Cell-based Therapies for Degenerative Disc Diseases

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Acknowledgements

This work was supported by General Funding from National Science Foundation of China (NSFC, No. 81371993), Small Project Funding of The University of Hong Kong (201409176191), and General Research Fund from Research Grants Council of Hong Kong (GRF, No. 17126615).

Abstract:

Intervertebral discs (IVDs) degeneration are responsible for the majority of cases of low back pain, which affects millions of people worldwide. Current clinical therapies for degenerative disc diseases (DDD) involve medications or physiotherapy for mild to moderate degeneration, and surgeries for severe degeneration. However, current treatments aim to remove symptoms sometimes at the cost of mobility instead of restoring the biological function. Future therapy for disc repair is in development by biological strategies, including protein/growth factor injections, gene therapy or cell therapy approaches. Cell-based therapies provides the greatest hope, as it is a continuous source of cells and cytokine production, and the safety is superior compared to gene therapy. The choice of cell source include chondrocytes, disc cells, embryonic stem cells, hematopoietic stem cells and mesenchymal stem cells (MSCs). Results of several clinical trials suggest that MSCs is the best choice of cell source for IVD regeneration. The remaining concern is that the harsh environment of the degenerated disc may have impact on the engraftment and differentiation of implanted cells. The timing of cell implantation may also have effect on the degree of disc regeneration, which may require a earlier intervention at mild to moderate level of disc degeneration to achieve satisfactory surgical outcome.

Keyword:

Intervertebral disc; Low back pain; degenerative disc diseases; Cell therapies; Mesenchymal stem cells;

Introduction:

Intervertebral discs (IVDs) are fibrocartilaginous tissues connecting the vertebral bodies, contributing about one-third of spinal length. IVDs are important to spinal function by providing stability while permitting motion between the vertebrae. Importantly, the complex structural features of IVDs also enable them to absorb and disperse loads from physical activities. IVD degenerates during time. Degenerative disc diseases (DDD) is a progressive disorder correlated with age. The prevalence is over 70% in the age group of 50 years or younger, and over 90% in those older than 60 (1). IVD degeneration may not necessarily cause pain. Depending on individual difference, it may sometimes be asymptomatic, but it can also be painful, which is termed discogenic pain. Discogenic low back pain accounts for 26%-42% of chronic LBP patients (2), which affects about 70% of the population at some point in their lives. DDD is a musculoskeletal disorder and can be indicative of severe conditions like disc herniation or prolapse, radiculopathy or spinal stenosis. It imposes an enormous socio-economic burden on the affected individual and the health care services, with increased medical expenditure and reduced productivity owing to loss of working hours.

Current clinical therapies for DDD involve symptomatic relief from pain by medications or physiotherapy for mild to moderate degeneration. For severe degeneration, surgeries such as disc arthroplasty, spinal fusion, and disc decompression are often employed as a last resort. However the effect is controversial

(3) (4) and it may cause complications such as adjacent level disease (5) that often warrants further surgical procedures. Such treatment modalities, however, neither arrest the progression of degeneration nor restore the native functional state of the IVD. Recent years, the advances in gene delivery techniques and the identification of adult stem cells has promoted the research on the possibility to treat disc degeneration with the transplantation of viable cells, especially stem cells. Expanding knowledge has enabled diverse therapeutic development and various techniques to manipulate and direct them to specific fates has promoted a stem cell-based regenerative approach to DDD.

IVD structure and Cell Biology

A healthy adult IVD is composed of three morphologically distinct components: the central gelatinous nucleus pulposus (NP), which contains chondrocyte-like cells, surrounded by a lamellated fibrous annulus fibrosus (AF) containing fibroblast-like cells, and capped by two cartilaginous endplates (EP) that anchor into the adjacent vertebrae. The IVD is rich in extracellular matrix predominantly represented by collagen and proteoglycans with distinct differences in each compartment (6). NP is rich in aggrecan and type II collagen, while AF contains type I collagen with little proteoglycans. In fact, collagenous proteins comprise 70% of the outer annulus dry weight, but only 20% are located in the central NP, while proteoglycans make up 50% of the NP (7). Although the component of NP matrix is similar to that of articular cartilage, there is much differences in the gene expression profile and matrix synthesis

(8). The density within the NP tissue is quite lower at around 5,000~6,000 cells/mm³ (9) compared with 15,000 cells /mm³ in cartilage (10). The proteoglycan : collagen ratio is also different: it is 27:1 in the NP, and 2:1 in the cartilage (11). High proteoglycan content is a important feature of the NP. The high positive charge of proteoglycans gives the NP a high osmotic potential, which draws in water and enable the NP to absorb mechanical shock and deliver it horizontally to AF. It also inhibits the ingrowth of nerve (12). Evidence has shown that the loss of proteoglycan lead to nerve and blood vessel ingrowth in an ovine annular lesion model of experimental disc degeneration (13). Due to the above mentioned reasons, NP is the most critical component of the IVD and is the region where degenerative changes first occurs. It is also the focus of IVD regeneration researches.

The Etiology of DDD

The etiology of DDD is complicated in origin. It can include occupational exposure, mechanical influences, injury, lifestyle, and genetic pre-deposition. Moreover, strong evidences have confirmed that the disturbance in the biology of the cells in the NP, AF and EP, which are significantly affected by the diffusion of nutrients and oxygen from the blood vessels (14,15), the soluble factors in the niche (16), the mechanical loads (17) as well as ageing or senescence (18,19), plays a key role in the regulation of the IVD degeneration. However, in spite of the pre-deposition factors and secondary causes, it is believed that mechanical overload is one of the initiating factors of the disorders of the IVD. The normal adult IVD is avascular and aneural

with the exception of the outer third of the AF. When IVD degenerates, the NP is disorganized, and the proportion and types of extracellular matrix proteins changes which makes the NP more eosinophilic. Overload leads to breakdown of the matrix of the NP with the formation of fissures that eventually extend into the AF. There is often also disruption of the collagen fiber orientation in the AF, traumatic damage to the end plate, and blood vessel (20) and nerve ingrowth (21) (22) into the inner AF and NP. Which is normally avascular and aneural.

Molecular Changes in the Degenerated IVD

Molecularly, DDD is characterized by cell death and degeneration of extracellular matrix. Normal NP cells are characterized by expression of type II collagen and proteoglycans. In disc degeneration, matrix remodeling (23) leads to an increase in collagens I and III and decreased production of aggrecan. In degeneration, the anabolic : catabolic balance is broken, there is a net increase in matrix-degrading enzyme activity over natural inhibitors of such activity, which leads to loss of disc matrix. Increased degraded fragments of proteoglycan (24) (25) and other matrix proteins (26) was reported, which has the potential to trigger further inflammatory changes (27) (summarized in (28)). Aggrecan has two cleavage sites, one acted upon by MMPs and the other by members of a group of enzymes called the ADAMs family (29). Matrix metalloproteinases (MMPs) and ADAMs have both been implicated in disc degeneration (30) (31).

Degenerated IVDs are demonstrated to be in a chronic inflammatory state with increased expression of multiple pro-inflammatory cytokines, including interleukin 1 (IL-1), matrix metalloproteinase 10 (MMP10) (32), IL-8, tumor necrosis factor- α (TNF- α) (33), IL-10 (34), IL-2, IL-4, IL-17, monocyte chemotactic protein-1 (MCP-1) and prostaglandin E2 (PGE2) (35,36). Of these, IL-1 is particularly interesting, because IL-1 plays more important role in mediating disc matrix degradation than TNF- α (16). IL-1b also leads to production of MMPs and pain mediators, such as the eicosanoid prostaglandin E2, by human IVD cells (37).

Potential Developing Treatments for Disc Degeneration

One of the problems of current treatments is their inability to repair the disc. Instead the aim of current treatments is more about removing symptoms such as pain and sometimes this is realized at the cost of mobility in the case of spinal fusion. As the future therapy for disc repair, protein/growth factor injections, gene therapy or cell therapy approaches is in continuous development.

Growth factors are small proteins with pleiotrophic effects on cells, including stimulation of cell differentiation and division. Several growth factors have been investigated for their effect on disc regeneration *in vitro* or *in vivo*. These include bone morphologic protein 2 (BMP2) (38), BMP7 (39), BMP12 (38), BMP13 (40), BMP14 (growth differentiation factor 5, GDF-5) (41), transforming growth factor beta (TGF-B) (42), Insulin-like growth factor 1 (IGF-1) (43) (44), basic fibroblast

growth factor (bFGF) (45), tissue inhibitor of metalloproteinase (TIMPs) (46), platelet-derived growth factor (PDGF) (43) (47), and so on. They are implied with positive outcomes suggesting restoration of the degenerated NP. The effect includes increased proteoglycan content and enhanced proliferation. Noteworthy, some BMPs may increase the risk of ectopic bone formation within the IVD (48), which may potentially be inhibited by BMP13 (49).

Gene transfer involves the delivery of exogenous genes to target cells, resulting in gene expression and subsequent production of proteins. Gene therapy is one way to modify the biological activity of the IVD cells. Retrovirus mediated transfer (50) of two different exogenous genes (bacterial b-galactosidase, LacZ; and human interleukin-1 receptor antagonist, IL-1Ra) to cultured bovine intervertebral end plate cells resulted in the production of IL-1Ra in 48 h, implicating the host tissues are capable of the synthesis of regeneration drugs. Adenoviral transduction of degenerate human NP cells with anti-catabolic enzyme, TIMP-1 or BMP-2, resulted in increased proteoglycan synthesis *in vitro* (46). Likewise, adenovirus mediated transfer of CTGF and TIMP-1 genes to NP cells resulted in increased proteoglycan and collagen II production (51) in rhesus monkey and rabbit animal models. In the latter study, transplantation of the modified cells back into the rabbit IVD resulted in a reversal of disc degeneration (52). Therefore, the ability of degenerate NP cells to produce the required matrix proteins could be revived through genetic modification, after which the transgenic cells can be transplanted into the degenerate IVD to restore its function.

Growth factor therapy and gene therapy has their disadvantages. The caveat with growth factor injection is that the therapeutic effects could not last long, and repeated injection may cause damage to the AF. Another concern is that at advanced levels of disc degeneration, the endogenous cells may no longer be responsive to such stimuli or there may be insufficient number of functional cells in the IVD to begin with (53) (54). The drawbacks of gene therapies are that viral vectors are often used for gene transfer, so their safety and effectiveness needs to be thoroughly evaluated before being employed in human trials. Conversely, cell based therapies offer an attractive solution to current treatments. Cell therapy does not have the problem of short-lived effect of growth factor injections. The cells transplanted is a continuous source of cytokine production. The safety is not an issue, as transplantation study has demonstrated their safety in other tissues or organs before. The only problem of concern is the viability and proliferation of the implanted cells, whether these cells can engraft and proliferate in the harsh environment of the degenerated disc. Numerous researches has been done in this aspect. The details were summarized as below.

Cell therapies for Disc Repair

In history, the investigated choice of cell source for disc repair include chondrocytes, disc cells, embryonic stem cells, and mesenchymal stem cells.

1. Chondrocytes

As adult NP cells are chondrocyte like cells, attempts have been made to regenerate the disc with cartilage cells. In a rabbit study (55), the nucleus pulposus was evacuated and replaced with tissue engineered autologous chondrocytes from auricular cartilage. The implanted cells survived well in the host for up to six months, and hyaline-like cartilage formed in the place of the NP. In a porcine model (56), allogeneic juvenile chondrocytes injection into denucleated discs demonstrated abundant cartilage formation at 3 months, and to a lesser extent at 6 and 12 months. Overall, transplantation of chondrocytes to regenerate the disc is feasible, but the generated tissue is cartilage like instead of nucleus pulposus like.

2. IVD Cells

Based on numerous *in vitro* and *in vivo* studies on rat, rabbit, canine, porcine, ovine, bovine and human, IVD cells, including notochordal cells and NP cells are potentially a good choice for transplantation procedures. Their ability to increase the proteoglycan content, viability upon transplantation and established protocols to expand them *in vitro* are advantageous for cell therapy (57) (58). For instance, implantation of autologous NP cells or entire NP retarded disc degeneration (59) (60) (61). Also, injection of immortalized human NP cells into degenerate rabbit disc preserved the matrix of NP and decelerated its degradation (58).

Despite positive results of animal studies of IVD cells, the acquisition of healthy

donor IVD cells either allogenic or autologous has for-see difficulty. It is noteworthy though that cells harvested from a degenerated IVD may not be suitable as they exhibit senescence with an altered molecular phenotype and distinct pathology (62). Similarly, autologous cells harvested from patients who are genetically susceptible to DDD may prove inferior for cell therapy since they are already pre-disposed to degeneration (63). More importantly, harvesting adult NP or AF cells from prospective healthy discs of the patients is also counterproductive as it could result in increased morbidity or further damage to the IVD. Allogenic sources may be the alternative; but there is lack of evidence in human studies. In addition, obtaining healthy notochord cells is not a feasible option owing to the absence of such cells in adults.

3. Embryonic Stem Cells

Stem cells possess multi-lineage differentiation potential and self-renew capacity. Stem cells hold great promise for regenerative therapy. They are unique in that they are pluripotent (e.g. embryonic stem cells, ESCs) or multipotent (e.g. mesenchymal stem cells, MSCs), possessing the ability to self-renew as well as differentiate into specific cell lineages. They could be utilized if they could be successfully directed to a NP or AF cell fate before transplantation.

ESCs are derived from inner cell mass of blastocysts, and have an advantage over MSCs as they can give rise to cells from all three germ layers (endoderm, mesoderm

and ectoderm) and exhibit extensive proliferation ability *in vitro*. In spite of this, their usage in clinical settings is hindered by a number of other factors such as: difficulties in acquisition owing to ethical concerns, immune rejection after allogeneic transplantation, risk of disease transmission and the possibility of teratoma formation. The use of ESC-derived chondro-progenitors may be an alternative option. In a rabbit study, at 8 weeks post-ESC derived chondro-progenitors implantation (64), islands of cell growth were observed.

4. Mesenchymal Stem Cells

MSCs is the most intensively investigated type of stem cells for intervertebral disc repair. MSCs are firstly derived in bone marrow but later found in many tissues or organs. They are characterized by their tri-potency (osteogenesis, chondrogenesis, adipogenesis) and marker expression (65). The safety and efficacy of MSC implantation for tissue regeneration has well been documented (66) (67). *In vitro*, MSC has been proved to be able to stimulate the proliferation, and the synthesis of collagen II and aggrecan, of cultured NP cells, in co-culture system (68) (69) (70). Numerous on-going studies by different research groups also highlighted the potential of MSCs to differentiate into NP-like phenotype (71) (72) (73) (74). *In vivo*, MSCs have been injected into mouse (75), rabbit (76), rat (77), canine (78), and porcine (79) IVD, with encouraging results. MSCs might be the most idea candidate of cell therapy for disc repair. This is because they have several advantages. First, They have no ethical concerns as ESCs have. Second, their safety is evidenced, they do not form

teratoma. They are adult stem cells which means they could be harvested easily from the patient self. Allogenic transplantation is also feasible due to the capacity of MSCs to escape immune surveillance due to the lack of HLA class II antigen presentation.

Published Clinical Trials

Cell therapy for disc repair has already marched into clinical phase. The outcome of several clinical trials has been published. The utilized cell source include HSCs, IVD cells, chondrocytes, and MSCs.

The effect of HSC on disc repair is minimum. In a HSC transplantation study with 10 patients enrolled (80), no improvement was recorded in pain assessment after 1 year post transplantation. However, the cell source used in this study is of doubt. As the author described, ‘A total of 5 cc of bone marrow aspirate was obtained. Next, 1 cc of HSCs were inserted into each of the problematic discs.’ It is not clear whether the authors have purified CD34+ HSCs from the bone marrow for the transplantation, or they just injected raw bone marrow. This is different as bone marrow not only contains HSCs. It also harbors MSCs, monocytes and contains various growth factors which may potentially affect the disc. Generally, HSC may not be a suitable source of stem cells for IVD repair as it is not reported to be a type of progenitor cells to give rise to cells of mesodermal origin.

Chondrocytes has been implicated for their effect in disc repair clinically. In a US

study (81), fifteen patients were treated with a single percutaneous delivery of $1\sim 2 \times 10^7$ NuQu juvenile chondrocytes with fibrin carrier. Pain and life quality, represented by the mean ODI (Oswestry Disability Index), NRS (Numerical Rating Scale), and SF-36 (36-Item Short Form Health Survey) physical component summary scores all improved significantly from baseline. At 6 months follow up, 77% of the examined patients showed MRI improvement, and 23% showed improvement in disc contour or height.

There is also one study documenting injection of disc cell could alleviate disc degeneration. In a Germany study, autologous disc cell transplantation (82) in 12 patients resulted in reduced pain, maintained disc height and water content at 2 years post transplantation, when compared to the control group constituted by 16 patients who received discectomy-only.

More clinical evidence support MSCs as the idea source of cell for IVD regeneration. In a Japan study (83), autologous bone marrow MSCs carried in collagen sponges was transplanted into the surgical discs of 2 patients. 2 year after the transplantation, low back pain and lower leg numbness was improved. Signal intensity on T2-weighted MRI was high, indicating high moisture content in the discs. However, the disc height has not changed and the sample size in this study was too small to confirm the long term effect of MSC transplantation clinically. Later, in another MSC transplantation study (84) in 10 patients, 9 patients achieved low back pain relief within 3 months,

accompanied by a parallel improvement in disability and physical life quality. Similar to Yoshikawa's observation, the disc height has not improved. But the ratio of fluid content increased at 12 month post-op. The analgesic effect of treatment with MSC approached 71% efficacy, which was explained as the potential induction of anti-inflammatory cytokines by the MSCs.

It is noteworthy that the therapeutic efficacy may be closely associated with the purity of MSCs within the injected material. A recent study investigated the effect of autologous bone marrow concentrate injection into surgical discs. At 2 years post injection, there was a significant reduction in pain. However, for degeneration grade as assessed by the modified Pfirrmann scale, only 40% of the patients improved by one grade, while the remaining twelve patients maintained their previous score. The outcome of this study is not as satisfactory as the other clinical trials using MSCs. As the same study suggested that the degree of pain relief was linked to the concentration of MSC (or fibroblastic colony forming units) in the bone marrow concentrate, the less than satisfactory outcome may be partially related with the fact that MSCs only exists in the bone marrow at a very low frequency (85), so the quantity of MSCs injected in this study is far less than the dose in the other studies.

Discussion

Because man is the only obligate bipedal vertebrate, replicating human disorders of the IVD in other animals has proved problematic. The results obtained in animal

studies may not be replicated in human. This endows importance of clinical studies for the efficacy of disc regeneration therapies.

Though chondrocytes have been proved to be effective in alleviating disc degeneration (81), using chondrocytes to repopulate the disc may be debated. In one way this may help to restore some of the disc spaces and function, in another sense this could be challenged, as the formation of hyaline cartilage instead of a gelatinous NP is against nature and may cause further long term problem. In theory and in practice, disc cells are a better source than chondrocytes. However based on the discussed reasons, there is difficulties and complications in the harvest of donor disc cells and the quality is a issue. ESCs derived chondro-progenitors may still have, but less, ethical issue as ESCs, besides the differentiation into NP like cells *in vitro* before transplantation is yet to be established. MSCs is, and will remain as, for the foreseeable future, the best choice of cell source for IVD regeneration.

Currently, the study on the utilization of MSCs in disc repair mostly focus on bone marrow derived MSCs. Nevertheless, MSCs can be isolated from many organs and tissues with distinct differences in characteristics and differentiation potential (reviewed in (86)). Bone marrow derived MSCs may not be the most superior source due to its high spontaneous osteogenic tendency (87). Instead, umbilical cord derived MSCs has been shown in our study to exert a higher regeneration effect than bone marrow derived MSCs in stimulating NP cell towards a healthier phenotype *in vitro*

(88). More researches on alternative MSC sources will be beneficial to reveal the ideal candidate source of MSC for IVD regeneration.

It is noteworthy that the implantation of MSCs into degenerated discs consensually generates satisfactory result on pain relief and disc moisture content, but has no effect on improving the disc height, as demonstrated in the three clinical studies above (83) (84) (85). These may be because the implanted MSCs need time to produce swelling matrix, but the mechanical load on the human IVD is too high that the implanted MSCs can only produce enough matrix to maintain but not increase the disc height. To get a better outcome with increased disc height, injection with an appropriate scaffold may be of use to help to alleviate the mechanical load on the disc space and injected cells and allow more time and space for cell propagation and matrix production, which are important to maintain disc height. A range of injectable scaffold can be considered as they have been tested in animal studies. These include fibrin carrier (81), atelocollagen gel (89), alginate capsules (90), amalgam (91) or chitosan-glycerophosphate hydrogel (92), etc. Thermogelling and in situ-forming scaffolds may be a good choice to alleviate implant expulsion under mechanical stress; The components are temperature sensitive, they remain as flowable at around room temperature, while change into compact hydrogel at around 37°C (93).

The disc microenvironment is hostile. The avascular situation, nutrient deficiency, low oxygen concentration, increased acidity, and mechanical loading, all affects the

survival, proliferation and differentiation of MSCs (94). Nutrient deprivation may present challenge but not a lethal threat to MSCs (95) (96). Hypertonicity, as well as increased acidity (95) (96), may inhibit the viability and matrix synthesis of MSCs. Despite the above, not all characteristics of IVD niche have bad influence for MSCs. Hypoxia is favorable for the chondrogenic differentiation of MSCs. Dynamic compression may promote NP-like differentiation (97), while radial compressive loadings may induce AF-like differentiation, in MSCs (98). Considering the timing for stem cell transplantation matters, that MSCs survives better in mild to moderate degenerated discs than severely degenerated ones (99), performing intervention of MSC implantation at earlier stage of disc degeneration may help to alleviate the stress of degenerated disc niche on MSCs and thus may promise a better clinical outcome.

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