions. Aqueous solutions of polymers containing amine and carboxylic acid groups can be crosslinked in this way. Tissue-adhering gels with different setting times and bond strengths could be formed by varying the NHS concentration (Iwata *et al.*, 1998; Kobayashi *et al.*, 2001; Wallace *et al.*, 2001).

Polysaccharides have inherent bioadhesive properties, functionalized polysaccharides are therefore of great interest for the preparation of tissue adhesives. A photocrosslinkable chitosan containing azo and lactose moieties produced an insoluble hydrogel in less than a minute that firmly adhered to meat tissue upon UV radiation (Ono *et al.*, 2000).

Glues from biological sources (fish, holothurians, insects, spiders, mussels and other sources) may also prove to be useful in AF repair strategies. Frog glue, for example, which is flexible, porous and non-toxic in nature, has been used in treating cartilage defects and can also be of great value in the non-surgical sealing of annular defects (Graham *et al.*, 2006). Also, mussel-mimetic tissue adhesives have potential in sealing AF defects. Dopamine-functionalized poly (ethylene glycol)-based hydrogels were used to seal 3 mm fetal membrane tissue defects (Haller *et al.*, 2011).

8. Conclusions

Regeneration of the IVD may be the future treatment for patients with a degenerated IVD suffering from low back pain. To date, most research has focused on regeneration of the NP. Many questions and challenges will have to be dealt with to develop strategies that lead to regeneration of the damaged AF.

Such strategies should focus on the development of scaffolding structures that enhance cell survival upon transplantation, their growth and their proper differentiation. Reproducing the complex and anisotropic structure of the native AF is a prerequisite for an effective treatment. So far, no studies have described support structures with mechanical properties and structural features matching those of the AF.

For the short term, novel approaches to close AF tears with a minimum chance of relapse may already be very beneficial to the patient. It should be realized, however, that sealing of the herniated disc will not always reduce low back pain, as the secretion of inflammatory cytokines may sensitize the nerve root or increase innervation (Freemont *et al.*, 1997; Kang *et al.*, 1996; Urban and Roberts, 2003).

Conflict of interest

The authors have declared that there is no conflict of interest.

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