

# Repair, regenerative and supportive therapies of the annulus fibrosus: achievements and challenges

Johannes Leendert Bron · Marco N. Helder ·  
Hans-Jorg Meisel · Barend J. Van Royen ·  
Theodoor H. Smit

Received: 21 June 2008 / Revised: 9 September 2008 / Accepted: 5 December 2008 / Published online: 23 December 2008  
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**Abstract** Lumbar discectomy is a very effective therapy for neurological decompression in patients suffering from sciatica due to hernia nuclei pulposus. However, high recurrence rates and persisting post-operative low back pain in these patients require serious attention. In the past decade, tissue engineering strategies have been developed mainly targeted to the regeneration of the nucleus pulposus (NP) of the intervertebral disc. Accompanying techniques that deal with the damaged annulus fibrosus are now increasingly recognised as mandatory in order to prevent re-herniation to increase the potential of NP repair and to confine NP replacement therapies. In the current review, the requirements, achievements and challenges in this quickly emerging field of research are discussed.

**Keywords** Annulus fibrosus Herniation Discectomy  
Repair Regeneration

## Introduction

Lumbar discectomy is an effective therapy for neurological decompression in patients suffering from an herniated nucleus pulposus (HNP), which can be safely performed via minimal invasive procedures [44, 128]. Current discectomy procedures, however, are not directed to treat the damaged intervertebral disc (IVD) and may even further aggravate existing damage [16, 22, 45]. It is therefore not surprising that successful neurological decompression is often followed by periods of persisting low back pain, severely affecting the quality of life [7, 8, 45]. Another serious problem in these patients is the high recurrence rates after discectomy, affecting up to 15% of the patients [7, 8, 16, 23, 42, 59, 63, 66, 98, 113, 115]. Since discectomy is still the most performed spinal surgical procedure worldwide and mainly affects the employed population, the resulting socio-economical consequences are dramatic [61]. This gives investigators the impetus to search for new strategies that also deal with the damaged IVD in patients treated for HNP [68, 74, 105].

During the last 5 years, increasing knowledge and technical advancements in the field of tissue engineering has resulted in numerous promising strategies to repair, replace or regenerate the herniated nucleus pulposus (NP) [45, 105]. None of these advancements, however, has yet resulted in a clinically proven effective therapy. One of the major limitations is the lack of effective strategies that deal with the damaged annulus fibrosus (AF) [125]. Since optimal regeneration of the NP should lead to restoration of the physiological intradiscal pressure, the surrounding AF

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Dedicated to the memory of Prof. Dr. Paul I. J. M. Wuisman.

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J. L. Bron · M. N. Helder · H.-J. Meisel · B. J. Van Royen  
Department of Orthopaedic Surgery,  
VU University Medical Center, Amsterdam,  
The Netherlands  
e-mail: jl.bron@vumc.nl

T. H. Smit (✉)  
Department of Physics and Medical Technology,  
VU University Medical Center, Post Box 7057,  
1007 MB Amsterdam, The Netherlands  
e-mail: th.smit@vumc.nl

J. L. Bron · M. N. Helder · B. J. Van Royen · T. H. Smit  
Skeletal Tissue Engineering Group Amsterdam  
(STEGA Foundation), Research Institute MOVE,  
Amsterdam, The Netherlands

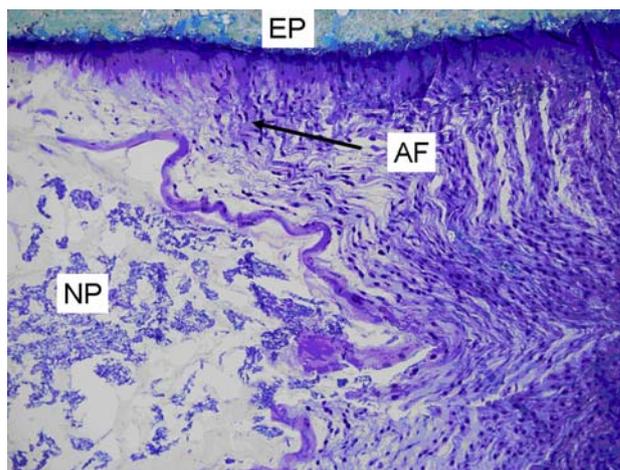
H.-J. Meisel  
Department of Neurosurgery, BG-Clinic Bergmannstrost,  
Halle, Germany

is generally of too inferior quality to withstand these forces. Without sufficient attention to the damaged AF, these treatments might be condemned to fail [5, 125]. Therefore, intervertebral disc engineering strategies are increasingly focusing on the regeneration or repair of the AF in order to reduce the number of re-herniations, increase the potential of NP engineering strategies and to mechanically assist NP replacement therapies [6, 125]. In the current review, we will discuss the requirements, achievements and challenges in this rapidly emerging field of research.

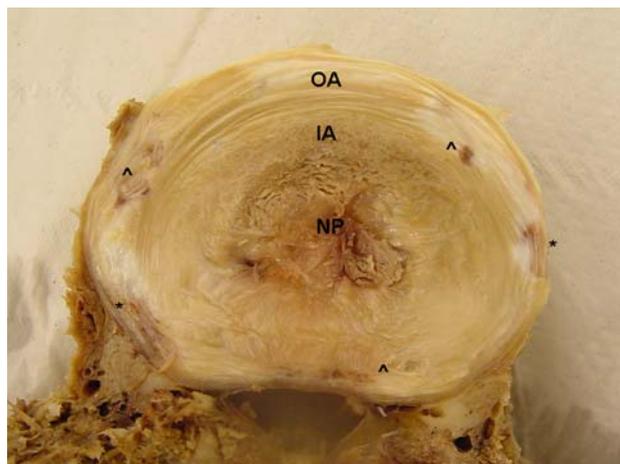
## Anatomy

### Structure of the annulus fibrosus

The IVD is confined by the two cartilage endplates and is composed of two distinct structures, the nucleus pulposus (NP), and the surrounding annulus fibrosus (AF) [53, 130]. Although the two cartilage endplates offer anatomical limitation to the vertebral bodies, morphology along the plate is distinguished by a central articular-like cartilage under the NP and a peripheral fibrocartilage appropriately associated with the AF. During embryogenesis, the AF develops from the mesenchyme, whereas the NP is derived from the notochord [120]. The AF consists of water (65–90%), collagen (50–70% dry weight), proteoglycans (10–20% dry weight) and noncollagenous proteins (e.g. elastin) [14, 114]. The AF has a laminate structure consisting of a minimum of 15 (posterior) to a maximum of 25 (lateral) concentric layers [71]. The layers are composed of type 1 collagen fibres that alternate in angles from 28° (peripheral AF) to 44° (central AF) with respect to the transverse plane of the disc [17, 71, 84]. The spaces between the separate layers of the AF are called interlamellar septae, and they contain proteoglycan aggregates and a complex structure of linking elements creating interlamellar cohesion [14, 89, 111]. At the periphery, some of the annulus fibres pass the endplates to penetrate into the bone of the vertebral body as “Sharpey’s fibres” [57]. Central fibres either insert into the cartilage of both endplates or bend with the NP (Fig. 1). The highly organised structure of the AF results in a complex anisotropic behaviour, with the tensile, compressive, and shear properties differing in the axial, circumferential, and radial directions [11, 106, 114]. Based on structural and cellular differences, the AF can be further distinguished into an inner and an outer part (Fig. 2) [14, 15, 71, 114]. The inner AF is a broad transition zone between the highly organised collagenous structure of the outer AF and the highly hydrated NP and consists of a mixture of extra cellular matrix (ECM) components of both [20, 130]. The inner AF is less hydrated than the NP and the layers are more widely spaced compared to the outer AF [52].



**Fig. 1** Histological image (toluidine blue) of the canine intervertebral disc revealing the relation between the nucleus pulposus (NP), annulus fibrosus (AF) and endplates (EP). Some of the most central AF fibres bend with the NP (arrow)



**Fig. 2** Sagittal section specimen of the L3–L4 intervertebral disc of a middle aged asymptomatic male subject. NP nucleus pulposus, IA inner annulus fibrosus, OA outer annulus fibrosus. Defects in the outer annulus (asterisk) and tears (hat symbol) are visible in the outer annulus, without a sign of herniation. The NP has a severely dehydrated appearance due to conservation techniques

Mechanically, the inner AF is more subjected to the high hydrostatic pressures of the NP than to the tensile forces in the outer AF [73, 112]. These differences have major consequences on ECM synthesis and turnover [52]. The proportion type 1 collagen increases from the inner part towards the outer annulus, whereas type II collagen follows a counterwise distribution [14, 20, 122, 130]. Other proteins that have a specific distribution include decorin and biglycan (mainly outer AF) and collagen type X [inner AF and (aged) NP] [55]. Elastin constitutes 2% of the dry weight of the AF, but plays an important role in the recoil properties of the AF [97, 129]. In the outer AF, long elastic

fibres are present within the lamellae, running parallel to each other and into the same direction as the collagen bundles. In the inner AF, the fibres are present between adjacent lamellae as well as more regularly organised within the lamellae [129]. These fibre networks couple adjacent lamellae together allowing them to work cooperatively during dynamic loading and prevent separation of lamellae during torsional compressive loading [76].

#### Annulus fibrosus cells

In mature subjects the cell density in the AF is about  $9 \times 10^6$  cells/cm<sup>3</sup>, which is over two times higher as compared to the NP [98]. Although all cells in the AF are derived from the mesenchyme, cells within the layers of the AF, the interlamellar spaces and the inner AF have their own morphology and synthesize a distinct ECM [14, 28, 52, 71, 90, 98, 130]. The cells experience not only differences in mechanical environment as described above, but also a rise in pO<sub>2</sub> and pH and a decrease in hydration from the central NP to the outer layers of the AF [50, 52, 94, 98, 118]. In the layers of the outer annulus, fusiform shaped cells, aligning with the collagen fibres and alternating with each lamella are found [71, 90, 106]. In the periphery of the outer annulus, these cells are interconnected by very long processes which results in a continuous communicating network [14, 77]. The processes are gradually reduced in length and increased in thickness towards the inner AF. In the most central part of the outer AF, the cells are completely isolated without any apparent physical, intercellular connections [14]. These outer annulus cells mainly produce type I collagen [130]. The cells in the interlamellar septae have a more flattened, disc-shaped morphology that show many similarities to the cells of the NP [14]. The predominant cell morphology in the inner annulus consists of spherical shaped cells with one or two short processes, having the highest frequency at the border with the NP [14]. These chondrocyte-like cells in the inner annulus mainly produce type II collagen. A recent study showed that cells derived from the human AF were able to differentiate into the chondrogenic and adipogenic lineages [95]. This suggests that cells in the AF could be skeletal progenitor cells that could be recruited under pathologic conditions such as herniation. Otherwise, progenitor cells from surrounding tissue might perhaps be capable to migrate into the intervertebral disc in the circumstances.

#### Pathophysiology

Tissue retrieved from a herniated disc is more often vascularised and is more highly innervated than healthy tissue [97]. Not surprising, this variant morphology also

demonstrates a proclivity to MMP and cytokine expression, each of which would be expected to contribute to further re-modelling [97]. Besides these clearly pathologic conditions, other structural changes do occur during ageing that are to certain extent physiological but might have consequences for its strength (Fig. 1.). In the ageing AF of rats, the number of distinct layers was found to decrease gradually and this loss of volume is compensated by increasing thickness of individual layers and thickening of the inner annulus [90]. In addition, the fibre bundles within the layers become more irregularly distributed with increased interbundle spaces [90]. The loss of distinct layers carries with it the inability for a sustained response to loading and support [1, 41]. Due to dehydration of the inner annulus, the compressive load is insufficiently converted in the integral of progressive recruitment of tensile support. The lack of annular tone in the degenerated disc results in a lag of mechanical conversion and the annulus comes under the force of axial compression, further reducing the anisotropic capacity for deformation in the normal, healthy disc [2, 49]. These changes have most significant impact on the posterolateral location of the AF, that has the highest frequency of layer interruption [71, 110]. This is also the region where the highest stresses are observed during loading [26] and where annular tears, fissures, protrusions, extrusion and/or sequestrations may develop [86]. Annular tears are seen in more than half of the patients in early adulthood and are invariably present in the elderly (Fig. 1) [119]. The degree of degeneration varies between subjects, for which genetic and environmental (e.g. physical loading, smoking) factors are held responsible [10, 13, 81, 88]. Patients with a genetic predisposition are more prone to disc degeneration under repeated mechanical loading [10].

The relation between loading and degeneration of the AF has been studied by several authors, but our knowledge is still only fragmented. Elvervig et al. [27] showed that shear stress increased the intracellular calcium concentration in AF cells. The sensitivity for shear stress was increased in the presence of the inflammatory cytokine IL-1 [27]. Rannou et al. [92] showed that static compression resulted in a significant increase of apoptotic cells in the inner AF in a mouse model. The authors also found an increased caspase-9 activity and decreased mitochondrial membrane potential following overload, suggesting that degeneration might be mediated through the mitochondrial apoptotic pathway [91]. Furthermore, vibratory loading has been associated with the activation of signalling pathways that regulate ECM destruction in the IVD [127]. Yamazaki showed that gene expression in AF cells for key ECM components such as aggrecan and type II collagen was suppressed following vibratory loading [126]. Lastly, cyclic tensile stretch was found to regulate the ECM by

decreasing proteoglycan production through a post-translational regulation involving nitrite oxide [92].

Gruber et al. hypothesized that the well-recognised reduction in cell number in the AF during ageing is an important factor for degeneration. This should result in a loss of cell–cell communication and hence a disruption of coordinated cell function [40]. Finally, many adult IVD's show signs of dehydration and “brown degeneration”, which is the result of post-translational collagen modification resulting in the formation of chromophores [83, 104]. In these discs, accumulated or enhanced oxidative stress of matrix proteins has resulted in glycoxidation of proteins [83]. Advanced glycosylation end products are further processed to carboxymethyl-lysine by free oxygen radicals, which can be detected by antibodies and used as a biomarker for oxidative stress [82].

### Intrinsic healing potential

The intrinsic capacity of the AF to cope with damage or degenerative changes has been studied in several animal studies [3, 29, 34, 43, 62, 75, 79, 85, 99, 109]. Key and Ford [62] studied the healing capacity of three different types of posterior annulus lesions in a dog model. The lesions included a square annular window, a transverse incision and puncture with a 20-gauge needle. At follow up, they found that the lesions were initially filled with extravasated blood, fibrin, bone and cartilage debris that was gradually replaced by a thin layer of fibrous tissue at later time points (up to 22 weeks). Some of the levels within the window and incision lesion group developed slowly progressive disc protrusion, which was most common in the transversely incised discs. The levels that underwent needle puncture revealed nothing abnormal and the site of puncture could not be identified after 22 weeks. A recent study, however, with rabbit discs in an organ culture model showed that needle puncture has immediate and progressive mechanical and biologic consequences that may lead to degenerative remodelling [65]. The findings of Key and Ford have been underscored and complemented in many studies afterwards [29, 43, 85, 97]. Smith et al. [109] further specified the healing process in three different phases. During the first phase, the outer AF heals, caused by a proliferative reaction in the fibrous tissue spreading from the lateral parts of the wound to the median parts. In the second phase, starting after a few weeks and lasting up to one year post-operative, changes occur in inner annular fibres. Similarly to the outer AF, the lateral parts of the inner AF layers gradually heal by a slow appositional spread in the median direction. During the last phase, there is an increase in the number of collagenous fibres in the NP tissue that has remained in the AF wound tract, which

becomes increasingly dense [109]. Similar findings were more recently obtained in sheep and dog studies [43, 85].

From the studies performed thus far it can be concluded that the AF has only a very limited regenerative capacity after annulotomy. Depending on the technique that is used, healing results in a thin layer of biomechanical inferior fibrous tissue [31]. One of the reasons for the limited healing capacity may be the fact that exterior repairs are not matched, or insulated to the demands of progressive recruitment of fibres to tensile force [41, 54]. The mechanical basis for shifting axial loading to circumferential tension requires that the nucleus volume remain elastic, deformable and contained. When the lateral aspects of the annulus are violated, or scarred the ability of the fibres to adequately contain the nucleus changes. In the case of static patient posture and prolonged loading, the disc will experience creep that is proportional to the stage of disc degeneration. In practice, disc degeneration results in a stiffer matrix that does not accommodate the modelling of a disc with normal morphology. If it is not possible to reduce the axial load, then the inevitability of sustaining increasing force in a stiffened matrix will lead to accelerated herniation and more rapid propagation of annular fissures [80]. Independent provinces of repair must therefore be placed in series to expect integration rather than in parallel plots where each will be insufficient to orchestrate the repair separately.

### Surgical strategies

The limited intrinsic healing capacity of the AF negatively affects the success rates of discectomies and NP replacement therapies. It also decreases the potential of intervertebral disc regenerative strategies. To dissolve this problem, attempts to preserve, repair, reinforce or regenerate the AF in addition to these surgical techniques are desired.

#### Annulus closure techniques

The most straight-forward solution is per operative suturing of the annular defect and this has been studied by Ahlgren et al. [3] in a sheep model. Although they found that sutured discs showed a tendency towards stronger healing, this was not significant [3]. Unfortunately, no further studies on this subject have been reported. The Xclose<sup>®</sup> and INclose<sup>®</sup> implants are now commercially available for annuloplasty and can be seen as modified sutures with anchors [12, 18]. Sutures, however, are fully directed to containment of the NP (replacement) and do not compensate the loss of annulus material nor reverse the biomechanical changes that have occurred in the damaged

AF. The Barricaid<sup>®</sup> is a commercial available implant used in adjunction to discectomies that fully bridges the defect in the AF [36]. This implant even reinforces the complete posterior annulus and would therefore even prevent contralateral herniation. Several other novel suture, seal and barrier techniques are currently being developed, resulting in an increasing attention at scientific workshops and conferences [9, 12, 16, 18, 36, 60, 108, 117]. More detailed analyses are therefore expected in peer reviewed journals in the near future. The momentum of acceptance, however, needs to be balanced in the proof of principle. Risks imposed by criticism need to be weighed in both short- and long-term successes. Clinical durability is the eventual arbiter of technology value, and open trials with clear data will be required.

### Regenerative strategies

Regeneration of the damaged AF is an attractive concept, since it allows restoration of all functions of the AF, but is exceptionally complex to achieve. Regenerative strategies can be divided into cell therapy, gene therapy and tissue engineering with scaffolds [45]. In case of the AF, however, direct mechanical strength and a certain volume to patch the defect seem required in order to contain the NP [125]. Ideally, it should combine direct closure of the defect as discussed in the preceding chapter with the potential for regeneration. Cell and gene therapies are therefore not suitable as standalone therapies, but should be combined with scaffolds. Below, these strategies are first discussed separately, followed by an overview of the studies performed with the necessary scaffolds.

#### Annulus cells

Annulus fibrosus cells that are used for AF tissue engineering are derived from humans or various other species (Table 1). The use of human disc cells as a cell source for tissue engineering is difficult because normal healthy disc tissue is not available for such a treatment strategy. In previous studies with tissue derived from herniated discs, an increased degree of cell senescence was found that accumulates over time [38, 96], thus hampering the applicability of this cell source for regenerative strategies [38]. Furthermore, isolation of the cells retrieved from human discectomy material does usually not allow division between inner and outer AF cells. Therefore, cells used for studying annulus regeneration are often harvested from IVD's from healthy small animals. To increase cell number, the AF cells are cultured *in vitro* first. These cells are isolated from native tissue and it is therefore important to realise that the environment differs greatly from that in

situ. Cells no longer have processes, a pericellular matrix and are isolated from each other, and are cultured in gels that do not always allow cellular sliding [25]. Annulus cells have shown to lose their phenotype during two-dimensional (2D) culturing. Chou et al. [20] showed that up to passage two, both inner and outer annulus cells are not different from freshly isolated cells. At later passages, however, both cell types became indistinguishable fibroblast-like with similar type I collagen expression and protein elaboration. The negative effects of monolayer culturing is currently further investigated with specialised 2D environments like collagen coatings, well inserts, or micro-grooved polycaprolactone membranes [21, 37, 58].

To prevent the loss of their phenotype, AF cells are usually cultured in three-dimensional (3D) environments, such as alginate, agarose or collagen hydrogels [4, 37, 39, 64, 100, 130]. Chou et al. [21] found NP and inner and outer AF cells to adopt similar phenotypes after two weeks of culturing in alginate. NP cells and AF cells displayed a rounded chondrocyte-like morphology, expressing high levels of type II collagen versus type I collagen and accumulation of sulphated GAG's. Indeed, the adopted phenotypes are typically NP-like and it was not investigated by these authors whether the changes are reversible [21]. Gruber et al. assessed the ECM expression of AF cells in different 3D culture environments including collagen sponge, collagen gel, agarose, alginate and fibrin [39]. Collagen sponges supported the most abundant ECM formation, whether the ECM production was nearly absent in fibrin gel. The ECM production, however, included types I and II collagen, aggrecan and chondroitin-6-sulfotransferase for all carriers and is this is not specific for AF cells. Moreover, although alginate might be appropriate for inner AF cells, outer AF cells do not survive well in alginate and show a different morphology and matrix expression as observed *in vivo* [52]. It can be concluded that the appropriate culture environment for AF cells has yet to be elucidated.

AF cells are very prone to pressure effects during culturing and this might be useful for tissue engineering strategies. Reza et al. [93] cultured inner and outer AF cells in PGA scaffolds to evaluate the effect of dynamic hydrostatic pressures (HP). Type II collagen production was enhanced in both cell types by the application of HP. This effect, but also the effects on ECM elaboration and organization, was more pronounced in the scaffold seeded with outer AF cells [93]. The value of these results for AF engineering however, may be questioned, since AF cells *in vivo* are more subject to tensile and shearing forces and mainly produce type I collagen. An attractive alternative, that would prevent the problems regarding senescence, limited supply and culturing of autologous AF cells, would be the use of mesenchymal stem cells [30, 51]. There are

**Table 1** Overview of the studies with scaffolds designed for regeneration of the annulus fibrosus

Author (year)	Scaffold	In vitro (1)/ in vivo (2)	Cells	Anisotropic	Biphasic	Follow-up (days)	Findings		
							Cell attachment, proliferation and differentiation	Mechanical findings	
Shao [107]	Wet-spun, lyophilized Alginate/chitosan	1	Canine AF cells	–	–	14	Cell growth in clusters and spread along fibres	Collagen I and II, aggrecan deposition and ECM deposition between alginate and alginate/chitosan scaffolds	Scaffolds have unidirectionally aligned fibres
Chang [19]	Porous silk fibroin	1	Bovine AF cells	–	–	56	35% cell attachment at 24 h	Tenfold increase in collagen deposition and small increase in proteoglycan content (at day 56)	
Mizuno [78]	Polyglycolic acid fibres mesh coated with 1.5% w/v polylactic acid solution	2	Ovine AF cells	–	–	102	<p>Significant increase cellularity only at day 56</p> <p>RGB decoration has no effect on cell attachment or morphology</p> <p>DNA content remained constant during first 8 weeks but increased between 12 and 16 weeks in vivo (in AF part)</p>	<p>Amount of hydroxyproline and GAG increased with time with GAG reaching native tissue level at 16 weeks</p> <p>Compressive modulus increased fivefold during implantation and hydraulic permeability decreased during implantation (at 16 weeks)</p>	
Nerunkar [84]	Electrospun poly-ε-caprolactone	1	Bovine AF cells	+	–	28	Elongated cells aligned along the pre dominant fibres direction	sGAG and collagen content increased during culturing	Anisotropic and non-linear mechanical behaviour during uniaxial tensile testing, comparable to native AF tissue.
Wan [121]	Poly(1,8 octanediol malate)	1	Murine AF cells	–	–	21	<p>No cellular differences between fibres angle groups</p> <p>Proliferation of cells with stellate and elongated morphology</p> <p>Cell penetration into the scaffold (confirmed by the presence of ECM)</p>	Increased gene expression for aggrecan and type II collagen	Tensile strength and degradation time both increased with polymerization time
Helen [47]	PDLLA/Bioglass (0.5 and 30 wt.%)	1	Human AF cells	–	–	28	More cell cluster attached to the pore walls of PDLLA/30BG and PDLLA/5BG compared to PDLLA/0BG	Deposition of sGAG and collagen highest on the PDLLA/30BG foam	Produced collagen is mainly type I collagen in all cultures

**Table 1** continued

Author (year)	Scaffold	In vitro (1)/ in vivo (2)	Cells	Anisotropic	Biphasic	Follow-up (days)	Findings	ECM expression	Mechanical findings
Wan [122]	Bone matrix gelatin (BMG)/poly (polycaprolactone triol malate) (PPCLM)	1	Rabbit chondrocytes	-	+	28	Chondrocytes attached to the scaffold, proliferated and penetrated into the inside (at 4 weeks)	Production of type II collagen and aggrecan could be detected in both parts	Tensile strength and degradation time both increased with polymerization time Incorporation of BMG in PPCLM enhanced compressive strength
Sato [102]	Type I atelocollagen	1, 2	Rabbit AF cells	-	-	84	Cells proliferated and retained spherical shape in vitro	Higher increases in type II collagen and GAG content than compared to monolayer content Accumulation of cartilage like matrix after insertion of cell-containing scaffold in vivo after 12 weeks	Significance less disc space narrowing in vivo

currently, however, no studies available demonstrating stem cells to differentiate into AF cells. The lack of conclusive phenotypic markers for both, AF cells and stem cells, makes it difficult to study this differentiation [30].

### Gene and bio-active factors

Extra cellular matrix production of AF cells can be influenced by various gene and bio-active factors [72, 93, 116, 126]. A few studies have addressed the effect of osteogenic protein-1 (OP-1) on AF cells cultured in alginate beads [72, 116]. Masuda et al. showed that continuous stimulation of rabbit AF cells cultured in alginate beads with recombinant OP-1 led to an increase in the total DNA, collagen content and a pronounced effect on proteoglycan synthesis. However, the authors also showed that this stimulation is more effective in NP cells, compared to AF cells [72]. Takegami et al. [116] showed that AF cells that were stimulated with OP-1 were able to repair the ECM that was depleted of sulphated glycosaminoglycans by chondroitinase ABC exposure. Since these studies show that OP-1 has greater effects on PG synthesis and on NP cells, it may be questioned if OP-1 really offers advantages for AF engineering. Zhang et al. studied the effects of several bone morphogenetic proteins (BMP's) and Sox-9 transfection on AF cells. They found that collagen synthesis could be enhanced by over-expression of BMP-13 and of the transcription factor Sox9 [131]. Although these in vitro results are promising, the effects of these growth factors upon application in animals or humans in vivo remain unknown.

### Scaffolds

The ultimate goal of AF engineering is to achieve both direct mechanical stability and to allow the formation of native tissue in the long term. In order to develop suitable scaffolds for tissue engineering, general principles should be taken into account including the immunogenicity, biocompatibility and biodegradability and method of graft delivery [67]. Specific requirements may be recognised for AF scaffolds. They should:

- Fill and/or repair the AF gap to contain the NP (replacement)
- Allow fixation to the surrounding structures, i.e. endplates and/or surrounding AF tissue
- Allow AF cells (or stem cells) to survive (differentiate), synthesize and secrete the native ECM
- Have the characteristic anisotropic behaviour, to maintain/restore the mechanical properties of a spinal motion segment
- Not irritate or adhere to the perineurium

Several scaffolds that could be used for AF tissue engineering have been proposed and evaluated in *in vitro* or in small *in vivo* studies. In Table 1 these studies are summarised, as well as to which extent they meet the aforementioned requirements. Without exception, strategies for delivery and fixation *in vivo* are lacking. Accurate mechanical characterizations are sparse. Only one study reported of a scaffold materials showing anisotropic behaviour, comparable to the AF [84]. However, since collagen sensitivity to lack of tension upregulates collagenase, and has been shown to be a factor specific in regulating cytokine activity, this factor requires further study [24, 35]. The biphasic appearance of the native AF has also been targeted by a single study. In this study, the inner and outer AF were simulated by bone matrix gelatine (BMG) and poly-caprolactone triol malate, respectively [122].

In general, these studies have been designed to investigate cell attachment, morphology, proliferation and ECM production on the scaffolds. Native outer AF cells have a typical elongated shape and this is observed in most scaffolds [47, 84, 107, 121]. Shao and Hunter, however, found spherical shaped cells in their scaffolds that agree with an inner AF cell morphology. Interestingly, most studies report of the production of type II collagen and aggrecan [19, 78, 84, 102, 107, 121, 122], instead of collagen type I [47, 48, 107, 124], while the latter is by far the most common ECM component of the AF. Ideally, the cells are seeded in scaffolds in a way they are equally distributed through the scaffold. The disadvantage of the silk and BMG scaffold is that the cells only can be seeded on top and invasion occurs only slowly [19, 122]. Chang et al. tried to improve cell attachment onto the silk scaffold by chemically coating the scaffold with the integrin binding motive RGD. RGD, however, did not result in enhanced cell attachment, but did result in higher levels of type II collagen and aggrecan [19]. Higher levels of type II collagen is an insufficient bridge to repair. Given the fact that type II collagen does not bundle or form fibrillated structures, expression obtained by using RGD peptides may be questioned. Using decorin, or small proteoglycans, which have known function is appropriately binding TGF- $\beta$  might be a separate consideration [70]. A critical structural entity of the annulus structure is the network of type I collagen forming fibrils oriented in sheets around the nucleus. A number of molecules present in the matrix regulate and direct the collagen fibril assembly by interacting with the collagen molecule and also the formed fibril. Several of these molecules bind by one domain to the collagen fibre and present another functional domain to interact either with other fibres or with other collagen matrix constituents such as type VI collagen. In this manner the collagen fibres are cross-linked into a network

that provides tensile strength and distributes load over large parts of the AF. Assembly occurs both by end-to-end and side-to-side associations. This process is catalyzed by both biglycan and decorin, where the combined effect of direct binding of the core protein to the collagen-6 N-terminal globular domain and the presence of the glycosaminoglycan side chain is essential. Diminished function in these cross-bridging molecules will lead to loss of mechanical properties of the collagen network and result in an impaired ability of the AF to resist forces delivered by compression of the disc and particularly the nucleus. Decorin has been shown in other systems to retard the TGF- $\beta$  affected fibrotic pathway and as such might limit fibrous scarring and impose tissue specific remodeling [32, 56].

Translation from *in vitro* results to the *in vivo* situation is difficult and the few studies that have assessed the scaffolds *in vivo* do provide important additional information. Mizuno et al. [78] implanted complete tissue engineered IVD constructs consisting of calcium alginate discs surrounded by a polyglycolic acid (PGA) ring seeded with AF cells in the dorsum of athymic mice. The tissue that was formed after 12 weeks follow-up tissue did not resemble native AF tissue with alignment of cells and tissue. Cell proliferation and viability was not quantified in this study. Sato et al. performed laser vaporization in rabbits and the lacunas in the NP and hole in the annulus were filled with an AF cell seeded atelocollagen honeycomb-shaped scaffold with a membrane seal (ACHMS-scaffold) [101–103]. They found a marked accumulation of cartilage like matrix inside and around the scaffold, which was histologically comparable to native AF tissue [102]. Although this combined NP/AF concept seems promising, it might be questionable if this technique is also feasible to be used to fill larger annulus defects. Novel strategies for delivery and fixation may be required.

### Alternative therapies

Wang et al. simulated a herniation in a swine model and delivered gelfoam, platinum coil, bone cement and tissue glue into the discs. Analysis was performed after two months by quantitative discomanometry. The gelfoam proved best in maintaining disc integrity with resistance to significant higher intradiscal pressures compared to the other groups. The gelfoam group was the only group that was not significantly weaker compared to the intact disc group. The authors conclude that gelfoam may be a potentially clinical applicable method to prevent re-herniation [123]. However, although the foam is safe to use according to the authors, it may be questioned how effective this method is in preventing re-herniation in larger

annulus defect than the 18 gauge lateral needle hole in the presented animal model.

## Discussion

The research on the AF as a target for novel therapies has only just started to evolve. There are several limitations and pitfalls in the research thus far that should be noted. Experimental AF lesions are generally made at the (antero)lateral region of healthy AF's (Fig. 2) and extrapolation of these studies to humans is difficult [87]. Repair mechanisms in animal studies may differ compared to patients with HNP due to the pathophysiological changes within the IVD that have occurred in the period prior to HNP (Fig. 1) [16]. Furthermore, it is important to realise that annulus fissures commonly develop bilaterally [2]. When successful patching of an AF defect allows restoration of the physiological high intradiscal pressures, the contralateral fissure may progress and become symptomatic. Complete annulus and nucleus tissue engineered constructs as for example of Mizuno et al. would offer a solution for this, but are even more difficult in terms of implantation.

There are striking discrepancies between AF closure techniques and regenerative strategies. Closure techniques are primarily focussing on restoration of the mechanical integrity of the AF and do offer clear solutions for delivery and fixation. These developments are mainly practised in vivo and scientific data is only sparse. Regenerative therapies, on the other hand, target the engineering of healthy and functional AF tissue, but lack strategies for implantation and fixation and thus for clinical application. Of course, a combination of strategies that offer direct mechanical stability and potential for remodelling AF tissue would be preferred.

## Future research

Now that the need for AF repair is increasingly recognised, many studies on this subject are expected to be reported in the scientific literature in the upcoming years. Both AF and NP engineering research are still in very early stages and combined repair strategies should be attempted. Patients undergoing discectomy should ideally benefit from a complete concept in which in one surgical procedure the neurological structures are decompressed and the damaged NP and AF are treated. The increasing knowledge on degradable (bio) polymers offers very encouraging future perspectives [69]. Regenerative matrix scaffolds, biopath materials, memory polymers, disc foams and synthetic gels to translate axial loads, and bioactive hybrid polymers with differential sacrifice to generate cyclic loading during integration efforts might open new paths to successful

treatment of patients suffering from disc herniation. These therapies might be further potentiated when combined cell supplementation, bioactive factors and cytokine modulation. The important role of mechanical loading in addition to IVD engineering is yet underexposed. The use of an interspinous implant for example, to favourably alter the motion, in addition to novel IVD engineering therapies in patients undergoing lumbar discectomy deserves attention [33].

AF cells are a phenotypically heterogeneous cell population. In many studies these differences are disregarded and a mixture of AF cells is used. If we could reveal the exact circumstances under which these cells elaborate, we might substitute these different cell types by stem cells and stimulate them to differentiate into all native cell types [46]. This should further prevent the inconveniences in the harvesting and culturing procedures needed for AF cells.

## Conclusion

Intervertebral disc regeneration offers promising perspectives for patients suffering for low back pain due to disc herniation treated with lumbar discectomy. Thus far, efforts for novel therapies have mainly been directed towards replacement or regeneration of the NP. The real challenge, however, might be the development of strategies that deal with the damaged AF, preferably in a combined approach with the NP. Regenerative therapies of the AF should always be accompanied by a clear vision for future clinical application.

**Acknowledgments** The authors are grateful to Timothy Ganey for his contributions to the manuscript.

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