Intervertebral disc regeneration: from the degenerative cascade to molecular therapy and tissue engineering

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Abstract

Low back pain is one of the major health problems in industrialized countries, as a leading source of disability in the working population. Intervertebral disc degeneration has been identified as its main cause, being a progressive process mainly characterized by alteration of extracellular matrix composition and water content. Many factors are involved in the degenerative cascade, such as anabolism/catabolism imbalance, reduction of nutrition supply and progressive cell loss. Currently available treatments are symptomatic, and surgical procedures consisting of disc removal are often necessary. Recent advances in our understanding of intervertebral disc biology led to an increased interest in the development of novel biological treatments aimed at disc regeneration. Growth factors, gene therapy, stem cell transplantation and biomaterials-based tissue engineering might support intervertebral disc regeneration by overcoming the limitation of the self-renewal mechanism. The aim of this paper is to overview the literature discussing the current status of our knowledge from the degenerative cascade of the intervertebral disc to the latest molecular, cell-based therapies and tissue-engineering strategies for disc regeneration. Copyright © 2013 John Wiley & Sons, Ltd.

Keywords intervertebral disc; disc degeneration; growth factors; stem cells; gene therapy; tissue engineering

1. Introduction

The most common clinical condition associated with musculoskeletal disorders is low back pain (BP). It represents an important economic problem and the main cause of disability in developed countries in people under 45 years of age (Luo et al., 2004). In the wide majority of patients, chronic BP is associated with degeneration of the intervertebral disc (IVD).

The IVD is composed of the inner nucleus pulposus (NP) and the outer annulus fibrosus (AF). The former is characterized by a gelatinous extracellular matrix (ECM) rich in proteoglycans, mainly constituted by aggrecan and type II collagen. Within the NP, a population of small chondrocyte-like cells is responsible for synthesizing and maintaining the ECM. The AF is mainly constituted by concentric dense lamellae of type I collagen and cells with fibroblasts morphology and phenotype.

Intervertebral disc degeneration (IDD) is an age-related process characterized by the decrease of proteoglycans and water content in NP, with consequent progressive loss of mechanical stability and shock absorber function, leading to osteophyte formation and decreased range of motion of spinal segments (Boni and Denaro, 1987). IDD and early degenerative changes are defined as an aberrant, cell-mediated response to progressive structural failure, whereas degenerative disc disease is a degenerated disc, which is also painful. The main cause of IDD is genetic inheritance, nutritional impairment, ageing and loading history, while the precipitating cause is structural disruption occurring from injury or fatigue failure (Adams and Roughley, 2006). Early signs of IDD have been also demonstrated among healthy young adults (Zobel et al., 2012). BP is often the first symptom of IDD, which may progress to other spinal disorders, such as disc herniation,
spondylolisthesis, spinal stenosis and segmental instability with associated neurological disorders (myelopathy and radiculopathy).

However, to better understand the pathophysiological links between degenerative disc disease and pain, it is fundamental to distinguish symptomatic from asymptomatic degeneration. Since IDD itself is not a basis for clinical intervention, identification of specific features underlying discogenic pain is essential to advance current treatments and identify novel therapeutic targets. Inflammation, innervation and hypermobility interact in ways that enhance and perpetuate the risk of discogenic pain.

The painful degeneration is characterized by ineffective healing of injury to the peripheral tissue. Because of the characteristic vascularization at the vertebral end plate and the outer part of the AF, these are the likely sites for focal damage, inflammation, neoinnervation and nociceptor sensitization. Therefore, while the NP is likely the main site of degenerative change, the end plate and peripheral part of the AF are more likely the source of patient discomfort (Lotz and Ulrich, 2006). Moreover, changes in architecture and biochemical composition lead to the impairment of the internal mechanical environment of the disc, with hypermobility in the early stages of IDD and hypomobility as it progresses (Mulholland, 2008).

Current treatments for IDD and related BP range from conservative therapies, such as bed rest, anti-inflammatory medication, analgesia and physical therapy, to invasive strategies, such as epidural steroid injections, ablation techniques or surgical therapies (discectomy, spinal fusion and disc replacement technologies) (Di Martino et al., 2005). Moreover, it is paramount to differentiate between the treatment of painful degenerative disc disease, which is not very successful, and treatments of stenotic diseases, which is very effective in the short and medium term but might be followed by painful degenerative disc disease. However, the efficacy and long-term outcomes of these therapies are neither reliable nor predictable, since these target clinical symptoms instead of the degenerative disc cascade. Patients operated on for lumbar disc herniations often develop BP due to disc degeneration months to years after surgery. Here, additional regenerative interventions would have a preventative intention, whereas interventions for painful degenerative disc disease, as an alternative to spinal fusion or disc arthroplasty, would be a curative approach (Hegewald et al., 2008).

This is the reason to support research towards new and more effective treatments for BP and IDD. The necessity to prevent, slow down or reverse the degenerative changes in IVD requires a careful knowledge and characterization of the degenerative cascade of the disc to develop novel therapeutic regenerative approaches.

2. The degenerative cascade

It is currently known that IDD is a progressive and chronic process whose aetiology is multifactorial. Recent findings on identical twins have demonstrated that environmental factors may explain only a small part of the IDD process and, as a matter of fact, genetics plays a dominant role in IDD (Battie et al., 2004; Fassett et al., 2009; Lee et al., 2010).

The genetic influence is confirmed by the analysis of several genes. Polymorphisms of the vitamin D receptor gene (Videman et al., 1998), collagen IX alleles (Annunen et al., 1999) and metalloprotease-3 (MMP-3) (Takahashi et al., 2001) and variations in the chondroitin sulphate-1 domain of the aggrecan gene (Kawaguchi et al., 1999) are demonstrated to be related to IDD. Moreover, single nucleotide polymorphisms that inhibit growth factors, such as transforming growth factor-β (TGFβ) (Seki et al., 2005), and alleles that code for interleukin-1 (IL-1) (Solovieva et al., 2004) have been also described.

The imbalance between synthesis and catabolism of crucial ECM components contributes to IDD (Smith et al., 2011). Disc cell metabolism is modulated by a wide variety of molecules, such as cytokines, enzymes, enzyme inhibitors and growth factors, that act in a paracrine and/or autocrine way (Masuda et al., 2004). Bone morphogenetic proteins (BMPs), TGFβ and insulin-like growth factor (IGF) are the main anabolic factors (Osada et al., 1996; Thompson et al., 1991). On the other hand, MMPs and aggrecanases act as catabolic factors, and are regulated by pro-inflammatory cytokines (Kang et al., 1997). Several studies have confirmed the correlation between elevated MMPs levels and progressive IDD. Indeed, it has been shown that the degenerated IVD presents increased levels of proteolytic enzymes, such as stromelysin-1 (MMP-3) and collagenase-1 (MMP-1) (Weiler et al., 2002), that belong to the disintegrin-like and metalloprotease with thrombospondin motifs (ADAMTS) family (Roberts et al., 2000). This process is mediated by the increase in IL-1 and tumour necrosis factor-α (TNFα) (Le Maitre et al., 2005) that modulate IDD onset and progression. On the other hand, the anabolic–anticatabolic imbalance may be worsened by the loss of tissue inhibitor of metalloproteinase-1 (TIMP-1), an anticatabolic tissue factor.

This imbalance in the anabolic–catabolic process reflects the altered function of NP cells and results in the progressive decline of the aggrecan content (Buckwalter, 1995) in the NP ECM (Sobajima et al., 2005b). Aggrecan consists of several negatively-charged sulphated glycosaminoglycans (GAGs), such as keratan sulphate and chondroitin sulphate, which bind water, affecting disc height and thus improving its load-bearing function (Urban and McMullin, 1985). In fact, the normal IVD distributes loads equally along all the directions because of its high water content (85–90% in young NPs).

Moreover, during ageing, the IVD undergoes significant changes in both the composition of the ECM and the cell density of the NP and AF. The cell density represents a crucial prerequisite for cell-based tissue engineering approaches and has been widely investigated. Sowa et al. (2008) showed in a rabbit model how ageing leads to a reduction in cell density and proteoglycans levels in the
ECM, with consequent NP dehydration, decreased disc height and loss of its load-bearing capacity. However, data from human studies do not fully support this statement (Hastreiter et al., 2001). Liebscher et al. (2011) demonstrated that cell density in the disc decreases significantly from 0 to 16 years, with the main changes occurring from 0 to 3 years for NP and AF, but no significant variations were observed thereafter.

Furthermore, abnormal distribution of forces across the IVD results in either cracking and fissuring of the AF, that may subsequently lead to disc herniation, or vertebral body pathological changes, such as subchondral sclerosis, end-plate ossification and osteophyte formation (Boni and Denaro, 1987).

The apparent inability of the IVD to self-repair after injury and ageing might be due to the low nutrition supply and metabolic activity caused by the inherent avascularity (Urban et al., 2004). Indeed, the adult IVD is the largest avascular tissue in the body, and the cells residing at the centre of the adult lumbar disc are about 8 mm from the nearest blood supply (Katz et al., 1986). Blood vessels in soft tissues can provide the needs of the outer AF cells, while the NP and inner AF cells rely on a capillary network in the subchondral end-plate, through which nutrients diffuse. The reduction of nutritional supply is demonstrated to be one of the leading causes of IDD. After the first decade of life, when the first IDD signs in magnetic resonance imaging (MRI) become evident (Zobel et al., 2012), the endplate capillary network decreases. Many factors, such as calcification (Benneker et al., 2005), vasoactive agents (noradrenaline and acetylcholine) (Wallace et al., 1994), mechanical stress (vibration) and smoking can cause the occlusion of vessels. The effect is an increased oxidative stress, acid pH and inadequate oxygen level leading to reduction of protein, proteoglycan synthesis and viable cells in the disc (Urban et al., 2004).

The loss of notochordal cells (NCs) has also been recognized to be involved in IDD pathophysiology. NCs are remnants of the embryonic tissue that guides the formation of the spine (Trout et al., 1982). These can be found in young human individuals and in adults of certain animal species, as a secondary population of large cells with granular cytoplasmic inclusions (Figure 1) (Oguz et al., 2007). These embryonic cells persist through most of adult life in some vertebrates, but they gradually disappear in other species during growth (Sowa et al., 2008). The mechanisms through which NCs decrease and disappear in the adult are still unclear, but the terminal differentiation of NCs into cells with a chondrocytic phenotype or into apoptotic cells has been proposed (Kim et al., 2009). Indeed, the observation of the high progression rate of IDD in the animal species where NCs disappear in IVDs in the early phases of growth has aroused great interest. The possible link between IDD and NCs is an intriguing possibility, for the reason that if residual NCs exist in the human spine, they may represent a powerful resource as a target for IVD regeneration strategies (Hunter et al., 2003).

3. Intervertebral disc regeneration strategies

Novel molecular and cell therapies aim to improve the IVD’s ability to restore proteoglycans levels in order to enhance disc hydration and biomechanics. It is possible to recognize three different approaches currently employed in IVD regeneration strategies, such as molecular therapies (growth factors and gene therapy), cell therapy and biomaterials-based tissue engineering.

3.1. Molecular therapy

Molecular therapies for IDD treatment are based on the upregulation of signals able to induce anabolic or anticas- tabolic responses on IVD cells. The recognition of the therapeutic potential of growth factors led to their use as a molecular therapy for IDD and other musculoskeletal disorders. These small peptide cytokines play a crucial role in the modulation of matrix synthesis and IVD cells proliferation, differentiation and migration.

Thompson et al. (1991) were the first to successfully use exogenous administration of growth factors (TGFβ/1), demonstrating an in vivo increase of proteoglycan synthesis by NP cells. Several in vitro studies afterwards demonstrated that other anabolic growth factors, such as BMP-2, BMP-7 and IGF-1, induce the synthesis of ECM components (Cao et al., 2003; Osada et al., 1996; Takegami et al., 2002). Moreover, the use of TGFβ/3 (Risbud et al., 2006), epidermal growth factor (EGF), basic fibroblast growth factor (bFGF) (Thompson et al., 1991) and growth differentiation factor-5 (GDF-5) (Li et al., 2004b) showed similar results. Platelet-rich plasma (PRP), extracted from...
peripheral blood, contains variable concentrations of several growth factors (Weibrich et al., 2002) and it has been demonstrated to be a promoter of IVD cell proliferation and ECM synthesis (Akeda et al., 2006).

The positive effects of growth factors in modifying the course of IDD have also been evaluated in animal models, confirming the positive effects of growth factors on IVD metabolism. Indeed, Masuda et al. (2006) observed the restoration of disc height and structural changes after BMP-7 injection in a rabbit model of IDD.

Although it has been proved that the biological function of NP cells can be widely modulated, the half-life of exogenous growth factors ranges from hours to days (Winn et al., 1999). Therefore, considering IDD as a chronic condition, long-lasting and increased local levels of growth factors would be necessary to obtain a long-term effect of this therapy. As a way to overcome this shortcoming, gene therapy has aroused significant interest as a potential treatment for IDD. Gene therapy allows modulation of gene expression in order to obtain a prolonged synthesis of a target gene product. It represents a great opportunity to act biologically and potentially reverse IDD. It is based on the transfer of exogenous genetic material (RNA or DNA) into a target cell, with the aim of overcoming the lack of a deficient protein or producing a beneficial one (Vadala et al., 2007a).

Basic strategies for gene delivery are in vivo gene therapy, in which vectors with appropriate genes are introduced directly into the body, and ex vivo gene therapy, in which target cells are isolated, cultured in vitro, genetically altered and then re-implanted. However, based on the biology of IVDs and the difficulty in harvesting the tissue for cell culture, in vivo gene transfer is the most suited to IVD gene therapy (Vadala et al., 2007a).

Exogenous genetic materials are delivered into the cells by viral and non-viral vector systems. Viral vectors can be subdivided into genome-incorporating [retrovirus-type (retrovirus and lentivirus)] and non-genome-incorporating [plasmid-type (herpes-, adeno- and adeno-associated virus)] (Hubert et al., 2008). A promising viral vector is adeno-associated virus (AAV), since it is safer and less immunogenic than the others. It has been used as an in vivo delivery model in dividing and non-dividing cells and, so far, has not been associated with any side-effects (Afiune et al., 1999; Leckie et al., 2012). Non-viral vectors are plasmid-based systems, which are non-pathogenic, relatively inexpensive to produce and without any of the viral vector side-effects. However, low efficacy in transducing target cells makes this strategy less used than viral vectors.

Nishida et al. (1999) were the first to perform transduction of a therapeutic gene in vivo. Human TGFβ1 was used to transduce healthy rabbit IVDs through an adenoviral vector, demonstrating a 100% increased proteoglycan synthesis in cells isolated after 1 week (Nishida et al., 1999). Afterwards, other studies demonstrated an improved ECM synthesis through the application of different target genes, such as BMP2 and IGF-1 (Li et al., 2004a).

Hence, after the success of single-agent gene therapy, multiple growth factors in a gene therapy cocktail have been studied to assess their effect. Combinations of TGFβ1, IGF-1 and BMP-2 showed an additive effect in amplifying proteoglycan synthesis compared to single-agent treatment (Moon et al., 2008). Recently, Leckie et al. (2012) injected AAV serotype 2 with BMP-2 and TIMP-1 into the NP of a stab model of IDD in rabbit. They demonstrated how this approach delays IDD changes, representing a potential alternative for the regeneration of the IVD.

The increase in disc matrix content can also be obtained by inhibition of local catabolic factors. In an in vitro study, Wallach et al. (2003) showed a five-fold increased proteoglycan synthesis in NP cells after TIMP-1 gene transfer. Moreover, Studer et al. (2007) proved that the decrease in proteoglycan synthesis caused by IL-1 and TNFα could be avoided by inhibition of p38 MAPK. Therefore, the inhibition of this signalling pathway may slow down IDD.

The physiological production of growth factors is the end-point of a complex cascade of signals mediated by transcription factors. These often upregulate multiple end products through diverging signalling cascades. Sox9 plays a significant role in chondrogenesis, upregulating key pro-chondrogenic factors. Paul et al. (2003) used Ad/Sox9 to demonstrate an increase in ECM protein synthesis. Moreover, Boden et al. (1998) demonstrated that LMP-1 (LIM mineralization protein), an important regulator of several BMP syntheses and osteoblast differentiation, has an anabolic effect on transduced IVD cells.

Gene therapy has proved its ability to beneficially modulate the biological processes of the IVD cells, both in vitro and in vivo. However, the expression of transgenic growth factors outside the IVD due to a misdirected injection has demonstrated toxicity, with potentially detrimental consequences (Levicoff et al., 2008). To overcome these problems, inducible systems have been proposed as efficient tools to modulate transgene expression, avoiding the toxicity that could derive from misdirected injections to the disc (Sowa et al., 2011; Vadala et al., 2007b).

Gene therapy and growth factor delivery systems also suffer from other limitations; indeed, an immediate structural and biomechanical alteration in the disc cannot be induced only by the upregulation of growth factors. In order to achieve a biological effect, disc cells need to be able to respond to the applied growth factor; however, in moderate and advanced stages of IDD the cell number is significantly decreased (Gruber and Hanley, 1998). To overcome this deficit, the adjunctive transplantation of healthy functional cells might be required.

3.2. Cell therapy

The introduction of exogenous cells into the IVD as a potential strategy for IDD treatment has been widely explored. Cell therapy primarily aims to supplement and/or replenish the local cell population already decreased by ageing and IDD (Richardson et al., 2010).
Different types of cells, such as disc cells, chondrocytes and progenitor cells, have been used. Transplantation of autologous NP cells has been used in the clinic and it is still under evaluation in clinical trials. Meisel et al. (2006) have shown their potential use to reduce disc narrowing and BP at 2 years follow-up. However, this approach is limited, due to the poor expansion rate and loss of native phenotypic features of NP cells during expansion in monolayer cultures. Moreover, this approach is only performed in patients undergoing surgical discectomy. Hence, stem cell therapy seems more attractive, given the simplicity of ex vivo cell expansion, the modulation of cell phenotype and lower harvest site morbidity.

Stem cells are undifferentiated cells with a high proliferation rate, capable of self-renewal and multilineage differentiation (Blau et al., 2001; Hiyama et al., 2008b). Stem cell types range from embryonic ones, which are considered totipotent albeit their use raises several ethical issues (Wobus, 2001), to adult stem cells that can be found in fully differentiated adult tissues such as skin (Toma et al., 2001), fat (Zuk et al., 2001), muscle (Lee et al., 2000) and bone marrow (Pittenger et al., 1999). The maintenance of functional features of each specific tissue is the main adult stem cells’ role. Theoretically, these have the capability to produce a small number of differentiated cells related to the embryonic origin of their tissue, and their use is not restricted by ethical questions, since they can be directly isolated from the patient. The common feature of these cells is the ability to differentiate into lineages of mesenchymal tissues, such as bone, cartilage, fat and muscle (Pittenger et al., 1999).

In the literature, the potential use of different adult stem cells for IVD regeneration has already been reported (Richardson and Hoyland, 2008). Bone marrow mesenchymal stromal/stem cells (MSCs) (Sakai et al., 2003; Vadala et al., 2008b), adipose tissue-derived stem cells (ASCs) (Li et al., 2005), muscle-derived stem cells (MdSCs) (Vadala et al., 2008a), synovial stem cells (Miyamoto et al., 2011), Wharton’s jelly stem cells (Ruan et al., 2011) and olfactory stem cells (Murrell et al., 2009) have been studied for this purpose. The use of adult stem cells for IVD regeneration has great therapeutic potential, in fact they have been used for this purpose in different ways (Figure 2). They can be injected directly into the disc: (a) as undifferentiated or predifferentiated cells; (b) delivered with hydrogels/scaffolds; or (c) as genetically modified with genes of interest and injected into the IVD as an ex vivo gene therapy strategy. Recently, a population of progenitor cells has been recognized in the degenerated human IVD (Risbud et al., 2007); therefore (d) the activation of these endogenous progenitors by the administration of suitable drugs/growth factors might promote IVD repair; moreover (e) the recruitment of circulating stem cells by chemotaxis (homing) could be another interesting strategy, as it has been recently demonstrated in an ex vivo organ culture model by MSC migration into the NP via the end-plate route (Illien-Junger et al., 2012).

The ability of adult human MSCs to differentiate towards NP cells has been the first step to be evaluated in the development of this strategy. Risbud et al. (2004) showed that MSCs, cultured under chondrogenic conditions, differentiated towards NP cells, demonstrating that this culture condition can be used as a preconditioning strategy before MSCs implantation into the IVD.

The therapeutic effects of stem cells have been also widely evaluated. In vitro studies suggested that the regenerative potential of MSCs may be due to interactions with NP cells, resulting in the upregulation of ECM synthesis. Co-culturing MSCs and NP cells in a pellet system, Sobajima et al. (2008) demonstrated an in vitro synergistic effect between the two cell types in terms of increased proteoglycan synthesis. MSCs from different origin have been used to demonstrate the same evidence. Li et al. (2005) co-cultured rabbit ASCs with NP cells in three-dimensional (3D) alginate beads, demonstrating an increase in the expression of type II collagen and

Figure 2. Representation of possible stem cell-based approaches for disc regeneration

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aggre can genes compared to NP cells alone. Moreover, our group showed higher proteoglycan levels in MdSCs and NP co-culture compared to NP cells cultured alone (Vadala et al., 2008a).

Stem cells and the microenvironment are linked by a mutual relationship. In fact, the microenvironment plays a crucial role in the differentiation of stem cells (Pittenger et al., 1999) and stem cells contribute to tissue repair, creating an environment that promotes local regeneration. The authors’ group analysed the interaction between NP cells from degenerating IVD and MSCs in a 3D culture system (Figure 3). Individual MSC or NP cell populations have been isolated from their co-culture and analysed to evaluate changes in gene expression profile. Indeed, MSCs showed a more chondrogenic phenotype and, on the other hand, NP cells demonstrated higher type II collagen gene expression (Vadala et al., 2008b). Yamamoto et al. (2004) reported higher cell viability and proliferation of NP cells in a co-culture system, due to cell–cell contact with MSCs. The mechanisms of this interaction have also been studied through the ASC population, showing changes in gene expression profile more similar to those of NP-like cells when co-cultured with NP cells (Lu et al., 2007). Furthermore, the authors’ group studied the possible role of cell fusion in the interaction between NP cells and MSCs. Cell fusion is considered to be the potential mechanism for the plasticity and regeneration ability of adult stem cells (Terada et al., 2002). Using a pellet co-culture system aimed to promote cell–cell interactions, the authors demonstrated that cell fusion does not occur in the interaction between NP cells and MSCs (Vadala et al., 2008b).

Although IVD represents a hostile environment, due to its avascularity, low oxygen concentration and acid pH, MSCs engraftment and long-term survival has been reported in several studies. Sobajima et al. (2008) transplanted MSCs into the lumbar IVDs of healthy rabbits, demonstrating cell survival up to 6 months after inoculation.

However, it is crucial to test all novel stem cell-based approaches in animal models that mimic the human pathological condition, in order to determine the efficacy in prevention or delay of IDD progression. Sakai et al. (2003, 2006) demonstrated an increase of proteoglycan content, disc height and hydration in a NP aspiration model of IDD after the injection of autologous MSCs delivered with a collagen type II-based carrier. MSC-injected IVDs maintained higher MRI signal intensity at 6 months follow-up when compared to control discs (Sakai et al., 2006). Nevertheless, these authors demonstrated site-dependent differentiation of MSCs into NP cells after transplantation in degenerated rabbit IVDs (Sakai et al., 2005). Furthermore, experiments in larger animal models have been performed, confirming the regenerative effect of MSCs in degenerated discs (Henriksson et al., 2009; Hiyama et al., 2008a).

Recently, a Phase I clinical study has been performed with autologous bone marrow MSCs expanded ex vivo. Orozco et al. (2011) treated 10 human patients affected by BP with MSCs injection into NPs. The patients showed a rapid improvement of pain and disability and at 1 year follow-up water content was significantly elevated, although disc height was not recovered.

Although these data are promising for a widely clinical use of this strategy, many other issues should be addressed. Indeed, a potential side-effect of this approach has been recently described. MSC transplantation in an IDD rabbit model showed an undesired cell migration from the injected site, which resulted in osteophyte formation (Vadala et al., 2012b). These data indicate that novel cell carriers and surgical techniques, such as the alternative transpedicular approach to the disc (Vadala et al., 2013), need to be employed for an effective stem cell-based therapy.

3.3. Biomaterials-based tissue engineering

IVD regeneration approaches are based on three different components, signals, cells and biomaterials, that can be used independently or in combination. Current knowledge confirms that the best treatment strategy is based on the disc degeneration stage. While growth factors might be indicated in the early stages of degeneration to stimulate NP cells, in the advanced stages, when the functionality and stability of the spine is compromised, biological tissue engineering therapies for disc regeneration cannot be an option and fusion strategies or total disc replacement implants should be considered. The middle stages, which go from the early onset of IDD to the more severe stages, might be considered an indication for the use of cell- and biomaterial-based treatments.

Harvesting and cultivating NP cells is one of the biggest issues in NP regenerative therapy, since it is difficult to obtain an adequate number of cells that, typically, are low proliferating during cultivation (Yang and Li, 2009). Therefore, the opportunity to use MSC composites with biomaterials represents an interesting approach. Scaffolds or matrices are artificial 3D frame structures that mimic

Figure 3. Representative histological section of a 3D co-culture system of MSC (green) and NP cells (red) that allows the study of cell-to-cell interaction (Vadala et al., 2008b)
the ECM, allowing cellular adhesion, migration and proliferation and therefore hosting tissue regeneration. In addition, a scaffold might deliver and progressively release drugs with the aim of promoting homing and producing progenitor cells (Spadaccio et al., 2009). The ideal scaffold for tissue repair should respond to important criteria that include: (a) manufacturing feasibility; (b) mechanical properties, in order to allow short-term function without affecting long-term function of the tissue; (c) low toxicity of degradation products, in terms of both local tissue response and systemic response; and (d) drug delivery compatibility.

The wide majority of materials used in IVD regeneration are biodegradable polymers (Leung et al., 2011). However, the selection of the most suitable biodegradable polymer should be targeted to the envisioned therapeutic approach and to the specific features of the damaged tissue. Considering the NP matrix high water content, the most suitable compounds for its regeneration are hydrogels, which are a 3D network of hydrophilic polymers, able to retain a large amount of water without being dissolved. Hydrogels and other polymeric scaffolds can be subsequently classified into biopolymers and synthetic polymers. The former have been extensively used in IVD and NP repair strategies and include cells carriers such as type I collagen, hyaluronan, fibrin, calcium alginate, chitosan and collagen type II. They represent a temporary 3D matrix for cells that produce new ECM while the hydrogel is reabsorbed.

As an alternative approach, biopolymers have been associated with synthetic polymers to mimic the mechanical and structural properties of the IVD (Mauth et al., 2009; Mizuno et al., 2004; Sha’ban et al., 2008). Synthetic polymers are not widely used in NP cell therapy, due to their lower cell-seeding efficacy. However, biodegradable poly(glycolic-co-lactic acid) (PLGA) scaffolds have been demonstrated to support NP cells proliferation and GAG production, but these are less efficacious than a fibrin gel matrix (Sha’ban et al., 2008).

Many hydrogels have been used to test NP regeneration strategies in vitro. Alginate- and agarose-based hydrogels have been widely used to study NP cells in 3D culture systems (Baer et al., 2001; Chiba et al., 1997). They stimulate NP cells to express type II collagen and grow with a rounded, chondrocyte-like morphology, rather than the fibroblast-like morphology observed in monolayer cultures. However, the stability and mechanical integrity of alginate hydrogels tend to progressively decrease over time, due to the loss of ions through diffusion (Baer et al., 2001) or depletion by the encapsulated cells.

Type I collagen may represent a scaffold for many cellular constructs and it has been considered for AF repair in association with other polymers (Bowles et al., 2010; Vadala et al., 2012a). Atelocollagen hydrogel has been used to culture human NP cells and, in fact, when compared to the alginate one, it provides a more suitable structure for cell adhesion and proliferation. Moreover, it has often been associated with other natural or synthetic polymers to produce hybrid tissue scaffolds for IVD regeneration (Alini et al., 2003).

GAGs and hyaluronan have been widely used for IVD tissue-engineering applications. NP, AF and MSCs have been cultured in vitro in hyaluronan with growth factors (Crevensten et al., 2004; Nesti et al., 2008). Injectable hyaluronan hydrogels have been widely studied by several research groups with the aim of optimizing their features for cell delivery to the NP, in situ polymerization and producing an ideal cell microenvironment for ECM restoration, progenitor cells differentiation and tissue regeneration. Indeed, hyaluronan can act as TGFβ3 by influencing IVD cells towards the NP phenotype and increase ECM formation in vitro (Haberstroh et al., 2009). Halloran et al. (2008) developed a hydrogel based on hyaluronan and atelocollagen type II, demonstrating that the crosslinked hydrogel has great potential as an injectable carrier for NP treatment in degenerative IVDs. Recently, a cytocompatible hydrogel with thermoreversible properties, obtained by grafting poly(N-isopropylacrylamide) on a hyaluronan backbone (HA-pNIPAM), has been developed, with a significant potential use in IVD regeneration, as it provides easy injectability and a mild gelling mechanism (by a physical crosslink). Indeed, it has low viscosity at 20°C and rapid gelling at 37°C with no volume change upon gelling (Mortisen et al., 2010; Peroglio et al., 2011; Peroglio et al., 2012). Carboxymethylcellulose (CMC), a water-soluble derivative of cellulose, is an alternative option to hyaluronan, and photocrosslinked CMC hydrogels are currently used for NP cell encapsulation (Reza and Nicoll, 2010).

PolymERIC hydrogels have also been extensively tested in animal models. Ruan et al. (2010) injected NP cells, cultured on PLGA scaffold before implantation, in a dog model. They showed how PLGA constructs might prevent or delay the degenerative process of the IVD.

Similarly, acellular hyaluronan gels have been injected into IVDs of primate, pig and rat models after nucleotomy, demonstrating a decrease in the degeneration rate (Pfeiffer et al., 2003; Revell et al., 2007). Better results have been obtained through injections of hyaluronan gels associated with MSCs in a rat model (Crevensten et al., 2004). Many other gels have been tested in association with MSC in vivo models, such as atelocollagen, fibrin and hyaluronan–fibrin gels (Bertram et al., 2005), hyaluronan–chondroitin sulphate hydrogel (Zhang et al., 2011), modified alginate and chitosan (Leone et al., 2008).

However, hydrogels used for NP regeneration should be non- or anti-angiogenic in order to mimic its features. Recently, Silva-Correia et al. (2012) investigated the anti-angiogenic response of gellan gum-based hydrogels. They stated that it can be used as an NP substitute in cellular and acellular strategies for IDD treatment, due to its adequate mechanical properties and ability to support cell encapsulation.

The present knowledge about biomaterials for NP regeneration demonstrates the necessity of a 3D environment and biological recognition of the material surface for cell attachment, survival, proliferation and matrix production. Other important scaffold properties capable of influencing NP cell behaviour, such as mechanics,
permeability and degradation, have been partially studied (Masuda and Lotz, 2010). Indeed, even though several promising biomaterials have been developed, they still need to be rigorously tested on validated animal models in order to assess their properties in terms of injectability, gelling capability, biomechanics and tissue regeneration.

3.4. Annulus fibrosus rescue strategies

The AF surrounds the NP with layers of unidirectional collagen lamellae. Across successive lamellae, collagen fibres alternate between directions of approximately +30° and −30° with respect to the transverse plane, but they have both intra- and interlamellar variations in fibre angle (Holzapfel et al., 2005).

The AF is characterized by a limited intrinsic healing capacity that negatively affects the success rate of discectomy and NP-replacement therapies. It also decreases the potential of IVD-regenerative strategies. Therefore, attempts to preserve, repair, reinforce or regenerate the AF are necessary. So far, AF surgical repair approaches aim to stop the NP from leaking out of the AF, but they cannot maintain the biological AF structure in the long term or stop AF degeneration. On the other hand, tissue-engineering approaches aim to close the injured AF, stop it from degeneration and prevent disc herniation. Cell and gene therapies are not suitable as stand-alone therapies, but should be associated with scaffolds. However, in contrast to NP, hydrogels are not good candidate for AF regeneration, since they stimulate NP cell differentiation and biomechanical properties not suitable for this aim. Indeed, the AF has the function to sustain its mechanical requirement to withstand the stresses and strains of different loading directions (Chan and Gantenbein-Ritter, 2012).

Shao and Hunter (2007) demonstrated that AF cells grow in clusters and spread along alginate/chitosan-aligned fibres expressing aggrecan and collagen II. Several synthetic and natural polymers have been widely tested for AF tissue engineering. Poly-DL-lactide (PDLLA)/bioglass scaffolds have been investigated for future treatments of IVDs with damaged AF regions (Helen and Gough, 2008; Wilda and Gough, 2006). Furthermore, the biodegradable malic acid-based polymer poly(1,8)-octanediol malate (POM) has shown good biocompatibility in rats (Wan et al., 2007). The same group also created a biphasic scaffold to simulate the inner and outer AF (Wan et al., 2008).

Recently, silk has been proposed as a biomaterial to suture, or a tissue construct to close, AF lesions and disc herniations (Chang et al., 2007, 2010). Moreover, Park et al. (2012) created a silk lamellar ring, demonstrating how porcine AF cells can support the native shape of these tissue-engineered AFs. However, the highly orientated AF structure is a challenge for tissue engineering. This problem has been successfully overcome by electrospinning poly-L-ε-caprolactone (PCL). It has been shown that cells seeded in these scaffolds elongated and aligned to the direction of the fibres, as in native tissue (Nerurkar et al., 2009). Recently, Vadalà et al. (2012a) produced, by electrospinning, a bioactive material made of poly-L-lactide scaffold (PLLA) releasing TGF/b1. It has shown significantly increased GAG and collagen synthesis by the seeded AF cells in vitro.

Up to now, efforts for novel treatments have mainly been directed towards replacement or regeneration of the NP. The real challenge, however, might be the development of strategies that deal with the damaged AF, preferably in a combined approach with the NP (Bron et al., 2009).

4. Towards clinical application

Although many issues remain unsolved, clinical Phase I studies are starting worldwide in order to test IVD regenerative strategies. Ex vivo expanded autologous bone marrow MSCs have been tested (Orozco et al., 2011) and recombinant growth factors and PRP are under investigation, yielding promising expectations. However, further studies are needed in order to evaluate long-term effects on IVD biology and new tissue formation, nutrition and biomechanical function.

Indeed, the complexity of IVD anatomy and pathophysiology raises several issues that need to be overcome. Because the nutrition supply to degenerated IVDs is poor (Urban et al., 2004), there is theoretical concern over the additional nutritional demand arising from increased metabolism of boosted cells by growth factors and/or higher total cell numbers derived from cell transplantation, with or without hydrogels. Therefore, envisioning the clinical applications, it might be useful to evaluate the nutritional supply of NP through new emerging technologies, such as functional magnetic resonance imaging (MRI) (Zobel et al., 2012), able to measure the endplate permeability, or microelectrodes able to evaluate oxygen or nitrous oxide diffusion (Bartels et al., 1998). These novel diagnostic methods would be crucial in selecting patients that could benefit from the regenerative treatments.

Another key issue is the delivery method of growth factors, cells and biomaterials into the NP space. The easiest and safer way to access the NP is through the AF; however, this is a fundamental anatomical and physiological structure that needs to be intact, since all the intradiscal pressure is relayed to it. Moreover, many animal models of IDD are triggered by lesions of the AF that induce a slow and progressive degenerative cascade in the NP (Sobajima et al., 2005a). Recently, a retrospective clinical study, with a 10-year follow-up, demonstrated that a small needle puncture used to perform discography, a diagnostic procedure based on the injection of contrast agent into the NP, induced accelerated disc degeneration with eventual disc herniation on the same side as the injection (Carragee et al., 2009). Taking into account that high-viscosity hydrogels for IVD tissue engineering need to be delivered through large-diameter needles, the
resulting AF lesion might impair the regenerative strategies, leading to further degeneration or leakage of the biological material delivered. Therefore, novel delivery methods (Vadala et al., 2013) and AF regeneration strategies should be further considered.

The problem underlying all these unanswered questions is the lack of a valuable preclinical model to rigorously test novel approaches. Many models have been used to study both aetiopathogenesis and the development of therapeutic strategies. However, there are several problems in inferring data obtained in animals to humans: (a) many models are induced by an acute injury, which is very different from the chronic and slowly progressive IDD in humans; (b) young animals are often used to study IDD, due to their large availability and affordability; (c) different anatomical and biomechanical features among species may often lead to misinterpretation of results; (4) moreover, NP cell populations are not homogeneous among different species – indeed, NCs are still present in many adult animals and they may affect the natural course of IDD progression (Alini et al., 2008). Therefore, standardizing a reliable large animal model is paramount in studying IVD regenerative strategies and it is supposed to be one of the most important objectives of the research community in this field.

The clarification of all these issues will lead to a more effective and safer clinical translation of these strategies as therapeutic tools that future spine physicians might choose to treat, prevent or delay IDD.

5. Conclusion

In the past few years, our understanding on IDD pathology has grown rapidly, due to increased knowledge of IVD biology and biochemistry. Although many mechanisms are still unclear, some key factors involved in this chronic process have been outlined, and it is clear that the cell loss and imbalance between anabolism and catabolism of ECM are the main pathological events.

This supports our effort on developing novel treatments targeted to regenerate the IVD. Patients with mild or moderate grades of IDD, in whom structural integrity is still preserved, could be the ones to benefit from molecular and stem cell-based therapies.

Recombinant human growth factors have gained more popularity in the treatment of musculoskeletal disorders since the recent successful application for bone regeneration. Animal studies have stated proof of efficacy for IVD regeneration and pilot clinical studies are ongoing. PRP is also widely used in the clinic to treat cartilage and tendon disorders, with promising results that are still under evaluation.

Gene therapy has received considerable interest over the past decade, considering its unique ability to provide sustained delivery of therapeutic agents and its potential use in IDD. Several studies have proved the feasibility of gene therapy in IVD. In addition, advances in the production of more satisfactory viral vectors make this new approach a powerful tool in future treatment for IVD regeneration.

The efficacy of MSCs transplantation in reproducible animal models and the feasibility of this technique have been widely proved. Indeed, the significant ability of MSCs to differentiate towards several cell lineages and secrete trophic cytokines, and recent clinical evidences, make stem cells therapy a real future treatment for IDD. The choice of the best progenitor cell type is a crucial factor for a successful outcome, as well as the more appropriate stage of NP damage. Further in vitro and in vivo studies should be carried out to better characterize the differentiation pathway, determine the effective cell dose, and find the best carrier and delivery method.

However, a cell carrier is paramount to effectively deliver progenitor cells into the NP, protecting them from the harsh environment of the disc, promoting engraftment, preventing cell leakage by in situ polymerization properties and restoring mechanical properties while the regeneration process takes place. New biomaterials are being developed with such properties, making them ideal for this purpose. However, their efficacy and safety need to be carefully proved on a validated animal model before clinical translation.

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