REVIEW

Strategies for regeneration of the intervertebral disc

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Low back pain resulting from degenerative disc disease is the most common cause of disability in the UK. Current low back pain treatments are aimed at either treating the symptoms of pain, or removing the source of pain itself, but do not address the biological basis of the disease. Our increasing understanding of the molecular biological basis for degenerative disc disease has enabled the development of strategies aimed at tackling the causes of degeneration. Here we review the progress that has been made in strategies using cells, biomaterials and growth factors aimed at regenerating the human intervertebral disc.

The socioeconomic impact of low back pain (LBP), as revealed by modern metrics, has increased its recognition as one of the leading causes of discomfort and disability in society today. It is estimated that two-thirds of adults suffer from LBP at some time [1], with point prevalence ranging from 12 to 35% [2] and an estimated 23 million episodes per year in the UK [3]. Whilst most sufferers spontaneously remit, it is estimated that 10% go on to develop chronic LBP. The economic costs imposed by LBP are staggering and the majority of medical costs arise from this minority of patients, who through failure of modern medicine to treat symptoms appropriately and relieve pain, become chronic users of the healthcare system. The direct cost to the NHS was estimated in 1991 to be £500 million annually [4], and these costs are compounded by disability benefits, insurance and work loss, resulting in a total economic burden of £12 billion per year [5].

The causes of LBP are undoubtedly multifactorial. However, the majority of cases involve the intervertebral disc (IVD) and include conditions such as disc herniation, myelopathy and spinal stenosis. Interestingly, imaging studies [6] and clinical studies with discography [7,8] have shown that when the IVD is examined, it shows features of IVD degeneration, and this is now thought to be one of the many causes of chronic LBP. In fact IVD degeneration is recognized as a cause of LBP in at least 40% of patients [6].

The normal IVD

The human spine contains 24 IVDs, which separate the bony vertebrae and lie in front of the spinal canal. The spine has two major functions: it is a loading structure that provides attachment for internal organs, supports the upper body and external loads, and transmits load from the upper to lower extremity; while permitting movement in all directions. Hence, the IVD is necessarily unique in its design in order to cope with these functional demands.

The IVD is composed of three distinct regions; the tough, collagenous annulus fibrosus (AF) surrounding the highly hydrated, gelatinous nucleus pulposus (NP), between two cartilage end-plates (CEPs). The AF consists of concentric rings of parallel collagen fibers, which insert into adjacent vertebral bodies. The collagen fibers run at 60° to the vertical with their orientation alternating in criss-cross fashion in adjacent lamellae, and each IVD consists of 10–25 lamellae [9]. This arrangement strongly unites the vertebral bodies and is suited to resisting tensile forces generated by twisting and bending. The NP is highly hydrated, composed predominantly of proteoglycans (PGs), and is more randomly organized and less mechanically stable than the AF. The boundary between the AF and NP is indistinct and known as the transition zone. CEPs cover opposing vertebral bodies and are predominantly composed of hyaline cartilage.

Extracellular matrix

The IVD extracellular matrix (ECM) is predominantly composed of PGs and collagens, and there are distinct differences in the ECM composition between the AF and NP. PGs are most abundant in the NP, where they are up to 27-times more abundant than collagens [10]. PG is hydrophilic with a high osmotic potential, which draws large amounts of water into the disc, causing expansion of the NP ECM and

Keywords: intervertebral disc, mesenchymal stem cell, nucleus pulposus, regenerative medicine, tissue engineering

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giving its classic 'hydrogel-like' properties. Several PGs are present, including the large aggregating PGs aggrecan and veriscan, which hydrate the IVD, and smaller nonaggregating PGs decorin, biglycan, fibromodulin, lumican and perglycan, which have a diverse and incompletely understood range of functions [11].

Aggrecan is the predominant PG within the IVD, although versican is also present but in lesser amounts than aggrecan and has a lower ability to bind glycosaminoglycan (GAG); its primary function is in cellular signaling and not NP hydration [12]. PG reversibly deforms under stress to give the IVD compressive strength [13], and there is a direct relationship between the number of GAG molecules bound to the core protein and the amount of compression that the IVD can withstand [14]. The large size, high number of GAG chains and the high concentration of PGs in the matrix all contribute to its compression-resistant properties. The balance of this complex structure is affected by any change in levels of synthesis or catabolism, exposing the IVD to greater risk of functional failure and degeneration.

Collagen forms an integral part of all connective tissue ECM; in the IVD, it accounts for 80% of the dry weight of the AF and 20% of the NP. Collagen types I and II account for 80% of total IVD collagen [15], and the ratio of type I to type II collagen is inverted, with the AF being mainly type I and the NP mainly type II. The remaining 20% of IVD collagen is made up by the minor collagens III, V, VI, IX and XI, which have a wide range of functions and are recognized as important IVD ECM components.

Cells

The NP forms from the embryonic notochord and is initially populated by notochordal cells, while mesenchyme forms the remaining tissues of the spinal column [16]. In humans after 4 years, notochordal cells are replaced by cells that morphologically and phenotypically resemble chondrocytes [17]. NP cells are phenotypically distinct from AF cells and cell morphology and metabolic activity varies between the AF and the NP. AF cells are fibroblast-like, being thin and elongated, lying parallel to the collagen fibers of the lamellae, whereas NP cells are rounded and chondrocyte-like [18]. *In vitro* studies show that NP cells incorporate ^{35}S-sulfate into PGs more rapidly than AF cells [19] and NP cells produce relatively more type II collagen than AF cells [20]. *SOX9*, a key regulatory gene of chondrocytic differentiation [21], is also expressed at higher levels by normal NP cells compared with normal AF cells.

Nutrition

Cells at the center of adult IVDs may be up to 8 mm away from the nearest blood vessels, and transport of essential nutrients, such as oxygen and glucose, and removal of metabolic waste, such as lactic acid, must be achieved via diffusion from vessels in the subchondral bone and in the outer margin of the AF [22]. This results in low oxygen tension and cells predominantly use glycolytic metabolism, which combines with slow removal of metabolic waste to lower the pH [23]. Cells are adapted to this environment and do not have a high mitotic rate. In addition, the IVD is sparsely populated, with only 4,000 cells/mm^3 and 9000 cells/mm^3 in the NP and AF, respectively, considerably less than the 15,000 cells/mm^3 found in articular cartilage. These nutrient supply pathways are vulnerable to erosion over time; both the peripheral AF and the CEP becomes less vascular [24], and the CEP itself ossifies impeding diffusion [25]. As nutrient supply routes are eroded, nutrient concentrations fall to levels inadequate to maintain cellular activity and viability.

Degenerative disc disease

Age-related IVD degeneration is common; by 50 years of age, 97% of lumbar discs show evidence of degeneration and 10% of these are severely degenerated [26]. Degenerate IVDs demonstrate radiographically obvious tissue abnormalities, including disc narrowing, bulging, osteophyte formation and end-plate sclerosis. Following the disappearance of notochordal cells during childhood, the IVD loses its translucency, becomes less hydrated, and in the young adult is white and opaque. Interestingly, some species that retain their notochordal cells in their adult life do not show age-related IVD degeneration [27]. In middle-age the nucleus becomes more collagenous and NP cleft formation often occurs [28]. Clefts may radiate towards the annulus, disrupting the collagen lamellae, and increase the likelihood of IVD prolapse. Repair involves scar-tissue accompanied by vascular in-growth, which is functionally inadequate and disposed to further degeneration [29]. The lamellae become more intricately arranged, fibers bifurcate and interdigitate, and the transition zone between AF and NP becomes less clear as the NP becomes more fibrous and less gel-like [30]. In the final

stages of degeneration the NP is replaced by disorganized scar and granulation tissue, and the IVD is distorted [28].

Biochemical changes underlie these macroscopic changes; central to age-related degeneration is altered composition of the NP matrix, most importantly a reduction in the amount of PG. PGs account for 70% of the total IVD dry weight in 15–25 year olds, but only 20% in 60–80 year olds [31], and this loss of PG is accompanied by water loss and reduced hydrodynamic pressure in the IVD, impairing its functional ability.

The presence of necrotic and apoptotic cells increases dramatically with age; necrotic cells account for only 2% of the fetal population but more than 50% of the adult cell population, and the total number of lacunae containing viable NP cells is reduced [32]. Although reactive cell proliferation occurs, increasing the total number of cells present [33], the characteristics of these 'replacement' cells are not fully known. Recent investigation has demonstrated cellular senescence [34]; it is likely that they are less well suited to maintaining the IVD ECM.

The functional integrity of the IVD is dependent on its highly intricate and complex ECM. However, the matrix is a dynamic structure and IVD cells continuously synthesise ECM macromolecules and secrete matrix metalloproteinases (MMPs) and aggrecanases, which break down the ECM. These proteases affect the balance between synthesis, degradation and accumulation of matrix macromolecules and are implicated in degenerative and age-related changes [35]. MMPs 1, 2, 3, 7, 8 and 13 have been found in the IVD; 1, 2, 3, 9 and 13 are particularly active in degenerated tissue [36,37], along with the A-disintegrin-like and metalloprotease with thrombospondin motifs aggrecanases [38].

Cytokines, such as IL-1α, IL-1β and TNF-α, which decrease PG production but increase MMP production [39,40], and the secretion of pain mediators [41] have been identified in the IVD. IL-1 is either endogenously produced by IVD cells [42], or diffuses into the IVD from outside [39], and this process may be facilitated by loss of PG in degenerate IVDs [43]. IL-1 may be particularly important as its increase in expression in degenerate discs is mirrored by increased expression of its receptor and not by its antagonist (IL-1Ra) [39,40].

Whilst IL-1 and TNF-α, amongst others, mediate damaging and degradative catabolic effects in the IVD, the TGF-β superfamily, which includes TGF-β itself, the bone morphogenic proteins (BMP)-2 and BMP-7 and cartilage-derived morphogenic protein-1, regulate IVD anabolism [44–47]. Mitogenic cytokines have also been identified in the IVD, for example, IGF-1 can prevent apoptosis of IVD cells [48] and stimulate the production of PGs [49].

Repair capacity & the effect of degenerative changes on IVD function & LBP

Investigation into the ability of the IVD to repair artificially induced AF lesions in animals have found little evidence of repair [50]. Any repair is confined to the better vascularized areas of the IVD, the outer AF, rendering the NP sensitive to damage and incapable of repair [51]. Not only is repair absent, but lesions induce progressive degenerative changes in both the affected IVD and adjacent IVDs [52].

Changes to the ECM affect the IVDs behavior in response to mechanical load. Loss of PG results in a lower osmotic pressure [53], the IVD is unable to maintain water content under load and the IVD bulges outwards, seen on radiographs as a reduction in IVD height [54]. When the IVD fails to behave hydrostatically in response to load, the stress distribution in the annulus and the CEP changes, and inappropriate stress concentration and mechanical damage results [55]. Abnormal force distribution induces compensatory contraction in surrounding muscles; spasm contributes to back pain. Therefore, LBP can be attributed to several possible sources; the IVD itself, surrounding muscles, joints, the outer AF and nerves accompanying vessels during repair. Importantly, each source may be remedied through the functional restoration of the IVD.

Current treatment of LBP

Treatments are aimed at pain control rather than treating the underlying pathology and are limited in their efficacy and cost–effectiveness. A vast range of pain-relieving monotherapies are employed, including both well-established pain-relieving pharmaceuticals (nonsteroidal anti-inflammatory drugs, paracetamol and opiods), and more novel therapies, such as *trans*-cutaneous electrical nerve stimulation, physical manipulation, exercise therapy and behavior therapy [56]. Despite large numbers of clinical trials and investigative studies, the efficacy of these interventions is still questioned [57]. Where the patient is unresponsive to conservative treatment and there

are easily identifiable and co-relatable diagnostic imaging signs and clinical symptoms, fusion surgery (arthrodesis), aimed at physically correcting the abnormalities and directly removing the source of pain, is the primary intervention [58], and is often effective in removing the source of pain [59]. However, problems may emerge at adjacent motion segments owing to biomechanical changes precipitated by the fusion, as well as resulting in reduced mobility and failure to return the sufferer to the pre-illness state.

IVD transplantation for severe degeneration aims to maintain motion segment function whilst preventing adjacent segment degeneration. Whole IVD or nucleus replacement with allogenic [60,61] or autogenic [62,63] IVD tissue using complicated surgical removal and re-implantation techniques has been successfully performed, but problems with the inserted tissue losing height, losing water and PG, becoming unstable, and issues regarding immunogenicity and IVD sourcing have directed researchers away from IVD tissue transplantation [64]. An alternative to tissue transplantation are nucleus replacement devices designed to mimic the NP, such as the prosthetic IVD nucleus (PDN; Raymedica Inc., Bloomington, MN, USA) or total disc replacements, such as Charite (DePuy Spine Inc., Warsaw, IN, USA) and ProDisc (Synthes Spine Inc., West Chester, PA, USA). Since 1996, several different versions of the PDN have been implanted in more than 400 people with varying degrees of success, while both Charite and ProDisc have demonstrated significant pain relief up to 24 months [65,66]. However, complications included device migration, extrusion and failure [58,67] and no study has shown that IVD replacement is superior to spinal fusion [68].

Potential therapies

Owing to the current lack of successful therapies, there is a demand for new treatments that tackle degeneration – not simply removing a mechanical pain stimulus but addressing the biological basis for disease, even preventing degeneration and the development of pain symptoms. Greater understanding of IVD cell and matrix biology and degeneration has led researchers to investigate a wide variety of novel strategies (Figure 1). It is thought that the stage of degeneration will determine the type of biological repair best suited. In early stages when the IVD is structurally intact, rescue of IVD cells through administration of growth factors (GFs) and preservation of IVD tissue using inhibitors of matrix degrading enzymes may be achievable. During later stages of degeneration, where the IVD structure is disrupted, biologic restoration of NP architecture with scaffolds or biomatrices containing cells may be best suited.

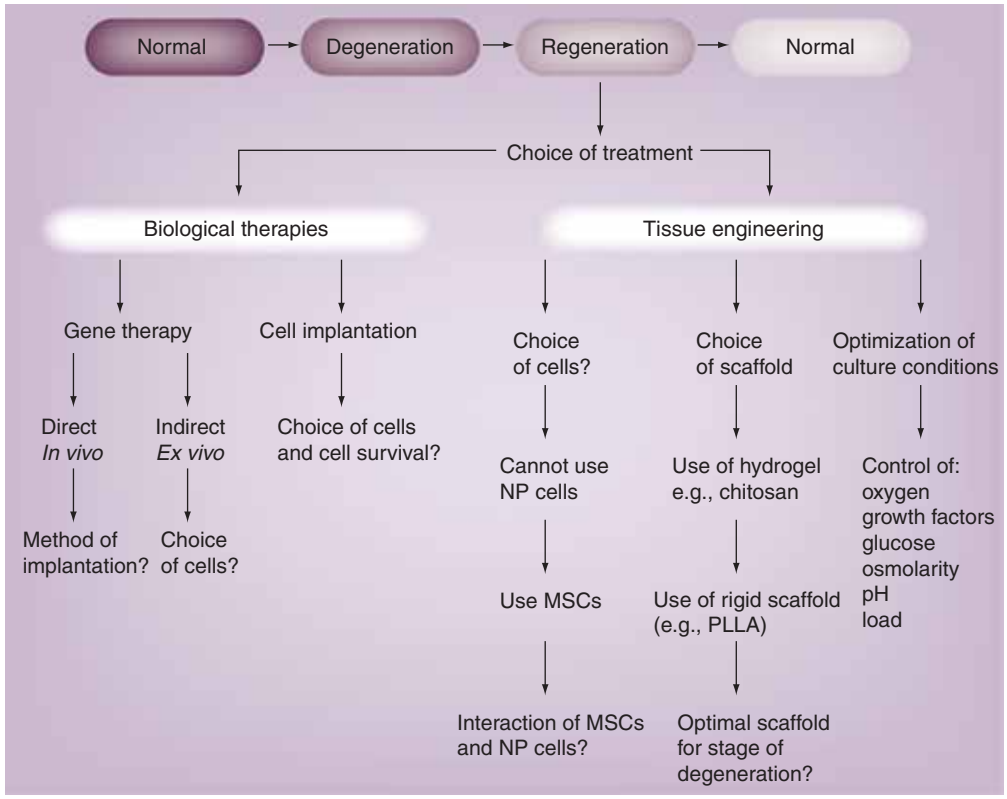
Direct administration of GFs

Direct administration of GFs to the IVD stimulates IVD cell-matrix production [49]. The TGF-β superfamily of GFs have well-established and reproducible effects on IVD cell anabolism [45–47,69]. *In vivo* studies demonstrate increased IVD height indicative of matrix production after injection with GF into both normal IVDs and IVDs artificially degenerated through needle puncture or static compression loading [70–72]. As our understanding of IVD biology improves, it may be possible to utilize not only anabolic proteins such as GFs, but manipulate the degenerative process through anticatabolic factors or inhibitors of degradative enzymes [37,39,73]. So far clinical data on this approach is lacking. An alternative approach has been trialled by Klein *et al.*, who used an 'IVD solution' consisting of matrix components and 'aiding components' [74]. Follow-up for 13 months was promising, with reduction in pain and disability scores, and this approach may prove to be suitable for early stages of degeneration, although leakage of injected components might be problematic in later stages of degeneration. These results encourage the possibility of GF administration for stimulation of repair and prevention of the progression of early degenerative change, but with the caveat that continuous or repeated delivery of GF may be required for maximum benefit, as the effects wore off with GF depletion over time. This problem may be addressed through the innovation of slow-release injectable formulations or employment of gene transfection.

Gene transfection

Gene transfection is the process whereby cells are directly infected with a viral vector carrying a target gene, the expression of which will have therapeutic benefit in the tissue of interest. Gene transfection of TGF-β into mouse IVDs has demonstrated a twofold increase in PG production and IVDs exhibited remarkable increases in TGF-β and PG production that was maintained over the duration of the experiment (a 6-week period). This is considerably longer than that with local administration alone [75]. Alternatives

Figure 1. Strategies for regeneration of the intervertebral disc.



Schematic overview of the biological therapies and tissue-engineering strategies under investigation for regeneration of the degenerate human intervertebral disc, including current options, unanswered questions and potential problems.
MSC: Mesenchymal stem cell; NP: Nucleus pulposus; PLLA: Poly-L-lactic acid.

to TGF-β include latent membrane protein-1 [76] and SOX-9 [77]. However, we need not be limited to therapy with anabolic factors; IVD cells transfected with IL-1RA, an IL-1 inhibitor, then injected into IVD explants resulted in reduced protease expression and matrix degradation [78]. Better results still might be achieved through the transfection of synergistically active GFs together with enzyme inhibitors [79].

The NP is a promising site for gene introduction, as its avascular encapsulated nature can protect the vector from the body's own immune system, preventing damaging immune reaction against the transfected cells and prolonging gene expression. Fas ligand is expressed in the IVD, providing a mechanism for the maintenance of immune privilege [80], and the isolated nature of the IVD ensures sequestration of the vector, reducing systemic spread and possible side-effects [81]. Questions remain as to the best method of gene delivery [82]. Adenoviruses are currently the most commonly used vector owing to their high transfection rate, although safety concerns preclude their use in clinical trials [83]. Alternatives include nonviral vectors such as the gene gun or liposomes, which deliver the gene as an episome into the host cell cytoplasm [84]. These methods have the disadvantage of reduced duration of gene expression and lower transfection success rate [85]. Investigation into viral vectors that fail to elicit an immune response and have no known pathogenicity, such as adeno-associated virus, may provide a solution [86–88], although currently an appropriate gene-delivery method does not exist, and animal *in vivo* experiments have caused spinal toxicity [82].

Tackling later stages of degeneration

The number of phenotypically normal viable IVD cells decreases dramatically with age and severity of degeneration [89]. This is a major problem in the use of 'rescue techniques' such as gene transfection and GF administration. Attempting to promote regeneration through stimulation of this small, necrotic and suicidal cell population may prove difficult, possibly requiring

the transplantation of healthy, functional cells [90,91]. The problems associated with stimulating repair in a tissue devoid of viable cells in more severe stages of degeneration has called for investigation into cellular-based repair strategies, with the possibility of repopulating the IVD with cells able to effect required matrix deposition.

Identifying a suitable cell source

Repopulating the IVD by implantation of autologous [90–92] or allogenic [93] IVD cells into animal models of IVD degeneration showed less degeneration relative to controls, and led these researchers to conclude that cell implantation retards IVD degeneration. It is important to note that these animal injury model studies use cells implanted prior to degeneration. They demonstrate reduced degeneration but do not demonstrate regeneration, and although illustrating the positive effect of a 'normal' population of IVD cells on the functional characteristics of the IVD, the source of IVD cells remains an unresolved issue. The ideal choice would be autologous IVD cells, but the relatively acellular nature of the IVD [94] makes removal of a large amount of tissue necessary in order to recover a significant number of cells. However, removal of IVD tissue is associated with accelerated degenerative changes and is not recommended [91,93].

To circumvent the problem associated with tissue removal Meisel *et al.* report a series of patients treated by re-implanting *in vitro*-expanded IVD cells isolated from patients undergoing discectomy for herniation [95]. Although preliminary, their results highlight the possibility of stimulating repair and preventing degeneration through autologous IVD cell therapy [96].

However, isolation of IVD cells is only possible in the case of acute traumatic injury, such as that resulting from herniation, when surgery is forced upon the patient and nuclear material is easily removed. Another doubt surrounds the use of cells isolated from severely degenerated IVDs; cells are of altered phenotype and demonstrate increased senescence [17,37,39]. Expansion of these cells *in vitro* to a number usable for tissue engineering might result in a population of 'abnormal' cells unsuitable for re-implantation.

Although IVD cell allografts may fail to elicit an immune response *in vivo* [93], safety concerns recommend caution in their use in humans, and further investigation is required before nonautogenic NP cells could be transplanted into humans.

The hypothesized benefits of increasing the population of viable IVD cells and increasing matrix production in regenerating the IVD and restoring function, alongside the difficulties associated with 'rescue' strategies or implantation of cultured NP cells, has led researchers to consider other sources of cells that might be able to affect biologic repair. Prominent among these suggestions are mesenchymal stem cells (MSCs), which can be easily isolated from a number of tissues and have both differentiation capacity and self-renewal ability. In addition to their osteogenic ability [97], these cells have since been shown to differentiate into several cell types of mesenchymal tissue, including cartilage [98], adipose tissue [99] and muscle [100].

IVD & tissue engineering

Although as yet no unique NP cell marker has been identified, differentiation of MSCs into NP-like cells have been demonstrated in a number of systems, including co-culture with NP cells [101,102], on 3D scaffolds with transfection with SOX-9 and culture with TGF-β [103], and in chitosan-based hydrogels [104]. The possibility of differentiating MSCs down an NP-like lineage, together with their ready extraction from bone marrow aspirates and *in vitro* expansion capacity, has led researchers to consider their application in regenerating the IVD [97]. Because transfection during culture is easily achievable, MSCs also offer a ready vector for gene delivery to the IVD [105]. However, for regeneration of the IVD to be successful, it is likely that implanted cells would require a support structure, both to protect the cells from the loads experienced within the disc and provide a template onto which cells could deposit newly synthesized ECM. Therefore, identification of a suitable scaffold or hydrogel will be vital for tissue engineering of the IVD.

Choice of scaffold materials

Implantable scaffolds can be divided into those derived from biological sources or those from synthetic sources. Biological sources (such decellularized autogenic, allogenic or xenogenic matrices, and collagen, fibrin, alginate or chitosan-based matrices) have the advantage of mimicking the native cellular environment and reduced possibility of immunogenicity [106–108], while synthetic polymers, such as polyglycolic acid, have readily modifiable material properties [109].

Several requirements are placed on a scaffold material, encompassing both biological and mechanical properties [110]. It should be porous to allow tissue growth, cellular communication and diffusion of nutrients and waste. The material should allow attachment, differentiation and possibly proliferation of cells. Its mechanical properties should mirror those of the tissue native to the implantation site. Lastly, its shape must conform to the site of implantation. These requirements are complicated by the extreme biomechanical forces exerted on the IVD, the harsh physiological conditions present and the relative isolation and inaccessibility of the IVD, thus the careful choice of scaffold is vital [111]. The IVD matrix has three principal functions; it anchors IVD cells in place and provides signals that mediate cellular metabolic and matrix remodelling activity, and it determines the mechanical properties of the IVD. Scaffolds used in tissue engineering must either mimic these properties, or encourage the formation of a matrix with such characteristics when implanted.

Application of tissue engineering to the IVD

Various different scaffold–cell combinations have been investigated in IVD applications ranging in complexity from a relatively simple collagen–hyaluronan matrix aimed at NP [112] or AF [113] regeneration, to development of an IVD composite [114], or an end-plate–NP construct [115]. Alini *et al.* seeded bovine coccygeal NP or AF cells on a collagen–hyaluronan scaffold, chosen because it mimics the native IVD ECM [112]. Over 60 days they noted PG production, although at levels considerably lower than are found in the native IVD. Although compounded by loss of PG by diffusion into the surrounding culture medium, this might not be a problem in the encapsulated IVD. Seguin *et al.* used a calcium polyphosphate bone substitute material [116]. PG production levels comparable to the native IVD were observed up to 6 weeks in culture. The construct's mechanical properties were compared with those of the native IVD, finding little difference in stiffness or ability to resist compression. Sato *et al.* showed cell viability and matrix production *in vitro*, then implanted atelocollagen honeycomb scaffold seeded with AF cells into rabbit IVDs with NP defects [117]. The cells survived and proliferated, and the treated group showed maintenance of IVD space height compared with an untreated control. More recently Yang *et al.* have investigated a novel scaffold seeded with human IVD cells, with promising results [118]. Several other synthetic chemical polymer formulations such as poly(D,L-lactide)/bioglass and poly-L-lactic acid (PLLA) have been developed and tested *in vitro* on their own [103,119], or in combination with natural polymers such as collagen or gelatin [120,121].

In moderate IVD degeneration, a potential problem with such constructs is the difficulty in implanting them whilst maintaining AF integrity; ideally, major, invasive surgical intervention for repair should be avoided. These strategies may be better tailored to treating later stages of degeneration, where considerable loss of NP tissue has occurred and mechanical strength of the construct is of paramount importance. However, whilst 3D scaffolds such as PLLA allow NP regeneration, questions over its suitability for application to the disc remain; during biological degradation lactic acid released might reduce the pH of the disc ECM, compromising native and implanted disc cells.

Hydrogels

These issues have led researchers to look away from traditional 3D scaffolds, often initially developed for engineering bone, towards alternatives with characteristics more tailored to the IVD, such as networks of crosslinked hydrophilic polymer chains that swell but do not dissolve in water, known as hydrogels. Developed in the 1950s as soft contact lenses, hydrogels are able to undergo a simple phase transition in water from liquid to gel, the sol–gel transition, often without any chemical reaction [122,123].

Our groups' work has focused on a polysaccharide hydrogel called chitosan, derived by alkaline deacetylation of chitin, found in the exoskeleton of crustaceans [124]. Chitosan provides an interesting example of the interdisciplinary development required in tissue engineering. Chitosan was initially developed as a bone substitute in the 1980s [125], then chemical modification by Chenite *et al.* through addition of polyol salts made chitosan thermosensitive (gelling at around 37°C) and heralded new interest as a biological polymer with the capacity to gel on implantation into the body [126,127]. In the IVD, this property could be employed to allow chitosan to flow into nuclear clefts prior to polymerization [128], and recent work has shown that chitosan has a similar loading properties compared with NP tissue [129]. Our work with

chitosan and MSCs has shown development of an NP-like phenotype *in vitro*, with expression of the key NP marker genes *SOX-9*, aggrecan and type II collagen [104]. Chitosan also has other characteristics that make it attractive for use in tissue engineering of the IVD: it may be modified to gel more or less rapidly *in vivo* and to be more mechanically stable; the tissue response to chitosan is limited [130]; it is bioresorbable [131] and bacteriostatic [132]; and its cationic nature allows interaction with GAGs, encouraging retention of PGs produced *in situ* [104]. Similarly, chitosan's analogous nature to native IVD ECM components such as hyaluronan might regulate the maintenance of the chondrocytic cell phenotype [133]. Other biological hydrogels that have shown promise include collagen–hyaluronan [64], collagen gel [134] and atelocollagen [135]. Several *in vivo* studies have investigated MSCs combined with hydrogels in animal models of IVD degeneration [136–138], and although current animal models do not mimic the loading environment experienced by cells in the human IVD, these studies demonstrate cell survival [102,136,137], cell migration into surrounding structures [102], maintenance of IVD structure and height, and evidence of ECM production [137,138]. Future studies with large animal models are now being used to investigate MSC regeneration of discs [139] and to determine the effect of complicated factors such as stage of degeneration on the regenerative capacity of MSCs [140]. Novel animal models of degeneration will be used to further determine the efficacy of interventions [141,142].

Synthetic hydrogels, such as poly(vinyl alcohol), polyvinyl pyrrolidone, and poly(ethylene glycol) (PEG) may be formed with specific chemical properties designed to mimic the NP, without the sourcing and reproducibility issues of biological hydrogels [143–145]. Although these nonbiodegradable polymers are highly hydrated and allow nutrient diffusion into the NP, they are not compatible with cell-based tissue regeneration. Cartilage defects have been regenerated with PEG hydrogels, but they have not been tested in the IVD [146].

Conclusion & future perspective

Understanding of IVD biology has increased dramatically; we know many of the important changes to the ECM that occur in degenerate IVDs, and are beginning to unravel the cellular processes responsible for degeneration and the cytokine and enzyme interplay driving these changes. Alongside improved understanding comes the possibility of arresting and reversing disruption to the IVD and treating LBP; numerous strategies are proposed to achieve this aim. Putting the information we have so far gained from *in vitro* experiments and *in vivo* animal studies in a clinical

Box 1. Application-focused problems to be addressed prior to clinical translation.

- Will implanted cells survive? For cell-based regeneration to be successful the degraded extracellular matrix (ECM) must be regenerated. To achieve this, implanted cells must survive and secrete matrix. However, the nucleus pulposus (NP) is a hostile environment and the calcification of the end-plates associated with disc degeneration results in a low pH, low oxygen tension, relatively high concentration of metabolic waste products and no blood supply. It may be that for implanted cells to survive intervention to improve the nutrient supply to the intervertebral disc (IVD) will be necessary. Therefore, there may also be a requirement to repair the end-plates as well as the degenerate IVD [147].
- How many cells should be implanted? Mesenchymal stem cells (MSCs) are readily expanded, but implantation of too many cells could catastrophically lower pH and oxygen levels and cause massive cell death. The caveat is that implantation of too few cells would be ineffective in regenerating the ECM.
- What biological factors should be employed? We understand the molecular mechanisms governing IVD cell biology but choosing the most appropriate biological factors for stimulating repair is a challenge. It may be that proteoglycan (PG)-specific factors, such as cartilage-derived morphogenetic protein-1 or IGF-1, will be the best option rather than factors such as TGF-β.
- Loading is an important consideration; human IVDs are subjected to load and extreme loading conditions have been shown *in vitro* to cause cell death and reduce the level of PG production [148]. More advanced *in vitro* studies of cell–scaffold implants are beginning to determine whether the implant has sufficient mechanical strength for implantation into the disc [129]. We must also determine whether implanted cells seeded on carrier scaffolds will survive under loading conditions experienced *in vivo.*
- Do implanted cells need to be differentiated prior to implantation (pre-differentiation) or could cells be harvested, expanded and then seeded in scaffolds and implanted immediately? Pre-differentiation allows the modification of cells prior to implantation but will require conditions that mimic the IVD such as osmolality [149], hypoxia and mechanical loading; a complex model, the exact parameters of which are yet to be defined. Conversely, immediate implantation followed by *in vivo* co-culture with NP cells could be sufficient to stimulate differentiation, but the exact interaction of MSCs with degenerate NP cells is yet to be established.
- What is the best method of implantation? Is it feasible to implant prefabricated 3D scaffolds, or should the focus be on injectable carriers that will result in the minimum possible disc disruption? However, severely degenerate discs may only be regenerated through the implantation of mechanically stable scaffolds.

context raises questions that need consideration before therapeutic intervention in humans is possible; we are now in a position to consider these application-focussed problems as listed in Box 1.

The application-focussed problems discussed in Box 1 are not easily addressed as there is no animal model for disc degeneration directly comparable with human degeneration. The answer may not be a one-size-fits-all approach: one degenerate disc may be best treated with cells predifferentiated in a loaded, hypoxic, nutrient-deficient environment, whereas another might be more suited to direct implantation of MSCs transfected with growth factors. How this will be determined is unclear at present; greater experience with regeneration strategies must be gained. Despite these challenges, our knowledge and understanding of both basic science and the potential of regenerative medicine strategies offers hope for the future repair of the degenerate IVD and treatment of LBP.

Financial & competing interests disclosure

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

No writing assistance was utilized in the production of this manuscript.

Executive summary

The normal intervertebral disc

- The intervertebral disc (IVD) is a unique and complex structure, highly specialized for its mechanical role. Its extracellular matrix has a high proteoglycan content that draws in water, hydrating the disc and allowing it to function as a shock absorber.

Degenerative disc disease

- Changes in the disc matrix, particularly loss of proteoglycan, are seen in age-related disc degeneration, which is a common cause of low back pain.

Current treatment of low back pain

- Current treatments are aimed at relieving the symptoms or the source of pain rather than treating the biological basis of the disease and are relatively ineffective in the long term.

Potential therapies

- Regeneration of the IVD through tissue-engineering strategies involving rescue of native disc cells, and repopulation of the disc with cells able to direct matrix synthesis could regenerate discs and treat sufferers of low back pain.
- Cell sourcing is difficult because removal of native disc cells precipitates degeneration. However, mesenchymal stem cells isolated from bone marrow offer an easily obtainable cell population with the capacity to generate disc matrix. Whether cells will need to be differentiated prior to implantation or will differentiate *in vivo* in the degenerate niche needs to be determined.
- Work is ongoing to identify suitable cell carriers for implantation into the disc. Biological hydrogel polymers such as chitosan have given promising *in vitro* results. However, in later-stage degeneration, rigid 3D scaffolds such as poly-L-lactic acid may be required as they confer mechanical strength that hydrogels currently do not offer.
- Investigation into cell survival *in vivo* following implantation and the optimum cell–scaffold–culture condition combination is needed before human trials will be possible.

Bibliography

Papers of special note have been highlighted as either of interest (•) or of considerable interest (••) to readers.

1. Deyo RA, Weinstein JN: Low back pain. *N. Engl. J. Med.* 344(5), 363–370 (2001).
2. Maniadakis N, Gray A: The economic burden of back pain in the UK. *Pain* 84(1), 95–103 (2000).
3. Macfarlane GJ, Thomas E, Croft PR, Papageorgiou AC, Jayson MI, Silman AJ: Predictors of early improvement in low back pain amongst consulters to general practice: the influence of pre-morbid and episode-related factors. *Pain* 80(1–2), 113–119 (1999).
4. Frymoyer JW, Cats-Baril WL: An overview of the incidences and costs of low back pain. *Orthop. Clin. North Am.* 22(2), 263–271 (1991).
5. Andersson GB: Epidemiological features of chronic low-back pain. *Lancet* 354(9178), 581–585 (1999).
6. Luoma K, Riihimaki H, Luukkonen R, Raininko R, Viikari-Juntura E, Lamminen A: Low back pain in relation to lumbar disc degeneration. *Spine* 25(4), 487–492 (2000).
7. Lim CH, Jee WH, Son BC, Kim DH, Ha KY, Park CK: Discogenic lumbar pain: association with MR imaging and CT discography. *Eur. J. Radiol.* 54(3), 431–437 (2005).
8. Barrick WT, Schofferman JA, Reynolds JB *et al.*: Anterior lumbar fusion improves discogenic pain at levels of prior posterolateral fusion. *Spine* 25(7), 853–857 (2000).
9. Marchand F, Ahmed AM: Investigation of the laminate structure of lumbar disc anulus fibrosus. *Spine* 15(5), 402–410 (1990).
10. Mwale F, Roughley P, Antoniou J: Distinction between the extracellular matrix of the nucleus pulposus and hyaline cartilage: a requisite for tissue engineering of intervertebral disc. *Eur. Cell Mater.* 8, 58–63 (2004).

11. Roughley PJ: Biology of intervertebral disc aging and degeneration: involvement of the extracellular matrix. *Spine* 29(23), 2691–2699 (2004).
12. Melrose J, Ghosh P, Taylor TK: A comparative analysis of the differential spatial and temporal distributions of the large (aggrecan, versican) and small (decorin, biglycan, fibromodulin) proteoglycans of the intervertebral disc. *J. Anat.* 198(Pt 1), 3–15 (2001).
13. Caterson B, Flannery CR, Hughes CE, Little CB: Mechanisms involved in cartilage proteoglycan catabolism. *Matrix Biol.* 19(4), 333–344 (2000).
14. Scott JE, Bosworth TR, Cribb AM, Taylor JR: The chemical morphology of age-related changes in human intervertebral disc glycosaminoglycans from cervical, thoracic and lumbar nucleus pulposus and annulus fibrosus. *J. Anat.* 184(Pt 1), 73–82 (1994).
15. Roberts S, Ayad S, Menage PJ: Immunolocalisation of type VI collagen in the intervertebral disc. *Ann. Rheum. Dis.* 50(11), 787–791 (1991).
16. Urban JPG, Roberts S, Ralphs JR: The nucleus of the intervertebral disc from development to degeneration. *Am. Zool.* 2000(40), 53–61 (2000).
17. Sive JI, Baird P, Jeziorsk M, Watkins A, Hoyland JA, Freemont AJ: Expression of chondrocyte markers by cells of normal and degenerate intervertebral discs. *Mol. Pathol.* 55(2), 91–97 (2002).
18. Lee CR, Sakai D, Nakai T *et al.*: A phenotypic comparison of intervertebral disc and articular cartilage cells in the rat. *Eur. Spine J.* 16(12), 2174–2185 (2007).
19. Horner HA, Roberts S, Bielby RC, Menage J, Evans H, Urban JP: Cells from different regions of the intervertebral disc: effect of culture system on matrix expression and cell phenotype. *Spine* 27(10), 1018–1028 (2002).
20. Chelberg MK, Banks GM, Geiger DF, Oegema TR Jr: Identification of heterogeneous cell populations in normal human intervertebral disc. *J. Anat.* 186(Pt 1), 43–53 (1995).
21. Lefebvre V, Huang W, Harley VR, Goodfellow PN, de Crombrugghe B: SOX9 is a potent activator of the chondrocyte-specific enhancer of the pro α1(II) collagen gene. *Mol. Cell Biol.* 17(4), 2336–2346 (1997).
22. Grunhagen T, Wilde G, Soukane DM, Shirazi-Adl SA, Urban JP: Nutrient supply and intervertebral disc metabolism. *J. Bone Joint Surg. Am.* 88(Suppl. 2), 30–35 (2006).
- Review covering the nutritional aspects of intervertebral disc degeneration and repair, highlighting the importance of glucose, nutrition, lactic acid and pH in the metabolism of intervertebral disc (IVD) cells.
23. Urban JP, Smith S, Fairbank JC: Nutrition of the intervertebral disc. *Spine* 29(23), 2700–2709 (2004).
24. Chandraraj S, Briggs CA, Opeskin K: Disc herniations in the young and end-plate vascularity. *Clin. Anat.* 11(3), 171–176 (1998).
25. Roberts S, Urban JP, Evans H, Eisenstein SM: Transport properties of the human cartilage endplate in relation to its composition and calcification. *Spine* 21(4), 415–420 (1996).
26. Miller JA, Schmatz C, Schultz AB: Lumbar disc degeneration: correlation with age, sex, and spine level in 600 autopsy specimens. *Spine* 13(2), 173–178 (1988).
27. Hunter CJ, Matyas JR, Duncan NA: The notochordal cell in the nucleus pulposus: a review in the context of tissue engineering. *Tissue Eng.* 9(4), 667–677 (2003).
28. Thompson JP, Pearce RH, Schechter MT, Adams ME, Tsang IK, Bishop PB: Preliminary evaluation of a scheme for grading the gross morphology of the human intervertebral disc. *Spine* 15(5), 411–415 (1990).
29. Vernon-Roberts B: Age related and degnerative pathology of the intervertebral discs and apophyseal joints. In: *The Lumbar Spine and Back Pain.* Jayson MIV (Ed.). Churchill Livingstone, Elsevier Ltd, Oxford, UK 17–35 (1992).
30. Roberts S: Disc morphology in health and disease. *Biochem. Soc. Trans.* 30(Pt 6), 864–869 (2002).
31. Antoniou J, Steffen T, Nelson F *et al.*: The human lumbar intervertebral disc: evidence for changes in the biosynthesis and denaturation of the extracellular matrix with growth, maturation, ageing, and degeneration. *J. Clin. Invest.* 98(4), 996–1003 (1996).
32. Gruber HE, Hanley EN Jr: Analysis of aging and degeneration of the human intervertebral disc. Comparison of surgical specimens with normal controls. *Spine* 23(7), 751–757 (1998).
33. Johnson WE, Eisenstein SM, Roberts S: Cell cluster formation in degenerate lumbar intervertebral discs is associated with increased disc cell proliferation. *Connect. Tissue Res.* 42(3), 197–207 (2001).
34. Le Maitre CL, Freemont AJ, Hoyland JA: Accelerated cellular senescence in degenerate intervertebral discs: a possible role in the pathogenesis of intervertebral disc degeneration. *Arthritis Res. Ther.* 9(3), R45 (2007).
35. Goupille P, Jayson MIV, Valat JP, Freemont AJ: Matrix metalloproteinases: the clue to intervertebral disc degeneration? *Spine* 23(14), 1612–1626 (1998).
36. Roberts S, Caterson B, Menage J, Evans EH, Jaffray DC, Eisenstein SM: Matrix metalloproteinases and aggrecanase: their role in disorders of the human intervertebral disc. *Spine* 25(23), 3005–3013 (2000).
37. Le Maitre CL, Freemont AJ, Hoyland JA: Localization of degradative enzymes and their inhibitors in the degenerate human intervertebral disc. *J. Pathol.* 204(1), 47–54 (2004).
38. Le Maitre CL, Pockert A, Buttle DJ, Freemont AJ, Hoyland JA: Matrix synthesis and degradation in human intervertebral disc degeneration. *Biochem. Soc. Trans.* 35(Pt 4), 652–655 (2007).
39. Le Maitre CL, Freemont AJ, Hoyland JA: The role of interleukin-1 in the pathogenesis of human intervertebral disc degeneration. *Arthritis Res. Ther.* 7(4), R732–R745 (2005).
40. Le Maitre CL, Hoyland JA, Freemont AJ: Catabolic cytokine expression in degenerate and herniated human intervertebral discs: IL-1β and TNFα expression profile. *Arthritis Res. Ther.* 9(4), R77 (2007).
41. Rannou F, Corvol MT, Hudry C *et al.*: Sensitivity of anulus fibrosus cells to interleukin-1β. Comparison with articular chondrocytes. *Spine* 25(1), 17–23 (2000).
42. Kang JD, Stefanovic-Racic M, McIntyre LA, Georgescu HI, Evans CH: Toward a biochemical understanding of human intervertebral disc degeneration and herniation. Contributions of nitric oxide, interleukins, prostaglandin E2, and matrix metalloproteinases. *Spine* 22(10), 1065–1073 (1997).
43. Urban JP, Roberts S: Degeneration of the intervertebral disc. *Arthritis Res. Ther.* 5(3), 120–130 (2003).
44. Yoon ST: Molecular therapy of the intervertebral disc. *Spine J.* 5(Suppl. 6), 280S–286S (2005).
45. Li J, Kim KS, Park JS, Elmer WA, Hutton WC, Yoon ST: BMP-2 and CDMP-2: stimulation of chondrocyte production of proteoglycan. *J. Orthop. Sci.* 8(6), 829–835 (2003).

46. Masuda K, Takegami K, An H *et al.*: Recombinant osteogenic protein-1 upregulates extracellular matrix metabolism by rabbit annulus fibrosus and nucleus pulposus cells cultured in alginate beads. *J. Orthop. Res.* 21(5), 922–930 (2003).
47. Zhang Y, An HS, Song S *et al.*: Growth factor osteogenic protein-1: differing effects on cells from three distinct zones in the bovine intervertebral disc. *Am. J. Phys. Med. Rehabil.* 83(7), 515–521 (2004).
48. Gruber HE, Norton HJ, Hanley EN Jr: Anti-apoptotic effects of IGF-1 and PDGF on human intervertebral disc cells *in vitro*. *Spine* 25(17), 2153–2157 (2000).
49. Thompson JP, Oegema TR Jr, Bradford DS: Stimulation of mature canine intervertebral disc by growth factors. *Spine* 16(3), 253–260 (1991).
50. Hampton D, Laros G, McCarron R, Franks D: Healing potential of the anulus fibrosus. *Spine* 14(4), 398–401 (1989).
51. Osti OL, Vernon-Roberts B, Fraser RD: 1990 Volvo Award in experimental studies. Anulus tears and intervertebral disc degeneration. An experimental study using an animal model. *Spine* 15(8), 762–767 (1990).
52. Melrose J, Ghosh P, Taylor TK *et al.*: A longitudinal study of the matrix changes induced in the intervertebral disc by surgical damage to the annulus fibrosus. *J. Orthop. Res.* 10(5), 665–676 (1992).
53. Urban JP, McMullin JF: Swelling pressure of the lumbar intervertebral discs: influence of age, spinal level, composition, and degeneration. *Spine* 13(2), 179–187 (1988).
54. Frobin W, Brinckmann P, Kramer M, Hartwig E: Height of lumbar discs measured from radiographs compared with degeneration and height classified from MR images. *Eur. Radiol.* 11(2), 263–269 (2001).
55. Adams MA, McNally DS, Dolan P: 'Stress' distributions inside intervertebral discs. The effects of age and degeneration. *J. Bone Joint Surg. Br.* 78(6), 965–972 (1996).
56. Bogduk N: Management of chronic low back pain. *Med. J. Aust.* 180(2), 79–83 (2004).
57. van der Roer N, Goossens ME, Evers SM, van Tulder MW: What is the most cost-effective treatment for patients with low back pain? A systematic review. *Best Pract. Res. Clin. Rheumatol.* 19(4), 671–684 (2005).
58. Errico TJ: Lumbar disc arthroplasty. *Clin. Orthop. Relat. Res.* 435, 106–117 (2005).
59. Hilibrand AS, Robbins M: Adjacent segment degeneration and adjacent segment disease: the consequences of spinal fusion? *Spine J.* 4(Suppl. 6), 190S–194S (2004).
60. Katsuura A, Hukuda S: Experimental study of intervertebral disc allografting in the dog. *Spine* 19(21), 2426–2432 (1994).
61. Nishimura K, Mochida J: Percutaneous reinsertion of the nucleus pulposus. An experimental study. *Spine* 23(14), 1531–1538 (1998).
62. Luk KD, Ruan DK, Chow DH, Leong JC: Intervertebral disc autografting in a bipedal animal model. *Clin. Orthop. Relat. Res.* (337), 13–26 (1997).
63. Frick SL, Hanley EN Jr, Meyer RA Jr, Ramp WK, Chapman TM: Lumbar intervertebral disc transfer. A canine study. *Spine* 19(16), 1826–1834 (1994).
64. Alini M, Roughley PJ, Antoniou J, Stoll T, Aebi M: A biological approach to treating disc degeneration: not for today, but maybe for tomorrow. *Eur. Spine J.* 11(Suppl. 2), S215–S220 (2002).
65. Blumenthal S, McAfee PC, Guyer RD *et al.*: A prospective, randomized, multicenter Food and Drug Administration investigational device exemptions study of lumbar total disc replacement with the CHARITE artificial disc versus lumbar fusion: part I: evaluation of clinical outcomes. *Spine* 30(14), 1565–1575 (2005).
66. Bertagnoli R, Yue JJ, Shah RV *et al.*: The treatment of disabling single-level lumbar discogenic low back pain with total disc arthroplasty utilizing the Prodisc prosthesis: a prospective study with 2-year minimum follow-up. *Spine* 30(19), 2230–2236 (2005).
67. Di MA, Vaccaro AR, Lee JY, Denaro V, Lim MR: Nucleus pulposus replacement: basic science and indications for clinical use. *Spine* 30(Suppl. 16), S16–S22 (2005).
68. Freeman BJ, Davenport J: Total disc replacement in the lumbar spine: a systematic review of the literature. *Eur. Spine J.* 15(Suppl. 3), S439–S447 (2006).
69. Imai Y, Miyamoto K, An HS, Thonar EJ, Andersson GB, Masuda K: Recombinant human osteogenic protein-1 upregulates proteoglycan metabolism of human anulus fibrosus and nucleus pulposus cells. *Spine* 32(12), 1303–1309 (2007).
70. Walsh AJ, Bradford DS, Lotz JC: *In vivo* growth factor treatment of degenerated intervertebral discs. *Spine* 29(2), 156–163 (2004).
71. An HS, Takegami K, Kamada H *et al.*: Intradiscal administration of osteogenic protein-1 increases intervertebral disc height and proteoglycan content in the nucleus pulposus in normal adolescent rabbits. *Spine* 30(1), 25–31 (2005).
72. Masuda K, Imai Y, Okuma M *et al.*: Osteogenic protein-1 injection into a degenerated disc induces the restoration of disc height and structural changes in the rabbit anular puncture model. *Spine* 31(7), 742–754 (2006).
73. Masuda K, Oegema TR Jr, An HS: Growth factors and treatment of intervertebral disc degeneration. *Spine* 29(23), 2757–2769 (2004).
74. Klein RG, Eek BC, O'Neill CW, Elin C, Mooney V, Derby RR: Biochemical injection treatment for discogenic low back pain: a pilot study. *Spine J.* 3(3), 220–226 (2003).
75. Nishida K, Kang JD, Gilbertson LG *et al.*: Modulation of the biologic activity of the rabbit intervertebral disc by gene therapy: an *in vivo* study of adenovirus-mediated transfer of the human transforming growth factor-β1-encoding gene. *Spine* 24(23), 2419–2425 (1999).
76. Yoon ST, Park JS, Kim KS *et al.*: ISSLS prize winner: LMP-1 upregulates intervertebral disc cell production of proteoglycans and BMPs *in vitro* and *in vivo*. *Spine* 29(23), 2603–2611 (2004).
77. Paul R, Haydon RC, Cheng H *et al.*: Potential use of *Sox9* gene therapy for intervertebral degenerative disc disease. *Spine* 28(8), 755–763 (2003).
78. Le Maitre CL, Hoyland JA, Freemont AJ: Interleukin-1 receptor antagonist delivered directly and by gene therapy inhibits matrix degradation in the intact degenerate human intervertebral disc: an *in situ* zymographic and gene therapy study. *Arthritis Res. Ther.* 9(4), R83 (2007).
79. Wallach CJ, Sobajima S, Watanabe Y *et al.*: Gene transfer of the catabolic inhibitor TIMP-1 increases measured proteoglycans in cells from degenerated human intervertebral discs. *Spine* 28(20), 2331–2337 (2003).
80. Takada T, Nishida K, Doita M, Kurosaka M: Fas ligand exists on intervertebral disc cells: a potential molecular mechanism for immune privilege of the disc. *Spine* 27(14), 1526–1530 (2002).
81. Cassinelli EH, Hall RA, Kang JD: Biochemistry of intervertebral disc degeneration and the potential for gene therapy applications. *Spine J.* 1(3), 205–214 (2001).
82. Wallach CJ, Kim JS, Sobajima S *et al.*: Safety assessment of intradiscal gene transfer: a pilot study. *Spine J.* 6(2), 107–112 (2006).

83. Ferber D: Gene therapy. Safer and virus-free? *Science* 294(5547), 1638–1642 (2001).
84. Wells DJ: Gene therapy progress and prospects: electroporation and other physical methods. *Gene Ther.* 11(18), 1363–1369 (2004).
85. Wiethoff CM, Middaugh CR: Barriers to nonviral gene delivery. *J. Pharm. Sci.* 92(2), 203–217 (2003).
86. Carter PJ, Samulski RJ: Adeno-associated viral vectors as gene delivery vehicles. *Int. J. Mol. Med.* 6(1), 17–27 (2000).
87. Lattermann C, Oxner WM, Xiao X *et al.*: The adeno associated viral vector as a strategy for intradiscal gene transfer in immune competent and pre-exposed rabbits. *Spine* 30(5), 497–504 (2005).
88. Levicoff EA, Gilbertson LG, Kang JD: Gene therapy for disc repair. *Spine J.* 5(Suppl. 6), 287S–296S (2005).
•• Review covering the application of gene therapy for treatment of intervertebral disc degeneration, including the risk factors involved and the potential for future clinical use.
89. Trout JJ, Buckwalter JA, Moore KC: Ultrastructure of the human intervertebral disc: II. Cells of the nucleus pulposus. *Anat. Rec.* 204(4), 307–314 (1982).
90. Okuma M, Mochida J, Nishimura K, Sakabe K, Seiki K: Reinsertion of stimulated nucleus pulposus cells retards intervertebral disc degeneration: an *in vitro* and *in vivo* experimental study. *J. Orthop. Res.* 18(6), 988–997 (2000).
91. Gruber HE, Johnson TL, Leslie K *et al.*: Autologous intervertebral disc cell implantation: a model using *Psammomys obesus*, the sand rat. *Spine* 27(15), 1626–1633 (2002).
92. Ganey T, Libera J, Moos V *et al.*: Disc chondrocyte transplantation in a canine model: a treatment for degenerated or damaged intervertebral disc. *Spine* 28(23), 2609–2620 (2003).
93. Nomura T, Mochida J, Okuma M, Nishimura K, Sakabe K: Nucleus pulposus allograft retards intervertebral disc degeneration. *Clin. Orthop. Relat. Res.* (389), 94–101 (2001).
94. Maroudas A, Stockwell RA, Nachemson A, Urban J: Factors involved in the nutrition of the human lumbar intervertebral disc: cellularity and diffusion of glucose *in vitro*. *J. Anat.* 120(Pt 1), 113–130 (1975).
95. Meisel HJ, Ganey T, Hutton WC, Libera J, Minkus Y, Alasevic O: Clinical experience in cell-based therapeutics: intervention and outcome. *Eur. Spine J.* 15(Suppl. 3), S397–S405 (2006).
96. Meisel HJ, Siodla V, Ganey T, Minkus Y, Hutton WC, Alasevic OJ: Clinical experience in cell-based therapeutics: disc chondrocyte transplantation: a treatment for degenerated or damaged intervertebral disc. *Biomol. Eng* 24(1), 5–21 (2007).
•• A clinical study of IVD regeneration using autologous cells implanted without a cellular scaffold demonstrating the technical feasibility of the technique and offering promising results.
97. Bruder SP, Jaiswal N, Haynesworth SE: Growth kinetics, self-renewal, and the osteogenic potential of purified human mesenchymal stem cells during extensive subcultivation and following cryopreservation. *J. Cell Biochem.* 64(2), 278–294 (1997).
98. Mackay AM, Beck SC, Murphy JM, Barry FP, Chichester CO, Pittenger MF: Chondrogenic differentiation of cultured human mesenchymal stem cells from marrow. *Tissue Eng.* 4(4), 415–428 (1998).
99. Pittenger MF, Mackay AM, Beck SC *et al.*: Multilineage potential of adult human mesenchymal stem cells. *Science* 284(5411), 143–147 (1999).
• Fundamental paper demonstrating the potential of adult human mesenchymal stem cells to differentiate down the chondrogenic, adipogenic and osteogenic lineages.
100. Ferrari G, Cusella-De AG, Coletta M *et al.*: Muscle regeneration by bone marrow-derived myogenic progenitors. *Science* 279(5356), 1528–1530 (1998).
101. Richardson SM, Walker RV, Parker S *et al.*: Intervertebral disc cell-mediated mesenchymal stem cell differentiation. *Stem Cells* 24(3), 707–716 (2006).
102. Sobajima S, Vadala G, Shimer A, Kim JS, Gilbertson LG, Kang JD: Feasibility of a stem cell therapy for intervertebral disc degeneration. *Spine J.* (2007) (Epub ahead of print).
103. Richardson SM, Curran JM, Chen R *et al.*: The differentiation of bone marrow mesenchymal stem cells into chondrocyte-like cells on poly-L-lactic acid (PLLA) scaffolds. *Biomaterials* 27(22), 4069–4078 (2006).
104. Richardson SM, Hughes N, Hunt JA, Freemont AJ, Hoyland JA: Human mesenchymal stem cell differentiation to NP-like cells in chitosan–glycerophosphate hydrogels. *Biomaterials* 29(1), 85–93 (2007).
105. Mosca JD, Hendricks JK, Buyaner D *et al.*: Mesenchymal stem cells as vehicles for gene delivery. *Clin. Orthop. Relat. Res.* 379(Suppl.), S71–S90 (2000).
106. Stock UA, Vacanti JP: Tissue engineering: current state and prospects. *Annu. Rev. Med.* 52, 443–451 (2001).
107. Shao X, Hunter CJ: Developing an alginate/chitosan hybrid fiber scaffold for annulus fibrosus cells. *J. Biomed. Mater. Res. A* 82(3), 701–710 (2007).
108. Halloran DO, Grad S, Stoddart M, Dockery P, Alini M, Pandit AS: An injectable cross-linked scaffold for nucleus pulposus regeneration. *Biomaterials* 29(4), 438–447 (2008).
109. Mouw JK, Case ND, Guldberg RE, Plaas AH, Levenston ME: Variations in matrix composition and GAG fine structure among scaffolds for cartilage tissue engineering. *Osteoarthr. Cartil.* 13(9), 828–836 (2005).
110. Kuo CK, Tuan RS: Tissue engineering with mesenchymal stem cells. *IEEE Eng Med. Biol. Mag.* 22(5), 51–56 (2003).
111. Anderson DG, Risbud MV, Shapiro IM, Vaccaro AR, Albert TJ: Cell-based therapy for disc repair. *Spine J.* 5(Suppl. 6), 297S–303S (2005).
112. Alini M, Li W, Markovic P, Aebi M, Spiro RC, Roughley PJ: The potential and limitations of a cell-seeded collagen/hyaluronan scaffold to engineer an intervertebral disc-like matrix. *Spine* 28(5), 446–454 (2003).
113. Wan Y, Feng G, Shen FH, Laurencin CT, Li X: Biphasic scaffold for annulus fibrosus tissue regeneration. *Biomaterials* 29(6), 643–652 (2008).
114. Mizuno H, Roy AK, Vacanti CA, Kojima K, Ueda M, Bonassar LJ: Tissue-engineered composites of anulus fibrosus and nucleus pulposus for intervertebral disc replacement. *Spine* 29(12), 1290–1297 (2004).
115. Hamilton DJ, Seguin CA, Wang J, Pilliar RM, Kandel RA: Formation of a nucleus pulposus-cartilage endplate construct *in vitro*. *Biomaterials* 27(3), 397–405 (2006).
116. Seguin CA, Grynpas MD, Pilliar RM, Waldman SD, Kandel RA: Tissue engineered nucleus pulposus tissue formed on a porous calcium polyphosphate substrate. *Spine* 29(12), 1299–1306 (2004).
117. Sato M, Asazuma T, Ishihara M *et al.*: An experimental study of the regeneration of the intervertebral disc with an allograft of cultured annulus fibrosus cells using a tissue-engineering method. *Spine* 28(6), 548–553 (2003).

118. Yang SH, Chen PQ, Chen YF, Lin FH: An *in vitro* study on regeneration of human nucleus pulposus by using gelatin/chondroitin-6-sulfate/hyaluronan tri-copolymer scaffold. *Artif. Organs* 29(10), 806–814 (2005).
119. Helen W, Gough JE: Cell viability, proliferation and extracellular matrix production of human annulus fibrosus cells cultured within PDLLA/Bioglass composite foam scaffolds *in vitro*. *Acta Biomater.* 4(2), 230–243 (2008).
120. Ma Z, Gao C, Gong Y, Shen J: Cartilage tissue engineering PLLA scaffold with surface immobilized collagen and basic fibroblast growth factor. *Biomaterials* 26(11), 1253–1259 (2005).
121. Cui YL, Hou X, Qi AD *et al.*: Biomimetic surface modification of poly (L-lactic acid) with gelatin and its effects on articular chondrocytes *in vitro*. *J. Biomed. Mater. Res. A* 66(4), 770–778 (2003).
122. Kopecek J: Polymer chemistry: swell gels. *Nature* 417(6887), 388–391 (2002).
123. Jeong B, Kim SW, Bae YH: Thermosensitive sol-gel reversible hydrogels. *Adv. Drug Deliv. Rev.* 54(1), 37–51 (2002).
124. Austin PR, Brine CJ, Castle JE, Zikakis JP: Chitin: new facets of research. *Science* 212(4496), 749–753 (1981).
125. Higashi S, Yamamuro T, Nakamura T, Ikada Y, Hyon SH, Jamshidi K: Polymer-hydroxyapatite composites for biodegradable bone fillers. *Biomaterials* 7(3), 183–187 (1986).
126. Chenite A, Chaput C, Wang D *et al.*: Novel injectable neutral solutions of chitosan form biodegradable gels *in situ*. *Biomaterials* 21(21), 2155–2161 (2000).
127. Li J, Xu Z: Physical characterization of a chitosan-based hydrogel delivery system. *J. Pharm. Sci.* 91(7), 1669–1677 (2002).
128. Mwale F, Iordanova M, Demers CN, Steffen T, Roughley P, Antoniou J: Biological evaluation of chitosan salts cross-linked to genipin as a cell scaffold for disk tissue engineering. *Tissue Eng.* 11(1–2), 130–140 (2005).
129. Cloyd JM, Malhotra NR, Weng L, Chen W, Mauck RL, Elliott DM: Material properties in unconfined compression of human nucleus pulposus, injectable hyaluronic acid-based hydrogels and tissue engineering scaffolds. *Eur. Spine J.* 16(11), 1892–1898 (2007).
130. Suh JK, Matthew HW: Application of chitosan-based polysaccharide biomaterials in cartilage tissue engineering: a review. *Biomaterials* 21(24), 2589–2598 (2000).
131. Hirano S, Tsuchida H, Nagao N: *N*-acetylation in chitosan and the rate of its enzymic hydrolysis. *Biomaterials* 10(8), 574–576 (1989).
132. Aimin C, Chunlin H, Juliang B, Tinyin Z, Zhichao D: Antibiotic loaded chitosan bar. An *in vitro*, *in vivo* study of a possible treatment for osteomyelitis. *Clin. Orthop. Relat. Res.* 366, 239–247 (1999).
133. Roughley P, Hoemann C, Desrosiers E, Mwale F, Antoniou J, Alini M: The potential of chitosan-based gels containing intervertebral disc cells for nucleus pulposus supplementation. *Biomaterials* 27(3), 388–396 (2006).
134. Gruber HE, Leslie K, Ingram J, Norton HJ, Hanley EN: Cell-based tissue engineering for the intervertebral disc: *in vitro* studies of human disc cell gene expression and matrix production within selected cell carriers. *Spine J.* 4(1), 44–55 (2004).
135. Sakai D, Mochida J, Iwashina T *et al.*: Atelocollagen for culture of human nucleus pulposus cells forming nucleus pulposus-like tissue *in vitro*: influence on the proliferation and proteoglycan production of HNPSV-1 cells. *Biomaterials* 27(3), 346–353 (2006).
136. Sakai D, Mochida J, Yamamoto Y *et al.*: Transplantation of mesenchymal stem cells embedded in Atelocollagen gel to the intervertebral disc: a potential therapeutic model for disc degeneration. *Biomaterials* 24(20), 3531–3541 (2003).
137. Crevensten G, Walsh AJ, Ananthakrishnan D *et al.*: Intervertebral disc cell therapy for regeneration: mesenchymal stem cell implantation in rat intervertebral discs. *Ann. Biomed. Eng* 32(3), 430–434 (2004).
138. Sakai D, Mochida J, Iwashina T *et al.*: Regenerative effects of transplanting mesenchymal stem cells embedded in atelocollagen to the degenerated intervertebral disc. *Biomaterials* 27(3), 335–345 (2006).
•• Demonstrates the ability of mesenchymal stem cells implanted in a collagen hydrogels to regenerate the IVD in an animal model.
139. Hiyama A, Mochida J, Iwashina T *et al.*: Transplantation of mesenchymal stem cells in a canine disc degeneration model. *J. Orthop. Res.* 26(5), 589–600 (2008).
140. Ho G, Leung VY, Cheung KM, Chan D: Effect of severity of intervertebral disc injury on mesenchymal stem cell-based regeneration. *Connect. Tissue Res.* 49(1), 15–21 (2008).
141. Zhou H, Hou S, Shang W *et al.*: A new *in vivo* animal model to create intervertebral disc degeneration characterized by MRI, radiography, CT/discogram, biochemistry, and histology. *Spine* 32(8), 864–872 (2007).
142. Hoogendoorn RJ, Helder MN, Kroeze RJ, Bank RA, Smit TH, Wuisman PI: Reproducible long-term disc degeneration in a large animal model. *Spine* 33(9), 949–954 (2008).
143. Allen MJ, Schoonmaker JE, Bauer TW, Williams PF, Higham PA, Yuan HA: Preclinical evaluation of a poly(vinyl alcohol) hydrogel implant as a replacement for the nucleus pulposus. *Spine* 29(5), 515–523 (2004).
144. Thomas J, Gomes K, Lowman A, Marcolongo M: The effect of dehydration history on PVA/PVP hydrogels for nucleus pulposus replacement. *J. Biomed. Mater. Res. B Appl. Biomater.* 69(2), 135–140 (2004).
145. Vernengo J, Fussell GW, Smith NG, Lowman AM: Evaluation of novel injectable hydrogels for nucleus pulposus replacement. *J. Biomed. Mater. Res. B Appl. Biomater.* 84(1), 64–69 (2008).
146. Williams CG, Kim TK, Taboas A, Malik A, Manson P, Elisseeff J: *In vitro* chondrogenesis of bone marrow-derived mesenchymal stem cells in a photopolymerizing hydrogel. *Tissue Eng.* 9(4), 679–688 (2003).
147. Boyd LM, Carter AJ: Injectable biomaterials and vertebral endplate treatment for repair and regeneration of the intervertebral disc. *Eur. Spine J.* 15(Suppl. 3), S414–S421 (2006).
148. Wang DL, Jiang SD, Dai LY: Biologic response of the intervertebral disc to static and dynamic compression in vitro. *Spine* 32(23), 2521–2528 (2007).
149. Takeno K, Kobayashi S, Negoro K *et al.*: Physical limitations to tissue engineering of intervertebral disc cells: effect of extracellular osmotic change on glycosaminoglycan production and cell metabolism. Laboratory investigation. *J. Neurosurg. Spine* 7(6), 637–644 (2007).