

CHALLENGES AND STRATEGIES IN THE REPAIR OF RUPTURED ANNULUS FIBROSUS

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Abstract

Lumbar discectomy is the surgical procedure most frequently performed for patients suffering from low back pain and sciatica. Disc herniation as a consequence of degenerative or traumatic processes is commonly encountered as the underlying cause for the painful condition. While discectomy provides favourable outcome in a majority of cases, there are conditions where unmet requirements exist in terms of treatment, such as large disc protrusions with minimal disc degeneration; in these cases, the high rate of recurrent disc herniation after discectomy is a prevalent problem. An effective biological annular repair could improve the surgical outcome in patients with contained disc herniations but otherwise minor degenerative changes. An attractive approach is a tissue-engineered implant that will enable/stimulate the repair of the ruptured annulus. The strategy is to develop three-dimensional scaffolds and activate them by seeding cells or by incorporating molecular signals that enable new matrix synthesis at the defect site, while the biomaterial provides immediate closure of the defect and maintains the mechanical properties of the disc. This review is structured into (1) introduction, (2) clinical problems, current treatment options and needs, (3) biomechanical demands, (4) cellular and extracellular components, (5) biomaterials for delivery, scaffolding and support, (6) pre-clinical models for evaluation of newly developed cell- and material-based therapies, and (7) conclusions. This article highlights that an interdisciplinary approach is necessary for successful development of new clinical methods for annulus fibrosus repair. This will benefit from a close collaboration between research groups with expertise in all areas addressed in this review.

Keywords: Annulus fibrosus rupture; disc herniation; disc biomechanics; biomaterial scaffold; pre-clinical model; interdisciplinary approach; annulus fibrosus tissue engineering; annulus fibrosus regeneration.

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Introduction

Intervertebral disc (IVD) herniation is a common condition that frequently affects the spine of young and middle-aged patients. The IVD is a complex structure composed of different but interrelated tissues: the central gelatinous highly hydrated nucleus pulposus (NP), the surrounding fibres of the annulus fibrosus (AF), and the cartilaginous endplates that connect these tissues to the vertebral bodies. The extracellular network of collagens and proteoglycans is maintained by sparse populations of cells with generally fibro-chondrocytic phenotypes and serves to transmit loads exerted on the spine. As a result of aging or degeneration, the normal extracellular matrix (ECM) turnover may be disturbed; if proteoglycan concentration decreases, disc hydration and disc height are diminished, increasing the strain on the fibres in the AF. This can lead to tears and fissures in the AF and ultimately to protrusion of the NP. On the other hand, injurious mechanical forces in combination with traumatic or degenerative failure of the AF may provoke herniation of disc tissue. Irrespective of aetiology, back pain may occur due to disc protrusions, whether they do or do not enter the spinal canal and exert pressure on the lumbar nerve roots. It is believed that the pain associated with lumbar disc herniation results from a combination of nerve root ischemia and inflammatory processes occurring at the site of extrusion (Takahashi *et al.*, 2008).

Surgical intervention has widely shown positive outcome in cases where conservative treatment including physical therapy, pain medication and epidural steroid injections are not successful. The majority of studies support earlier relief of pain-related symptoms and conceivably superior restoration of function in patients who undergo surgery (Guilfoyle *et al.*, 2007; Spengler *et al.*, 1990; Weber, 1983; Weinstein *et al.*, 2006b; Weinstein *et al.*, 2008). In fact, lumbar disc herniation is the pathological condition for which spinal surgery is most often performed. The incidence of disc surgery is 160/100,000 inhabitants in the USA and 62/100,000

in Switzerland, indicating large geographic variations (Andersson and Deyo, 1996; Berney *et al.*, 1990; Weinstein *et al.*, 2006a).

Lumbar discectomy is the most common surgical procedure performed for patients suffering from back pain and sciatica, with over 300,000 operations done yearly in the USA (Atlas *et al.*, 1996; Deyo and Weinstein, 2001). While standard discectomy provides favourable results in the majority of the cases, conditions with unmet needs in terms of treatment include large disc protrusion with only minimal disc degeneration and adolescent disc protrusion. In both conditions, the partial removal of the protruding herniation tissue is indicated, and the breach in the annulus removes the safe constraints encasing and maintaining pressurisation of the gel-like nucleus pulposus. In these cases, recurrent herniations are quite frequent, necessitating revision surgery (McGirt *et al.*, 2009; Lebow *et al.*, 2011). Furthermore, an average disc height loss of 25 % has been reported after discectomy, which has been associated with increased back pain and disability (Barth *et al.*, 2008; Yorimitsu *et al.*, 2001; Loupasis *et al.*, 1999).

Another problem after discectomy is the so-called 'post-discectomy syndrome' involving recurrent herniation with return of symptoms, motivating surgeons to remove a greater portion of disc tissues during the original herniation procedure. A recent investigation suggests that the main source for chronic low back pain after surgical discectomy is discogenic and that annular fissures may be the primary cause for the painful symptoms (DePalma *et al.*, 2012). This finding indicates the need for repairing annular fissures after discectomy.

To prevent recurrent symptoms, novel strategies towards annular repair are compulsory. Efficient AF repair could significantly improve the presently limited surgical outcome, with the largest improvement likely to occur in cases of contained disc herniations but otherwise minor degenerative changes, which mainly occur in relatively young patients. With interdisciplinary collaborations, we are evaluating innovative and clinically relevant approaches to treat AF ruptures, focusing on improving cell-biomaterial interaction for application in disc herniation. Ideally, such a biological construct will, upon implantation, provide immediate closure of the defect and maintain the mechanical properties of the disc, while the cellular component will start the regenerative process.

This article reviews the clinical problems, current treatment options and needs, cellular and biomechanical considerations, and biomaterials required for AF repair. Furthermore, *in vitro* and *in vivo* preclinical models commonly used to test newly developed cell- or materials-based therapies are outlined. The ultimate goal of our research is to offer the surgeon an off-the-shelf biological solution for treating disc herniations with a single intervention.

Clinical Challenge

Clinical pathology of the AF

As an integral part of the functional spine unit (FSU), the annulus fibrosus is involved in almost any pathological

condition of the degenerating spine. There are two specific clinical situations for which the impaired function of the AF plays a crucial role: as the source of discogenic low back pain (LBP) and, in the case of insufficiency, as the origin of disc herniation. Both conditions are very common and have an enormous socio-economic impact with no established satisfactory treatment options to date (Gore *et al.*, 2012; Mehra *et al.*, 2012; Parker *et al.*, 2010; Weinstein *et al.*, 2006a; Weber, 1994).

Discogenic low back pain is believed to arise from acute tears or fissures of the AF and from focal defects of the outer AF. These defects result in a repair process where granulation tissue is formed along with neovascularisation and concomitant ingrowth of nerve fibres (Melrose *et al.*, 2002; Freemont *et al.*, 1997; Aoki *et al.*, 2006). Although the AF has not fully lost its main function to withstand the hydrostatic pressure from the NP and to stabilise the FSU, discogenic low back pain has a high likelihood to develop chronicity and often needs medical treatment.

Acute and chronic disc herniations not only generate local pain from the disc but can also create pain and loss of function of the segmental nerves by direct compression and irritation from local inflammatory processes. As this condition has a lifetime prevalence of 1-3 % and often affects active, working persons of 30-50 years of age, the socioeconomic impact due to medical treatment and long-term absence from work are enormous (Weinstein *et al.*, 2006a; Weber, 1994).

Diagnostics and classification

In the case of disc herniation, a correct diagnosis is usually made due to the irritation of the segmental nerves, which is frequently accompanied by low back pain observed during a clinical examination. The clinical symptoms of discogenic low back pain on the other hand are less specific, which requires a diagnosis that is mostly based on imaging methods and diagnostic infiltrations. Although some typical findings may be present on plain radiographs and computer tomograms, magnetic resonance imaging (MRI) and discography are more sensitive and specific. High intensity zones (HIZ) are believed to be the radiological correlate of the innervated granulation tissue described above; HIZ are found in the outer layers of the posterior AF, mostly radially oriented and show a high prevalence in patients with acute LBP (Kang *et al.*, 2009). However, a large proportion of asymptomatic individuals present HIZ in MRI (among various other abnormalities) (Jensen *et al.*, 1994a; Stadnik *et al.*, 1998; Carrino *et al.*, 2009; Cheung *et al.*, 2009) and therefore confirmation of the diagnosis is performed by provocative discography. Discography has the advantage of better visualisation of the annular defect morphology and provoking a typical pain pattern when increasing the intradiscal pressure (Carragee and Alamin, 2001). As a consequence of recent investigations that have demonstrated the risk of accelerated disc degeneration after discography, the use of this invasive method is currently more restricted and control-discographies of adjacent, healthy, segments are becoming obsolete (Carragee *et al.*, 2009).

For the detection of location and severity of disc herniations and annular tears, MRI is without doubt the

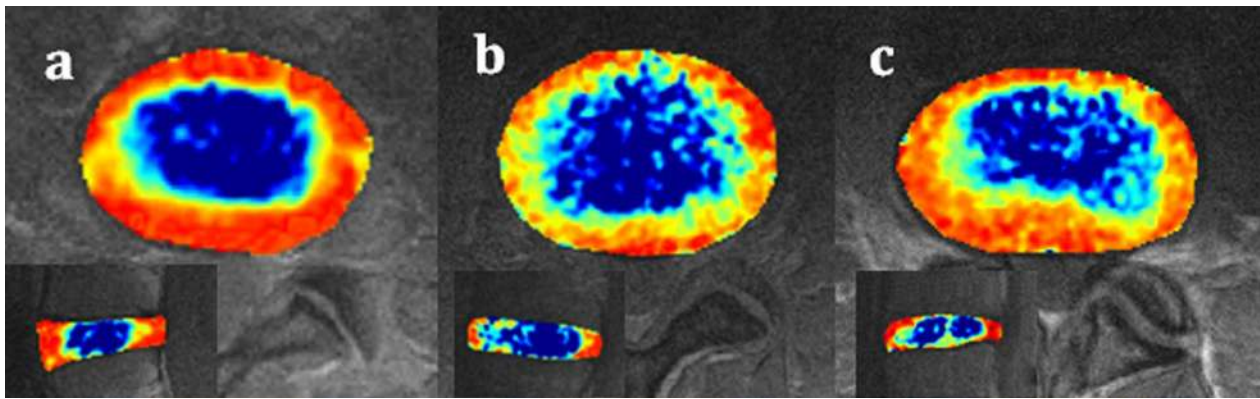


Fig. 1. Axial and mid-sagittal T2-mappings of L4/5 intervertebral discs. **a)** Healthy disc (grade 1) with clear demarcation of NP/inner/outer annulus; the annulus shows a uniform thickness. **b)** Moderately degenerated disc (grade 2); demarcation becomes unclear, loss of orientation of annulus fibres, uniform thinning of the AF. **c)** Moderately degenerated disc (grade 2) with unilateral, posterior thinning of the AF and relatively healthy contralateral AF (Watanabe *et al.*, 2007).

method of choice, as all relevant structures are visible with one non-invasive investigation. There are various classifications of disc herniations, mostly based on their location (central, paramedial, posterolateral, lateral; foraminal or extraforaminal) or degree of protrusion (normal, bulge, extrusion, protrusion, sequestration) (Jensen *et al.*, 1994b). The type of annular tear is usually classified based on sagittal MR images by orientation and localisation of the lesion (concentric, transverse and radial) (Yu *et al.*, 1988). With regards to annulus repair strategies, it is obvious that transverse tears (rim lesions), which represent a disruption of the annulus from the underlying bone, will need a different implant design from the more common radial tears, which are a rupture within the AF itself. The Dallas discogram classification describes the extensions of radial tears within the AF (Sachs *et al.*, 1987). A more complete classification from Carragee *et al.* (2003) combines the type/extent of herniation and the extent/size of the underlying radial tear and is, therefore, also superior in terms of prognostic value.

Unfortunately, all current classifications focus more on the amount and localisation of herniated NP material and less on the underlying morphology and grade of degeneration of the AF, which would provide essential knowledge in creating a successful AF repair. Modern, quantitative MRI techniques on high field units show a variety of different AF morphologies and have the capability to detect degenerative changes at earlier stages (Watanabe *et al.*, 2007; Hoppe *et al.*, 2012; Zobel *et al.*, 2012). Such individual differences need to be considered in AF repair strategies (Fig. 1).

Current treatment options

There are several options available today for the treatment of discogenic low back pain. Segmental fusion or prosthetic replacement of the painful disc are established and present satisfactory short-term results for restrictive indication. However, these techniques are invasive, costly and have the potential to generate new problems when the biomechanical properties of the spine are altered, creating

potential adverse effects on the adjacent levels (Ekman *et al.*, 2009). Less invasive methods were developed with the intention to denervate the observed pathological ingrowth of nerve fibres into the dorsal AF by thermocoagulation. Techniques like PIRFT (percutaneous intradiscal radiofrequency thermocoagulation) or IDET (intradiscal electrothermal therapy) are still performed, although there is only low quality evidence regarding effectiveness and possible complications such as radiculopathy and infection (Ercelen *et al.*, 2003; Freeman *et al.*, 2005; Kvarstein *et al.*, 2009; Pauza *et al.*, 2004). A less destructive, regenerative treatment is in clear clinical demand, though such a therapy would also have to address the problem of pathological innervation.

Discectomy has been shown to be an effective treatment for acute disc herniation with regard to neurological symptoms, but fails to address the altered biomechanical properties of the segment and the resulting annular defect. In this situation, the surgeon faces the dilemma of how extensive a discectomy should be performed. If only the extruding material of the NP is resected, a relevant risk of recurrent disc herniation is well documented. However, if all or most of the NP is resected, there is also a significant chance that lost biomechanical function leads to instability or collapse of the segment (Moore *et al.*, 1994a; Kambin *et al.*, 1995; Yorimitsu *et al.*, 2001; Suk *et al.*, 2001; Vucetic *et al.*, 1997). NP replacement or regeneration to restore the biomechanical function of the disc as a shock absorber will only be successful in the presence of a functional AF to withstand the necessary intradiscal pressure (Veres *et al.*, 2008; Thompson *et al.*, 2000; Fazzalari *et al.*, 2001). Several attempts to close an AF defect after discectomy have been undertaken, of which the most obvious method of direct suture of the AF is technically very demanding – due to limited space and potential injury to the proximal neurological structures. To our knowledge no clinical studies have been reported, and in animal models no sufficient reinforcement could be shown after experimental AF defects were sutured without the use of sealants such as fibrin glue (Ahlgren *et al.*,

2000; Heuer *et al.*, 2008b). Despite this, all-inside sutures with anchors that allow this type of minimally invasive application in human patients are already commercially available. In addition, although early promising short-term results have been presented at scientific meetings, there are still no peer-reviewed publications available that would demonstrate safety and effectiveness of these sutures over a longer period (Bourgeault *et al.*, 2007; Bailey *et al.*, 2010; Guyot and Griffith, 2010). Disc herniations that result from larger radial tears (>3 mm) with more damage to the inner annulus have a higher risk for recurrence and limited healing potential. For these types of defects, an obvious approach is to introduce an implant that seals and reinforces the AF defect by either suturing to the remaining AF (e.g. InClose®) or anchoring into the adjacent vertebral bone (e.g. Barricaid®) (Carragee *et al.*, 2003; Osti *et al.*, 1990). Again, despite early promising reports at scientific meetings, there is still a lack of published data that would confirm its safety and efficacy (Bajanes *et al.*, 2007; Ledic *et al.*, 2007). The difficulties in proof of efficacy for AF repair and recent reports of implant dislocation or occurrence of hernias on the contralateral side imply that purely mechanical repair may not be sufficient for all kinds of defects and that biology and morphology of the AF need to be respected (Maestretti *et al.*, 2012).

Biomechanical Demands for AF Repair

Native AF mechanical properties

The design of successful AF repair strategies requires a clear understanding of the functional biomechanics of the healthy and diseased disc. The structural and mechanical properties of the healthy disc change from the AF to the NP. The NP is highly pressurised and the AF prevents radial disc bulge by generating large hoop stresses. The annulus also resists large tensile and compressive strains as the disc undergoes 6 degree of freedom motion. The cartilaginous endplate is an interface tissue connecting the disc to the adjacent vertebral bodies, and functions to distribute stresses between the disc and vertebrae and to act as a gateway for nutritional transport in the avascular disc. Therefore, AF repair strategies need to withstand the high tensile hoop stresses generated from NP pressurisation and tensile and compressive stresses resulting from spinal motion.

Changes in the IVD and AF structure in degeneration and disease

Natural AF aging

During aging, there is a loss of NP pressurisation, which shifts the load carriage mechanisms in AF and NP regions. ECM breakdown is slow, and degenerative changes accumulate chronically as opposed to an instantaneous or acute insult. Although alterations in the AF may occur later or with less severity than those in the NP during aging, they are very significant and loss of AF integrity may greatly accelerate the rate of degeneration. The range of mechanical properties in the healthy and degenerative disc can be found in Table 1. In general, there is an increase in the compressive modulus due to tissue compaction, a

moderate increase in the shear modulus, and an increase in radial permeability (Iatridis *et al.*, 2006). With aging, the width of the annulus is found to increase by 80 %, with compressive peak stresses increasing by 160 % (Adams *et al.*, 1996). In addition, there is an alteration to the crosslinking of the fibres (Roughley, 2004), gradual decrease in the number of lamellae and a loss of organisation in these layers, which leads to higher localised shear strains (Iatridis and ap Gwynn, 2004) and a greater opportunity for discontinuities in fibre arrays and cleft formation (Schollum *et al.*, 2010).

Accelerated degeneration or disease

The age-related changes described previously hold true for the diseased state and many of the processes of aging and degeneration occur in parallel. Abnormal loading not only affects tissue wear but also fluid flow and disc nutrition. Certain areas of the disc, such as the posterolateral region, are subjected to greater stresses and are more susceptible to micro-failure or herniation.

Acute injuries caused by overloading or a remodelling of the disc as a result of altered loading, such as immobilisation, can both result in a degenerative cascade and disease progression (Iatridis *et al.*, 2006). Therefore there exists a healthy window for loading, where too little loading (immobilisation) or too much loading (overloading) can both lead to remodelling with abnormal disc composition, structure and mechanical properties (Stokes and Iatridis, 2004). Acute annular injuries observed in puncture models affect not only the fibre structure but also the pressurisation and fluid flow (Hsieh *et al.*, 2009; Michalek and Iatridis, 2012). While the laminated fibre network in the healthy annulus is highly effective at arresting crack propagation, multiple failure patterns occur with fibre breaks being a likely failure mode, resulting from extreme or abnormal loading conditions (and/or when the collagen network is degraded), whereas delaminations are likely a result of damage accumulation due to annular shearing and loss of NP pressurisation (Iatridis and ap Gwynn, 2004).

Lifestyles exposing people to heavy physical work or vibrations can lead to higher incidences of disc degeneration (Pope *et al.*, 1998; Williams and Sambrook, 2011). However, epidemiological studies have also shown that these lifestyle exposures could play less of a role than genetic influences (Battie and Videman, 2006). It is likely that genetic influences interact with biomechanics and other environmental factors, for example by diminishing AF or NP tissue quality, or through alterations in anthropometric factors, spine shape or muscle attachments that also modify the stress state in the disc (Williams and Sambrook, 2011).

Motion segment dysfunction associated with IVD degeneration

Changes to the spinal motion segment during degeneration begin with a period of hypermobility, which leads to increased tissue stiffness and consequently results in hypomobility. Fujiwara and co-workers observed this with increasing segmental motion through degeneration grade IV, but decreased motion with grade V (Fujiwara *et al.*, 2000). The IVD must withstand large amounts

Table 1. Biomechanical demands for an AF repair: healthy and degenerative properties of the human lumbar disc.

	Property	Disc Grade	Property Range	Reference
Organ Level / Motion Segment (<i>in situ</i> testing)	Disc height	Healthy	11.1 ±2.5 mm 11.3 ±0.3 mm	(Chen and Wei, 2009) (O'Connell <i>et al.</i> , 2007)
		Degenerative	9.2 ±2.9 mm (spondylo-listhetic disc height)	(Chen and Wei, 2009)
	Intradiscal pressure	Healthy	0.5 MPa (leakage pressure) 1.9 MPa (300 N load pressure) Single patient 0.1-2.3 MPa	(Panjabi <i>et al.</i> , 1988) (Adams <i>et al.</i> , 1996) (Wilke <i>et al.</i> , 1999)
		Degenerative	0.2 MPa (leakage pressure) 1.3 MPa (300 N load pressure)	(Panjabi <i>et al.</i> , 1988) (Adams <i>et al.</i> , 1996)
	Torsional mechanics	Healthy	0.7° - 0.8° (with 8° pelvic rotation) 3°max axial rotation 3.18 ±0.89 N·m/°	(Blankenbaker <i>et al.</i> , 2006) (Haughton <i>et al.</i> , 2002) (Costi <i>et al.</i> , 2007) (Showalter <i>et al.</i> , 2012)
		Degenerative	1.8° - 3.2° (with 8° pelvic rotation)	(Blankenbaker <i>et al.</i> , 2006) (Haughton <i>et al.</i> , 2002)
Tissue Level (<i>in vitro</i> testing)	Compressive (confined compression)	Healthy	0.56 ±0.21 MPa (H_A , aggregate modulus)	(Iatridis <i>et al.</i> , 1998)
		Degenerative	1.10 ±0.53 MPa (H_A , aggregate modulus)	(Iatridis <i>et al.</i> , 1998)
	Tensile (circumferential samples)	Healthy	12.7 MPa (E, tensile modulus)	(Acaroglu <i>et al.</i> , 1995)
		Degenerative	9.4 MPa (E, tensile modulus)	(Acaroglu <i>et al.</i> , 1995)
	Shear	Healthy	25 < G_{eq} (kPa) < 110	(Iatridis <i>et al.</i> , 1999)
		Degenerative	25 < G_{eq} (kPa) < 140	(Iatridis <i>et al.</i> , 1999)

of compression, measured to be more than 200 % body weight on the lumbar disc during sitting and standing at a 20° angle (Nachemson, 1966). A successful AF repair would reduce excessive spinal motions, recover nonlinear stiffness behaviours of the motion segment and restore NP pressurisation. Such repair has the potential to inhibit progression of biomechanical alterations in the disc, including tissue compaction, stiffening and pathological remodelling from altered mechanobiology.

Biomechanical Demands for AF Repair

The repair of a damaged disc is complicated for many reasons, including the risk of reoccurrence as well as the prevention of stress shielding in the tissue, both of which could exacerbate the clinical problem the AF repair strategy is trying to mitigate. From the many mechanical tests that have been performed on AF tissue and described in this review, we parameterise many target tissue and motion segment level biomechanical behaviours. Tissue mechanics remains an important technique for future studies optimising biomaterials by 'tuning' them to match the biomechanical behaviours of native AF tissues. Motion

segment mechanics provides another technique required for future studies in order to characterise the capacity of the AF repair to restore biomechanical function of the motion segment.

Finite element models

While tissue-scale testing can provide target properties for AF repair materials and organ-scale experiments give basic insight into the mechanical response of the disc, they are limited for the study of the internal mechanics of an annular defect and the performance of a repair. Finite element (FE) modelling can provide a more precise description of the local mechanical environment that exists within the damaged tissue and can quantify the mechanical design constraints required for successful AF repair.

The response of the disc to complex loading, and the critical loading cases that determine the risk of prolapse, have been the subject of several simulation studies. Shirazi-Adl (Shirazi-Adl, 1989) provided an early estimate of failure limits of the annulus, showing that asymmetric lifting with bending and rotation produces annulus fibre strains over 20 %. Fibre rupture initiated in the inner

posterolateral annulus and propagated as a radial tear. Disc degeneration also influenced the risk of prolapse in an advanced model incorporating the spatial variation in annulus fibre orientation (Schmidt *et al.*, 2007). These studies present a consistent conclusion that complex loading (i.e., combined flexion plus compression, or torsion plus bending) leads to high fibre strains, especially in the posterolateral annulus (Wang and Li, 2005).

The process of creating and validating a numerical simulation itself can provide valuable insight into the micro- and macro-mechanical response of the annulus. Complex loading of disc specimens, following a stepwise reduction of the structure (posterior elements and ligament transection, anterior and posterior longitudinal ligament transection, nucleotomy) (Heuer *et al.*, 2008a), has shown the important dependence of the annulus on the nucleus for internal support. These data were incorporated into the validation of the intrinsic material properties of the annulus in simulation models (Schmidt *et al.*, 2006), where only one unique combination of ground substance and fibre properties was valid for all loading directions and magnitudes, which provides a potential design target for AF repair materials. In a bottom-up approach, starting with known individual fibre properties (Malandrino *et al.*, 2012), the predicted optimal region-specific fibre orientation for normal annulus function is consistent with previous anatomical and biomechanical descriptions (Schmidt *et al.*, 2007).

A thorough understanding of the mechanical consequences of annular lesions is critical for the development of repair strategies. Surprisingly little information is available. The effects of radial and circumferential lesions have been analysed in a non-linear segmental model where injury was modelled by element removal in the posterolateral portion of the disc (Goel *et al.*, 1995). Interlaminar shear stresses were higher in the posterolateral regions of intact discs under compression and increased with annular injury. Furthermore, stresses and disc bulge were also sensitive to circumferential injuries. The effects of rim, radial or circumferential annular lesions were investigated, with or without the simultaneous loss of nuclear pressurisation (Little *et al.*, 2007). Loss of nucleus pulposus pressure had a greater effect on the disc mechanics than the presence of annular lesions, implying that the development of annular lesions alone (prior to degeneration of the nucleus) had minimal effects on disc mechanics. However, nucleus depressurisation likely leads to an inward bulge of the annulus, consistent with *in vitro* findings (Heuer *et al.*, 2008a) and accelerates annulus damage, thus restoration of the nucleus should be considered a parallel design goal of AF repair.

Simulation tools to assess functional performance of AF repair strategies are rare. While suturing techniques for AF repair have been proposed for decades, simulation of the retention of intradiscal pressure following annular suturing has only recently been reported (Chiang *et al.*, 2012). In a segmental model, a modified purse string suture technique was compared to two simple horizontal sutures or a single continuous crossed suture. Closure of a 4.8 mm transverse cleft was simulated and the modified suturing technique showed a substantially higher sealing potential.

This was validated in a subsequent *in vitro* trial, where higher nuclear pressures could be retained under load. The only other reported simulation of AF repair evaluated the performance of a biomimetic composite disc implant consisting of an artificial polymer nucleus contained within a fibre-reinforced textile annulus (Noailly *et al.*, 2012). Parametric variation of the material properties and fibre orientation was performed with the goal of optimising implant design to match global biomechanical properties of the natural disc. While the material properties were shown to play an important role, fibre orientation was especially relevant for the control of torsional loading (and by association annulus loading). This finding highlights how simulation models offer an important and underutilised tool to rapidly screen repair strategies and advance the design of AF repair methods.

Cells of the AF

Cell morphology and ECM

The mechanical function of the AF strongly depends on the structure of the ECM, which is maintained by a sparse population of cells; the average cell density in human AF approximates 9000/mm³ (Maroudas *et al.*, 1975). AF cells are derived from the mesenchyme and have been described as fibroblast-like cells with spindle shaped morphology (Roberts *et al.*, 2006). However, marked regional variations in cell morphology exist that appear to correspond with the local mechanical environment (Bruehlmann *et al.*, 2002). Morphologic and molecular studies also provide evidence for the presence of a distinct cell polarity in the AF cells (Gruber *et al.*, 2007a). Molecules with roles in cell polarisation, e.g. PAR 3, were expressed more abundantly in outer compared to inner AF and to NP cells and may be important for the synthesis of the specialised lamellar structure.

The inter-lamellar septae consist of proteoglycan aggregates with water-binding characteristics similar to those in the NP, whereas the lamellar layers contain monomeric proteoglycan molecules that interact with collagen fibres (Ortolani *et al.*, 1988). The microfibrillar network is suggested to play a crucial mechanical role (Yu *et al.*, 2007). Without distinguishing regional variations, the glycosaminoglycan (GAG) to hydroxyproline ratio within the AF of human lumbar discs was found to be on average 1.6:1, with little variations among age or degeneration levels (Mwale *et al.*, 2004). This is in contrast to the NP, where a GAG to hydroxyproline ratio of approximately 25:1 was found in young healthy discs, while this ratio was decreased to about 5:1 in degenerative NP.

Cellular phenotype

Expression ratios of matrix proteins have also been considered to distinguish between cellular phenotypes in NP, AF and articular cartilage (AC). While both COL2/ACAN and COL2/COL1 ratios were highest in AC, COL2/ACAN was higher in AF than NP and COL2/COL1 higher in NP than AF cells (Clouet *et al.*, 2009). This study with rabbit cells also found significantly increased expression of collagen type V in AF compared to both

Table 2. Selection of matrix and phenotypical characteristics of the annulus fibrosus.

Marker molecule	Characterisitic (Method used)	Species	Reference
Glycosaminoglycan / Hydroxyproline ratio	Ratio 1.6:1 (biochemical assay)	Human	(Mwale <i>et al.</i> , 2004)
Elastin	Co-localisation with fibrillin-1 (immunohistochemistry)	Human; bovine	(Yu <i>et al.</i> , 2007)
Decorin, Lumican	Decreased in aged (>70 y) AF (Western Blot)	Human	(Singh <i>et al.</i> , 2009)
Collagen II / Collagen I ratio	AF<NP<AC (RT-PCR)	Rabbit	(Clouet <i>et al.</i> , 2009)
Collagen V	AF>NP=AC (RT-PCR)	Rabbit	(Clouet <i>et al.</i> , 2009)
Collagen I; Collagen III; Collagen V; Cadherin-13; Decorin; Versican v3	NP/AF<0.1 (Microarray)	Rat	(Lee <i>et al.</i> , 2007)
Laminin B1; Collagen I; Collagen XIV; Aquaporin 1; CD 163; Caveolin 3; Haemoglobin beta	NP<AF (Microarray)	Canine	(Sakai <i>et al.</i> , 2009)
Tenomodulin; TNFAIP6; FOXF1; FOXF2; Aquaporin 1	AF>NP and AC (Microarray, RT-PCR)	Bovine	(Minogue <i>et al.</i> , 2010)

NP and AC cells, suggesting that type V collagen could potentially be used as a phenotypic marker for AF cells. To explore the phenotypic characteristics of IVD cells more comprehensively, microarray analyses were performed comparing IVD with the phenotypically similar AC cells. Although often greater emphasis was put on the expression profiles of NP cells, these studies are also relevant for AF cells. In rat cells, 10 genes with NP/AF intensity ratios ≤ 0.1 were found, including the proteoglycan decorin (Lee *et al.*, 2007). In a similar investigation on beagle dogs, 77 genes with a NP/AF signal log ratio of -1 or lower were identified, including collagen XIV, cell adhesion molecules and integrin precursors (Sakai *et al.*, 2009). Tenomodulin, a member of the small proteoglycan family, was proposed as a marker for the AF cell phenotype, being expressed at higher levels in AF compared to NP and AC cells in both bovine and human species (Minogue *et al.*, 2010). However, inter-species variations have generally been observed, and difficulties to detect clear differences between AF and NP cells have often resulted in the general classification of a gene as marker for the IVD phenotype (Rutges *et al.*, 2010; Minogue *et al.*, 2010; Power *et al.*, 2011). Table 2 summarises some characteristics of the cellular phenotype and the extracellular matrix of the AF that are known to date. Further research will be necessary to more precisely define the AF cell, since the maintenance or acquisition of the correct cell type is a crucial requirement for successful cell therapy. This is particularly important for differentiation of therapeutic MSCs towards the AF cell phenotype.

Phenotype changes during degeneration

During IVD degeneration, changes in levels of proteoglycans can be observed (Cs-Szabo *et al.*, 2002; Singh *et al.*, 2009). Especially, AF cells may respond to early degeneration by increasing biosynthetic processes, while in severely degenerated tissue a decline in aggrecan and increases in concentrations of small proteoglycans, such as decorin,

biglycan and fibromodulin, may account for the failure of effective repair. Furthermore, tenomodulin expression was found to be increased in degenerative human AF cells (Minogue *et al.*, 2010). Other correlations between the expression of certain genes and age or degeneration state were identified, such as an increase in pleiotrophin mRNA in AF cells with increasing patient age (Rutges *et al.*, 2010), which might be related to an increased vascular ingrowth in degenerate AF (Johnson *et al.*, 2007). Higher levels of glypican-3, cytokeratin-19, matrix gla protein and pleiotrophin were also found in aged compared to young tissue in the rat (Lee *et al.*, 2007).

In order to investigate changes in gene expression profiles of AF cells during degeneration, genome-wide microarray analyses were performed. Laser capture microdissection was used to harvest cells from paraffin-embedded sections of human AF tissue from different degeneration grades (Gruber *et al.*, 2007b). Genes significantly altered during degeneration included cell senescence, cell division, hypoxia-related and other genes important for cell survival. Similarly, in AF cells cultured in 3D carriers, differences in gene expression patterns between healthier and more degenerative discs were observed for a variety of molecules involved in AF metabolism (Gruber *et al.*, 2010). Important genes included those related to ECM synthesis or degradation, cell proliferation, apoptosis, growth and differentiation, or inflammation. Further data also showed that gene expression patterns related to mitochondrial dysfunction were present in the human AF from more degenerative discs (Gruber *et al.*, 2011). In particular, changes were identified in genes involving apoptosis, oxidative stress and senescence.

Cell nutrition

Given that blood supply is restricted to the borders of the cartilaginous endplate and the AF, the two possible ways for nutrient transport are the endplate and the peri-annular

route. For the latter, the amount of nutrients transported depends on the transport properties and the cellular metabolic rates of the tissue. The diffusion of nutrients through the AF may depend on the strain and the direction of diffusion, i.e. the anisotropy of the tissue (Jackson *et al.*, 2008). There are marked gradients in oxygen concentration across the avascular disc, dropping to as low as 1 % O₂ in the centre of a large disc (Urban *et al.*, 2004). While low oxygen is known to promote the NP cell phenotype, AF cell phenotype and metabolic activity do not seem to be affected by changes in O₂ levels (Mwale *et al.*, 2011). Nonetheless, organ cultures have shown that impaired glucose supply can be detrimental for the survival of both NP and AF cells (Junger *et al.*, 2009; Illien-Junger *et al.*, 2010).

Regenerative potential

Although the IVD is considered to have a poor intrinsic repair capacity, minor self-repair processes have been observed, especially in the outer AF (Melrose *et al.*, 2007). Chemotactic mechanisms may play a role in AF repair, as AF cells can be recruited by chemokines, and chemokine receptors have been identified on human AF cells (Hegewald *et al.*, 2012). Recent studies have confirmed continuous cell proliferation in different parts of the IVD, though the proliferation rate is suggested to be low (Henriksson *et al.*, 2009). Findings of distinct stem/progenitor cell niches in the AF border to ligament zone and the perichondrium region also suggest that these cells may be recruited to migrate into the disc (Henriksson *et al.*, 2009), where they may act together with local progenitor cells and potentially with bone marrow-derived mesenchymal stem cells (MSCs) to maintain regenerative processes. Potential migration routes from these stem cell niches towards the AF and the inner parts of the disc have recently been reported (Henriksson *et al.*, 2012). There is also increasing evidence that progenitor cells with characteristics of MSCs are present in the NP, the AF, and the cartilaginous endplate of healthy and degenerated human adult discs (Risbud *et al.*, 2007; Blanco *et al.*, 2010; Feng *et al.*, 2010; Liu *et al.*, 2011). This intrinsic repair capacity might not be sufficient or might be disturbed by inflammatory and catabolic processes that accelerate matrix degradation (Wuertz *et al.*, 2012). Therapeutic approaches have therefore addressed both the repopulation of the disc with autologous cells and supplementation of anabolic, anti-catabolic or anti-inflammatory agents. For AF repair, autologous AF cells could potentially be obtained from a surgical sample and used for regenerative therapy with or without a suitable biomaterial scaffold. However, as such procedures are technically demanding and AF cells from degenerating discs often show signs of senescence, the focus has been directed towards the application of MSCs derived from the bone marrow or potentially from adipose tissue. Although MSC-based IVD regeneration has been well documented, treatments have generally aimed to restore the NP rather than the AF (Grad *et al.*, 2010; Orozco *et al.*, 2011). Co-culture of MSCs and AF cells *in vitro* indicates stimulatory effects in terms of ECM synthesis (Le Visage *et al.*, 2006), and it can be postulated that both paracrine factors released by MSCs and MSC differentiation may contribute to tissue regeneration. Based on the highly

oriented structure and the mechanical function of the AF, the structural and mechanical environment is suggested to be a critical determinant of the cell phenotype that also may guide the differentiation of MSCs (See *et al.*, 2011; Nerurkar *et al.*, 2011a).

In conclusion, further research is needed to identify the optimal cellular environment that supports the AF cell phenotype, whereby inner and outer AF should be distinguished. Moreover, although it is well recognised that apoptosis, senescence and increased synthesis of inflammatory and catabolic mediators play a role in degeneration of the human disc, there is still very little understanding of the underlying causes at the cellular and molecular level. Finally, additional studies are required to characterise the progenitor/stem cell types that reside in the IVD and adjacent tissues and to find ways for *in situ* mobilisation of these immature cells for tissue repair.

Biomaterials

An ideal AF biomaterial structure achieves instant and prolonged mechanical stability and allows new competent tissue to form. Current biomaterial-based strategies aimed at the regeneration of AF tissue focus either on developing an implant that restores the mechanical function of the damaged or diseased AF; on preparing a scaffolding architecture that supports AF and other relevant cells; or on delivering biologics where biologically active compounds and cells promote ECM production and new tissue formation.

Biomaterials-mediated delivery of biologics

The rationale for using biomaterials to deliver biologics is to transport exogenous biological material (cells, proteins, genes, drugs) to a target site where these biomolecules can provide the desired therapeutic effect on the targeted pathology. The AF tissue consists of a variety of cell populations and tissue microstructures that reflect the different physiological stresses experienced. This complicates tailoring a suitable delivery system to the AF (Fig. 2).

Cell delivery

Cell delivery addresses the problem of increased cell death, senescence and lack of paracrine factors in the degenerative disc. Cells delivered to the degenerative disc are envisaged to repopulate the region where the native cells and ECM have been lost. The transplanted cells deposit new matrix proteins, enabling the disc to recover its function. There have been no *in vivo* studies that have attempted to deliver cells to the degenerative AF. The cell choice remains a critical issue, given that the application of bone marrow-derived MSCs might lead to unwanted bone formation (Vadala *et al.*, 2012), in particular in combination with growth factors. A recent *in vitro* study investigating the suitability of AF cells, chondrocytes and bone marrow-derived cells supplemented with recombinant human bone morphogenetic protein 2 (rhBMP2), concluded that chondrocytes are the best candidates for cell delivery to the disc based on sGAG production, mRNA and protein

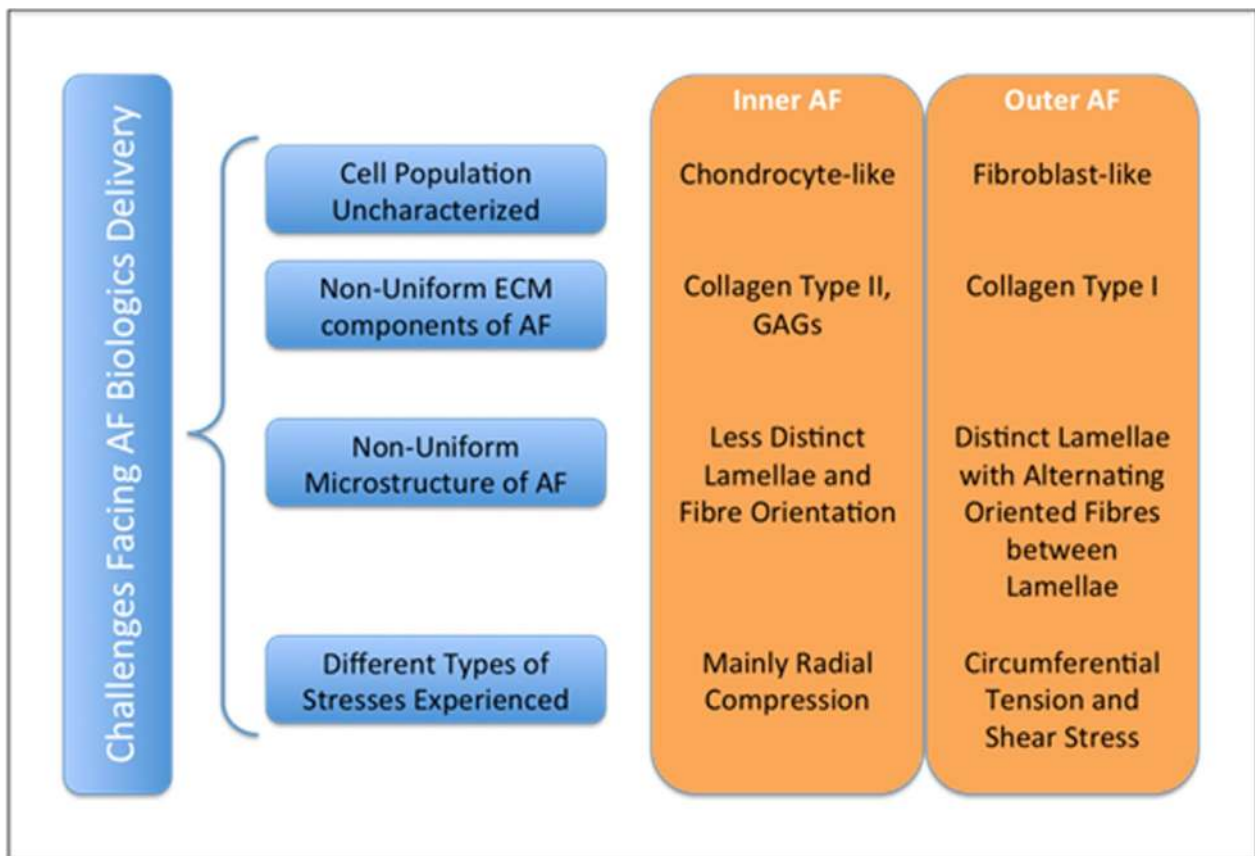


Fig. 2. Schematic of the challenges facing AF biologics delivery.

expression of aggrecan and collagens (Kuh *et al.*, 2009). Yet, as previously discussed, AF cells are currently not well characterised; moreover, the study performed in 2D cell culture limits the relevance of the findings for cell delivery approaches. Significance will increase with the use of appropriate 3D cell delivery vehicles in conjunction with established cell phenotypes (Collin *et al.*, 2011). Ultimately, only longer term organ culture and *in vivo* studies that also take the clinical applicability into consideration will reveal advantages and limitations of different cell therapies.

Protein delivery

The delivery of proteins encompasses growth factors, enzymes and cytokines. Ideally, biomaterials-based delivery systems allow for proteins to have a sustained effect on the target system, such that repeated administration of the proteins can be obviated. Despite the number of studies that have reported the delivery of proteins for therapeutic intervention, none have studied the feasibility of using a biomaterial-based platform to deliver proteins to the AF. In fact, the efficacy of these systems could be drastically improved by incorporation in a non-cytotoxic biomaterial tailored to release the protein in a timely and spatially controlled manner. In addition, the biomaterial can protect and maintain the functionality of the protein over the sustained release time period. The development of such delivery vehicles requires deep understanding of the growth factor functions in AF therapy. The only delivery

system that has been reported to have a prolonged effect on the cells (up to a few weeks) is viral gene delivery (Gilbertson *et al.*, 2008; Zhang *et al.*, 2007; Zhang *et al.*, 2009).

Gene delivery

There are two main methods of gene delivery, i.e. virus-mediated and non-viral methods. For AF gene therapy, most studies have used adenovirus as the vector. Gilbertson *et al.* (2008) and Zhang *et al.* (2007; 2009) both reported the efficacy of various BMPs for ECM production. Virus-mediated gene transfer, delivering growth and differentiation factor-5 (GDF-5) (Liang *et al.*, 2010), and LIM mineralisation protein-1 (LMP-1) (Yoon *et al.*, 2004) were both reported to enhance matrix deposition in *in vivo* models. Lentiviral shRNA silencing of CHOP (C/EBP homologous protein), an apoptosis regulated gene, was shown to inhibit stretch-induced apoptosis in AF cells. Intradiscal injection of this shRNA improved MRI and histologic scores in a rat model, confirming the potential of anti-apoptotic gene therapy (Zhang *et al.*, 2011d).

However, all viral gene delivery systems have the inherent problem of potentially becoming infectious. Non-viral delivery systems might therefore be the preferred delivery vehicle. The drawback of non-viral gene delivery systems is the low transfection efficiency. Many studies are now investigating more efficient protocols that use naturally occurring ECM components like elastin, chitosan and hyaluronan to aid in delivery of genes (Dash *et al.*,

2011; Mahor *et al.*, 2011; Rethore *et al.*, 2009). These systems have the flexibility to alter the rate at which exogenous DNA is released to the surrounding cells and also demonstrate an improvement in gene expression. With viral gene delivery systems becoming more unattractive due to the possibility of inherent complications, these newer non-viral controlled gene delivery systems will play an increasing future role in gene therapy for AF regeneration.

Biomaterial scaffolding and support structures

Natural materials and biological considerations

To create extracellular matrix analogues and provide substitute materials for AF repair (Gruber *et al.*, 2006), tissue-engineering scaffolds have been prepared from natural materials. Investigators have prepared biomimetic matrices based on collagen, a major component of the AF ECM. Collagen is used in the form of gels or as three-dimensional porous structures and has several advantageous properties, such as a weak immunogenicity and the ability to support a large variety of cell types (Gruber *et al.*, 2004). In AF tissue regeneration, mainly collagen type II has been used. When compared to collagen type I, which is also abundantly present especially in the outer parts of the AF, scaffolds prepared from collagen type II showed better maintenance of cell numbers and higher production of ECM (Saad and Spector, 2004). These scaffolding structures were also combined with glycosaminoglycans, such as chondroitin-6-sulphate and hyaluronan, to mimic more closely the extracellular matrix composition (Liu *et al.*, 1999; Schneider *et al.*, 1999; Yannas *et al.*, 1989; Alini *et al.*, 2003). Analogous gels have also been prepared from other natural hydrogels, such as alginate (Bron *et al.*, 2011; Chiba *et al.*, 1997; Maldonado and Oegema, Jr., 1992; Wee and Gombotz, 1998), agarose (Gruber *et al.*, 1997; Gruber *et al.*, 2004) and chitosan (Shao and Hunter, 2007). The remarkable mechanical properties of silk, combined with a very high propensity for cell attachment, make it a material of great interest for use in AF tissue engineering (Chang *et al.*, 2007; Chen *et al.*, 2003).

Soluble atelocollagen from collagen type II has been considered for IVD regeneration (Sakai *et al.*, 2003; Sakai *et al.*, 2006; Sato *et al.*, 2003a; Sato *et al.*, 2003b). In this form, compositions containing cells have been investigated for AF tissue regeneration (Alini *et al.*, 2003; Alini *et al.*, 2002; Sakai *et al.*, 2006; Sakai *et al.*, 2003). To create and regulate circumferential fibril alignment, cell-induced contraction of collagen type I gels was achieved using AF cells, and the feasibility of this technique for tissue engineering of entire IVDs was demonstrated *in vitro* and in a rat model *in vivo* (Bowles *et al.*, 2010; Bowles *et al.*, 2011). Tissue regenerating compositions that can be injected into the degenerative disc offer advantages over the more invasive surgical approaches required when implanting (cell seeded) scaffolds.

The reported biomaterials and scaffolds adequately support cells, but unfortunately they are until now inherently mechanically weak compared to AF tissue. Thus, research has focused on the necessity to create scaffolds with mechanical properties that better resemble those of the natural AF.

Synthetic materials

To obtain scaffolding structures with more appropriate mechanical properties than those of most natural materials available, many studies have been conducted on the use of synthetic biodegradable polymeric materials. Synthetic polymeric biomaterials have proven to be useful in many tissue engineering applications, possessing important characteristics such as their highly reproducible syntheses and predictable properties, their lack of immunogenicity and their ease of processing into desired structures and implants (Freed *et al.*, 1994). Poly(ϵ -caprolactone) (PCL), poly(lactide) (PLA) and poly(glycolide) (PGA) and copolymers prepared from the respective monomers are synthetic polyesters that have been studied for AF tissue regeneration. Mizuno *et al.* (2006) used a nonwoven mesh of PGA fibres coated with PLA to generate AF scaffolds for construction of composite tissue engineered IVDs. Several of these biodegradable polymers have also been used to prepare composite materials with ceramics (Blaker *et al.*, 2005; Helen *et al.*, 2007; Wilda and Gough, 2006) or with natural polymers such as hyaluronic acid (Choi *et al.*, 2009; Nesti *et al.*, 2008) and fibrin (Moutos *et al.*, 2007; Sha'ban *et al.*, 2008a; Sha'ban *et al.*, 2008b). In this manner, the scientists aimed to enhance integration with tissue and biological activity of the structure. Furthermore, potentially detrimental effects of the polyester degradation products could be counteracted (Coombes *et al.*, 2002; Sherwood *et al.*, 2002).

Polyurethane and polyamide scaffolds presenting polar surfaces might improve cell adhesion and tissue integration (Attia *et al.*, 2011; Santerre *et al.*, 2005; Yang *et al.*, 2009). Also, the visco-elastic properties and flexibilities of these synthetic biodegradable polymers resemble those of the AF without the highly complex architecture of the tissue, which makes them very attractive as biomaterials for AF repair.

Oriented scaffolding structures

To engineer annulus fibrosus with properties similar to that of native tissue, the design and preparation of scaffolds which recapitulate some of the mechanical features of the complex structural anisotropy of the AF and can induce orientation of newly produced ECM are being investigated (Johnson *et al.*, 2006). Numerous AF scaffolding structures have been prepared using a variety of techniques, which include freeze-drying (Cole *et al.*, 1985; Rong *et al.*, 2002; Sato *et al.*, 2003a; Sato *et al.*, 2003b; Schneider *et al.*, 1999), salt-leaching (Wan *et al.*, 2007; Wan *et al.*, 2008), weaving and non-weaving (Mizuno *et al.*, 2006; Moutos *et al.*, 2007), thermally induced phase separation (TIPS) (Blaker *et al.*, 2005) and electrospinning (Koepsell *et al.*, 2011; Nerurkar *et al.*, 2009; Nesti *et al.*, 2008).

Electrospinning is the most often used method to prepare porous membranous structures for use as AF tissue engineering scaffolds. It allows the preparation of highly porous structures composed of large numbers of fused oriented nanofibres that mimic the oriented collagen fibres in the native AF (Fig. 3). There is in fact a major trend toward the design and preparation of oriented biomaterials.

Nerurkar *et al.* (2007; 2009; 2010; 2011a; 2011b) demonstrated the potential of electrospinning PCL to

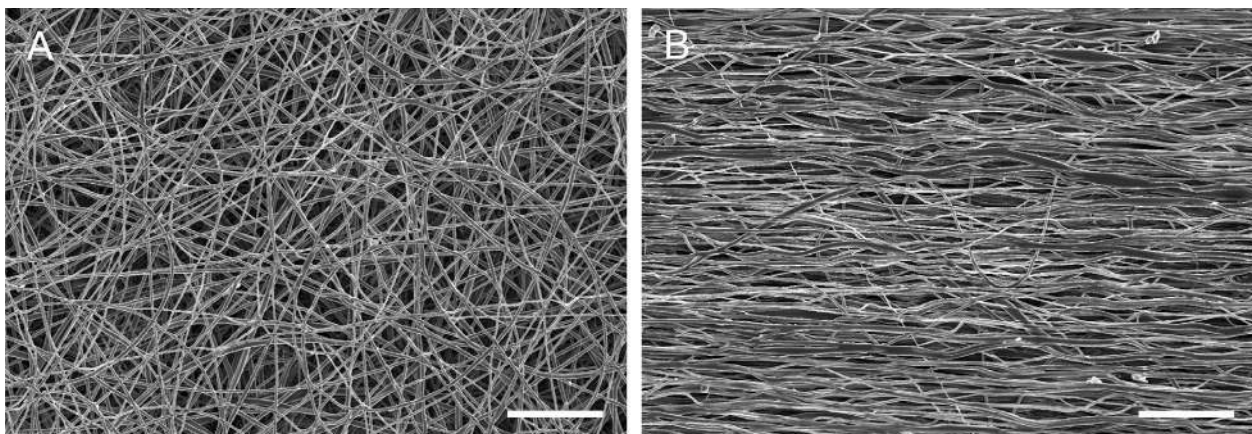


Fig. 3. Scanning electron microscopy images of the surface of non-oriented (A) and oriented (B) electrospun biodegradable segmented poly(ϵ -caprolactone)-isosorbide poly(ester-urethane) fibrous scaffolds. Scale bar = 20 μm .

generate AF-like tissue. Their scaffolds were composed of layers of nanofibres oriented at specific angles similar to the native tissue. As the scaffold mimics the anisotropy and non-linearity of AF tissue, it is a most suited substrate for AF cell and/or MSC adhesion and the oriented ECM production that is necessary to obtain the proper mechanical properties. To date, the most promising results in terms of recapitulating AF mechanical properties and directing cell behaviour have been obtained with these anisotropically oriented scaffolds.

The growing interest in IVD repair and AF tissue engineering in recent years has provided fundamental understanding for the rational conception of regenerative biomaterial implants for AF repair. A biomaterial-based regenerative therapy for the damaged or diseased AF has not yet been reported, but this biomaterial will probably allow for new tissue formation and integration with the host *via* the delivery of biologics. At the same time it will provide the necessary structural and mechanical stability for functional recovery, preventing re-herniation and expulsion of the NP.

Preclinical Models

There is a general agreement that the ideal model that reflects the degenerative changes occurring in the human IVD does not exist to date. Nevertheless, both *in vivo* models and organ culture systems have provided an important insight into the feasibility and mechanisms of new treatment methods to repair or regenerate a damaged disc. Every model has its limitations, and these need to be carefully taken into account in the experimental design and the interpretation of study results (Alini *et al.*, 2008). While novel cell- and material-based NP regeneration has been addressed in various investigations, work that has focused on AF repair is fairly limited. This appears surprising, given that an induced IVD disease model often implies an AF defect.

Small animal models

For basic studies investigating the metabolic and phenotypic responses of endogenous cells or therapeutic

cell populations to changes in their environment, small animal models are useful and can provide important fundamental understanding. Genetic modifications such as the use of mice deficient in a distinct structural or regulatory protein have revealed the specific roles of these molecules. For example, it was found that GDF-5 was essential for the normal lamellar architecture of the AF and the structure of the NP (Li *et al.*, 2004), and that a deletion of type IX collagen led to premature onset of IVD degeneration (Boyd *et al.*, 2008). Recently, the DNA repair deficient mouse model of accelerated aging, which had been reported to exhibit age-related IVD degeneration, was used to explore the role of the NF κ B signalling pathway in the onset of age-dependent degenerative changes (Nasto *et al.*, 2012).

Mechanical injury models have also been described in mice for proof of concept of gene therapy approaches. Adenoviral delivery of GDF-5 was able to restore the function of a disc in which degeneration had been induced by needle puncture of the AF (Liang *et al.*, 2010). Mechanical intervention has frequently been used to induce degenerative changes also in the rat-tail model. Increased or decreased loading or motion and annular injury have been demonstrated to affect the IVD integrity (Yurube *et al.*, 2012; Zhang *et al.*, 2011a; Wuertz *et al.*, 2009). Furthermore, rat models have been employed to investigate the potential of cell therapy (Wei *et al.*, 2009a) and of gene therapy/gene modulation (Zhang *et al.*, 2011d). While in the latter work gene silencing was proposed to prevent AF cell apoptosis, studies specifically focusing on AF repair are rare. Recently, the feasibility of replacing a native rat caudal IVD by an engineered whole IVD composite was documented (Bowles *et al.*, 2011). The tissue engineered construct could maintain disc height, accumulated newly synthesised matrix, integrated into the spine, and showed dynamic mechanical properties similar to native discs. Nevertheless, the composites lacked the lamellar structure of the AF and the native distribution of collagen types I and II, and major challenges remain regarding the translation of such a concept to larger animals and into clinics.

Rabbits have commonly been used as models for the evaluation of new IVD regenerative therapies. The most widely applied surgical intervention is to injure the

AF. Total annular puncture causes NP avulsion and the occurrence of disc degeneration within a relatively short time. Therefore, this model has been used to study the effect of injection of growth factors, such as GDF-5 or osteogenic protein 1 (OP-1) (Masuda *et al.*, 2006; Chujo *et al.*, 2006; Kim *et al.*, 2005; Sobajima *et al.*, 2005). In a rabbit nucleotomy model, injection of autologous MSCs embedded in atelocollagen gel not only restored the NP but also the inner AF structure, significantly improving disc height and MRI signal intensity (Sakai *et al.*, 2006). Nevertheless, a critical risk of annular puncture, that should not be underestimated, is the leakage of injected MSCs that may induce serious side effects such as osteophyte formation, finally leading to the formation of mineralised tissue. Such observations highlight the need not only for stable cell carrier materials but also for effective annulus repair technologies (Vadala *et al.*, 2012).

Large animal models

Larger animal models with disc size, loading and nutrition conditions close to the human are required to assess the efficacy of new procedures or materials for IVD regeneration and in particular for AF repair. The effect of directly repairing three different annular incisions on the healing strength was tested in a sheep model (Ahlgren *et al.*, 2000). When annular defects, followed by partial discectomy, were either repaired directly or left unrepaired to heal spontaneously, no significant differences in the healing strength were noted. In another sheep model an annulotomy was created and repaired by grafting a patch and plug made of small intestinal submucosa (Ledet *et al.*, 2009). Although the treated levels did not maintain the MRI signal intensity of intact levels and the newly formed AF tissue was sparsely organised without specific orientation and lamellar-like structure, the annular closure was improved and resulted in a functional recovery compared to the untreated annulotomy levels.

A number of studies have utilised the ovine annular rim-lesion model since its development in 1990 (Osti *et al.*, 1990). Such superficial lesions are considered clinically relevant, as connections between lesions and radiating tears have been found in aged spines (Vernon-Roberts *et al.*, 2007). Degeneration after a partial AF lesion is slower than after total AF puncture and involves active degenerative processes. Fixation failed to stabilise the affected IVD and could not prevent subsequent degenerative changes (Moore *et al.*, 1994b). Controlled outer AF lesions 5 mm deep by 5 mm wide have generally led to (i) early spontaneous repair of outer AF defects, (ii) propagation of inner AF defects, i.e. delamellations, circumferential, radial or transverse tears resulting in severe distortion of the AF structure, loss in disc height and degeneration, and (iii) vascular changes in the endplate and adjacent vertebrae (Melrose *et al.*, 2008). A recent investigation revealed that deregulation of metalloproteinases is involved in the changes ultimately leading to mechanical destabilisation (Melrose *et al.*, 2012). The efficacy of a single injection of rhBMP13 at the site of a controlled annular stab injury in an ovine model was recently demonstrated (Wei *et al.*, 2009b). BMP13 treatment resulted in prevention of cell loss (or cell mobilisation), prevention of neo-vascularisation,

deposition of collagen fibres in the AF, production of proteoglycans in the NP, and retention of the original disc height.

A model of annular injury was also described in miniature pigs, where consistent sequential and progressive degeneration was observed that did not improve by the final time point of 39 weeks (Yoon *et al.*, 2008). Furthermore, a porcine annular puncture model was utilised to test the efficacy of non-cell-based materials to prevent the recurrence of disc herniation (Wang *et al.*, 2007). Disc injuries could not be recovered within a 2-months healing period and the implantation of gelfoam material improved the disc integrity.

Goat models of disc degeneration have been described, including annular injuries with a surgical blade or a 4.5 mm drill bit, whereby the latter resulted in more significant histological changes (Zhang *et al.*, 2011b; 2011c). A reproducible, mild but progressive degeneration of a goat disc can be achieved by injection of a defined concentration (0.25 U/mL) of chondroitinase ABC (Hoogendoorn *et al.*, 2008). In general, these models have recently been employed to evaluate novel NP regenerative therapies, while reports of AF repair strategies are limited to date.

An attractive approach is the use of specific breeds of dogs that show spontaneous IVD degeneration as a translational model (Bergknut *et al.*, 2012). In chondrodystrophic breeds, signs of degeneration are already observed in young animals (<1 year), while in non-chondrodystrophic breeds only older animals (5-7 years) show degenerative changes. Close similarities in the gross pathology and histology can be observed between humans and dogs. Spontaneous models are more difficult to use for evaluation of therapy approaches, as progression cannot be controlled and large variations between animals, even from the same breed, are expected. Ultimately, all animal models can only answer specific research questions regarding methodology, materials or cellular responses, while the human pathology cannot be reproduced.

Organ culture models

As a striking alternative to *in vivo* studies, organ culture models have been developed that enable the maintenance of large animal IVDs with endplates in a controlled *ex vivo* environment. This approach allows testing of cells, nutrients and supplements, and mechanical impact on host disc cells that are maintained in their physiological surroundings (Fig. 4). In optimised bioreactor systems, caudal or lumbar discs from calf, sheep, or goat can be cultured under simulated physiological nutrition and loading conditions for up to several weeks without significant loss in cell viability. On the other hand, degenerative conditions can be simulated by restricting the nutrient supply, applying non-physiological load, or injuring the AF (Junger *et al.*, 2009; Illien-Junger *et al.*, 2010; Haglund *et al.*, 2011; Paul *et al.*, 2012; Illien-Junger *et al.*, 2012). The limitations of explant cultures are that only acute effects can be evaluated and the whole range of interactions of local and systemic factors cannot be reproduced. Nevertheless, whole IVD bioreactors will significantly contribute to the reduction of animal experiments, as preliminary proof of concept studies can be performed in a controlled system

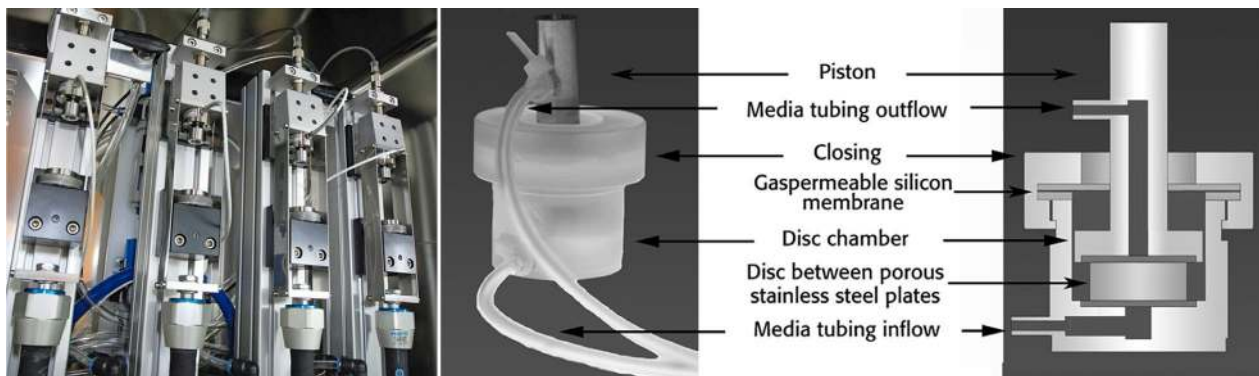


Fig. 4. Bioreactor system for culture of large animal (ovine/bovine) IVD. Four station loading device installed in a cell culture incubator (**left**), and polycarbonate chamber for culture of whole IVD with endplate designed to go with the loading system (**right**) (Junger *et al.*, 2009; Illien-Junger *et al.*, 2010).

prior to verifying an optimised biomaterial or cell therapy approach *in vivo*. Finally, the loading pattern not only can be precisely controlled, but may also be more relevant to the human condition than the load that is experienced in an animal disc *in vivo*.

Conclusions

Disorders related to back symptoms, including disc degeneration, low back pain and radiculopathy, are commonly not distinguished when economic considerations are addressed; it is therefore difficult to assess the economic impact of symptomatic disc herniation. Nonetheless, the health care costs, lost days at work, and decreased productivity account for an enormous economic burden. Back related conditions are a frequent cause of disability leading to annual health care costs of over \$1 billion in the USA. Health care spending on lumbar discectomies has been estimated to exceed \$300 million annually (Schoenfeld and Weiner, 2010).

Complementary or alternative surgical options would significantly improve the chances for the long-term benefit of discectomy procedures. Specifically, present surgical approaches to annulus repair are unsatisfactory, and novel strategies towards annular repair are needed. A substantial deficiency in the area of disc repair, which we aim to address, is the fragmented and often narrowly focused research efforts. We have established an interdisciplinary collaboration addressing biomaterial, biologic, mechanical and surgical needs. This consortium is run as a network of scientists working on the same clinical problem through different approaches. The inclusion of cellular and molecular signalling experts together with specialists in biomaterials, biomechanics, preclinical and clinical areas within the research program will add strength and complementary skills to the whole consortium. Contributions from all these areas are necessary to develop a biological construct which will, upon implantation, provide immediate closure of the defect and maintain the mechanical properties of the disc, while the cellular and biomolecular components will prevent further degeneration and enhance the endogenous regenerative process. Such an

interdisciplinary approach is required to address the highly complex problem of providing an intra-operative procedure which could lead to reduced re-herniation of repaired AF tissue and decrease long term pain for patients.

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Discussion with Reviewers

Reviewer II: Does the existing data support the need to replicate the native tissue structure, or native tissue biochemistry, in identifying a suitable AF repair?

Authors: It is generally recognised that in load-bearing tissues an interrelation between composition, structure and function exists. A number of *in vitro* studies have demonstrated that close replication of the structural organisation of the native AF is beneficial for the phenotype of incorporated cells, the accumulation of appropriate matrix molecules, and the mechanical properties of a tissue engineered construct. These data provide substantial evidence for the success of such systems *in vivo*. However, to date there is no clinical or pre-clinical *in vivo* data available that clearly indicate to what extent the natural composition and/or structure is needed to support long term AF repair. Large animal *in vivo* studies and eventually clinical studies will be necessary to ultimately corroborate the importance of the implant design for functional AF repair.