REVIEW ARTICLE

Repair and Regenerative Therapies of the Annulus Fibrosus of the Intervertebral Disc

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Abstract

Degeneration of the intervertebral disc is implicated as the main cause of low back pain. Current treatment strategies for degenerative disc disease, such as conservative treatments and surgeries, only relieve the symptoms of low back pain without treating the causes of underlying degeneration. Surgical treatments cannot reverse the degeneration of the intervertebral disc degeneration, and may even accelerate the degeneration. The development of tissue engineering and regenerative therapeutic strategies have brought new hope for repair and regeneration of the degenerated intervertebral disc. These strategies have been developed mainly targeting to the repair and regeneration of the nucleus pulposus of the degenerated but intervertebral disc. Although many studies that focused on the nucleus pulposus repair have achieved successes in laboratory settings but disc repair without giving much regard to annulus fibrosus could not recover the normal mechanical environment, which might make the disc degenerative change continuously exacerbate. Lately, the strategy to simultaneously repair the damaged annulus fibrosus and nucleus pulposus has attracted more attention, which could be considered to slow the disc degenerative rate and obtain better repair effect. An extensive literature search up to March 2015 for annulus fibrosus repair and regeneration *in vitro* or *in vivo* studies and clinical trials with the key words of "annulus fibrosus, repair, regeneration, tissue engineering, intervertebral disc and scaffold" were performed through PubMed, China National Knowledge Infrastructure and China Biology Medicine. The goal of this paper was to review the current research progress of annulus fibrosus repair and regeneration, and also suggest directions for future research.

Key Words: *Annulus fibrosus tissue engineering. Disc herniation. Biomaterial scaffold. Annulus fibrosus regeneration.*

INTRODUCTION

Degeneration of the Intervertebral Disc (IVD) is usually implicated as the main cause of low back pain (LBP), which is a big economic burden for a country. The current treatments for Disc Degenerative Disease (DDD) could be divided into three kinds: conservative treatments, surgical treatments, and repair and regenerative strategies. Conservative treatments include exercise, medication, physical therapy, and other non-operative therapy. Surgical treatments include discectomy, spinal fixation and fusion, and artificial intervertebral disc.[1,2] Conservative treatments and surgeries only relieve the symptoms of LBP without treating the cause of underlying degeneration. Meanwhile, surgical treatments could not reverse the IVD degeneration, even it further aggravate the existing damage. Repair and regeneration of the damaged IVD is an attractive concept, because it might treat DDD by restoring normal physiological structure and functions of the IVD, without increasing the disc injury. Repair methods could be divided into cell therapy, bioactive factors therapy and Tissue Engineering (TE).[3] Previous researches mainly targeted the Nucleus Pulposus (NP) repair, seldom simultaneously considering to repair Annulus Fibrosus (AF). Although many researchers reported good results in *in vitro* or *in vivo* studies to repair NP alone,[1,2] but the long-term results were unsatisfactory without repairing AF, for the abnormal mechanical environment caused by injured AF. These days, IVD regenerative strategies have been increasingly focusing on repairing the damaged AF and NP together, in order to prevent or postpone the degenerative change of repaired NP.

In this review, the authors discussed the therapeutic strategies and achievements for repair and regeneration of AF, and also suggested the directions for future research.

METHODOLOGY

The literature search was limited to Chinese and English language articles only; thus literature search was performed over PubMed, China National Knowledge Infrastructure (CNKI: 1979 to March 2015), and China Biology Medicine (CBM: 1978 to March 2015) databases. Proceedings of Annual Meeting of Orthopaedic Research Society (ORS) were searched. The search strategy was conducted by searching "repair, regeneration, biomaterials, tissue engineering and scaffolds" combined with the key words "annulus fibrosus, intervertebral disc", etc. Only articles focusing on the AF or IVD repair and regeneration *in vitro*, *in vivo*,

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and in clinical trials were included. With this strategy, 71 studies were found, out of which 14 studies were excluded because they had suboptimal information related to this research purpose, and 7 studies were outdated or repeated. Thus, a total of 50 studies were finally included in this review.

Intrinsic healing potential: The damaged AF has a very limited regenerative capacity. Hegewald *et al*. investigated the role of chemokines CXCL7, CXCL10, CXCL12, CCL25, and XCL1 in AF homeostasis and repair.[4] They found that AF cells expressed the chemokine receptor CXCR3 and that the corresponding chemokine CXCL10 effectively recruited AF cells, which suggested that CXCL10 were involved in AF homeostasis and spontaneous AF repair. Henriksson *et al*. investigated IVD cell regeneration and localized stem cells within the IVD[5]. Detection of cell regeneration zones and label-retaining cells were done by 5-bromo-2-deoxyuridine (BrdU) labelling in 18 rabbits. BrdU positive cells were found at the early and later time in most regions of the AF and NP, demonstrating slow ongoing cell regeneration. In the AF border to ligament zone and the perichondrium region, a stem cell niche-like pattern was determined, which might be important for the development of treatment strategies. GDF-5 attracted high interest because of its potential therapeutic efficacy in the treatment of disc degeneration. Helen *et al*. found the presence of GDF-5 in human outer and inner AF tissues.[6] Microarray analysis of AF cells showed significant upregulation of GDF-5 expression in herniated IVD. They used an *in vitro* model to test AF cells growth exposed to IL-1β or TNF-α in 3D culture. IL-1β and TNF-α were two proinflammatory cytokines known to be elevated in the degenerating disc. They found that the expression levels of GDF-5 in cultured cells showed a significant downregulation in cells exposed to TNF-α and IL-1β. It suggested that the high proinflammatory cytokine levels might limit expression of GDF-5, resulting in poor innate regenerative capacity.

Surgical treatments:

Intradiscal Electrothermal Therapy (IDET) and disc Pulse Radiofrequency (disc PRF): IDET or disc PRF was a minimally-invasive technique performed in the outpatient setting, offering an intermediate intervention between conservative treatments and surgeries. It was reported that in IDET a temperature-controlled thermal resistive coil was used, providing conductive heating of the AF in the temperature range, which provided local denaturing of collagen fibrils, cauterized granulation tissue, and coagulated nerve fibers.[7] The principle of disc PRF was similar to IDET; but it used radiofrequency as an energy source. A carefully selected group of fifty (50) consecutive patients with LBP were identified, underwent IDET treatments and were followed prospectively for 2 years. The findings of that study suggested that durable clinical improvements were realized after IDET in highly selected patients with imaging evidence of mild AF rupture.[8] IDET was ineffective for cases of complete AF rupture. Sei Fukui *et al*. choosed 15 patients with LBP who underwent disc PRF, and 16 cases with IDET to compare the representative outcomes of disc PRF and IDET in terms of pain relief. The study showed that disc PRF was an alternative to IDET for patients with LBP.[9] Undergoing IDET or disc PRF procedure, did not eliminate the possibility for extensive spinal surgery at a later time, if disc degeneration progressed.

Annulus closure techniques: The most straightforward solution was operative suturing of the AF defect. Ahlgren *et al*. firstly investigated the effect of repairing sheep AF defects with sutures on the healing strength of the IVD.[10] They performed level, cross and window incision on AF, then sutured and observed for 2, 4 and 6 weeks to compare the healing strength of AF. It showed that direct repair by suturing AF defects did not significantly change the healing in the IVD. Michalek *et al*. demonstrated residual tensile strains existing at the outer periphery of the AF, which became large residual compressive strains at the inner periphery of the AF.[11] The release of residual tension in the outer AF by herniation or incisions, made the closure difficult and might accelerate degeneration of the surrounding tissue. The Xclose and Inclose implants were commercially available for annuloplasty and could be seen as modified sutures with anchors.[12] The Xclose implants closed the incision in the AF by putting T-Anchors on the sides of the opening and suturing the incision. The Inclose implants were designed as a barrier and a scaffold for the repair of the AF. They were inserted in their closed forms by a disposable delivery tool and expanded beneath the defect in the AF. Then they were anchored using non-absorbable sutures. The Barricaid annular reconstruction device was inserted through the incision in the AF after discectomy to create a strong barrier between the AF and the NP.[12] However, preliminary studies have shown that those devices were not effective for a long time and did not help the healing of the AF.

Some other novel sutures, seal and barrier devices, were also being developed, based on PGA-HA, ACD, high-density collagen gel and a biodegradable shape-memory polymer network.[13-15] These devices had a major flaw of not recreating the lost parts of the AF. Moreover, their long-term consequences were not characterized. A biodegradable glue was bio-mechanically tested for AF closure using goat IVDs. The glue increased the force at which NP protrusion occurred, and limited herniations. The study provided a low-cost assessment for AF repair strategies. However, the clinical efficacy needed to be further addressed using long-term *in vivo* studies.[16]

Tissue Engineering (TE): TE included three major components: the cells, the scaffolds, and the bioactive factors. In brief, AF TE was based on the concept of producing a TE, constructed by cells+scaffolds, bioactive factors+scaffolds, or cells+bioactive factors+ scaffolds, implanted using minimally invasive surgery, such as injection, to induce histologically differentiated in situ regeneration of the AF tissue. In the past decade, TE strategies have been developed mainly targeting the regeneration of the NP of the IVD, and lack of effective strategies for the AF. Without giving attention to the AF, these treatments for the NP might fail. Focusing on the repair and regeneration of the AF, increased the potential of NP TE strategies. Below, the three components of AF TE were discussed separately.

Cells: Autologous or allogeneic AF cells and stem cells were all reported to use for AF TE. However, AF cells accelerated the aging when transplanted and were difficult to plant. The previous studies showed that the stem cells, origined from bone marrow, adipose and synovium, were all feasible.[17,18] Guo *et al*. explored the feasibility of using transforming growth factor-β3-mediated bone marrow stem cells (tBMSCs) for AF TE.[19] They found that tBMSCs had strong tendency to differentiate into various types of AF cells and presented gene expression profiles, similar to AF-derived stem cells (AFSCs), thereby establishing a rationale for the use of tBMSCs in AF TE. Saraiya *et al*. found that reversine could induce AF cells plasticity and promote their differentiation along mesenchymal lineages. It showed the possibility that reversine could be used to generate cells, expressing the AF characteristics.[20] Tsai *et al*. cocultured multipotent human MSCs and IVD cells to enhance the differentiation of hMSCs into hAF and hNP cells.[21] They found that hAF cells and hMSCs in the ratio of 2:1 cultured in nanofibers showed the closest mRNA expression levels of hAF-related markers to positive control hAF cells. Their approach provided a favourable cue through cellmatrix and cell-cell interactions to enhance IVD generation. In the past years, NP TE was paid more attention than AF TE. Table I summarises the similarities and differences of cell sources used for AF TE, and NP TE in order to find more appropriate cell sources.

Bioactive factors: Bioactive factors are essential in moderating tissue formation and maintenance by acting through the endocrine, paracrine, or autocrine systems. Bioactive factors applied to construct TE IVD could trigger signal pathway reactions, promote key gene expression, cell proliferation and intrinsic cell migration to the target region, and increase the formation of local ECM. Various studies demonstrated that many growth factors had the ability to stimulate matrix production of AF cells.[23-26] TGF-β1 elevated the expression of Smad2/3, preserved the expression of TGF-β1 receptors, and decreased aggrecan turnover in AF cells.[23] BMPs and Sox9 increased the proteoglycan and collagen expression in AF cells.[24] GDF-5 augmented anabolic metabolism of AF and NP cells.[22] bFGF stimulated the proliferation of AF and NP cells.[25] PDGF-AA stimulated the proliferation, differentiation and migration of AF and NP cells and the production of ECM.[25,26] IGF-1 stimulated GAG, type I and II collagen expressions in AF cells.[27] Osteogenic protein-1 increased proteoglycan and collagen contents in AF cells.[24] Pirvu found that injection of Platelet-Rich Plasma (PRP) into the AF defect increased the matrix production and AF cell number and promoted AF repair.[28] In addition, Gonzales *et al*. found that extracellular ATP promoted and increased the energy supply for ECM biosynthesis and the intracellular ATP level in AF and NP cells.[29] The gene expression of aggrecan and type II collagen in AF and NP cells was also upregulated by extracellular ATP. AF TE and NP TE used the same bioactive factors (e.g., IGF-1, TGF-β1 and BMP-7) to trigger gene expression, promote cell migration, and secretion of ECM.[23-27] Table II summarises the role of common bioactive factors applied for AF/NP cells.

Scaffolds: The goal of AF TE was to achieve both direct mechanical stability and to allow the formation of native tissue for a long term. The scaffold played quite an important role in AF TE. Important considerations in the

Table I: The similarities and differences of cell sources used for AF TE and NP TE.

	Advantages	Disadvantages	Conclusion
AF TE			
hAF cells[21]	No immune response	1. Not available in sufficient amounts 2. Accelerate the degeneration	hAF cells and hMSCs in the ratio of 2:1 cultured in nanofibers showed the closest mRNA expression levels of AF-related markers to positive control hAF cells.
AFSCs[17]	1. Minimal immune response 2. Available in sufficient amounts	Lack of definitive phenotype markers	AFSCs might potentially be an ideal candidate for DDD treatments using TE approaches.
NP TE			
hNP cells[21]	No immune response	1. Not available in sufficient amounts. 2. The surviving implanted cells in a bad state. 3. Phenotype change	NP cells and hMSCs in the ratio of 1:2 cultured in hydrogels showed the closest expression levels of NP-related markers to positive control hNP cells.
Cartilage endplate-derived stem cells (CESCs)[18]	1. Minimal immune response 2. Available in sufficient amounts	Lack of definitive phenotype markers	CESCs might act as an efficient seed cell source for NP TE.
Chondrocytes[22]	Available in sufficient amounts	Phenotype difference	Chondrocytes in a porcine model produced NP-like tissue regeneration.

Table II: The role of bioactive factors on AF/NP cells.

Examples	Role
IGF-1[27]	Stimulations of AF and NP cells proliferation and ECM synthesis
bFGF[25]	Stimulations of the proliferation of AF and NP cells
PDGF-AA[25,26]	Stimulations of the proliferation, differentiation and migration of AF and NP cells and the production of ECM
BMP-2, BMP-7 and BMP-12[24]	Stimulations of AF and NP cells differentiation
GDF-5[22]	Augmenting anabolic metabolism of AF and NP cells
TGF-β1[23]	Upregulating GAG, type II collagen and ECM of AF cells
TIMP[24]	Inhibitory effect on degradative enzymes of AF and NP cells
Sox9, Link N and LMP-1[24]	Regulating cellular differentiation, and function downstream of the molecules of AF and NP cells
PRP[28]	Stimulations of AF cells proliferation
ATP[29]	Promote and increase energy supply for ECM biosynthesis and the intracellular ATP level in AF and NP cells

Table III: Summary of biomaterials for AF TE and NP TE.

Author	Material	Technique	Cell source	Important results
AF TE				
Shao[32]	Alginate/chitosan	Wet spinned	Canine AF cells	Cell growth well and ECM deposition.
Chang[37]	Porous silk fibroin	Salt leaching	Bovine AF cells	RGB decoration can result in higher level type II collagen and aggrecan.
Mizuno[38]	PGA	Non-woven mesh	Ovine AF cells	DNA content, hydroxyproline and GAG increased with time.
Nerunkar	PCL	Electrospinning	Bovine AF cells	GAG and collagen content increased during culturing.
Wan[44]	Poly (1,8 octanediol malate)	Crosslinking/Salt leaching	Murine AF cells	Increased gene expression for aggrecan and type II collagen.
Helen[45]	PDLLA/Bioglass	TIPS	Human AF cells	Deposition of GAG and collagen highest on the rate PDLLA/ Bioglass =1:30. Produced collagen is mainly type I collagen.
Wan[44]	BMG/PPCLM	Crosslinking	Rabbit chondrocytes	Production of type II collagen and aggrecan could be detected.
Sato[42]	ACHMS	Gelation	Rabbit AF cells	Cell growth well and type II collagen and GAG content accumulation.
Le[31]	SIS	\	Pork AF cells	Cell adhesion well.
Nesti[46]	HANFS	\	Human MSC cells	hMSCs differentiation into chondrocytes-like cells.
Vadala[47]	PDLLA/TGF-β	TIPS	Bovine AF cells	Collagen and glycosaminoglycan deposition.
NP TE				
Ruan[48]	PLGA	Electrospinning	Beagle NP cells	The scaffolds had significantly higher disc height and less instability.
Huang[33]	Type II collagen	Gelation	Rabbit NP cells	Type II collagen deposition. Better stability mechanical properties.
Mauth[40]	PU	Electrospinning	Human NP cells	Cell adhesion well.
Abbushi[49]	PGA/HA	Non-woven mesh	Pork bone marrow cells	The implant immersed in serum after discectomy induces regeneration, resulting in improvement of the disc water content.
Ganey[50]	HA	Hydrogel crosslinked	Dog adipose stem cells	Recovery of aggrecan, T2 intensity and disc height.

design of a scaffold included mechanical properties, biocompatibility, biodegradability, and delivery of the scaffold into the implantation site. The technical methods of designing of a scaffold included freeze-drying technology, salt-leaching technology, woven and non-woven technology, Thermally Induced Phase Separation (TIPS) technology and electrospinning technique.[29] The aim of optimizing biomaterials of scaffold was to match biomechanical demands for AF repair. The biomaterials demanded good biocompatibility, biodegradability, and low immunogenicity. Specific requirements for AF scaffolds included that it filled and/or repaired the AF gap to contain the NP, allowed fixation to the surrounding structures, allowed AF cells to survive, synthesized and secreted the native ECM, and had the characteristic of anisotropic behaviour, in order to maintain or restore the mechanical properties of a spinal motion segment.[30] The biomaterials, that investigated as a scaffold for AF TE, were divided into three kinds: native biomaterials, polymer synthetic biomaterials, and composites. Different from NP TE, the choice of biomaterials used in AF TE was determined by the physico-mechanical properties of the AF. In Table III, common biomaterials used in AF TE and NP TE are summarised, in order to get a better understanding of AF TE.

Native biomaterials: Native biomaterials were widely applied in the AF TE. The advantages included good biocompatibility and low cytotoxicity. Poor biomechanics and immunogenicity were their disadvantages. Native biomaterials that investigated as a scaffold included alginate, chitosan, agarose, collagen, fibrin gel, proteoglycans, fibroin, demineralized bone matrix, and Small Intestinal Submucosa (SIS).[31] Some native biomaterials scaffolds had a good prospect. Shao *et al*. performed an alginate/chitosan scaffold and found better growth of AF cells and the production of type II collagen and aggrecan.[32] Type II collagen, one of the main components of ECM of the inner AF, was an ideal native biomaterial.[33] Bowles *et al*. implanted collagen gel to plant AF cells, and they found that the arrangement and

shape of AF cells were similar to live disc.[34] However, whether fibrin gel was used to repair the AF or not was still controversial. Gruber *et al*. respectively implanted sponge-like collagen, fibrin gel, collagen gel, alginate and agarose with AF cells. They found that AF cell in fibrin gel could not secrete aggrecan or chondroitin-6-sulphate sulfotransferase which was necessary for growth. Fibrin gel was not suitable for the AF TE, whereas collagen and sponge agarose induced AF cells to produce the essential components of cells growth.[35] Schek *et al*. found that a genipin crosslinked fibrin gel was created with a modulus in the range of native AF tissue. This material was compatible with the *in vitro* growth of AF cells when genipin:fibrin ratio was 0.25:1 or less, although AF cell proliferation was slower.[36] The study showed that genipin crosslinked fibrin gels remained adhered to the AF tissue pieces at strains exceeding physiological levels and might be suited as a sealant for AF defects.

Silk had good flexibility and biocompatibility, but had immunogenicity. Silk fibroin resulted from silk without immunogenicity and was proved to be the strongest known natural fiber. Chang *et al*. seeded bovine AF cells on porous silk fibroin scaffolds.[37] They found that AF cells attached to porous silk fibroin scaffolds, proliferated and synthesized and accumulated extracellular matrix. This study showed that porous silk fibroin scaffold was an appropriate scaffold on which AF cells grow.

Polymer synthetic biomaterials: Various biodegradable polymer synthetic biomaterials were investigated. The advantages of polymer synthetic biomaterials included good mechanical properties, repeatability, controllability, no immunogenicity, and easy processing. The disadvantages included lack of bioactivity, poor cell affinity, and tissue aseptic inflammation. Polymer synthetic biomaterials for AF TE were thought on behalf of the aliphatic polyesters, including polylactic acid (PLA), polyglycolic acid (PGA), polylactic/glycolic acid (PLGA), polycaprolactone (PCL), polyoxymethylene (POM), poly (polycaprolactone triol malate) (PPCLM), polyamide, polyurethane (PU) and so on. Mizuno *et al*. seeded AF cells with PLGA scaffold.[38] They found that the formation of the organization in general morphology, histology, biochemistry, biomechanics and physiological state was similar to the live IVD, and it also maintained the AF cells phenotype with producing ECM; but the degradation of PLGA scaffold could cause certain cytotoxicity. Nerurkar reported an electrospinning polycaprolactone nanofiber scaffold, which successfully simulated the AF multilayer structure, similar in the histology, biochemistry and biomechanics of live IVD.[39] Mauth *et al*. successfully performed a biodegradable polyurethane (PU) nanofiber scaffold, which showed good cell adhesion.[40] Then, Yang *et al*. improved this PU nanofiber scaffold with a novel Anionic Dihydroxy Oligomer (ADO).[40] The new scaffold enhanced the AF cell attachment. Collagen accumulation was also modulated by increasing ADO content. Kandel *et al*. demonstrated that a PU scaffold containing an anionic dihydroxy oligomer promoted the maintenance of AF disc cell phenotype, as examined to date, with the outer AF and inner AF cells accumulating different ECMs.[41] Inner AF cells accumulated more versican and type II collagen than outer AF cells, similar to the native disc. The study suggested that there were fundamental differences between inner AF and outer AF cells; thus raised the possibility that maintaining these features, perhaps through appropriate scaffold selection, might be critical for bioengineering a functional and phenotypically correct IVD.

Composites: Combine native biomaterials with polymer synthetic biomaterials could improve the strength of the scaffolds. But the processing was quite complicated with high cost. Depending on the materials, composites with divided into three kinds: native/native composites, synthetic/synthetic composites, and native/synthetic composites.[42] Composites included collagen/hyaluronic acid (HA), PGA/HA, alginate/collagen, bone matrix gelatin/PPCLM (BMG/PPCLM), poly (DL-lactic acid)/bioglass (PDLLA/Bioglass), silk fibroin/hydroxybutyl chitosan (SF/HBC) and hyaluronic acid gel/polylactic acid nanofibers (HANFS). Alini *et al*. seeded bovine AF cells with type I collagen/HA, and they found that the AF cells grew well, and produced and accumulated type I collagen and proteoglycans.[43] Wan *et al*. extracted BMG from the bone combining with PPCLM. The new scaffold had good organizational and mechanical response tested by a mechanical tensile trial.[44] Helen *et al*. combined PDLLA with Bioglass to perform a biological scaffold. They found that the new scaffold decreased cytotoxicity brought by degradation of PDLLA.[45] The scaffold also improved cell adhesion and maintained the AF cells phenotype, and AF cells produced dextran sulphate and collagen. Furthermore, the mechanical response and degradation rate of the new scaffold were controlled by regulating the concentration and temperature of PDLLA. Nesti *et al*. designed a biodegradable HANFS scaffold by electrospinning technique.[46] When MSCs was seeded into the HANFS scaffold with TGF-β, histology, biochemistry, immunohistochemistry and Serial Analysis of Gene Expression (SAGE), all showed that MSCs differentiated into AF-like cells. In addition, cytokines were added in the making of the composite scaffold. For example, adding TGF-β avoided cytokine release concentration effect and extended the duration to play a better role.[47] Now there are still many challenges remain in AF TE, including limited cell source, culture difficulties, lack of AF cell markers, implantation complication, low cells survival rate, and lack of official animal model.

CONCLUSION

Despite the promising results in AF TE, there will be so much work to be done regarding further clinical applications. Novel strategies for delivery and fixation will be required. The previous studies mainly focused on small animals, and it was questionable whether the technique was effective in repairing larger animals. In the future, it should be discussed how to achieve better cells source, implantable technology and improve the transplanted cells survival rate.

REFERENCES

1. Smith LJ, Nerurkar NL, Choi KS. Degeneration and regeneration of the intervertebral disc: lessons from development. *Dis Models Mech* 2011; **4**:31-41.
2. Hudson KD, Alimi M, Grunert P. Recent advances in biological therapies for disc degeneration: tissue engineering of the annulus fibrosus, nucleus pulposus and whole intervertebral discs. *Curr Opin Biotechnol* 2013; **24**:872-9.
3. Sharifi S, Bulstra SK, Grijpma DW. Treatment of the degenerated intervertebral disc; closure, repair and regeneration of the annulus fibrosus. *J tissue Engineer Regenerat Med* 2015; **9**:1120-32.
4. Hegewald AA, Neumann K, Kalwitz G. The chemokines CXCL10 and XCL1 recruit human annulus fibrosus cells. *Spine* 2012; **37**:101-7.
5. Henriksson HB, Thornemo M, Karlsson C. Identification of cell proliferation zones, progenitor cells and a potential stem cell niche in the intervertebral disc region: a study in four species. *Spine* 2009; **34**:2278-87.
6. Gruber HE, Hoelscher GL, Ingram JA. Growth and differentiation factor-5 (GDF-5) in the human intervertebral annulus cells and its modulation by IL-1ß and TNF-α *in vitro*. *Experiment Molecul Pathol* 2014; **96**:225-9.
7. Block JE. Intradiscal electrothermal therapy (IDET) for the treatment of discogenic low back pain: Patient selection and indications for use. *Pain Physician* 2008; **11**:659-68.
8. Assietti R, Morosi M, Migliaccio G. Treatment of discogenic low back pain with Intradiscal Electrothermal Therapy (IDET): 24 months follow-up in 50 consecutive patients. *Springer Vienna* 2011; **108**:103-5.
9. Fukui S, Nitta K, Iwashita N. Results of intradiscal pulsed radio-frequency for lumbar discogenic pain: comparison with intra-discal electrothermal therapy. *Korean J Pain* 2012; **25**:155-60.
10. Ahlgren BD, Lui W, Herkowitz HN. Effect of anular repair on the healing strength of the intervertebral disc: a sheep model. *Spine* 2000; **25**:2165-70.
11. Michalek A J, Gardner-Morse M G, Iatridis J C. Large residual strains are present in the intervertebral disc annulus fibrosus in the unloaded state. *J Biomech* 2012; **45**:1227-31.
12. Schutgens EM, Tryfonidou MA, Smit TH. Biomaterials for intervertebral disc regeneration: past performance and possible future strategies. *Eur Cells Mat* 2015; **30**:210-31.
13. Bron JL, van der Veen AJ, Helder MN. Biomechanical and *in vivo* evaluation of experimental closure devices of the annulus fibrosus designed for a goat nucleus replacement model. *Eur Spine J* 2010; **19**:1347-55.
14. Sharifi S, van Kooten TG, Kranenburg HJ. An annulus fibrosus closure device based on a biodegradable shape-memory polymer network. *Biomaterials* 2013; **34**:8105-13.
15. Grunert P, Borde BH, Hudson KD. Annular repair using high-density collagen gel: a rat-tail *in vivo* model. *Spine* 2014; **39**: 198-206.
16. Vergroesen PPA, Bochynska AI, Emanuel KS. A biodegradable glue for annulus closure: evaluation of strength and endurance. *Spine* 2015; **40**:622-8.
17. Liu C, Guo Q, Li J. Identification of rabbit annulus fibrosus-derived stem cells. *Plos One* 2014; **9**:e108239.
18. Wang H, Zhou Y, Huang B. Utilization of Stem Cells in Alginate for Nucleus Pulposus Tissue Engineering. *Tissue Eng Part A* 2013; **20**:908-20.
19. Guo Q, Liu C, Li J. Gene expression modulation in TGFβ-3-mediated rabbit bone marrow stem cells using electrospun scaffolds of various stiffness. *J Cell Molecul Med* 2015; **19**: 1582-92.
20. Saraiya M, Nasser R, Zeng Y. Reversine enhances generation of progenitor-like cells by dedifferentiation of annulus fibrosus cells. *Tissue Eng Part A* 2010; **16**:1443-55.
21. Tsai TL, Nelson BC, Anderson PA. Intervertebral disc and stem cells co-cultured in biomimetic extracellular matrix stimulated by cyclic compression in perfusion bioreactor. *Spine* J 2014; 2127-40.
22. Stoyanov J V, Gantenbein-Ritter B, Bertolo A. Role of hypoxia and growth and differentiation factor-5 on differentiation of human mesenchymal stem cells towards intervertebral nucleus pulposus-like cells. *Eur Cell Mater* 2011; **21**:533-47.
23. Li B, Su YJ, Zheng XF. Evidence for an important role of smad-7 in intervertebral disc degeneration. *J Interferon Cytokine Res* 2015; **35**:569-79.
24. Cho H, Lee S, Park SH. Synergistic effect of combined growth factors in porcine intervertebral disc degeneration. *Connective Tissue Res* 2013, **54**:181-6.
25. Wismer N, Grad S, Fortunato G. Biodegradable electrospun scaffolds for annulus fibrosus tissue engineering: effect of scaffold structure and composition on annulus fibrosus cells *in vitro*. *Tissue Eng Part A* 2014; **20**:672-82.
26. Moon HJ, Yurube T, Lozito TP. Effects of secreted factors in culture medium of annulus fibrosus cells on microvascular endothelial cells: elucidating the possible pathomechanisms of matrix degradation and nerve in-growth in disc degeneration. *Osteoarthrit Cart* 2014; **22**:344-54.
27. Jin L, Shimmer A L, Li X. The challenge and advancement of annulus fibrosus tissue engineering. *Eur Spine J* 2013; **22**: 1090-100.
28. Pirvu TN, Schroeder JE, Peroglio M. Platelet-rich plasma induces annulus fibrosus cell proliferation and matrix production. *Eur Spine J* 2014; **23**:745-53.
29. Sharifi S. The development of novel biodegradable polymeric biomaterials for use in the repair of damaged intervertebral discs. *University of Groningen*; 2012. p.202.
30. Lei B, Shin KH, Noh DY. Nanofibrous gelatin-silica hybrid scaffolds mimicking the native extracellular matrix (ECM) using thermally induced phase separation. *J Materials Chem* 2012; **22**:14133-40.

31. Le Visage C, Yang SH, Kadakia L, Sieber AN, Kostuik JP, Leong KW. Small intestinal submucosa as a potential bioscaffold for intervertebral disc regeneration. *Spine* 2006; **31**: 2423-30.

32. Shao X, Hunter CJ. Developing an alginate/chitosan hybrid fiber scaffold for annulus fibrosus cells. *J Biomed Materials* Res 2007; **82**:701-10.

33. Huang B, Zhuang Y, Li CQ. Regeneration of the intervertebral disc with nucleus pulposus cell-seeded collagen II/hyaluronan/chondroitin-6-sulfate tri-copolymer constructs in a rabbit disc degeneration model. *Spine* 2011; **36**:2252-9.

34. Bowles RD, Gebhard HH, Dyke JP. Image-based tissue engineering of a total intervertebral disc implant for restoration of function to the rat lumbar spine. *NMR Biomed* 2012; **25**: 443-51.

35. Gruber HE, Hoelscher G, Ingram JA. Culture of human anulus fibrosus cells on polyamide nanofibers: extracellular matrix production. *Spine* 2009; **34**:4-9.

36. Schek RM, Michalek AJ, Iatridis JC. Genipin-crosslinked fibrin hydrogels as a potential adhesive to augment intervertebral disc annulus repair. *Eur Cells Mat* 2011; **21**:373.

37. Chang G, Kim HJ, Vunjak-Novakovic G, Kaplan DL, Kandel R. Enhancing annulus fibrosus tissue formation in porous silk scaffolds. *J Biomed Materials Res* 2010; **92**:43-51.

38. Mizuno H, Roy AK, Zaporojan V, Vacanti CA, Ueda M, Bonassar LJ. Biomechanical and biochemical characterization of composite tissue-engineered intervertebral discs. *Biomaterials* 2006; **27**:362-70.

39. Nerurkar NL, Mauck RL, Elliott DM. Modeling interlamellar interactions in angle-ply biologic laminates for annulus fibrosus tissue engineering. *Biomechanics Modeling Mechanobiol* 2011; **10**:973-84.

40. Yang L, Kandel RA, Chang G. Polar surface chemistry of nanofibrous polyurethane scaffold affects annulus fibrosus cell attachment and early matrix accumulation. *J Biomed Materials Res* 2009; **91**:1089-99.

41. Kandel R, lu J, Santerre P. Inner and outer annulus fibrosus cells exhibit differentiated phenotypes and yield changes in extracellular matrix protein composition *in vitro* on a polycarbonate urethane scaffold. *Tissue Eng Part A* 2014; **23**: 3261-9.

42. Sato M, Asazuma T, Ishihara M, Kikuchi T, Masuoka K, Ichimura S, *et al.* An atelocollagen honeycomb-shaped scaffold with a membrane seal (ACHMS-scaffold) for the culture of annulus fibrosus cells from an intervertebral disc. *J Biomed Materials Res* 2003; 64:248-56.

43. Alini M, Li W, Markovic P, Aebi M, Spiro RC, Roughley PJ. The potential and limitations of a cell-seeded collagen/hyaluronan scaffold to engineer an intervertebral disc-like matrix. *Spine* 2003; **28**:446-53.

44. Wan Y, Feng G, Shen FH. Biphasic scaffold for annulus fibrosus tissue regeneration. *Biomaterials* 2008; **29**:643-52.

45. Helen W, Gough J E. Cell viability, proliferation and extracellular matrix production of human annulus fibrosus cells cultured within PDLLA/Bioglass composite foam scaffolds *in vitro*. *Acta Biomaterialia* 2008; **4**:230-43.

46. Nesti LJ, Li WJ, Shanti RM. Intervertebral disc tissue engineering using a novel hyaluronic acid-nanofibrous scaffold (HANFS) amalgam. *Tissue Eng Part A* 2008; **14**:1527-37.

47. Vadalà G, Mozetic P, Rainer A, Centola M, Loppini M, Trombetta M. Bioactive electrospun scaffold for annulus fibrosus repair and regeneration. *Eur Spine J* 2012; **21**:20-6.

48. Ruan DK, Xin H, Zhang C. Experimental intervertebral disc regeneration with tissue-engineered composite in a canine model. *Tissue Eng Part A* 2010; **16**:2381-9.

49. Abbushi A, Endres M, Cabraja M. Regeneration of intervertebral disc tissue by resorbable cell-free polyglycolic acid-based implants in a rabbit model of disc degeneration. *Spine* 2008; **33**:1527-32.

50. Ganey T, Hutton W C, Moseley T. Intervertebral disc repair using adipose tissue-derived stem and regenerative cells: experiments in a canine model. *Spine* 2009; **34**:2297-304.