REVIEW

Hydrogels in acellular and cellular strategies for intervertebral disc regeneration

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Abstract

Low back pain is an extremely common illness syndrome that causes patient suffering and disability and requires urgent solutions to improve the quality of life of these patients. Treatment options aimed to regenerate the intervertebral disc (IVD) are still under development. The cellular complexity of IVD, and consequently its fine regulatory system, makes it a challenge to the scientific community. Biomaterials-based therapies are the most interesting solutions to date, whereby tissue engineering and regenerative medicine (TE&RM) strategies are included. By using such strategies, *i.e.*, combining biomaterials, cells, and biomolecules, the ultimate goal of reaching a complete integration between native and neo-tissue can be achieved. Hydrogels are promising materials for restoring IVD, mainly nucleus pulposus (NP). This study presents an overview of the use of hydrogels in acellular and cellular strategies for intervertebral disc regeneration. To better understand IVD and its functioning, this study will focus on several aspects: anatomy, pathophysiology, cellular and biomolecular performance, intrinsic healing processes, and current therapies. In addition, the application of hydrogels as NP substitutes will be addressed due to their similarities to NP mechanical properties and extracellular matrix. These hydrogels can be used in cellular strategies when combined with cells from different sources, or in acellular strategies by performing the functionalization of the hydrogels with biomolecules. In addition, a brief summary of therapies based on simple injection for primary biological repair will be examined. Finally, special emphasis will focus on reviewing original studies reporting on the use of autologous cells and biomolecules such as platelet-rich plasma and their potential clinical applications. Copyright © 2011 John Wiley & Sons, Ltd.

1. Human spine and intervertebral disc (IVD)

The human spine plays an important role in loading the structures where the internal organs are attached supporting the superior part of the body and transmitting loads between superior and inferior extremities (Pye *et al.*, 2007). Body weight and muscle activity are the main factors responsible for such loads on the spine. The protection of

the nerves along this structure is provided from the brain to the periphery (arms and legs) (Bao *et al.*, 1996; Kalson *et al.*, 2004; Shankar *et al.*, 2009). Occupying one-third of the spine height, the linked spine structure contains 24 intervertebral discs (IVDs) separating the bony vertebrae. Body motion is controlled by the IVDs in three distinct planes: lateral bending, axial rotation, and flexion-extension (Twomey and Taylor, 1985; Roberts *et al.*, 1989).

IVD is a fibrocartilaginous structure composed of three morphologically distinct regions (nucleus pulposus, annulus fibrosus, endplates) working together to absorb and disperse energy and transfer loads within the spine (Nerurkar *et al.*, 2010; Lotz *et al.*, 2002). Simultaneously, IVDs provide flexibility, enabling movements such as bending, flexion, and torsion. During heavy lifting activities, forces up to 17 kN have been estimated in lumbar discs. All types of loads, mainly caused by

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torsion, can induce early degeneration in the disc (Cholewicki *et al.*, 1991).

The central gelatinous core of the IVD, the nucleus pulposus (NP), is composed of 80% water during youth and progressively loses water content throughout the years (Coventry *et al.*, 1945). Unlike other IVD regions, the NP develops from endoderm. In adulthood, the NP contains an amorphous matrix of proteoglycans (PGs) positioned in a network of randomly organized collagen (mainly type II) and elastin. The collagen crosslinking provides form and tensile strength to the NP, whereas the PGs (mainly aggrecan) maintain their viscoelastic properties, resistance to compression, and stiffness, since they sustain the osmotic pressure by which water enters and nutrient exchange occurs. The capacity to absorb and release water is related to the capacity to absorb shocks (Walker and Anderson, 2004; Singh *et al.*, 2009; Woods *et al.*, 2010). Two distinct regions are present in the annulus fibrosus (AF): the outer and the inner part, which have quite different characteristics. In the outer AF, long and elastic collagen fibres lie parallel within each lamella orientated at 60° to the vertical axis (Shankar *et al.*, 2009). The outer AF arrangements provide resistance to tensile forces from body motions such as twisting and bending (Kalson *et al.*, 2008). The inner AF is a transition zone between the highly organized outer AF and the highly hydrated NP. The characteristics of outer AF and NP are present in inner AF, which then becomes a mixture of ECM components from both. The inner AF contains lower water content compared to NP. Moreover, inner AF is composed of layers that are more widely spaced than those of outer AF. Regarding mechanical properties, inner AF is more exposed to NP hydrostatic pressures than to outer AF tensile forces.

At the cellular level, NP and AF are morpho- and phenotypically distinct; therefore possessing dissimilar metabolic processes. IVD is populated by different cell phenotypes that are present in different cell numbers. NP is composed of rounded and chondrocyte-like cells at a density of 5×10^3 cells/mm^3, whereas AF contains 9×10^3 cells/mm^3 and possesses an elongated fibroblast like morphology (Eyre *et al.*, 2002; Roughley, 2004; Roberts et al., 2006a, 2006b; Kalson *et al.*, 2008). The two cartilage endplates act as a morphological and functional junction zone occupying 90% of the interface between the IVD and the vertebrae. These structures are composed mainly of hyaline cartilage similar to articular cartilage. The endplates serve as the main route for metabolites exchange due to their dense capillary network. The cells that populate these structures show a fibroblast like morphology (Weishaupt *et al.*, 2001; Roberts *et al.*, 2006a, 2006b; Woods *et al.*, 2010).

1.1. Biochemistry of the IVD

The cellular metabolism of IVDs greatly depends on the finely balanced equilibrium assured by the action of biomolecules such as growth factors (GF), tumour necrosis factor (TNF), cytokines, and insulin like growth factors families (IGF). The extracellular matrix (ECM) of the distinct morphological IVD regions varies in composition. There are quite significant differences between NP and AF ECMs. The natural IVD balance is maintained by the action of different biomolecules such as cytokines and GF, which are responsible for controlling catabolic and anabolic processes. The process of intervertebral disc degeneration (IDD) leads to an increase in production of cytokines and matrix degrading enzymes (metalloproteinases; MMPs), leading to a pre-dominance of catabolism over anabolism (Roberts et al., 2006a, 2006b; Clouet *et al.*, 2009; Woods *et al.*, 2010).

PGs, the glycosylated proteins that are strongly hydrophilic and commonly found in connective tissues are also abundant in NP. These proteins contribute to draw significant quantities of water into the disc. When PGs are present, the NP ECM expands to become a hydrogel-like structure. Several types of PGs are present in the IVD. Aggrecan and versican are aggregated PGs largely responsible for NP hydration. Non-aggregating PGs such as decorin, biglycans, fibromodulin, lumican, and proglycan still do not have a well-defined function. The aggregated PGs aggrecan and versican are capable of binding glycosaminoglycans (GAGs) to the core, playing a role in cellular signaling. The mechanical properties of IVD depend directly on the number of GAG molecules linked to proteins, which in turn is directly related to the compression-resistance that IVD can load (Kalson *et al.*, 2008). The protein Sox-9 (an essential regulatory gene for chondrocyte differentiation) is also expressed in healthy NP.

1.2. Degenerative disc diseases; pathophysiology

Mild microscopic IVD degenerative changes can be noticed with aging (Boos *et al.*, 2002). In fact, it is the first body tissue to reveal signs of degeneration. IDD should be considered as a multi-factorial process dependent on genetic and environmental mechanisms. Studies on genetic predisposition to IDD, namely on cervical and lumbar regions, have suggested that there is a strong heritable component associated to this degenerative process (Sambrook *et al.*, 1999). Most patients suffering from lower back pain (LBP) present different pathological conditions such as disc herniation, spinal stenosis, and myelopathy (Walker and Anderson, 2004; Kalson *et al.*, 2008).

The healthy IVD structure is the largest avascular organ in the human body (Woods *et al.*, 2010). As a result, the supply of essential nutrients and metabolites are dependent on the process of diffusion. Small blood vessels can be found in the outer region of the AF and sub-chondral bone but they are limited to 1–2 mm in depth. In a non-pathological condition, IVD has a diminished diffusion capacity that becomes even more reduced with aging and degenerative processes. The mechanism by which small molecules can penetrate into the disc is simple diffusion,

which can compromise cell viability. However, cells are adapted to this environment, resulting in a low mitotic rate.

Another important issue related to the process of IDD is the anaerobic metabolism carried out by cells. In this respect, the relatively acidic pH from a non-pathological IVD (pH 6.9-7.2) decreases to pH 6.1 under stress conditions (pathological condition). Under pathological conditions, inhibition of cell metabolism occurs, leading to matrix deterioration. These alterations, in combination with the poor innervations of the IVD (found only in the surface of the outer AF), make regeneration of this structure a real challenge (Buckwalter, 1995; Clouet et al., 2009).

The number of large aggregates of PGs present in the NP decreases throughout the years, contributing to a reduction of the water-binding capacity. Consequently, the desiccation of NP leads to the formation of clefts and tears in the AF. The asymmetric distribution of loads does not only affect the capacity of the disc to absorb and dissipate compressive loads but also diminishes the foramen dimensions, thereby leading to nerve compression (Boos et al., 2002; Hukins, 2005).

1.3. Intrinsic healing process: current treatments

LBP can be associated with IDD, since it is present in about 40% of patients (Luoma et al., 2000). A possible explanation for this is related to the proximity of surrounding muscles, joints or even the innervations of the outer AF. The self-repair capacity of IVD is confined only to the vascularized regions. ECM modifications lead to drastic effects in the overall IVD structure. This structure is aimed to compensate the abnormal distribution of forces when IDD occurs. Nevertheless, the IVD structure can occasionally be subjected to inappropriate stress, causing mechanical damage to the disc. Histological changes at the cellular level are manifested by an increase in cell proliferation and cell-cluster formation. Moreover, an increase in cell death (necrosis and apoptosis) can occur. The natural balance between anabolic and catabolic processes is strongly affected by degeneration (Trout et al., 1982; Gruber and Hanley, 1998; Johnson et al., 2001).

Randomized trials of therapeutic approaches are an option to relieve symptoms such as symptomatic medical treatments such as non-steroidal anti-inflammatory drugs (NSAIDs), paracetamol, and opioids that act as muscle relaxants. Last resources, including the use of anti-neuropathic drugs and invasive surgical methods are also a possibility. Nevertheless, these treatments have limited efficacy and cost-effectiveness (Deyo and Weinstein, 2001; Richardson et al., 2007; Kalson et al., 2008; Freimark and Czermak, 2009).

Current conservative practices for treating patients with acute or sub-acute LBP are directed towards removing the damaged tissue and/or stabilizing the resultant instability by surgery. The most common spinal surgery intervention is neurological decompression by discectomy, a procedure where the NP that is causing pain by stressing the spinal cord or radiating nerves is removed (Yeung and Yeung, 2007). Consequently, the disc height cannot be restored and the capacity to bear loads is dramatically reduced, thereby affecting the biomechanics of the IVD. Another surgical intervention commonly performed is bone fusion, wherein adjacent vertebrae are fused. To promote the growth of new bone to connect both vertebrae, bone grafting is usually performed. Recently, the use of bone morphogenetic protein 2 (BMP-2) in humans to promote the anterior lumbar inter-body fusion has been approved by the Food and Drug Administration (FDA) (Masuda, 2004). However, after bone fusion, flexibility was reduced and degenerative changes could be induced in adjacent vertebral bodies. Partial or total disc excision also presents serious contraindications and it has been performed followed by posterior replacement with artificial discs. There are no data reporting their long-term efficacy (Woods et al., 2010).Synthetic prostheses to replace IVD are now commercially available. The total IVD structure, or part of it, can be also removed to proceed with the insertion of allogeneic or autogeneic tissues. Both procedures present several difficulties and are thus not very feasible. In fact, when performing such types of interventions, height is reduced and water and PGs can also be lost. Finally yet importantly, there is the possibility of immunogenicity but low tissue availability could be an issue (Kalson et al., 2008; Kandel et al., 2008). Regenerative therapies can be divided into cell therapy, gene therapy, and tissue engineering. More recently, approaches using functionalized scaffolds showed to be a feasible therapy to restore the IVD (Hegewald et al., 2008).

2. Biomaterials based therapies

2.1. Artificial disc: disc prosthesis

Various devices to restore normal disc height and support loads have been extensively investigated. However, these devices lack some requirements to be successful in IVD restoration. Device migration, extrusion, and failure are some of the problems that can occur, thereby making these devices unsuitable. In addition, these types of devices have limited life-spans in vivo and require revision surgeries (Bao et al., 1996).

Total disc replacement could be performed using three different artificial disc options: metallic disc replacements, non-metallic materials, or a combination of both. Metallic devices take advantage of their high fatigue strength and of being capable to withstand loads up to 100 million constant amplitude cycles. As a result, the devices have a life-span of 40 years. However, strategies involving metallic replacements remain unable to replicate the complex motion of the disc. Metallic devices constructed from alloys (stainless steel, titanium, cobalt-chromium) show high biocompatibility and are therefore being used in orthopaedic surgeries. However, the growth

of fibrous tissue around the devices can compromise their application. Additionally, the friction between the device components can generate wear debris, leading to immune responses and even failure of the system (Boyd and Carter, 2006; Goins *et al.*, 2005).

Non-metallic devices are composed of polymers and elastomers that possess mechanical similarities to the natural disc. The combination of metallic and non-metallic devices results in a sandwiched structure composed by metal-polymer-metal. Some strategies have focused on developing prosthesis to restore NP that easily resembles the IVD structure. However, in advanced stages of degeneration, those devices are not viable (Bao *et al.*, 1996). Most prosthesis applications are created immediately after open surgery or percutaneous nucleotomy. The prosthetic disc nucleus (PDN) is one example of these devices and is comprised of two sections: the first is made up of a non-degradable hydrogel pellet (polyacrylamide) and the second, which surrounds the hydrogel, is a polymer mesh or jacket composed of polyethylene (Di Martino *et al.*, 2005). Aquarelle® (Stryker® Orthopaedics, Mahwah, NJ, USA) is another example of an implant that is primarily composed of polyvinyl alcohol. Different kinds of prostheses have been reported such as Newcleus Spiral Implant, composed by a polymer with memory and NuCore™ (SpineWave Inc., Shelton, CT, USA), a copolymer of silk and elastin. The number of patents for injectable biomaterials aimed for NP restoration has increased in the last decade. In this respect, a wide range of biomaterials have been studied including chitosan, injectable synthetic polymers, recombinant bioelastic materials, cross-linkable silk elastin copolymer, and polyurethane-filled balloons (Boyd and Carter, 2006). Prosthetic discs are not seen as a good strategy to regenerate IVD since they lack the ability to remodel and sustain cyclical stress (Woods *et al.*, 2010). Such devices require improvements in surgical techniques to avoid implant migration. Moreover, their efficacy requires extensive further research to be validated by clinical data (Goins *et al.*, 2005).

2.2. Polymeric-based Designs

Many novel therapies have focused on restoring not only the IVD structure but also its functioning. Apart from focusing on pain suppression, new therapies should address IVD preservation, repair, reinforcement, and regeneration. The ultimate goal is to achieve mechanical stability and formation of neo-tissue resembling natural IVD over a long term. When considering the restoration of natural IVD function, it is important to define IVD stages of degeneration. Due to the huge number of loading cycles that the spine often bears, the potential substitute should be able to support significant physical loads. Regarding biocompatibility, the material should be able to degrade in balance with matrix synthesis without inducing acute immune responses or any deleterious cytotoxic effects. The sterilization process that both

enables the preservation of original bioactivity and chemical composition and avoids bacteriological contamination appears to be a good procedure for hindering immune responses or even infections (Boyd and Carter, 2006). The best suited approaches may require a simple strategy or a more intricate design such as tissue engineering that can combine scaffolds, biomolecules, and cells, (Bron *et al.*, 2009; Clouet *et al.*, 2009). Advances in the field of tissue engineering and regenerative medicine (TE&RM) have been targeting the replacement or repair of injured tissue by creating a structural and functional tissue mimicking the native one (Slaughter *et al.*, 2009).

New insights on IVD substitute designs have been performed throughout the years. In the 60's, scientists (Nachemson and Evans, 1968) suggested the injection of vulcanized silicone into the degenerated disc. Later, Shneider *et al.* (Schneider and Oyen, 1974a, 1974b) proposed the use of silicone elastomer as an IVD substitute. Over 40 years, several IVD substitutes have been designed to restore IVD functioning. Currently, many substitutes have been produced by means of using synthetic, natural materials, and blends. Due to the many similarities between joint cartilage ECM and NP ECM, different scaffolds developed for joint regeneration could be an optimal choice in IVD tissue engineering (Clouet *et al.*, 2009). Similarly, therapeutic strategies used in other types of cartilage such as articular cartilage and meniscus could also be considered for IVD regeneration. This is mainly due to the similarities of these tissues in terms of the harsh biologic and mechanical loading conditions (Setton *et al.*, 2007).

Interestingly, some studies on cartilage repair could serve alternatively as guidelines in the design of new strategies for IVD regeneration. Elisseeff *et al.* (Elisseeff *et al.*, 2000) encapsulated bovine and ovine chondrocytes in a poly(ethylene oxide)-dimethacrylate and poly(ethylene glycol) semi-interpenetrating network. The hydrogel constructs were obtained by photopolymerization and investigated for their mechanical properties and biological performance. Results showed that the chondrocytes were viable after 1 day of culturing and dispersed within the hydrogel. Over a period of 2 weeks, biochemical analysis revealed an increase in proteoglycans and collagen, leading to a functional ECM.

A 3D network to promote interaction between cells and ECM is crucial for the regulation of tissue morphogenesis. As reported elsewhere (Khetan *et al.*, 2010), besides 3D environment remodeling ability, an appropriate material should be able to support cellular functions. Hyaluronic acid (HA) has been proposed as a material possessing such features but it has also been chemically modified for improving its biostability. In fact, HA hydrogels have been synthesized using multiple types of crosslinking. Using this strategy, encapsulated mesenchymal stem cells (MSCs) were able to spread in a controlled manner. The spatial control could be vital for developing complex microenvironments. As a result, the use of hydrogels in a clinical setting presents many advantages such as the possibility of being implanted using minimally invasive

techniques. Besides, due to their gel-like behaviour, it is possible to predict an efficient filling of the complete defect area, which is an advantage from a materials integration point of view. An interesting study involving promising hydrogels has been reported by the Fan group (Fan *et al.*, 2010). In their study, MSCs isolated from rabbit synovial tissue were encapsulated within Gellan gum hydrogels (GG). The constructs were cultured *in vitro* and proliferation and chondrocyte gene/protein expression were investigated over a period of 42 days. Results showed that the MSCs were viable and produced cartilaginous-like matrix (collagen and GAGs). Moreover, cells expressed chondrogenic markers such as collagen type II, Sox-9, biglycan, and aggrecan. Approved by the FDA, GG is a promising biomaterial when considering tissue-engineered cartilage. The pioneering studies performed by Oliveira *et al* (Oliveira *et al.*, 2009a, 2009b; Oliveira *et al.*, 2010) revealed that GG hydrogels have an adequate biological performance for finding a potential application in cartilage tissue engineering. As reported elsewhere (Oliveira *et al.*, 2009a, 2009b), GG hydrogels not only allowed cell encapsulation but were shown to support human chondrocyte viability *in vivo*. Type II collagen and aggrecan were found to be expressed. In fact, a functional cartilage tissue was formed in nude mice after 4 weeks of subcutaneous implantation. Other interesting studies addressing IVD regeneration have been performed to evaluate the interaction of cell biomaterial *in vitro*. Unfortunately, few *in vivo* studies have been performed to date. Table 1 summarizes different approaches of cellular polymeric-based designs for IVD regeneration that show promise for future clinical application.

The three main components of IVD aimed at tissue regeneration are: 1) AF (Sato *et al.*, 2003; Chang *et al.*, 2007; Helen and Gough, 2007; Nerurkar *et al.*, 2007; Shao and Hunter, 2007; Wan *et al.*, 2007; Nerurkar *et al.*, 2008; Wang *et al.*, 2008; Nerurkar *et al.*, 2009); 2) NP (Baer *et al.*, 2001; Sakai *et al.*, 2003; Seguin *et al.*, 2004; Bertram *et al.*, 2005; Wilke *et al.*, 2006; Roughley *et al.*, 2006; Cloyd *et al.*, 2007; Chou *et al.*, 2008; Halloran *et al.*, 2008; Richardson et al., 2008; Vernengo et al., 2008; Bron *et al.*, 2009; Chou *et al.*, 2009; Sawamura *et al.*, 2009; Calderon *et al.*, 2010; Silva-Correia *et al.*, 2010a; Silva-Correia *et al.*, 2010b; Reza and Nicoll, 2010;); and 3) both NP and AF by means of using biphasic scaffolds (Mizuno *et al.*, 2004; Hamilton *et al.*, 2005; Mizuno *et al.*, 2006; Nerurkar *et al.*, 2008; Nesti *et al.*, 2008). It has been proposed that for producing a tissue resembling natural IVD, total IVD replacement could be required. In this respect, strategies focused on the injection of cells and biomolecules alone or in combination have been proposed. However, when NP restoration is considered, it might not be possible to reverse all the IVD modifications (calcification observed in endplates), even when using approaches involving cell/scaffold constructs. The restoration of all AF functions is complex to achieve. Some specific requirements are required to validate the AF engineered scaffolds. The design should first not irritate or adhere to the perineurium; rather, it

should have an anisotropic behaviour and secrete the native ECM while allowing the fixation of surrounding structures. It should also support direct mechanical strength. As a result, the lamellar and fibrocartilaginous framework of the AF will demand a highly organized and oriented tissue into distinct lamellae. The presence of collagen (mainly type I) in AF makes it resistant to tensile loads. Several strategies aimed at regenerating AF use different types of material, processing techniques, cells source, and phenotypes. Besides, it should be mentioned that the mechanical properties of such implants should resemble those of the native AF (Setton *et al.*, 2007; Bron *et al.*, 2009). This explains in part why strategies for AF regeneration are mainly focused on synthetic materials and their processing onto porous scaffolds and electrospun nets (Sato *et al.*, 2003; Wan *et al.*, 2007; Helen and Gough, 2007; Nerurkar *et al.*, 2007; Nerurkar *et al.*, 2008a, 2008b; Nerurkar *et al.*, 2009; Chang *et al.*, 2007; Wan *et al.*, 2008).

Research within this field has been limited to date to fundamental *in vitro* and *in vivo* studies aimed to investigate cell attachment, proliferation, phenotypes, and ECM production in AF. For example, Sato *et al.* (Sato *et al.*, 2003) demonstrated that AF cells isolated from rats retained a fibrocartilaginous phenotype and produced more ECM in 3D cultures compared to 2D (monolayer). To obtain scaffolds with unidirectional aligned fibres, Shao *et al.* (Shao *et al.*, 2007) produced wet-spinned composite scaffolds of alginate and chitosan. The AF cells isolated from dogs were found to survive and proliferate. Moreover, both types of collagen (I and II) and aggrecan were found to be synthesized after 14 days. Similarly, Wan *et al.* (Wan *et al.*, 2007) harvested AF cells from rats and *in vitro* studies revealed that these cells proliferated, leading to expression of type II collagen. The subcutaneous implantation of these constructs showed no immunogenic response. The degradation and tensile strength increased with a corresponding increased time of photopolymerization. Another study by Wan *et al.* (Wan *et al.*, 2008) assessed the possible regeneration of outer and inner parts of AF using a biphasic construction. The outer AF was made up of bone matrix gelatine (BMG) and the inner AF was comprised of polycaprolactone triol malate (PPCLM). The incorporation of BMG in PPCLM improved the compressive strength of the construct. The outer AF structure was seeded with chondrocytes harvested from rabbits. After 4 weeks of culturing, the chondrocytes attached to the scaffolds, proliferated well, and migrated into the BMG. In both structures, production of type II collagen and aggrecan was observed over a period of 28 days.

Another study (Helen *et al.*, 2007) carried out an *in vitro* study where scaffolds composed by poly DL-lactic acid (PDDLA) and Bioglass®/HDPE (at different ratios) were processed as foams. The human cells seeded on these foams formed clusters attached to pores of PDDLA/30BG and PDLLA/5BG. The PDLLA/30BG foam showed the highest amount of GAGs formation, and in all cultures, the presence of collagen (mainly type I), was observed. Nerurkar and coworkers (2007; 2008; 2009) performed some studies involving electrospun

Table 1. Comparative pelvimetry of ancient populations

Intended tissue	Scaffold material	Processing method	Mechanics measured	Native benchmark	Cell type	Reference
ANNULUS FIBROSUS [AF]						
	Atelocollagen honeycomb	Scaffold	N/A	N/A	AF cells (lapine)*	Sato et al. (2003b)
	Alginate/Chitosan	Hybrid fibers	N/A	N/A	AF cells (canine)	Shao and Hunter (2007)
	Poly(1,8-octanediol malate)	Bone matrix gelatine scaffold (outer AF) and salt-leaching PPCLM (inner AF)	Compressive modulus 0.12- 0.25 kPa Ultimate tensile stress 7–15 MPa	440–750 kPa	AF cells (rat)	Wan et al. (2007)
	Poly (caprolactone triol malate) (outer AF) and Bone matrix gelatine (inner AF)	Scaffold	Ultimate tensile stress 1.3 MPa Ultimate compressive stress 3.5 MPa	N/A N/A	Chondrocytes (lapine)	Wan et al. (2008)
	Poly (DL- lactid) acid/Bioglass	Foam	N/A	N/A	AF cells (human)	Helen et al. (2007)
	Polycaprolactone	Electrospun nanofibers	Unaxial tensile 5.8 MPa Anisotropic and non-linear	5.6 MPa	AF cells (bovine)	Nerurkar et al. (2007)
			Uniaxial tensile modulus 50 MPa	80–120 MPa	AF cells (bovine)	Nerurkar et al. (2008b)
			Uniaxial tensile modulus 14.5 MPa	18 MPa	MSCs (bovine)	Nerurkar et al. (2009a)
	Silk	Porous scaffold	N/A	N/A	AF cells (bovine)	Chang et al. (2007)
NUCLEUS PULPOSUS [NP]	Scaffold material	Processing method	Mechanics measured	Native benchmark	Cell type	Reference
	Alginate	Drop-wise beads	Compression and torsion $\delta= 6°\text{-}14°$	23-30°	Inner AF (bovine)	Baer et al. (2001)
		Hydrogels	N/A	N/A	Inner AF, Outer AF, NP cells (bovine)	Chou et al. (2008)
	Photo-crosslinked Alginates	Hydrogels	N/A	N/A	NP cells (bovine)	Chou et al. (2008)
		Hydrogels	Unconfined pressure $E_{eq}= 4.3$ kPa	5.4 kPa	NP cells (bovine)	Chou et al. (2009)
	Calcium polyphosphate substrate	Substrate	Unconfined Compression $|G^*| = 0.2\text{-}0.6$ kPa $E_{eq}= 8.3$ kPa	7.4- 19kPa 5.4 kPa	NP cells (bovine)	Seguin et al. (2004)
	Type I collagen gel	Hydrogel constructs	Torsional shear $\delta= 6.5°\text{–}8.5°$	23°-30°	N/A	Bron et al. (2009)
	Type I collagen scaffold Photo-crosslinked carboxymethylcellulose	Matrix implant Hydrogels	N/A Unconfined compression $|G^*| = 2\text{–}10$ kPa $E_{eq}= 4.3$ kPa	N/A 7.4- 19kPa 5.4 kPa	N/A NP cells (bovine)	Wilke et al. (2006) Reza an Nicoll (2010b)
	Atelocollagen® type II	Hydrogel constructs	N/A	N/A	MSCs (rabbit)	Sakai et al. (2003); Halloran et al. (2008)
	Branched-copolymer PNIPAAM-PEG	Hydrogel constructs	Unconfined uniaxial compressiom $50\approx120$ kPa	50kPa	N/A	Vernengo et al. (2006)
	Chitosan-Glycerosphospahte	Hydrogel constructs	N/A N/A	N/A N/A	MSCs (human) NP cells (bovine)	Richardson et al. (2008) Roughley et al. (2006)

Scaffold material	Processing method	Mechanics measured	Native benchmark	Cell type	Reference
Gelatin	Microspheres (oil phase)	N/A	N/A	N/A	Sawamura et al. (2009)
Fibrin	Photopolymerized Hydrogels	N/A	N/A	N/A	Bertram et al. (2005)
Type II collagen and hyaluronic acid	Hydrogels	Confined compression 171.9 kPa	N/A	NP cells (rabbit) IVD cells (rabbit)	Calderon and Pandit (2010)
Hyaluronic acid	Hydrogels	Unconfined compression 21.90 kPa	5.39 kPa	MSCs (rat)	Cloyd et al. (2007)
Methacrylated Gellan gum	Hydrogel constructs	Shear modulus 89.5 ± 7.4 kPa	20–60 kPa	Lung fibroblast cell line (rat)	Silva-Correia et al. (2010, 2010)
AF AND NP [BIPHASIC SCAFFOLDS]					
Polyglycolic acid (AF) and Alginate (NP)	Nonwoven mesh scaffold (AF) and alginate hydrogel (NP)	N/A	N/A	AF and NP cells (ovine)	Mizuno et al. (2004)
Calcium polyphosphate	Substrates	N/A	N/A	Articular chondrocyres (ovine)	Hamilton et al. (2005)
Polycaprolactone (AF) and Agarose (NP)	Mesh (AF) and hydrogel (NP)	Unconfined compression 2MPa Torsion 250 kPa	3–10 MPa 2–9 MPa	AF cells and MSCs (bovine)*	Mizuno et al. (2006); Nerurkar et al. (2008a)
Poly(L-lactid) acid (AF) and hyaluronic Acid (NP)	Electospun nanofibrous scaffold (AF) and hydrogel (NP)	N/A	N/A	MSCs (human)	Nesti et al. (2008)

*In vivo studies; N/A, Not Available; δ, phase shift; E_{eq}, equilibrium stress; $|G^*|$, complex shear modulus.

polycaprolactone (PCL). Different types of cells were seeded on the scaffolds but all of them supported cell alignment along the fibres. Biologically, GAGs and collagen increased during the culture period, showing that these scaffolds efficiently supported cellular functions.

2.2.1. Hydrogels in cellular strategies for disc regeneration

Nucleus pulposus (NP). Hydrogels have been attracting a great deal of attention in IVD regeneration due to the possibility of developing biomaterials that structurally resemble the ECM. A wide range of hydrogels have been studied for their wide physical and chemical properties. The hydrogel designs contemplate the performance of the implants in terms of structural integrity, biodegradability, biocompatibility, molecular response, solute transport and cellular behaviour, mechanical strength, and low viscosity.

Natural hydrogels are particularly appealing for such applications, mainly due to their inherent biocompatibility. However, synthetic hydrogels allow for large-scale production and offer consistent and highly tunable properties, which are important advantages. However, since most synthetic procedures involve harsh reagents, there is a need to ensure that contaminants and unreacted reagents are completely removed (Slaughter et al., 2009). This issue is extremely important to avoid possible cytotoxic and immunogenic effects.

The 3D network of hydrogels makes them the best option to allow the interaction of cells and ECM, leading to a dynamic tissue morphogenesis (Khetan et al., 2010). Hydrophilic polymers (co-, homo-, or macromers) are able to be crosslinked to improve their bio-stability *in vitro* and *in vivo*. The hydrogels are typically soft and elastic after crosslinking (Slaughter et al., 2009). These injectable biomaterials are most advantageous since they allow the incorporation of cells and biomolecules alone or in combination. The possibility of injection by a small puncture, which can reduce the surgical damage and allow good dispersion of cells and/or biomolecules within the material, makes hydrogels suitable substitutes for NP. In this respect, the NP substitutes should not only be able to completely fill the shape and size of the damage but also be well fixed in the NP space (Boyd and Carter, 2006). The amplification of the repair process can be enhanced through GF-, TNF-, or IGF-functionalization of the injectable materials. As a result, injectable and functionalized hydrogels that can be ionic and/or chemically crosslinked appear to be a promising alternative for restoring natural NP functions. Another challenge that has been addressed concerns the mechanical properties of NP, which is a quite soft tissue (5 kPa) due to its unconfined compression (Nerurkar et al., 2010). In this respect, hydrogels are also very versatile and by varying the type of material, molecular weight, degree of crosslinking, chemistry, and functionalization, it could be possible to mimic the mechanical properties of the native tissue.

Several strategies have been examined to replace NP healthy functions. These are summarized in Table 1 (Baer *et al.*, 2001; Sakai *et al.*, 2003; Seguin *et al.*, 2004; Bertram *et al.*, 2005; Wilke *et al.*, 2006; Roughley *et al.*, 2006; Cloyd *et al.*, 2007; Chou *et al.*, 2008; Halloran *et al.*, 2008; Richardson et al., 2008; Vernengo et al., 2008; Bron *et al.*, 2009; Chou *et al.*, 2009; Sawamura *et al.*, 2009; Calderon *et al.*, 2010; Silva-Correia *et al.*, 2010; Silva-Correia *et al.*, 2010; Reza and Nicoll, 2010;).

Similarly, Baer (Baer *et al.*, 2001) investigated the mechanical behaviour of NP in terms of compressive and torsional shears. Alginate hydrogels cultured with porcine cells isolated from inner AF were maintained for 16 weeks *in vitro*. Posterior assessments revealed the maintenance of NP-like phenotype. Although a decrease of shear stress was observed, the values remained in the same order of magnitude compared to native tissue. Another study performed by Bron *et al.* (Bron *et al.*, 2009) demonstrated that type I collagen gels presented a lower phase angle compared to native tissue but showed quite favourable results concerning shear modulus. Reza and Nicoll (Reza and Nicoll, 2010) studied different methods to obtain photocrosslinked hydrogels by modifying carboxymethyl-cellulose with methacrylate groups. Bovine NP cell-encapsulated hydrogels were viable at 7 days of culturing. Moreover, the developed hydrogels showed good values to elastic modulus. Histological assessments showed that cells possessed a round morphology and production of proteoglycans was observed after 14 days of culturing.

In vivo effects of platelet-rich plasma-impregnated gelatine hydrogels microspheres were developed to find applications for the treatment of IDD. Chitosan has been proposed as a suitable cell-based scaffold (Roughley *et al.*, 2006). In that study, NP cells were encapsulated in different formulations of chitosan-glycerophosphate gels. *In vitro* results showed proteoglycans (PGs) production by NP cells. These were found to be entrapped into the hydrogels rather than released into the culture medium. Their studies also demonstrated that water content in such constructs was preserved. The production of PGs core proteins assessed by mRNA synthesis was increased compared to other constructs. Similarly, type II collagen production also increased. However, cells in the proliferating state were not found in NP and inner AF. A significant decrease in apoptotic cells in NP was observed. A study by Calderon *et al.* (Calderon *et al.*, 2010) proposed different polymers to regenerate NP. In that study, two types of materials, type I collagen and HA, were seeded with MSCs. The hydrogels demonstrated to be stable *in vitro*. The level of crosslinking was found to have a significant influence on both swelling properties and cellular proliferation. They also evaluated the differentiation of MSCs into chondrogenic lineage. The current study group (Silva-Correia *et al.*, 2010a, 2010b) has proposed the use of novel chemically modified Gellan gum hydrogels with the hope of finding applications for NP regeneration. The methacrylation of Gellan gum (GG-MA) allowed the synthesis of both ionic- and photo-crosslinkable hydrogels for *in situ* gelification. Moreover, the ability of these hydrogels to be photo-crosslinked by UV light lead to the production of hydrogels with improved mechanical properties that ultimately could mimic NP. This *in vitro* study showed that the hydrogels (GG-MA) were non-cytotoxic over L929 cells. Since the chemical modification GG improved the physicochemical and biological versatilities of the hydrogels, the produced GG-MA appeared as a suitable biomaterial to be used in NP regeneration (Cloyd *et al.*, 2006).

Recently, this study group (Pereira *et al.*, 2011) proposed a substitute to regenerate NP by means of using Gellan gum hydrogel matrices with cell-loaded microparticles (MPs). Two forms of Gellan gum (High and Low acyl) were combined in different ratios and processed as matrices and MPs. The matrices were reinforced with MPs possessing the same formulation. Mechanically, the formulation of 25% HAGG: 75% LAGG incorporated with MPs at 50 mg/ml showed the best mechanical performance compared to native NP. All hydrogel formulations were tested *in vitro* to screen for possible cytotoxicity. The cell culture studies revealed that the hydrogels did not have a cytotoxic effect over L929 cells. Moreover, the cell-loaded MPs were efficiently produced and L929 cells remained viable over a period of 72 hours.

Biphasic scaffolds for AF and NP.
To restore IVD, therapeutic approaches should address not only the anatomic aspects and mechanical behaviour of the tissue, but primarily ensure that the natural functions of IVD are recovered. The complex and heterogeneous IVD needs to be well defined and should be the main goal when tissue engineering this tissue. As a result, strategies addressing the different components of IVD structure, NP, and AF, could be required to restore IVD functions. As shown in Table 1, few studies have shown the efficacy of synthetic and natural polymers to regenerate AF and NP (Mizuno *et al.*, 2004; Hamilton *et al.*, 2005; Mizuno *et al.*, 2006; Nerurkar *et al.*, 2008; Nesti *et al.*, 2008). However, a study performed by Mizuno *et al.* (Mizuno *et al.*, 2004) revealed the efficacy of an integrated biphasic composite for AF and NP regeneration. The polymers used were polyglycolic acid seeded with AF cells and alginate scaffolds cultured with NP cells. The *in vivo* study was performed in rats for 12 weeks. Despite the innovative character of the study, results showed that the implant only allowed the synthesis of half the amount of PGs and collagen compared to native tissue. In another integrative tissue engineering study, Hamilton *et al.* (2005) developed a calcified tissue cartilaginous endplate NP. The first approach was to generate a hyaline cartilage tissue layer seeded with NP cells on calcium polyphosphates substrates. The NP cells were able to synthesize the PGs that are found in natural discs but did not produce collagen. More research is required to integrate all IVD structures.

Other studies (Mizuno *et al.*, 2006; Nerurkar *et al.*, 2008) developed an interesting strategy to replicate the micro-structural organization of AF by using scaffolds made of aligned PCL nanofibers. The centre of the PCL scaffold was filled with agarose to resemble NP. The compressive properties of agarose increased by reinforcement with the

multilamellar PCL nanofibers. The frameworks resembling the natural ones allowed cells to adopt opposing orientations of $\pm 30°$. Consequently, a deposition of collagen into multilamellar angle-ply organization was observed. Similarly, a study performed by Nesti (Nesti *et al.*, 2008) consisted of coupling HA hydrogel with electrospun nanofibers of poly(L-lactic) acid (PLLA). The HA was used to encapsulate MSCs that were injected at the centre of the non-aligned nanofibrous mesh. The nanofibrous mesh was also seeded with MSCs to produce cells possessing a different phenotype. In the AF region, cells developed a fibroblastic like phenotype. A chondrogenic phenotype was observed in cells encapsulated in the HA hydrogel. As a result, that study reported the production of disc-like tissue by means of using MSCs seeded in biphasic scaffolds.

Cell Source. The cells that were investigated to engineer tissues can be obtained from different sources and have different origins, namely, human and animal (canine, ovine, bovine, rabbit). The lack of healthy human disc cells impairs their use in tissue engineering strategies. Some studies (Roberts *et al.*, 2006; Gruber *et al.*, 2007; Le Maitre *et al.*, 2007) reported a high degree of senescence of the cells when herniated discs were the source of the cells. Cells from discs showing degeneration may not be an optimal source of cells for tissue engineering strategies (Gruber *et al.*, 1998). The isolation of cells from healthy human IVDs raises important issues that should be taken in account: the natural environment of cells is significantly changed after isolation and the removal of tissues to isolate healthy cells can lead to IDD (Woods *et al.*, 2010). To deal with this drawback, cells recovered from healthy IVDs of small animals show promise as a feasible alternative. To obtain a sufficient number of cells for tissue engineering and regenerative medicine (TE&RM) applications, these cells need to be first expanded *in vitro* for several weeks.

Currently, notochordal cells and MSCs have been well investigated for their regenerative potential. Studies involving their use in the regeneration of osteoarticular tissue encourage researchers to explore similar cellular strategies for IVD regeneration. These cells can differentiate into different phenotypes, namely NP cells (Vernengo *et al.*, 2008) and chondrocytes (Merceron *et al.*, 2008; Vinatier *et al.*, 2009). Richardson *et al.* (Richardson *et al.*, 2006) showed that co-cultures of normal human NP and normal human MSCs with and without cell-cell interaction resulted in MSCs with an NP-like phenotype. It was shown by an increase in aggrecan and type II collagen genes. Studies of bone marrow and adipose tissue have also been performed. Vadala *et al.* (Vadala *et al.*, 2008) performed a study with MSCs co-cultured with NP cells. The study revealed an altered gene profile expression and the up-regulation of ECM synthesis.

Besides MSCs, nothocordal cells have been proposed as a good choice for use in TE&RM. These types of cells are not produced in adult human IVDs; however some animals express these cells over their life time. Aguiar *et al.* (Aguiar *et al.*, 1999) carried out a study involving co-cultures of

nothocordal cells with NP cells. The *in vitro* studies showed an increase in PG synthesis. However, when using these cells, the risk of tumour growth could not be discarded in both cases and its screening should be well assessed (Sakai *et al.*, 2008).

To efficiently regenerate injured tissues, the cells seeded into the materials should demonstrate characteristics similar to those found in normal tissues. As a result, cells cultured *in vitro* often require the presence of specific morphogenetic factors. The characteristic phenotype of each cell type can be maintained by mean of culturing in a 3D environment (Benya and Shaffer, 1982; Vinatier *et al.*, 2005). The vast amount of research efforts on cartilage tissue engineering will certainly improve knowledge in this field and hopefully will be applied in IVD TE&RM (Clouet *et al.*, 2009). Chou *et al.* (Chou *et al.*, 2006) reported the negative effect of cell cultures of inner and outer AF in 2D. That study showed that cells lost their phenotype up to passage two. In later stages, both types of cells became indistinguishable. Reza *et al.* (Reza and Nicoll, 2008) showed that cells from inner and outer AF were able to express collagen type II when seeded in poly-glycolic acid (PGA) scaffolds and cultured under hydrostatic pressure. The scaffolds seeded with outer AF cells expressed a more pronounced and organized ECM. However, it was reported that cells were able to express mainly collagen type I *in vivo* (Bron *et al.*, 2009). As a result, further studies are required to develop adequate TE&RM strategies for obtaining the production of a native-like ECM.

2.2.2. Hydrogels in acellular strategies for disc regeneration

Cell-free scaffolds have also been investigated for their potential as IVD substitutes. The advantages of these substitutes are mainly associated with mechanically stability and present less shipping constraints and longer shelf life compared to cell-based scaffolds (Woods *et al.*, 2010). As reported by Abbushi *et al.* (2008) and Endres *et al.* (2010), implantation of PGA-HA scaffolds, which have been shown to be suitable for proteoglycans-rich tissues, induces the regeneration of NP. The migration of cells into the defect area is responsible for the increase of the amount of PGs within a period of 12 months. To produce cell-free scaffolds capable to restore the IVD, Waldman *et al.* (2002) and Seguin *et al.* (2004) showed that calcium phosphates ould be used as substrates to engineer NP and endplates *in vitro*. The success of this strategy relies on the similarity to native tissue; once the PGs are being synthesized and the biomechanical properties closely resemble the natural ones.

Biomolecules to functionalize hydrogels. Scaffold performance can be improved by functionalization of the biomaterial. The capacity of polymers to bind or be linked to biomolecules has been attracting a great deal of attention in IVD regenerative strategies. To recruit and protect growth factors such as bone morphogenetic proteins (BMPs), basic fibroblastic growth factors (bFGFs) and vascular endothelial growth factors (VEGFs), heparin

functionalization has been explored by several authors (Nie and Wang, 2007; Joung *et al.*, 2008). Heparin functionalization could be a good strategy due to its ability to bind to biomolecules. In this way, a wide range of methods have been developed towards functionalized scaffolds with heparin (Fugita *et al.*, 2004). Hydrogel constructs functionalized with heparin and fluvastin have also been proposed for bone repair. Benoit (Benoit *et al.*, 2007) showed that the developed scaffolds were able to promote osteogenic differentiation of human MSCs. Similarly, Edlund *et al.* (2008) reported that BMP-2 loaded in heparin functionalized polymers improved adhesion and proliferation of murine MSCs. Another study (Fujita *et al.*, 2004) showed that biologically active FGF-2 was stimulated by chitosan/periodate-oxidized (IO_4) heparin hydrogels. The studies reported by Fujita and Edlund (Fujita *et al.*, 2004; Edlund *et al.*, 2008) showed that functionalized materials are effective in promoting IVD regeneration. Covalent linking of heparin to scaffolds has been described as the most attractive method to functionalize materials (Yang and Li, 2009). Nevertheless, a novel approach to design scaffolds by heparin carrying microcapsules was reported by Li *et al.* (2009). The microcapsules were grafted to the material, which ensured minimal loss of bioactivity and kept the heparin molecules intact.

Chang *et al.* (2007) developed a strategy to improve cell attachment on the silk porous scaffolds by chemical bonding to the RGD, an integrin binding motif. The study showed that improvement of cell adhesion was not achieved but that collagen type II and aggrecan production increased. However, the increase of collagen type II and aggrecan observed was not suitable for the regeneration of AF since collagen type II is not able to form or bundle fibrillated structures. Therefore, the use of biomolecules such as decorin or small PGs could be addressed when functionalization of biomaterials is considered. These biomolecules are known to be capable to bind TGF-β and have increasingly been used in fundamental studies (Macri *et al.*, 2007).

3. Direct injected-based therapies

3.1. Cell therapy

The continuous investigation of IVD biology and its degenerative cascades has created novel concepts for developing functional solutions at a cellular level (Woods *et al.*, 2010). Frequently, IDD appears at an early age, especially during the second decade of life. The abnormal cell-mediated response leads to injuries at the cellular level (Woods *et al.*, 2010). As a result, it has been proposed to use of cellular strategies to efficiently regenerate IVD. The reinsertion of autologous cells can limit the application of cell therapies. Human IVD lacks in nothocordal cells and differentiated cells harvested from degenerated IVDs show an altered phenotype impairing their use in clinics. Moreover, surgical procedures to harvest cells can induce degeneration in healthy discs. Degenerated discs show cells possessing different phenotypes due to altered biochemical processes, which lead to increased rates of senescent cells. The clinical application of such cells can induce a degenerative cascade of the IVD. The regeneration of the IVD by reinsertion of allogeneic cells poses the risks of immune rejection, allergy, or disease transmission. Despite being non-vascular and non-innervated, IVD pathological conditions are associated with an increase in neovascularization and innervations. Stem cells lack the specific markers responsible for determining the cell lineage (Woods *et al.*, 2010). As a result, stem cells could be excellent candidates to repair IVD when considering the use of cellular approaches. In a study reported by Sobajima *et al.* (Sobajima et al., 2008), human mesenchymal stem cells (hMSCs) were co-cultured with human nucleus pulposus cells (hNPCs) to assess the feasibility of stem cells in restoring IVD. The transplanted stem cells showed excellent viability and migration through different IVD structures and differentiated in several phenotypes. However, some specific markers were not expressed in the used cell type. The presence of molecules such as aggrecan, collagen type II, and SOX-9 allows us to distinguish the IVD cells. However, these markers are non-specific and are also used to identify articular chondrocytes, for example (Risbud *et al.*, 2004).

3.2. Biomolecules therapy

One approach to stimulate IVD regeneration is based on the direct injection of biomolecules acting as activators for ECM production. IVD cells are differently influenced by several growth factors and enzymes, which can be injected as pure protein solutions or combined with matrices that allow their controlled release. Most studies involving the use of BMPs in IVD were aimed at stimulating ECM production (Zhang *et al.*, 2007). In the study reported by Zhang et al. (Zhang *et al.*, 2007), AF cells were stimulated with different BMPs and by Sox-9 transfection studies. The collagen synthesis was increased by over-expression of such molecules. Interestingly, another study (Takegami *et al.*, 2005) reported the repair of ECM by stimulation of AF cells with OP-1 (also known as BMP-7). Masuda *et al.* study (Masuda *et al.*, 2003) showed a significant increase in total DNA, PG synthesis, and collagen content by continuous stimulation of AF cells with BMP-7. In addition, the study was able to establish the best performance of BMP-7 on NP compared to AF results. Currently, BMP-7 is widely used in studies related to bone and cartilage tissue engineering. The chondrocyte differentiation results in a stimulation of matrix production, mainly PGs and collagen type II (Kong *et al.*, 2008). BMP-2 has been shown to exert potent effects both in articular chondrocyte ingrowth capacity and anabolic processes. Although, some *in vivo* studies showed an increase of IVD degeneration in rats treated with BMPs (Huang *et al.*, 2007).

Recently, some studies proposed the use of platelet-rich plasma (PRP) to stimulate cell proliferation and ECM metabolism of the IVD cells. The activated platelets were able to release multiple growth factors directly involved in regulation of cell proliferation, differentiation, and morphogenesis. Akeda et al. (Akeda et al., 2006) showed the effect of using PRP in stimulating cell proliferation and ECM metabolism. PRP cultured in alginate beads up-regulated PGs and collagen syntheses, while accumulated PG amounts were higher compared to platelet-poor plasma. In other studies, Nagae et al. (Nagae et al., 2007) and Sawamura et al. (Sawamura et al., 2009) investigated the in vivo effect of PRP-impregnated gelatin hydrogel microspheres (PRP-GHM). Both studies presented a significant suppression of the progression of IVD degeneration. Similarly, Nagae et al. (Nagae et al. 2007) proposed a different strategy in which PRP was injected directly into the NP of degenerated IVD, instead of being impregnated into gelatin hydrogel microspheres. The progressive degeneration of the IVD was suppressed over 8 weeks of PRP administration. Sawamura et al. (Sawamura et al., 2009) reported an increase of IVD height after treatment with PRP-GHM. The presence of water was also preserved in PRP-GHM administration. Moreover, the mRNA expression of PGs core protein and type II collagen increased after treatment with PRP-GHM. Although no proliferating cells were found in the IVDs treated with PRP-GHM, there was a decrease in the number of apoptotic cells in the NP. However, the lack of studies in animals or humans does not make this approach feasible yet. The regeneration of IVD by direct injection of biomolecules is limited since it is only of short-term action. This option could be feasible only for mild or moderate degenerated discs (Freimark and Czermak, 2009).

3.3. Gene therapy

Gene expression profiles can be changed by means of gene therapy. To envisage a long-term stimulation of the disc, activators can be introduced into the IVD cells genome. Two main methods are commonly used (Kalson et al., 2008). The in vivo method uses exogenous therapeutic genes to target cells throughout injectable vectors. In the ex vivo method, the targeted cells are removed and expansion of the cells is performed in vitro and only then are re-implanted. The second method is preferred and it involves a virus or a non-viral carrier that aims to serve as vehicle to integrate the gene of the activator (Freimark and Czermak, 2009). However, adenoviruses can induce strong immune reactions in vivo. To diminish such a response, it has been proposed to use adeno-associated viral vectors. Such strategies have shown positive results. The synergistic effect of using adeno-mediated therapies might be helpful in restoring the IVD functions.

An experiment carried out for 6 weeks in a IVDs mouse model (Nishida et al.,1999) demonstrated that gene transfection of TGF-β had a two-fold increase in PG synthesis. A considerable increase of PGs and TGF-β were present during the experimental period. The successful integration of genes of BMP-2 and IGF-1 by adenovirus vector has also been reported in vitro (Wallach et al., 2003; Moon et al., 2008). Both studies revealed an increase in the synthesis of intradiscal PGs in a dose-dependent manner. Nevertheless, the paucity of studies focusing on the unlimited and uncontrolled release of biomolecules makes gene therapy approaches questionable (Freimark and Czermak, 2009). Many issues are raised: namely, the choice of the vector, method and timing of delivery, course of action, safety of gene transfer, and therapeutic gene biology. An interesting study (Grunhagen et al., 2006) reported a contraindication of this therapy, suggesting a potential degeneration effect due to the increase of cellular metabolism in a harsh environment. However, gene therapy has been proposed as an alternative to large surgical interventions (Woods et al., 2010).

4. Future outlooks

Over the past two decades, significant efforts have been made to develop novel regenerative strategies targeted to IVD. The important socio-economical impacts of LBP in society became a serious problem with large annual expenses. Human disability and suffering are uncomfortable conditions and current treatments cannot solve these problematic on a long-term basis. The stage of IVD damage should be accurately assessed in order to design the most suitable substitute(s). Many problems are continuously emerging while strategies to restore/regenerate IDD are under development. The pathological state as cause of degeneration is not well defined. A considerable knowledge achieved in cartilage tissue repair is nowadays used for IVD restoration. However, the high complexity and heterogeneity of IVD require an increase in the fundamental knowledge to understand all processes involved in IVD degeneration. To be feasible, an IVD regenerative strategy should require integration among native tissues and generation of functional neo-tissue. Therapies using cells, biomaterials, biomolecules, or gene therapy by direct injection are performed as standalone therapies. Nevertheless, the short-term success and the unclear long-term consequences become a pitfall. Meanwhile, such strategies are used to restore a single tissue. Novel approaches are being studied focusing on restoration of two dissimilar tissues, the AF and NP. All approaches involve the use of cells/biomolecules/genes alone or combined with biomaterials, mainly hydrogels. Significant research should be conducted to clarify both common and specific cellular responses in human IVD. Autologous cells and growth factors have shown great promise for developing a new generation of biological-based therapies. Mimicking the natural environment outside the body can be a huge challenge in some fields of engineering, especially those focusing on replicating the in vivo mechanical stimuli.

Regenerative therapies can only succeed if guided by a clear vision of their future clinical applications. To efficiently translate TE&RM strategies into clinical practice, scientists should perform *in vivo* studies using an appropriate animal model. Animal IDD can occur spontaneously or in an induced way; but by proceeding with the latter, the experiments could be compromised. When animal IVD is punctured to induce IDD, the pathological condition can rise up intensively after the procedure. Different species exhibit different biochemistry and biological behaviours. The unclear knowledge about such features in animals can result in erroneous conclusions. Inferring the data obtained in animals to humans is also a difficult task. The most used animals in the *in vitro* studies involve rat, rabbit, and sheep models. Similarly to humans, rabbits lose their nothocordal cells and due to their anatomic position, resemblance makes them a suitable model for *in vivo* studies. Despite these pitfalls, TE&RM provides hope to IVD and a consensus on which strategies could be developed to treat LBP might not be too far.

Acknowledgements

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