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Cell and molecular biology of intervertebral disc degeneration: current understanding and implications for potential therapeutic strategies

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Abstract

Intervertebral disc degeneration (IDD) is a chronic, complex process associated with low back pain; mechanisms of its occurrence have not yet been fully elucidated. Its process is not only accompanied by morphological changes, but also by systematic changes in its histological and biochemical properties. Many cellular and molecular mechanisms have been reported to be related with IDD and to reverse degenerative trends, abnormal conditions of the living cells and altered cell phenotypes would need to be restored. Promising biological therapeutic strategies still rely on injection of active substances, gene therapy and cell transplantation. With advanced study of tissue engineering protocols based on cell therapy, combined use of seeding cells, bio-active substances and bio-compatible materials, are promising for IDD regeneration. Recently reported progenitor cells within discs themselves also hold prospects for future IDD studies. This article describes the background of IDD, current understanding and implications of potential therapeutic strategies.

Introduction

Intervertebral disc degeneration (IDD) is perhaps best defined as a cascade that begins with changes to the local cellular microenvironment and progresses to impairment of their structure and function (1). Prominent changes to IDD are characterized by reduction in active cell numbers, depletion of extracellular matrix (ECM), altered phenotype of normal disc cells, and presence of pro-inflammatory cytokines and mediators (2,3). These cell and molecular changes impose a profound influence on progress of IDD and further impair normal function of the intervertebral disc (IVD) and a patient's quality of life. Aetiology of IDD is difficult to precisely characterized, as the degenerative progression can be attributed to multiple factors (4). Ageing, living conditions and biomechanical loading are often related to it; genetic factors can also result in disc degeneration (5). This review article attempts to summarize current understanding of molecular and cellular biology in normal and pathological IVD, as well as to account for potential biological therapeutic strategies for clinical application.

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Overview of IDD

According to recent statistics, low back pain (LBP) affects more than half our population during their lives and impacts heavily on the economy and quality of life of the patients (6,7). With progression in understanding of IDD, many techniques including examination, imaging investigations (8) and trials of intervention (9) have been applied from different directions, to discover causes of the syndrome and to alleviate the pain. IDD can play a pivotal role in LBP and correlates with disc structural breakdown and dysfunction.

Disc degeneration often occurs with advances in ageing, and water content and proteoglycans reduce gradually (10). Lower aggrecan content leads to dehydration which in turn also impairs mechanical functions (11,12). In addition, genetic, mechanical, environmental and behavioural factors must also be taken into consideration (13).

Over the last two decades, underlying mechanisms of IVD metabolism have benefitted from significant progress. New techniques to examine human tissues, such as immunohistochemistry (14), *in situ* zymography (15), *in situ* hybridization (16) and quantitative image analysis

(17) have helped to update understanding of mechanisms of IDD.

Current therapeutic strategies to assuage disc degeneration mainly lie in conservative therapies, including physiotherapy and anti-inflammatory medication. Although these conservative strategies are often able to alleviate symptoms, actual causes of the degeneration are not addressed. When considering spinal surgery, however, adjacent spine segments may experience high risk of accelerated degeneration post-operatively (18,19); thus novel therapeutics have emerged and brought new light to address the issue. With the advent of biotherapy, innovative methods may help to restore disc structure and mechanical function.

Structure and function of the IVD

The IVD is a viscoelastic weight-bearing 'cushion' which plays a major role in maintaining flexibility and stability of the spine (20). Anatomically, the outer region [annulus fibrosus (AF)] is composed of lamellae mainly of bundles of type I collagen. The AF is oriented to alternate lamellae to form an angle-ply structure (21,22). Central region of the IVD [nucleus pulposus (NP)] consists of type II collagen and aggrecan, but networks of the type II collagen are less organized compared to type I collagen of the AF.

The space-filling proteoglycan accounts for the most part of the NP and forms large macromolecular aggregates enclosed by the type II collagen network (23). Versican is a further kind of IVD proteoglycan, found in regions between adjacent annular lamellae, which may lubricate collagen bundles (24–26). These networks effectively mobilize water content of the disc and maintain its internal hydrostatic pressure. There are two thin cartilaginous endplates (EP). EP extend superiorly and inferiorly over the inner AF and NP to separate vertebral bodies and supply nutrients to discs by diffusion (27). The EP provides resilience to prevent collision between vertebral bodies and absorbs load transmitted.

The main function of the IVD is mechanical, it transfers load and provides spinal mobility. Biomechanics play an important role in the process of IDD; unusual complex injury or trauma are often considered to be its major risk factors. Interactions between NP and AF structures contribute to distribution and transmission of the loads between the vertebral bodies (28). When a disc is under high load, hydrostatic pressure is generated within the NP then conducted to the outer AF, generating circumferential stress within the lamellar structure (29). Pressure can also be supported by the inner AF, and proteoglycans may serve as a cushioned pad to slow this process (30). High loads are generated by the human upright posture and load-bearing activities. Under these, the IVD deforms and its hydrostatic pressure increases. Fluid is slowly squeezed out of the disc which further results in higher osmolarity and lower pH. Loading affects IVD cell metabolism, as changes in physical microenvironment of cells, including fluid content, osmolarity and pH, change the supply of nutrients and bioactive factors within the disc.

Cell and molecular biology of the normal IVD

Cell and molecular biology of IDD are not totally clear, but most experts believe that it is caused by many factors. Increase in pro-inflammatory cytokines, reduction in disc cell numbers and impaired cell viability are normal changes that occur to ageing discs. These lead to modifications in cell and molecular composition of the IVD. A better understanding of normal cell and molecular development of the IVD will help us correct current errors and discover ideal therapeutic strategies.

Cell components of IVD and their inter-relationships

The cell population executes crucial machinery for synthesizing and maintaining the IVD matrix. Most cells found in adult NP are small and chondrocyte-like. However, in both juveniles and adults, a further cell type has been proposed to function in renewal and homoeostasis of the IVD. These are large vacuolated cells of notochordal origin (31). Loss of notochordal cells coincides with the onset of IDD which suggests that this cell population may be involved in maintenance and regeneration of the IVD (32). Human notochord cells have been observed to gradually disappear with ageing, and their depletion correlates with disc degeneration (33,34). It should also be acknowledged that during the process of disc degeneration, there is remoulding of NP and AF tissues. Based on previous reports, early degenerate adult discs may preserve a population of skeletal progenitor cells. To make this perspective more clear and to assert whether this kind of progenitor/stem cell really exists in human IVDs, innovative studies have confirmed their presence (35,36). These studies indicated that progenitor cells were present in AF and NPs and express a repertoire of membrane markers common to bone marrow stem cells. Further investigations ensured their presence and defined tissue zones of the skeletal progenitors in the mature rat (37).

More recently, a study undertaken by Sakai *et al.* (38) identified populations of progenitor cells that were Tie2 positive (Tie2+) and disialoganglioside 2 positive (GD2+) in NP from mice and from humans. This study has far-reaching impact on our present understanding of

cell types of the IVD. They express type II collagen and aggrecan and also they can differentiate into mesenchymal lineages and induce reorganization of NP tissues. Tie2, GD2 and CD24 were proposed to be important markers for recognition of hierarchy of progenitor cells isolated from NP. The sequence originated from Tie2+GD2-CD24- cells, followed in order by Tie2-GD2+CD24- cells and Tie2-GD2-CD24- cells. Of these, Tie2-GD2+CD24- cells had better self-renewal capacity and NP tissue reorganization potential. This improved our current information in understanding IVD cell biology.

ECM components of IVD and their role

Structure of the IVD changes with advancing age, specially in composition of disc ECM; these eventually can lead to IDD. Type I and II collagens are the main components of the IVD, accounting for in the region of 80% of IVD collagen between them. Type I collagen makes up major parts of the outer AF and plays an important role in anti-stretching and repairing damaged tissue (39). Type II collagen is mainly located in inner layers of the AF and NP with the functions of maintaining water content, withstanding and absorbing conductive pressure (40). The major proteoglycan of the disc is aggrecan which occounts for osmotic properties and helps maintain disc height and ability to withstand compression (41,42).

Molecular biology of the IVD: mechanisms of IVD homoeostasis

The molecular biology of the IVD is complex, with many growth factors, genes and proteinases involved. These molecules coordinate with each other and maintain homoeostasis (Fig. 1). Transforming growth factor-β (TGF-β) is comprised of a series of peptides and is considered to have the highest relative relationship with collagen metabolism. TGF-β not only regulates synthesis of collagen and proteoglycan, but also affects IVD metabolism. PR-PCR techniques suggest that TGF-β is a key factor in maintenance and degenerating processes of the IVD (43). Current study has revealed that TGF-β signalling is essential for endplate cartilage growth over post-natal life (44). Bone morphogenic protein (BMP) is also a multi-functional growth factor, belonging to TGF-β superfamily. BMP receptors are found in the IVD, further indicating that its cells react to growth factors of the BMP superfamily (45). Further investigation indicated that the ligand–receptor model is the way in which BMP plays a pivotal role to regulate events of the IVD, such as increasing synthesis of proteoglycan, upregulating mRNA expression of type II collagen and serving as mitotic agent (46,47). Under hypoxic conditions, NP cells upregulate expression of vascular endothelial growth factor-A (VEGFA) and its receptor and membrane-bound vascular endothelial growth factor receptor-1 (mbVEGFR-1), which plays an important role in survival of NP cells (48). Currently, Ang-1, a ligand of Tie2 was reported to have an innate anti-apoptotic effect on hNP cells by Sakai *et al.* (38). According to their study, when added the Tie2-blocking antibody to the serum-free culture medium, the number of apoptotic NP cells increased about 2-fold.

Molecular biology of the IVD is complex, with many growth factors, genes and proteinases involved. These coordinate with each other to promote synthesis of matrix and survival of disc cells, which help to maintain IVD homoeostasis.

The *Sox9* gene is a member of the *Sox gene* family and is an important transcription factor in the process of type II collagen synthesis. In development of cartilage, *Sox9* is an enhancer of type II collagen and remains the most promising gene target for IVD regeneration. When transfected with adenovirus-mediated *Sox9*, IVD cells proliferate, and synthesis of proteoglycan and type II collagen follows an upward trend (49,50).

The IVD is the largest human avascular organ which lacks all immune cells. Destruction of the immune status may lead to degeneration of the IVD. Fas is a type I transmembrane glycoprotein of disc cells. When the Fas ligand of type II transmembrane glycoprotein binds to FasL antibody, death signals are passed into the cell

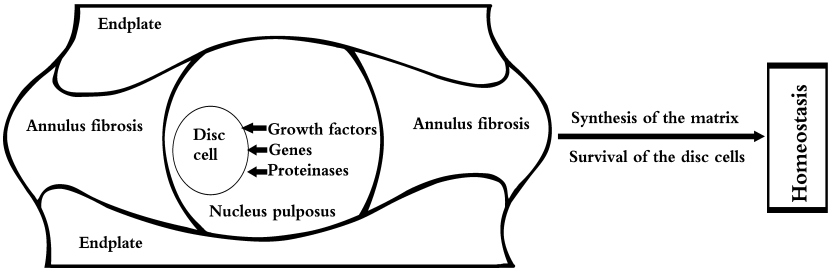

Figure 1. Sagittal structure and homoeostasis of the intervertebral disc.

which further results in degeneration of the IVD (51,52).

Matrix metalloproteinases (MMP) are proteolytic enzymes with metal ions structured within them. They can be divided into five subfamilies, of which MMP-3 is a matrix enzyme, playing a significant role in degradation of the ECM; imbalance of these enzymes and their inhibitors may lead to IDD (53–55). Interleukin (IL) is mainly derived from bone marrow stromal cells, monocytes, macrophages, myeloma cells and osteoblasts. IL-1, a single nuclear factor, is composed of both IL-1α and IL-1β. IL-1 may initiate nerve root pain induced by other inflammatory factors. In addition, IL-1β can stimulate the IVD to release MMPs which degrade proteoglycan, with concentration and time dependency. In addition, IL-1β increases the body's sensitivity to pain and plays an important role in metabolism of the cartilage matrix (56). Other ILs are also found in the IVD, such as IL-6, which may induce sciatica and aggregate inflammatory responses in it.

Cell and molecular hallmarks of IDD

Degenerative changes to the IVD are accompanied by cell and molecular changes. Wide genetic studies have been able to prove the importance of heredity in processes of IDD (57–59), and at least 14 genes have been shown to be associated with disc degeneration, although their specific functions at the moment remain unknown. Recent investigations have revealed that *IL6, SKT and CILP* are involved in aetiology of IDD in young adults (60–62). According to work by Seki *et al.* (63), *CILP* protein plays an important role in IVD homoeostasis, based on controlling TGF-β signals. Several other genes, such as those that code for aggrecan, MMP-3 and type-IX collagen, may affect structure and progression of IDD, which still need further investigation.

With harsh conditions of nutrient diffusion through the cartilaginous EP, IVD may undergo continuous increase in levels of cell death (64). However, proliferation is also often seen in degenerating IVD, specially amongst chondrocyte-like cells (65). Cell death is so commonly observed that it has been taken as an indicator of IDD; as there are no efficient phagocytes in the disc, dead cells are not promptly removed and remain in the matrix for relatively long periods of time. Dead cells, together with proliferating cells eventually increase cell density (66–68). Meanwhile, numbers of viable cells do not increase with cell density, their number decreases with ageing and degeneration (69). Degenerate disc cells generally have an altered phenotype which includes changes in morphology, metabolism gene expression and more. These features of degenerate cells have been observed as accelerated cell senescence (70). It has been reported that degenerate annular cells become more rounded and chondrocytic, whereas under normal conditions, they are more spindle shaped (71). Annular cells have stellate appearance during degenerative events, with multiple, branching cytoplasmic processes extending into their surrounding matrix (72).

During ageing, numbers of notochordal cells reduce and vary towards more chondrogenic phenotype, observed in the NP region (73). Although the mechanism of disappearance of notochordal cells is unknown, apoptosis induced by antocrine or paracrine Fas-mediated counterattack are suspected in the process (74). Without sufficient number and activity of cells, the IVD is not able to produce and maintain large matrix molecules which further aggravate loss of proteoglycans and shift in collagen synthesis. Specifically, type I collagen, rarely present in the NP begins to be expressed, while type II collagen, the main NP collagen, is sharply down-regulated (75).

Up to now, progenitor cells within discs have been poorly characterized. Tie2+ cells in NP tissues have recently been proven to be progenitor cells, with self-renewal capacity and multiple differentiation potential (38). Frequency of Tie2+ NP cells reduces with increasing age and is correlated to extent of disc degeneration, which strongly suggests that exhaustion of these cells may cause IVD degeneration itself.

Relevance of cell and molecular biology to potential therapies for IDD

During the process of disc degeneration, reduction in number of viable cells and phenotypic changes to live ones need to be taken into consideration when designing any biological therapeutic applications. Direct injection of active substance therapy, gene therapy and cell transplantation therapy remain to be promising biological strategies.

Active substances therapy

The most direct technique of delivering active substances to disc cells is injection into the IVD; promising results have been reported. Direct injection aims to promote synthesis of proteoglycan, and restoration of disc height. Many active substances, including multiple cytokines and growth factors, are able to move a cell catabolic state to an anabolic one, which is significant in maintaining IVD homoeostasis. Taking clinical applications into consideration, active substance injection into discs is less invasive compared to traumatic surgery, specially when under guidance of fluoroscopy.

A range of studies *in vivo* has confirmed efficacy of bio-active substances, when used to repair and regenerate degenerate discs (Table 1). In an early murine IDD model, Walsh *et al.* (76) compared regenerative effects of a variety of growth factors, including growth and differentiation factor (GDF)-5, TGF-β, insulin-like growth factor (IGF)-1 and basic fibroblast growth factor (bFGF). IGF-1 and TGF-β induced expansion of inner annular fibrochondrocyte populations which actively expressed aggrecan and type II collagen mRNA. The growth factors tested increased cellularity and improved proliferation and a statistically significant increase in disc height was measured after GDF-5 treatment. In a rabbit IDD model, GDF-5 was also proven to be an effective bioactive substance for its regeneration potential, including restoration of disc height, improvement in magnetic resonance imaging scores and histological grading scores (77). Osteogenic protein (OP)-1 is a promising growth factor for its potential in stimulating production and formation of the ECM. Kawakami *et al.* (78) have demonstrated that OP-1 injection into degenerate rat discs increased ECM and inhibited pain-related behaviour.

Table 1. Growth factors confirmed to have regenerative potential in degenerate intervertebral discs: *in vivo* studies

Growth factors	Carriers	Species	Animal model of disc degeneration	Outcome	Study	Year
TGF-β	(−)	Adult, male Swiss Webster mice	Caudal disc compression	Greater percentage of proliferating cells and increased population of anular fibrochondrocytes	Walsh *et al.* (76)	2004
IGF-1	(−)	Adult, male Swiss Webster mice	Caudal disc compression	Upward trend in cell density	Walsh *et al.* (76)	2004
bFGF	(−)	Adult, male Swiss Webster mice	Caudal disc compression	Upward trend in cell density	Walsh *et al.* (76)	2004
OP-1	(−)	Male Sprague-Dawley rats	Caudal disc compression	Increased extracellular matrix without mechanical hyperalgesia	Kawakami *et al.* (78)	2005
OP-1	(−)	New Zealand white rabbits	Age-related disc degeneration	Increased disc height index and proteoglycan content	An *et al.* (79)	2005
OP-1	(−)	New Zealand white rabbits	Needle puncture	Restoration of disc height; increase in water content and proteoglycan content	Masuda *et al.* (80)	2006
OP-1	(−)	New Zealand white rabbits	Needle puncture	Restoration of disc height and biomechanical properties; increase in proteoglycan and collagen content	Yoshiyuki *et al.* (81)	2006
OP-1	(−)	New Zealand white rabbits	Enzymatic digestion by chondroitinase ABC injection	Reverse of reduction in disc height	Imai Y *et al.* (82)	2007
GDF-5	(−)	Male Swiss Webster mice	Caudal disc compression	Upward trend in cell density and increase in disc height	Walsh *et al.* (76)	2004
GDF-5	(−)	New Zealand white rabbits	Needle puncture	Restoration of disc height, improvement of magnetic resonance imaging scores, and histological grading scores	Chujo *et al.* (77)	2006
PRP	Gelatin hydrogel microspheres	Male Japanese white rabbits	Nucleus pulposus aspiration (0.005–0.008 ng)	Suppressed progress of IVD degeneration	Nagae *et al.* (84)	2007
PRP	Gelatin hydrogel microspheres	Male Japanese white rabbits	Nucleus pulposus aspiration (0.005–0.008 ng)	Preserved disc height and water content; higher mRNA expression levels of PG core protein and type II collagen	Sawamura *et al.* (85)	2009
PRP	(−)	Sprague–Dawley rats	Needle puncture	Fewer inflammatory cells and higher fluid content on MRI	Gullung *et al.* (86)	2011
PRP	(−)	New Zealand white rabbits	Needle puncture	Restoration of disc height; higher quality of chondrocyte-like cells	Obata *et al.* (87)	2012
PRP	(−)	New Zealand white rabbits	Needle puncture	Increased production of extracellular matrix and maintained MRI signal intensity	Hu *et al.* (88)	2012

Active substances listed above have exhibited promising potential in regeneration of degenerate discs, indicating potential for active substance therapy.

TGF-β1, transforming growth factor-β1; IGF-1, insulin-like growth factor-1; bFGF, basic fibroblastic growth factor; OP-1, osteogenic protein-1; GDF-5, growth and differentiation factor-5; PRP, platelets-rich plasma.

Subsequently, in the rabbit IDD models, several studies have shown the regenerative efficacy of OP-1 in degenerate discs (79–82).

However, single growth factor injection may have limitations, as it seems that no single growth factor is potent enough to reverse degenerative trends. As a natural carrier of multiple growth factors, platelet-rich plasma (PRP) has been introduced into the field of IVD regeneration and enjoys some popularity. PRP is used as a fraction of autologous plasma with high platelet concentration. When activated, platelets from PRP release multiple growth factors, including PDGF, GDF-5, TGF-β, vascular endothelial growth factor, bFGF, endothelial growth factor and connective tissue growth factor, among others (83). Some studies have proposed that PRP might be an ideal active substance to serve as a cocktail in a strategy of multiple growth factors (84–88). Nagae *et al.* (84) injected PRP carried by gelatin hydrogel microspheres into rabbit degenerate discs, prepared by NP aspiration. Growth factors released successfully suppressed progress of IVD degeneration. Further work by this team has confirmed that PRP injection preserved disc height and water content and expression levels of proteoglycan core protein and type II collagen were also upregulated (85). In a further study, Gullung *et al.* (86) showed by MRI that PRP reduced inflammatory cells, while increasing fluid content. In a rat IDD model, Obata *et al.* (87) suggested that PRP was able to restore disc height and increase numbers of chondrocyte-like cells. A study from our team using PRP in an early rabbit IDD model, also confirmed its regenerative efficacy (88).

Gene therapy

When genes encoding active growth factors are transfected into disc cells, they stably express the corresponding gene products which help to promote cell proliferation and ECM accumulation. Compared to bioactive substance injection therapy, gene therapy is superior due to its continuous effect of stimulating the sustained expression of ECM. Gene carriers are mainly of two types, viral vectors and non-viral vectors. In one animal study, Seki *et al.* (89) evaluated suppressive effects of injections of ADAMTS5 small interference RNA (siRNA) oligonucleotide and results confirmed its efficacy as a potential non-viral vector for gene therapy of IDD. Non-viral vectors are safer, but transfection rates end to be rather low. Thus, viral vectors are superior in current gene therapies. Sai *et al.* (90) constructed an adeno-associated virus expression system for TGF-beta3, and confirmed its efficacy in enhancing proteoglycan synthesis of earlier and later dedifferentiated NP cells. For AF gene therapy, lentiviral shRNA silencing of CHOP (C/EBP homologous protein), which is apoptosis regulated, was proven to inhibit stretching-induced apoptosis in AF cells and to improve MRI and histological scores in a rat model (91). Moon *et al.* (92) examined biological effects of 'cocktail' therapeutic gene transfer into human IVD cells in three-dimensional cultures. The recombinant adenovirus bore *TGF-beta1* gene (Ad/*TGF-beta1*), *IGF-1* gene (Ad/*IGF-1*) and *BMP-2* gene (Ad/*BMP-2*). Results confirmed that human IVD cultures with triple gene combination transfer demonstrated synergistic amplification effect in proteoglycan synthesis. However, even if production of the respective gene product could be achieved, it should be noted that only if surrounding, starving cells are able to properly respond can an improved matrix be produced. Moreover, with utilization of adenovirus vectors, viruses can transfect other tissues when delivered into the discs (93). As a potential therapy, safety of gene transfer needs to be further investigated, but at the moment, for clinical purposes, non-virus transfection is safer.

Cell transplantation

Cell transplantation is an ideal approach for IVD regeneration, as this type of therapy can increase both number of viable cells and accumulation of matrix components. Complete structure of the IVD is considered to play an important role in limiting the immune response after cell transplantation. Cells from the NP have been reported to express Fas ligand (FasL) which is immune privileged (94,95). Thus, some studies considered the ideal cell candidate may be disc cells themselves (96,97). However, preparation of NP cells for re-implantation is limited as autologous transplantation requires large numbers of cells, and harvesting them from healthy discs may create unnecessary degeneration of the donor's discs.

As a potential substitute for native disc cells, mesenchymal stem cell (MSC) transplantation holds a better prospect for IVD regeneration (98,99). MSCs are capable of long-term self-renewal and can differentiate into a variety of specialized cells. Also, injection of stem cells into the IVD has confirmed them to migrate to the inner AF for repair and regeneration (100). Synergistic effects of certain active growth factors have been reported to be effective in induction of chondrogenic differentiation of bone marrow mesenchymal stem cells (BMMSCs) *in vitro* for potential application in IVD repair (41). When co-cultured with NP cells, MSC exhibited enhanced proliferation and telomerase activity over NP cells cultured alone (101,102). Thus, NP cells and MSCs may be mixed together as seeding cells for disc regeneration applications. Some studies have illustrated stimulatory

effects of notochordal cell conditioned medium on native IVD cells and chondrocytes (103–106). Results clearly demonstrated the ability of notochord-conditioned media to direct differentiation of MSCs. Makarand *et al.* (107) reported evidence for skeletal progenitor cells in degenerate human IVDs. This finding suggested that these endogenous progenitors may be applied to repair degenerate discs. Further investigations revealed locations of these progenitor cells within the IVD (37). A further study identified MSCs from degenerate human NP and indicated that these NP-derived MSCs were similar to MSCs from bone marrow (108). These findings suggest that stimulating endogenous MSCs of the IVD may be a new target for IDD regeneration strategies.

Recently, tissue engineering strategies based on cell transplantation have enjoyed more popularity (109,110). Tissue engineering is the combined use of seeding cells, biological scaffolds and bioactive substances. The biological scaffold provides a more suitable micro-environment to help retain cell morphology and provides primary mechanical stability. In a recent study, a biodegradable AF closure system comprised of a diisocyanate glue, based on polyethylene glycol-PTMC triblock copolymers, a supporting membrane and an adhesive material, was proven to be a promising potential tissue engineering method to restore function of herniated discs (111). Above, we mentioned PRP as a bio-active substance being a promising choice for its regenerative properties – when activated, it forms a gel-like substance. Thus, PRP itself may serve as a good bio-scaffold for cell implantation, as well as being an activator for cell proliferation and differentiation. In the future, combined use of most suitable seeding cells, best biocompatible materials, and active substances to maintain normal cell phenotype, or directional differentiation, seem to be promising for IDD regeneration strategies.

Summary and perspectives

During the process of degeneration, the IVD undergoes multiple cell and molecular changes, including altered phenotype, cell proliferation, cell density, as well as loss of ECM. To maintain stability and metabolic balance of the IVD, multiple cell and molecular factors function together and compromise changes. Now, the most promising therapeutic strategies lie in three fields active substance injection, gene therapy and cell transplantation. With advanced understanding of cell composition of the IVD, multiple strategies can be jointly applied for better regeneration in the near future. However, pathological mechanisms of IDD still remain unclear. Currently discovered stem/progenitor cells within discs have advanced our knowledge of cell biology of the IVD and how these cells might be related to IDD. In the future, cell mechanisms, and how biological therapies affect endogenous stem/progenitor cells within discs need to be further investigated. Further, current studies are mainly focused on regenerative efficacy of biological strategies, but possibility of adverse effects need to be further addressed when applied in clinical use.

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Competing interests

The authors declare that they have no competing interests.

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