Stem Cell Therapy for Intervertebral Disc Degeneration

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Abstract

Low-back pain is one of the major health problems in western society and is a leading source of disability in people under 45 years of age; intervertebral disc degeneration (IDD) has been identified as a main cause of this problem. Recent advances in our understanding of the biology of the intervertebral disc (IVD) have led to increased interest in the development of novel treatments. Stem cell therapy might support IVD regeneration by overcoming the limitation of self-regeneration, which is considered the main cause of degeneration-mediated functional loss of the IVD. The potential use of different adult stem cell types, such as undifferentiated and pre-differentiated bone marrow mesenchymal stem cells, adipose tissue stem cells, and muscle-derived stem cells, has been described, with promising prospects. This article reviews the current existing literature on the status of stem-cell-based therapy for IDD and the critical issues that remain to be resolved before clinical application can be realized.

Keywords

Intervertebral disc degeneration, mesenchymal stem cells, nucleus pulposus, tissue engineering, differentiation

Pathophysiology of Intervertebral Disc Degeneration

Back pain (BP) is a common clinical condition that leads to high morbidity with significant psychosocial and economic effects. It is the leading cause of disability in people under 45 years of age, and it results in enormous national economic losses in developed countries. The wide majority of BP is associated with degenerative change of the intervertebral disc (IVD).

The IVD is composed of the inner nucleus pulposus (NP) and the outer annulus fibrosus (AF). The AF is a fibrocartilaginous ring composed of concentric dense lamellae of highly oriented collagen type I fibers in which there are cells with the morphology and phenotype of fibroblasts. The NP is a less structured gelatinous extracellular matrix (ECM) that is rich in proteoglycans, mainly aggrecan, and type II collagen. Aggrecan comprises a great number of negatively charged sulfated glycosaminoglycans that attract and imbibe water. The high level of hydration helps to maintain disc height and contributes to the load-bearing ability of the IVD. Small chondrocyte-like cells are scattered within the NP and are responsible for synthesizing and maintaining the ECM.

Intervertebral disc degeneration (IDD) is an age-related chronic process that is characterized by a progressive reduction of proteoglycans and water content in NP with loss of the disc’s ability to resist compressive forces ensuring stability. BP is the first symptom of IDD that may progress to multiple spinal disorders such as disc herniation, degenerative spondylolisthesis, instability, and spinal stenosis associated with neurological symptoms such as radiculopathy and myelopathy. Current treatment options for BP and IDD range from conservative measures such as bed rest, anti-inflammatory medication, analgesia, physical therapy, and bracing to invasive procedures such as epidural steroid injections and ablation techniques, as well as surgical procedures such as discectomy, total disc replacement, and spinal fusion. However, these treatment modalities have limited efficacy and do not produce predictable and reliable outcomes. In fact, they target the clinical symptoms as opposed to targeting the pathophysiology involved in the degenerative process. Therefore, there is a clear need for more effective early treatment of BP that may prevent, slow down, or reverse the degenerative changes in the IVD. Exciting advances in the fields of molecular and cellular biology have allowed spine researchers to better characterize the degenerative pathways of the IVD and to develop novel regenerative therapeutic approaches in order to alter the course of IDD and possibly lead to disc repair and recovery of function.
the IVD during the degenerative process. At the biochemical level, the diminished proteoglycan content reflects an imbalance in the normal anabolic and catabolic functions of the NP cells resulting from decreased synthesis, increased catabolism, or a combination of these two processes. In addition, a progressive decrease in cell density is associated with aging and IDD, and has been thought to compromise the disc’s ability to compensate for these changes by producing and maintaining a functional ECM. The result of reductions in the proteoglycan content of the NP is dehydration, decreasing disc height, and alteration of its load-bearing capacity. Anomalous distribution of forces across the disc results in cracking and fissuring of the AF, disc herniation, and vertebral body pathology, including subchondral sclerosis, end-plate ossification, and osteophyte formation. The inherent avascularity, isolation, and low metabolic activity of the IVD may be key reasons for the disc’s apparent inability for self-repair following injury and degeneration.

Potential Biological Treatments

Recovering the disc’s ability to repair the ECM and re-establish the proteoglycan content may have therapeutic effect by increasing disc hydration and thereby improving its biomechanics. Several genes that have been found to have a significant impact on ECM synthesis and catabolism within the disc may be targeted to alter this balance by using recombinant growth factors or gene therapy technologies. Intra-discal injection of growth factors has been shown to raise the proteoglycan level of the disc.

However, the short half-life of exogenous growth factors has led to increased interest in the potential use of gene therapy in the treatment of IDD as a means to overcome this potential shortcoming. Gene therapy allows modulation of gene expression in order to obtain a prolonged production of a gene product. Numerous reports have shown that anabolic factors, antianabolic factors, and gene regulators are able to modulate the activity of disc cells, thereby boosting proteoglycan disc content. However, work on more efficient gene delivery systems is ongoing, and the safety issues surrounding gene therapy make it an emerging technology that still requires more research before clinical application.

The progressive cell loss seen in IVD aging and IDD may be treated by introducing exogenous cells to supplement and replenish the disc cell population. A cell therapy approach has been described using different type of cells such as disc cells, cartilaginous chondrocytes, and progenitor cells. Autologous NP cells are currently under clinical investigation, and two-year follow-up has shown a decrease of BP and prevention of disc narrowing. However, this approach is limited by the poor expansion rates or the loss of phenotypic characteristics when expanded in monolayer cell culture, and it is applicable only when discectomy is required. Therefore, stem cell therapy is more attractive due to the low harvest-site morbidity, ease of ex vivo cell expansion, and favorable modulation of the cell phenotype before or after transplantation. In this review we will discuss the potential use of stem cells for disc repair.

Stem-cell-based Therapy

By definition, stem cells are undifferentiated cells with high proliferation capability, being capable of self-renewal and multi-lineage differentiation. There are different stem cell types, ranging from embryonic to adult stem cells. Embryonic stem cells are considered to be totipotent, but ethical and practical questions encumber their use in regenerative medicine. Adult stem cells reside in fully differentiated adult tissue such as bone marrow, fat, muscle, and skin, to name just a few. Their function is related to the maintenance of the functional characteristic of each specific tissue. Theoretically, these cells are capable of producing a limited range of differentiated cells related to the embryonic origin of their tissue. The use of these cells does not raise ethical problems in regenerative medicine, as they can be isolated from the patient.

The potential use of different adult stem cell types, such as bone marrow-derived mesenchymal stem cells (MSCs), adipose tissue-derived stem cells (ASCs), and muscle-derived stem cells (MdSCs), has been described for IVD regeneration. All of these adult stem cell types share the characteristic of undergoing differentiation to lineages of mesenchymal tissues, including bone, cartilage, fat, and muscle. Recently, although bone marrow MSCs have been identified as sinusoidal adventitial reticular cells, ASCs and MdSCs have been shown to share their phenotype with pericyte/perivascular cells. Therefore, it may be speculated that all the stem cells of mesenchymal origin, such as bone marrow MSCs, ASCs, and MdSCs, derive from the perivascular wall.

There are several possible therapeutic strategies for IVD regeneration using adult stem cells:

- they can be injected directly into the IVD as undifferentiated or pre-differentiated cells (see Figure 1);
- they can be used in conjunction with a visco-elastic scaffold, as popularized by tissue engineers; or
- they can be modified genetically by transfecting genes of interest into them and injecting the transfected stem cells into the IVD as ex vivo gene therapy strategy.

Recently, a population of progenitor cells has been identified in the degenerated human IVD. Therefore, a final strategy for treatment is to recruit these endogenous progenitors to orchestrate IVD repair by administration of suitable drugs/growth factors.
Stem Cells for Intervertebral Disc Degeneration

In the past few years, several in vitro studies have been conducted to evaluate the use of adult stem cells for possible use in the treatment of IDD. The capacity of adult human MSCs to differentiate towards NP cells has been one of the first steps in the evaluation of their use as a cell source for IVD regeneration. Indeed, MSCs have been cultured under conditions conducive to chondrogenesis, thereby demonstrating differentiation along a phenotype consisting of NP cells. These methods have been considered a pre-conditioning system to direct MSCs into NP-like cells before they are implanted into the IVD.

The stem cell therapeutic effect has also been studied. In vitro studies suggest that the regenerative potential of MSCs may result from MSCs and NP cell interactions that upregulate ECM protein synthesis. We carried out experiments whereby bone marrow MSCs were co-cultured with NP cells in a 3D pellet culture system. The data from this study revealed a synergistic effect between NP cells and MSCs, yielding the greatest increase of the proteoglycan synthesis rate and glycosaminoglycan (GAG) content compared with a culture of NP cells and MSCs alone.

The therapeutic effect has also been tested using a co-culture system with other adult stem cell types. Li et al. demonstrated that rabbit ASCs co-cultured in 3D alginate beads with NP cells showed an increase in expression of type II collagen and aggrecan genes compared with NP cells alone. In addition, our group showed that MdSCs co-cultured with NP cells resulted in a significant increase in GAG content compared with NP cell culture alone. Thus, the adult stem cells of mesenchymal origin favorably interact with disc cells, inducing an enhancement of the ECM component of the disc.

Identification of the mechanisms of the observed interaction between MSCs and NP cells has been the target of several recent studies. Stem cells undergo differentiation under stimuli that come from the surrounding microenvironment. In addition, they contribute to tissue repair, also creating a microenvironment that promotes the local regeneration of cells endogenous to the tissue. Richardson et al. co-cultured human MSCs with normal NP cells in a monolayer with or without cell-to-cell contact. MSCs co-cultured with contact underwent a change in gene expression profile similar to NP-like cells, as demonstrated by an increased expression of aggrecan and collagen type II genes. However, the low cell density in the NP makes direct cell-to-cell contact a rare event if stem cells are implanted in the disc.

Therefore, our group studied the mechanisms of the interaction between human NP cells from degenerating discs and MSCs in a 3D culture system that allows short distance paracrine interactions typical of the NP tissue. Using a double labeling cell system (see Figure 2), changes in gene expression profile were analyzed on the individual MSCs or NP cell populations isolated from their co-culture. MSCs acquired a more chondrogenic gene expression profile and influenced messenger RNA levels of the human NP cells. MSCs secrete a variety of cytokines and growth factors that have the potential to stimulate mitosis and tissue-intrinsic reparative potential of the host cells. This trend was only partly reflected in our gene expression data. Although we observed an increase in collagen type II by NP cells after co-culture with MSCs, we observed a downregulation of aggrecan. Accordingly, with the trophic effect, Yamamoto et al. reported increased cell viability and proliferation of NP cells induced by cell-to-cell contact with MSCs in a co-culture system. However, as positive effects have been observed in the new synthesis of proteoglycans under co-culture conditions, further studies must be carried out to determine whether the exposure of degenerated NP cells to MSCs has a net trophic effect.

The mechanisms of the interaction between stem cells and NP cells were also studied with an ASC population. ASCs undergo a change in gene expression profile more similar to NP-like cells when cultured with NP cells by paracrine interactions.
Cell fusion has been found to be potentially responsible for the plasticity and tissue regeneration potential of adult stem cells. The possible occurrence of cell fusion in the interaction between MSCs and NP cells has been studied by our group. Cell fusion was examined in a pellet co-culture system to accentuate cell-to-cell interactions, demonstrating that cell fusion does not occur in the interaction between MSCs and NP cells. 48

**Intradiscal Stem Cell Transplantation in Animal Models**

The IVD is the largest avascular organ of the body, with low pH and oxygen partial pressure. MSC engraftment and long-term survival in this harsh environment of the normal disc tissue has been demonstrated by several studies. 30, 34, 36 Our group assessed MSC survival in the IVD for six months after transplantation in healthy rabbit lumbar IVD. 30

However, to determine the efficacy of a stem cell therapy approach in preventing or delaying the progression of IDD, it is critical to test rigorously the proposed strategies in animal models of IDD that closely simulate the human condition. Sakai et al. showed that autologous bone marrow MSCs embedded in Atelocollagen®, a collagen type II-based carrier, and injected in an NP aspiration model of IDD enhanced proteoglycan content, disc height, and hydration. 29, 30 A longitudinal magnetic resonance imaging (MRI) follow-up for a six-month period showed that the MSC-injected disc maintained an MRI signal intensity, whereas the degenerating disc control group underwent an MRI signal intensity reduction. 41 In addition, Sakai et al. demonstrated that undifferentiated MSCs transplanted into degenerated discs in rabbits proliferated and differentiated into cells expressing some of the major phenotypic characteristics of NP cells, suggesting site-dependent differentiation of MSCs. 48

Recently, MSC injection has been tested in a larger animal model. Hiyama et al. evaluated the effect of autologous MSC transplantation on the suppression of disc degeneration and preservation of immune privilege in a canine model of IDD. 48 MSC injection effectively led to the regeneration of degenerated discs and contributed to the maintenance of IVD immune privilege. 48

Henriksson et al. transplanted human MSCs in injured porcine spinal discs. This xenotransplantation model in minipigs showed that human MSCs survive in a degenerated disc for up to six months and express SOX9 and collagen II after transplantation, indicating differentiation toward disc-like cells. 30 In addition, these authors showed that a 3D hydrogel carrier seems to facilitate the differentiation of transplanted MSCs.

**Toward Clinical Trials**

In order to move toward successful human clinical trials, it is critical to test rigorously the long-term effects of stem-cell-mediated strategies in animal models of disc degeneration that closely simulate the human condition on disc biology, nutrition, and biomechanical function, such as larger animal models or primates.

The patients who could be expected to benefit from stem-cell-mediated therapy are those with mild or moderate grades of IDD in whom the structural integrity of the disc remains preserved (see Figure 3). Because the nutrition supply to many degenerated discs is poor, there is theoretical concern over the added nutritional demands arising from the increased number of metabolic active cells into the disc after transplantation. 31

Therefore, evaluation of the nutrition transport into the IVD using microelectrodes able to evaluate oxygen or nitrous oxide diffusion 31 may be useful in order to select the patients who could benefit from the treatment.

**Conclusion**

Thanks to the research efforts aimed at further developing our knowledge of the biology and biochemistry of the IVD, our understanding of the process of IDD is growing rapidly. Identification of the importance of cell loss within the disc has led to a focus on novel treatments aimed at regenerating the degenerating tissue. With adult stem cell therapy’s unique ability to differentiate into different cell types and to secrete a range of trophic cytokines, it has received considerable interest and shown much promise in terms of treating chronic conditions such as IDD.

Multiple studies have demonstrated the feasibility of adult stem cell therapy for IDD, and recent studies have demonstrated proof of efficacy of MSC transplantation in reproducible animal models. Adult stem cell therapy has shown promise in becoming a powerful tool in the future treatment of IDD.

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**References**