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Review

Intervertebral disc biology, degeneration and novel tissue engineering and regenerative medicine therapies

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Summary. Degeneration of the intervertebral disc (IVD) is a major cause of low back pain affecting a large percentage of the population at some point in their lives. Consequently IVD degeneration and its associated low back pain has a huge socio-economic impact and places a burden on health services world-wide. Current treatments remove the symptoms without treating the underlying problem and can result in reoccurrence in the same or adjacent discs. Tissue engineering offers hope that new therapies can be developed which can regenerate the IVD. Combined with this, development of novel biomaterials and an increased understanding of mesenchymal stem cell and IVD cell biology mean that tissue engineering of the IVD may soon become a reality. However for any regenerative medicine approach to be successful there must first be an understanding of the biology of the tissue and the pathophysiology of the disease process. This review covers these key areas and gives an overview of the recent developments in the fields of biomaterials, cell biology and tissue engineering of the IVD.

Key words: Intervertebral disc, Nucleus pulposus, Mesenchymal stem cell, Tissue engineering, Regenerative medicine

Introduction

Low back pain (LBP) affects between 60% and 80% of the population at some point in their lives (McFarlane et al., 1999; Sive et al., 2002), making it one of the most important public health issue for westernised societies today. It accounts for approximately 15% of all sickness leave (Freemont et al., 2002) and costs the UK alone over £12 billion in lost production, disability benefits, medical and insurance costs each year (Maniadakis and Gray, 2000). Until recently very little has been known about the pathogenesis of LBP and while the causes are thought to be multifactorial, research on intervertebral discs (IVDs) from patients with LBP has shown signs of degeneration in almost every case (Freemont et al., 2002).

Degeneration of the IVD impacts not only on the disc, but also on the surrounding tissues such as the muscles and ligaments and affects the spines ability to cope with the physiologically normal loads it experiences during a daily routine. As well as accelerating degeneration these changes also cause pain and reduce the mobility of the spine.

Current treatments for IVD degeneration are usually either symptomatic medical treatments or invasive surgical methods such as discectomy or spinal fusion. As the global population ages the incidence of IVD degeneration and LBP will increase, therefore new treatments are needed which restore full disc function and normalise disc cell biology. This review will discuss the novel therapies currently being developed to achieve these objectives.

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Normal IVD morphology

The intervertebral disc comprises 3 morphologically distinct regions:

1. The end-plates cover the cortical bone surfaces of the superior and inferior vertebral bodies. The tissues are cartilaginous, resembling articular cartilage and experiments have shown that they are the weakest part of the disc (Holmes et al., 1993).

2. The nucleus pulposus (NP) of the IVD lies between the adjacent end-plates and forms the hydrogel-like core of the disc. The primary component of the NP are the proteoglycans (PGs), in particular that of aggrecan. The high level of hydrophilic PGs serves to hydrate the tissue and produces its gel-like consistency.

Within this matrix are also randomly organised type II collagen and elastin fibres, although they are neither as organised or as mechanically stable as those found in the other regions of the disc.

3. The annulus fibrosus surrounds the NP and comprises between 15 to 25 concentric rings (lamella) of highly organised collagen fibres. The predominant collagen is type I, although types II and III are also present. The fibres are orientated approximately 60° to the vertical axis and run parallel within each lamella, but alternate between adjacent lamellae. Elastin fibres and PGs are found between each lamella (Yu, 2002) and these may aid in flexion/extension of the disc during movement.

The IVD is a relatively acellular environment, with the NP and AF having approximately 5000 cells/mm^3 and 9000 cells/mm^3 respectively (Maroudas et al., 1975). The cells within the NP have a rounded morphology and are phenotypically similar to articular chondrocytes (Sive et al., 2002) while cells of the AF are morphologically and phenotypically fibroblastic, being thin, elongated and aligned with the collagen fibres. Our work has shown that the phenotype and morphology of both cell types is interchangeable, dependent on culture conditions. NP cells in monolayer become fibroblastic, decrease type II collagen production and increase type I collagen production. Conversely, AF cells cultured in alginate increase production of type II collagen and the chondrocyte differentiation factor SOX-9 (Le Maitre et al., 2005).

Normal IVD biochemistry

Along with collagen types I and II and aggrecan, the extra-cellular matrix (ECM) of the disc contains many other collagens, in particular types III, V, VI, IX and XI; proteoglycans, such as biglycan, decorin and fibromodulin; and glycoproteins such as fibronectin. While the role of many of these molecules has not yet been fully investigated the biomechanical and functional characteristics of the IVD are dependant on the coherent interactions of the matrix molecules and the maintenance of a stable ECM.

The cells of the adult disc are constantly involved in both the breakdown of the ECM component molecules and the formation of new matrix in a process that is exquisitely controlled to ensure an even balance between matrix degradation and new matrix formation.

Proteinases such as members of the matrix metalloproteinase (MMP) family have been implicated in the breakdown of matrix molecules and these enzymes have the ability to cleave collagens (MMPs 1, 8 and 13), gelatins (MMPs 2 and 9) and other matrix macromolecules (MMPs 3 and 7). The high aggrecan content of the disc (50% and 20% of wet weight in the NP and AF respectively) has led investigators to study the aggrecanolytic members of the ADAMTS family, including ADAMTS-1, 4, 5, 8, 9 and 15. As a result of these investigations aggrecanolytic activity has been identified in the IVD during degeneration (Le Maitre et al., 2004; Pockert et al., 2006) and these enzymes are suggested to be critically involved in proteoglycan turnover in the disc.

The regulation of MMP and ADAMTS production (and hence matrix degradation) and matrix macromolecule production is achieved primarily by a number of cytokines and growth factors. Of particular importance in IVD matrix homeostasis are the members of the IL-1 family (catabolism) and TGF-ß (anabolism) superfamily, which will be discussed later.

Degeneration of the IVD

While degeneration affects all areas of the IVD, the most constant and noticeable changes occur in the NP. In early degeneration there is disruption of the matrix, in particular increased degradation of both aggrecan and collagens, in particular denaturation of the fibrillar collagens such as type II. While the ability of the cells to produce aggrecan and collagen is not affected there is a change in the production of small PGs and a change in the type and distribution of collagen produced.

As degeneration progresses there is increased matrix degradation and the formation of ‘slit-like’ spaces that permeate through the NP and into the AF. The collagen fibre arrays of the AF are disrupted and both collagen and elastin networks become more disorganised. There is often also traumatic damage to the end-plate and the invasion of blood vessels and accompanying nerves into the inner AF and, by late-stage degeneration, the NP.

The number of viable cells in early degeneration decreases, although as the disease progresses there is an increase in lacunae containing cell clusters and this may result in an overall increase in number of cells. An increasing body of literature has demonstrated some form of programmed cell death (apoptosis) within the degenerate IVD and as a recent review by Zhao et al. (2006) demonstrates, this cell death may be responsible for a number of the features of IVD degeneration and involved in disease progression. Additionally degenerate NP and AF cells also exhibit signs of cellular senescence, which affects cellular function.

In addition, cell physiology also changes, as evidenced by an increase in MMP and ADAMTS production (Le Maitre et al., 2004; Pockert et al., 2006). The cells also increase synthesis of catabolic cytokines and are also more susceptible to these molecules. In particular, our work has shown that levels of IL-1 increase in degeneration over normal, but that degenerate discs show no concomitant increase in its antagonist IL-1Ra (Le Maitre et al., 2005, 2006). The catabolic effects of IL-1 have also been shown to be accentuated in degenerate disc cells *in vitro* (Le Maitre et al., 2005).

Altered disc nutrition as a factor in degeneration

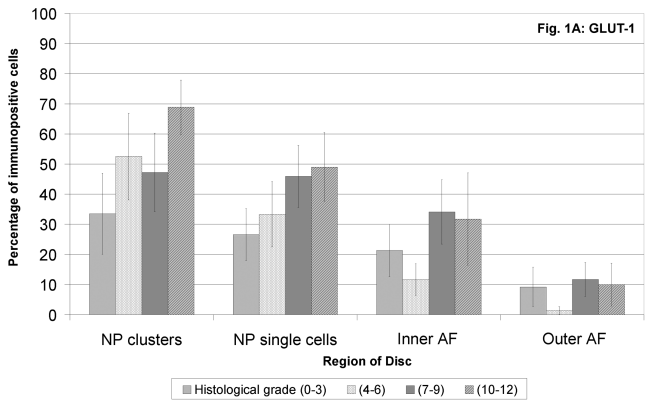
The IVD is a large, avascular environment and cells

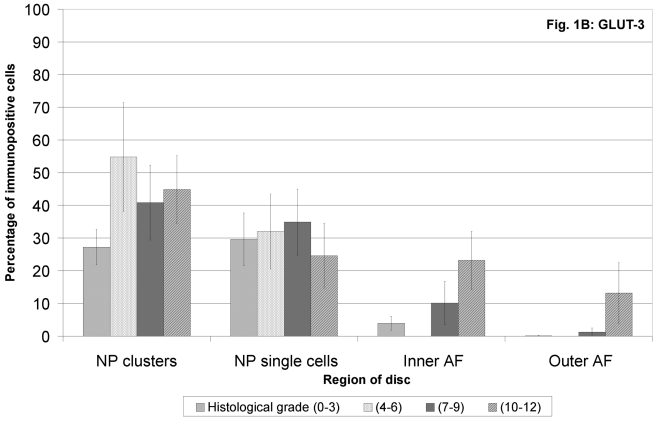
rely on diffusion of nutrients such as glucose and oxygen from capillaries adjacent to the cartilaginous end-plates. Disc cells cultured *in vitro* are sensitive to both oxygen levels and pH, both of which are affected during degeneration. As either oxygen tension or pH falls, ECM synthesis rates and cell viability decrease and this mirrors changes seen in degenerate discs. Research has also shown that degeneration significantly decreases the discs ability to transport nutrients and solutes. NP cells cultured *in vitro* with 1% oxygen showed significantly lower levels of PG production than did cells cultured in an oxygen concentration of 5% and this correlated with an increase in lactic acid production (Ishihara and Urban, 1999). The failure of the nutrient supply to the disc has been proposed as a cause of disc degeneration and whether this failure is the cause of, or an effect of, degeneration the evidence points to nutrient supply as a key regulator in the progression of disc degeneration.

As a result of this, combined with the ability of NP and AF cells to survive within the harsh physiochemical environment of the IVD has lead to groups, ours included, investigating the molecular mechanisms involved. We have recently demonstrated that NP cells (Fig. 1), like articular chondrocytes, express a range of glucose transporters (GLUTs), a number of which are hypoxia-regulated (Richardson et al., 2003; Mobasheri et al., 2005). Furthermore, we have shown that, in addition to differences in GLUT protein expression in different regions of the disc, there are also changes with degeneration. It is therefore possible that a change in GLUT expression may play a role in the alteration of disc nutrition in degeneration (Fig. 1).

The need for novel therapies

While new surgical treatments for IVD degeneration are evolving, spinal fusion is still one of the most common procedures for treatment of end-stage degeneration, accounting for over 200,000 procedures in the United States per annum (Boden, 2002). While the efficacy of autograft material has been demonstrated, the harvesting of this material has been associated with a number of complications and in many cases the amount of material available is insufficient (Summers and Eisenstein, 1989; Kurz et al., 1989; Murata et al., 2002). As with many of the other surgical interventions they remove the symptoms of degeneration, rather than treating the route cause of the problem and in most cases the procedure results in a loss of movement or function at that disc level and can have deleterious effects on adjacent discs. This is evidenced by an increase in degenerate phenotypes in adjacent discs, both above and





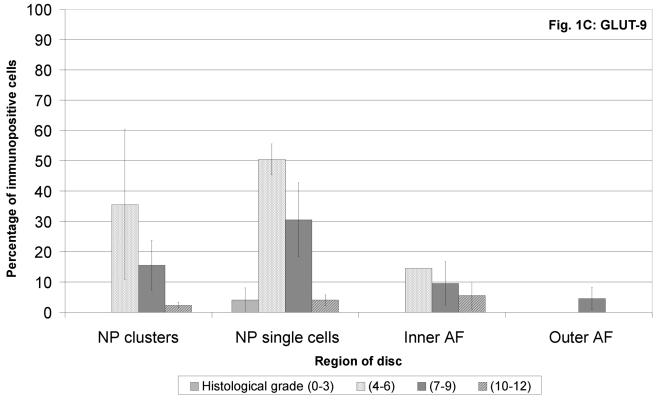


Fig. 1. Results of immunohistochemical studies on GLUT-1, 3 and 9 expression in human intervertebral disc. **A-C** demonstrate the percentage of cells which were immunopositive for GLUT-1 **(A)**, GLUT-3 **(B)** and GLUT-9 **(C)** protein in each region of the IVD.

below the operated level, following many types of spinal surgery.

Alongside the current conservative and surgical management approaches, a range of NP replacement devices have been developed. The aim of these devices is to restore normal disc height and load distribution, as well as to limit degenerate changes in adjacent discs. This is achieved in a variety of ways, usually involving the implantation or injection of a biocompatible, biomechanically stable, non-biodegradable hydgrogel encased within a polymer mesh or jacket. These devices are currently in varying stages of clinical or pre-clinical testing, with Prosthetic Disc Nucleus (PDN; Raymedica Inc., Bloomington, MN, USA) seemingly the most advanced. PDN comprises a hydrogel pellet encased in a polyethylene jacket, where the hydrophilic (polyacrylamide) component of the central hydrogel attracts up to 80% of its weight in water and swells, while the low elasticity of the polyethylene jacket restrains this swelling pressure. Since 1996 a number of versions of PDN have been implanted in just over 400 patients with varying degrees of success. The main complications with this, and other similar devices, are device migration, extrusion and failure (Di Martino et al., 2005).

There is obvious clinical interest in these devices, however they remain a minor component of the current surgical caseload and there is currently no long-term clinical follow-up. As with many surgical implants they are likely to have only a limited life-span in vivo before either migration or failure results in revision operations.

These current clinical limitations, combined with a rapid increase in the understanding of the cellular and matrix biology of both IVD development and degeneration, has led many researchers to investigate novel, cell-based strategies for IVD regeneration. As the fields of gene therapy and tissue engineering advance it is becoming more feasible to develop strategies for treatment of IVD degeneration that will restore full disc function and protect adjacent discs over many years, rather than just treat the symptoms of degeneration.

The NP, with its single cell population and simple matrix structure, means tissue engineering a new NP should theoretically be a relatively simple task. However, as the remainder of this review will discuss, there are numerous obstacles to overcome, particularly the choice of cells and the choice of cell carrier for implantation.

The problem of cells

The requirements of any cell used in tissue engineering are its ability to survive within the graft site and produce a suitable and functional matrix which mimics, or even improves on the original tissue. Autologous chondrocyte implantation (ACI) has been shown to be effective in the treatment of osteoarthritic cartilage lesions, producing a type II collagen and proteoglycan-rich matrix which restores function to the joint (Gillogly et al., 1998). Similarly, implantation of chondrocytes within a 3-dimensional carrier system such as a collagen gel has also been shown to produce a cartilaginous matrix which could be of clinical use (Gavenis et al., 2006; Sakai et al., 2006). Meisel and coworkers (2006) recently demonstrated that autologous cultured disc-derived chondrocytes reimplanted into the dogs from which they were sampled survived, integrated and produced a cartilaginous extra-cellular matrix, albeit not of morphtypic NP matrix. This suggested that cell implantation was feasible and a current clinical trial is aiming to elucidate the effectiveness of human disc cell reimplantation. However, investigations by our group and others into the cellular and pathophysiological changes which occur during IVD degeneration have lead to the suggestion that autologous NP cells may not be the ideal choice for engineering of a new disc. Perhaps most significantly we have shown that cells from the degenerate disc show a senescent phenotype with an altered cellular phenotype (Le Maitre et al., 2007). Senescent cells show a decreased rate of matrix synthesis and an increase in the production of both MMPs and aggrecanases, which may exacerbate the problem. As with any tissue engineering strategy a large number of cells is required and these cells need to be able to produce a functional matrix. Even during *in vitro* culture senescent NP cells have both a reduced proliferation rate and a reduced lifespan, suggesting that there would not be a significant increase in cell number for reimplantation. After even short periods in culture these cells also failed to show a redifferentiated phenotype following culture in alginate beads (a standard method for redifferentiating chondrocyte-like cells). In addition to this work we have shown decreased responses of degenerate NP cells to growth factors such as TGF-ß, when compared to normal NP cells as well as increased susceptibility to catabolic cytokines, such as IL-1 (Le Maitre et al., 2005). In addition to the effects of senescence on cellular proliferation, Gruber and coworkers (2006) recently showed that patient demographics can have a significant impact on the ability of NP cells to proliferate, with age and familial history being major influences in proliferative capacity.

One alternative to overcome the problems associated with degenerate NP cells is to use normal NP cells and these have been shown to produce an NP-like matrix (comprising of high levels of proteoglycans and type II collagen) in tissue engineering systems. However, when considering normal NP cells as a potential source of cells, the major problem is that of acquisition. To isolate normal cells would require the removal of tissue from a normal disc level and this has been suggested to lead to the induction of degenerate changes at that disc level and would therefore cause as many problems as it alleviates. The use of allogeneic cells also has its limitations, since there is always the risk of an allergic response and tissue rejection. While the normal IVD is avascular and aneural, degenerate IVD show both neovascularisation and innervation, which means that the degenerate disc

could be susceptible to immune rejection of implanted allogeneic cells.

As a result of these problems many groups, including our own, are now concentrating on the use of either notochordal cells or bone marrow-derived mesenchymal stromal stem cells (MSCs). Auguiar et al. (1999) showed that notochordal cells and NP cells interact during in vitro co-culture to cause an increase in proteoglycan synthesis. Although in species where notochordal cells persist throughout life there is no evidence for disc degeneration, in humans notochordal cells do not usually persist past the age of 10 years (Trout et al., 1982), which removes the possibility of their use for treatment of adult human IVD degeneration.

Therefore MSCs offer the best potential as a cell source as these cells can be isolated without major surgery, purified simply and expanded rapidly in culture. Studies have demonstrated that MSCs can differentiate down a number of lineages, including the chondrogenic cells such as those found within the NP of the disc. We used a novel co-culture system of normal human NP cells and normal human MSCs both with and without cell-cell contact to investigate the potential differentiation of MSCs into NP-like cells (Richardson et al., 2006a). This study demonstrated that co-culture with cell-cell contact produces MSCs with an NP-like phenotype as demonstrated by increased expression of aggrecan and type II collagen genes, amongst others. What is not clear at present in whether implantation of normal human MSCs into a degenerate IVD would alter the differentiation of the MSCs and we are currently conducting co-culture experiments to elucidate any effects degenerate NP cells might have on MSC differentiation. The fact that autologous MSCs are currently in clinical trials for the treatment of a variety of pathological conditions suggests that these cells may be the optimal choice for tissue engineering of the IVD.

Development of tissue engineering strategies

As NP cells and articular chondrocytes share some common phenotypic markers, in particular the transcription factor SOX-9 and the matrix molecules aggrecan and type II collagen (Sive et al., 2002), lessons can be learnt from the more established field of cartilage tissue engineering. As with NP cells, chondrocytes cultured in monolayer revert to a more undifferentiated state. Their morphology becomes more spindle-shaped and fibroblastic and their phenotype changes from expression of type II collagen to types I and III and they decrease the expression of SOX-9 (Mayne et al., 1976; Benya et al., 1977, 1978). For many years researchers have overcome this problem by culturing chondrocytes in micromass/pellet systems (Kato et al., 1988; Farquharson and Whitehead, 1995) or encapsulated in semi-solid, 3-dimensional matrices such as sodium alginate, which maintain chondrocyte morphology and phenotype (Benya and Schaffer, 1982; Guo et al., 1989). Ma et al. (2003) demonstrated that human MSCs, seeded in low-viscosity sodium alginate differentiate into chondrocyte-like cells and that this differentiation was improved by addition of TGF-ß1 and dexamethasone. Our work has also shown that MSCs cultured in alginate differentiate into chondrocytic cells that express a matrix similar to that of monolayer-cultured NP cells encapsulated in alginate and that this differentiaiton is improved by addition of recombinant TGF-ß1 and dexamethaxone.

Many of the gels used for retention of cell phenotype *in vitro*, such as alginate, are not capable of withstanding the loads experienced within the IVD and therefore are not suitable for tissue engineering. However, in many instances an injectable hydrogel is the ideal choice for NP replacement. The main reason for this is that it allows implantation of the cell/carrier without disrupting the annular collagen lamellae and this has lead to investigation of a wide range of hydrogels. In addition to a gels ability to withstand load and allow the embedded cells to persist and maintain a differentiated phenotype, they must have a sol-gel transition mechanism that is suitable for clinical application. The sol-gel transition is the point at which a liquid becomes a gel. This is important as for both ease of cell dispersion and ease of injection the hydrogel should be in a liquid form and be capable of gelling rapidly in vivo post injection. A number of natural systems have been developed for this, most notably type I collagen gels and chitosan glycerophosphate (chitosan-GP), along with a number of synthetic polymer-based hydrogel systems, mainly involving polyethylene glycol (PEG).

Chitosan is a biocompatible, biodegradable polymer with a similar structure to glycosaminoglycans, that is derived from chitin, found in the exoskeleton of arthropods. Purified chitin is deacetylated to form chitosan and the extent of this deacetylation, combined with the method of fabrication allow the chemical and physical properties of the resulting scaffold to be controlled. It has been used in tissue engineering applications for a number of tissues, including skin, liver, bone and cartilage and for the delivery of proteins (such as growth factors) in a controlled manner (Zielinski and Aebischer, 1994; Ruel-Gariepy et al., 2000; Suh and Matthew, 2000; Nettles et al., 2002; Lai et al., 2003; Hoemann et al., 2005). Chitosan has pH-dependant solubility, being insoluble at a pH over 7 and soluble below ~ pH 5. While chitosan has many characteristics that make it suitable for use in treating degenerate IVDs, it is limited by the pH-dependant nature of its gelling since a decrease in pH is associated with disc degeneration. To overcome this problem, Chenite et al. (2000) developed a pH-dependant, thermally sensitive hydrogel by combining chitosan with a polyol salt (glycerophosphate). The addition of a phosphate salt means that at room temperature in a physiological pH the solution remains as a liquid, but when heated to body-temperature the solution turns into a gel. This method overcomes the pH problem of chitosan and allows cells to be mixed with liquid

chitosan, then injected into the affected disc where it will gel. This concept was recently developed further by Roughley and coworkers (2006), who successfully cultured NP cells with chitosan-based thermosensitive hydrogels and produced an NP-like matrix rich in proteoglycans. Our work using chitosan-GP has found that, by altering percentage of chitosan and glycerophosphate molarity, gelling characteristics can be altered without affecting cell viability. MSCs cultured in chitosan-GP for 4 weeks maintained around 80% viability and showed a rounded, chondrocytic morphology, expressing aggrecan, type II collagen and SOX-9 but no osteogenic genes (Fig. 2).

While natural hydrogels, such as alginate and chitosan, have the ability to maintain a differentiated phenotype in embedded MSCs or NP cells, they have problems with variability of production, mechanical properties and degradation rates. Synthetic hydrogels, however, can be formed with high reproducibility and allow intimate control of structure and degradation rates. These properties mean that a hydrogel can be formed to match the gelatinous nature of NP tissue. Recent studies have used poly vinyl alcohol (PVA; Allen et al., 2004) and PVA/polyvinyl pyrrolidone (PVA/PVP; Thomas et al., 2004) hydrogel implants to replace the NP and have shown that they can survive for upto 24 months without systemic toxicity. These non-biodegradable hydrogels have a relatively high water content (around 55% for PVA) and allow diffusion of nutrients from the cartilaginous end-plates of the disc (Allen et al., 2004), however they are designed as long-term NP replacements rather than tissue regeneration strategies. To achieve regeneration the majority of the work has focussed on the use of poly(ethylene glycol) (PEG) hydrogels and its copolymers for the treatment of cartilage defects (Sawhney et al., 1993; Metters et al., 1999; Bryant and Anseth., 2003; Williams et al., 2003; Bryant et al., 2004a,b), however to date there is no data on their use in degenerate IVD treatment.

Alongside the work on hydrogels a number of rigid natural and synthetic scaffolds have been proposed for later stage degeneration when initial restoration of mechanical stability is essential. Most commonly used are poly-lactic acid (PLA), poly-glycolic acid (PGA) and their co-polymer poly(lactic-*co*-glycolic) acid (PLGA) which have been used for a number of tissue engineering applications in a number of forms, including fibre meshes and foams, and the choice of polymer depends on the requirements of the tissue (Vacanti et al.,1988; Freed et al., 1993, 1994; Mikos et al., 1993; Maurus and Kaeding, 2004; Wang et al., 2005; Li et al., 2006). Other polymers, such as poly-caprolactone (PCL), polyethylene glycol (PEG) and hyaluronan-based biomaterials are also being considered. Our group has used poly-l-lactic acid (PLLA) scaffolds, seeded with untransfected or SOX-9 transfected human MSCs, and shown NP-like differentiation after 2 and 4 weeks in culture with a specialised differentiating medium (Richardson et al., 2006b). While cell adhesion to these scaffolds is lower than corresponding PGA-based scaffolds they offer more mechanical stability and slower degradation rates, meaning they will persist and support the disc while a stable matrix is formed by the embedded cells.

Mizuno et al. (2004) recently produced a composite IVD using an alginate-based nucleus encapsulated by a fibrous PLA/PGA AF. When AF cells were seeded onto the cage and NP cells into the alginate and cultured for 12 weeks they showed morphological, histological and biochemical similarities to native disc tissue, with

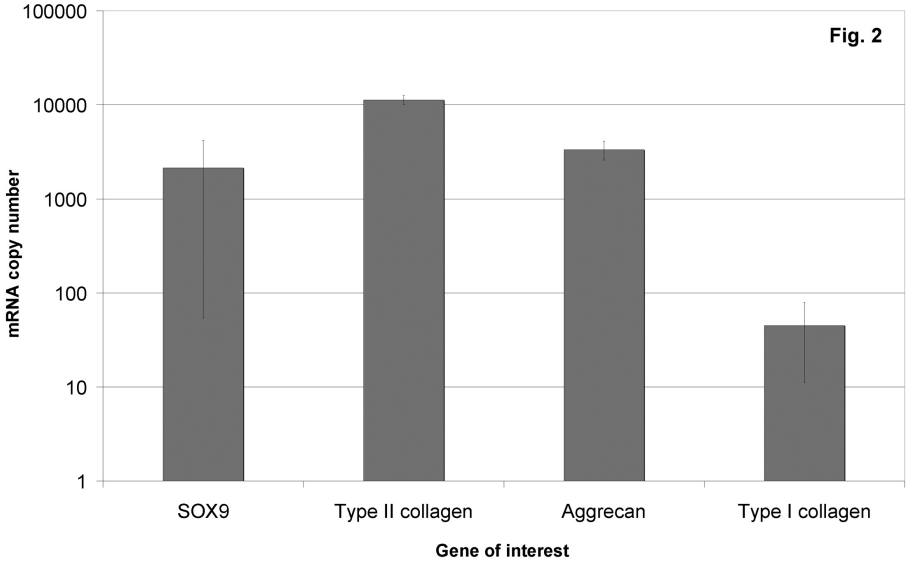


Fig. 2. Quantitative real-time PCR for NP marker genes SOX-9, type II collagen and aggrecan, and the fibroblastic marker gene type I collagen, in human MSCs cultured for 4 weeks in chitosan/glycerophosphate. Expression was normalised to 18s and copy number was calculated using a genomic DNA standard curve.

regional differences in collagen type between alginate (NP) and PLA/PGA (AF) zones. Similar results have also been shown by Caterson et al. (2001), who cultured MSCs in an alginate/PLLA amalgacaffolds with collagen (Ma et al., 2005), gelatin (Cui et al., 2003a), and chitosan (Cui et al., 2003b), although these studies have used chondrocytes rather than MSCs.

Other factors involved in development of a successful strategy

Whether a rigid scaffold or a hydrogel is used for tissue engineering, ensuring even cell distribution and high cell viability is essential. While cell distribution within a hydrogel in its liquid phase is relatively straight-forward, scaffolds present a more difficult challenge. While most scaffolds have an open framework and high pore interconnectivity, the seeding method adopted will impact on the number of cells reaching the centre of the scaffold. Static seeding is the easiest method; however cells will preferentially adhere to the scaffold surface. Therefore various groups have suggested alternate seeding strategies, either by agitating both cells and scaffold in an open system such as on an orbital shaker or within a rotating bioreactor, or by forcing cells and media through the scaffold in a closed bioreactor system. These systems show higher cell numbers on and within scaffolds and have also been demonstrated to improve matrix synthesis and deposition of cartilaginous matrices when compared to static systems.

The media composition is also key to ensuring, PDGF-BB, BMP-7 and CDMP, have been shown to aid chondrogenesis of MSCs or improve maintenance of NP phenotype in a variety of systems, as has reduction of oxygen tension. As the IVD is normally avascular, NP cells exist in a hypoxic environment and studies have shown that both NP cells and MSCs maintain a more NP-like phenotype under hypoxic conditions. Similarly the IVD is exposed to a constantly changing mechanical stresses, in particular load and studies have shown that cyclical compression is also important for both retention of NP phenotype and induction of a chondrocyte-like phenotype in MSCs.

Conclusions and potential application to IVD degeneration

What is clear from the current literature is that, while there has been a sharp rise in the knowledge of IVD development, pathophysiology, MSC biology and tissue engineering, regeneration of even such a seemingly simple structure as the NP is a complex process. Compared to other systems, tissue engineering of the IVD is still in its infancy, however the increasing interest suggests that a cell-based strategy for repair of the degenerate IVD will be realistic possibility in the future. Much work is still required in development of better hydrogels and scaffolds, more refined culture systems and strategies for injection or implantation that do not disrupt the surrounding tissues. It is also clear that a clinical perspective is needed to keep focus on an output and to define a streamlined process for cell isolation, expansion, possible predifferentiation and final implantation that has both practical clinical, as well as scientific merits.

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