MESENCHYMAL STEM CELLS FOR INTERVERTEBRAL DISC REGENERATION

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Low back pain (LBP) is one of the most common disabling symptoms affecting the adult population throughout the industrialized world. The main cause underlying this condition is intervertebral disc degeneration (IDD), which is characterized by progressive decrease of the proteoglycan content within the nucleus pulposus (NP), leading to disc dehydration and loss of its morpho-functional and biomechanical properties. To date, LBP treatment is based upon conservative and invasive procedures which are not capable of restoring the degenerative alterations of the disc, as they only help relieve the symptoms and/or slow down disc degeneration and are, nonetheless, characterized by significant comorbidities, costs and secondary risks. The potential use of different mesenchymal stem/stromal cells (MSCs) for treating IDD has been promisingly tested *in vitro* and *in vivo*. The combination of different cell types, preconditioning culture conditions, engineered scaffolds and delivery systems have yielded proof of disc matrix reconstitution, increased cell viability and tissue regeneration in several experimental settings. This article reviews the current literature on stem cell-based therapy for IDD and the outcomes that diverse approaches have achieved.

Low back pain (LBP) is a musculoskeletal symptom affecting more than 80% of the general population throughout their life, leading to high morbidity with great psychological, social and economic burdens. LBP attests itself as the first cause of disability in people under 45 years of age, especially among female individuals, hence resulting in huge national economic losses in developed countries (1).

LBP is mainly caused by degeneration of the intervertebral disc (IVD). The IVD consists of three highly specialized tissues: the inner nucleus pulposus (NP), the outer annulus fibrosus (AF) and the cartilaginous end-plate, which connects the disc with the adjacent vertebrae. The AF is a fibrocartilaginous ring, composed of concentric, dense lamellae of highly orientated type I collagen fibers which constitute an organized matrix hosting fibroblast-like cells. The NP is an amorphous, gelatinous matrix rich in proteoglycans (mainly aggrecan) and type II collagen. Aggrecan is a large-aggregating proteoglycan which is capable of interacting with hyaluronan and is provided with several negatively charged sulfated glycosaminoglycans (namely chondroitin sulfate and keratan sulfate) that are able to bind water. The high level of hydration produces a swelling pressure which is responsible for maintaining the disc height and contributes to provide the disc with mechanical resistance to twisting, bending and compression. Small chondrocyte-like cells can be found within the NP and are responsible for synthesizing the proteoglycans thus maintaining the matrix environment (2).

Intervertebral disc degeneration (IDD) in an age- and disease-related chronic process which is characterized by a progressive decrease of the proteoglycan (and consequently water) content in the NP, with the subsequent loss of the disc ability to resist compressive forces, leading to instability (3).

IDD may evolve to several spinal disorders such

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as disc herniation, degenerative spondylolisthesis and spinal stenosis associated with neurological symptoms including radiculopathy and myelopathy. Current treatment options for LBP and IDD range from conservative approaches such as rest, analgesic and anti-inflammatory medication and physical therapy, to invasive procedures, including epidural steroid injections and ablation techniques as well as surgical solutions, e.g. discectomy, total disc replacement, laminectomy and spinal fusion (4). However, these options have provided limited efficacy and do not produce preventable and reliable outcomes. Indeed, they act in order to relieve clinical symptoms instead of targeting the pathophysiology that underlies the degenerative process. For this reason, there is an emerging need to find an early treatment for LBP that may prevent, slow down, halt or even reverse the degenerative alterations of the IVD.

Significant advances in the fields of stem cell therapies have helped spine to develop innovative regenerative therapeutic approaches aiming to alter the natural course of IDD and possibly leading to disc repair/regeneration and recovery of function. The aim of this review is to discuss the potential use of mesenchymal stem/stromal cells (MSCs) from bone marrow for disc regeneration.

Pathophysiology of intervertebral disc degeneration

The detailed aetiology and pathophysiology of IDD still remains poorly understood. However, the progressive loss of aggrecan and free water content in the NP is known to be the major structural change in the IVD involved with the degenerative process (2). This seems to alter the normal balance between anabolic and catabolic functions of the NP cells, resulting in decreased synthesis, increased turnover or a combination of these two processes within the NP matrix (5).

In addition, the progressive decrease of cell number and density in the NP has been proven to be associated with both aging and IDD. This has been hypothesised to compromise the disc ability to compensate for these changes by producing and maintaining a functional extracellular matrix (6).

The direct consequence of the reduction in proteoglycan content within the NP is dehydration, which decreases disc height and alters its load-bearing capacity (7). Anomalous distribution of forces across the disc results in cracking and tearing of the AF, disc herniation and vertebral body pathological changes including subchondral sclerosis, end-plate ossification and osteophyte formation. The relatively isolated anatomical location, the inherent avascularity and low metabolic activity of the IVD may be the key reasons for the apparent disc inability for self-repair after injury and degeneration (1).

Stem cell-based therapy

Recovering the disc potential to restore the matrix and re-establish the original proteoglycan content may therapeutically lead to increased disc hydration, thus improving its biomechanical properties (1). The progressive cell loss seen in IVD aging and degeneration may be treated by injecting exogenous cells in order to supplement and restore the original disc cell population. This cell therapy approach has been investigated using different type of cells, such as disc cells (8) and progenitor cells (9).

Autologous disc chondrocyte transplantation is currently under clinical evaluation as a possible approach for preventing degenerative sequelae after lumbar disc surgery. To date, this method has been shown to be safe and to decrease LBP, to reduce disc narrowing and partially restore the physiological NP environment (10). However, this approach is only feasible after performing discectomy and is limited by low expansion rates and the loss of phenotypic characteristics when expanded in a monolayer cell culture. A recent study has demonstrated that this latter limitation could be overcome by coculturing autologous NP cells with bone marrow mesenchymal stem cells. This process allows to obtain activated NP cells that have shown the ability to improve type II collagen and proteoglycan synthesis and restore the disc height (11).

On the other hand, stem cell therapy is characterized by low harvest site morbidity (which is a major concern in spine surgery), ease of *ex vivo* expansion and favourable phenotype modulation before or after transplantation.

Stem cells are defined as undifferentiated and

uncommitted cells characterized by high proliferation rate, self-renewal ability and multilineage differentiation (1). Adult stem cells can be harvested from completely differentiated tissues, including bone marrow, adipose tissue, muscle, skin, periosteum, blood, pericytes, synovial membrane and trabecular bone (12). Their main function is to replace existing senescent and/or damaged cells following a lineage-committed differentiation pathway, in order to guarantee and maintain the physiological homeostasis of the tissues in which they reside. As they can be isolated from the patient's tissues, the application of adult stem cells in regenerative medicine does not raise any ethical concern.

The potential use of adult stem cells, such as bone marrow-derived MSCs, muscle-derived stem cells (MdSCs), adipose tissue-derived stem cells (ASCs), hematopoietic stem cells (HSCs), olfactory membrane stem cells (OFs), synovial stem cells and disc stem cells has been taken into account for IVD regeneration (12). All these cell types have demonstrated differentiation towards mesenchyme-like tissues lineages, including fat, cartilage, bone and muscle. In addition, the aforementioned cytotypes have all been shown to originate from the cells residing within the perivascular wall, such as pericytes and bone marrow sinusoidal adventitial reticular cells (12).

There are several methods to utilize adult stem cells for IVD regeneration: (i) they can be harvested, minimally manipulated in the operating room and injected; they can be isolated and expanded *ex vivo* and then transplanted into the IVD as (ii) undifferentiated cells or (iii) pre-differentiated using growth factors or co-culture methods; (iv) they can be engineered and combined with a visco-elastic hydrogels; (v) they can be transfected with genes of interest and then injected into the IVD. (Fig. 1). Stem cells can be also transplanted allogenically as a product off-the self (13).

Systemic delivery of bone marrow-derived MSCs has also been tested in an animal model: although no significant changes were reported in the extracellular matrix composition, this approach resulted in diminished hypoxic response, risk of herniation and increased synthesis of cytokines, that may help disc degeneration treatment (14).

Therapeutic effect of stem cells for disc regeneration

In the past ten years numerous *in vitro* studies have raised the possibility to evaluate the use of adult stem cells for the treatment of IDD. This has been postulated after adult human MSCs were acknowledged to be able to differentiate towards NP chondrocyte-like cells if cultured under discogenic conditions (i.e. providing cultures with chondrogenic growth factors and/or coculturing MSCs with NP cells) (15). Coculture studies have demonstrated a synergistic effect and a cross-talk between MSCs and NP cells that may enhance NP extracellular matrix protein synthesis and induce MSC differentiation toward NP cells (15, 16).

Our group studied the mechanism of interaction between MSCs and NP cells harvested from a degenerating disc by coculturing them in a 3-dimensional culture system characterized by such a short distance that allowed paracrine interactions between cells, which is typical of the NP. Gene expression profiles were analysed on both cell types and showed that MSCs acquired a chondrocyte-like phenotype and influenced mRNA levels in human NP cells, which resulted in an increase of type II collagen expression by NP cells, while aggrecan synthesis was downregulated (15).

A recent study confirmed our previous data (15) that NP cells alone and MSCs cocultured with NP cells showed higher expression of type II collagen and aggrecan, as well as increased GAG content, if compared to MSCs-only cultures. However, the presence of degenerative media conditions dramatically upregulated the expression of catabolic genes in NP cells-only groups, while this detrimental effect had only a slight influence on coculture groups, which proved to be more resistant to inflammation and hypoxia. Configuring a coculture micropellet instead of culturing individual cells showed to decrease the expression of catabolic genes despite hypoxic and inflammatory media conditions (17).

Shim *et al.* further investigated this paracrine interaction by isolating MSCs, AF and NP cells from the same donor's vertebrae and then culturing them together. Cocultures were characterized by increased mRNA levels of matrix components (including type II collagen, aggrecan and versican), growth

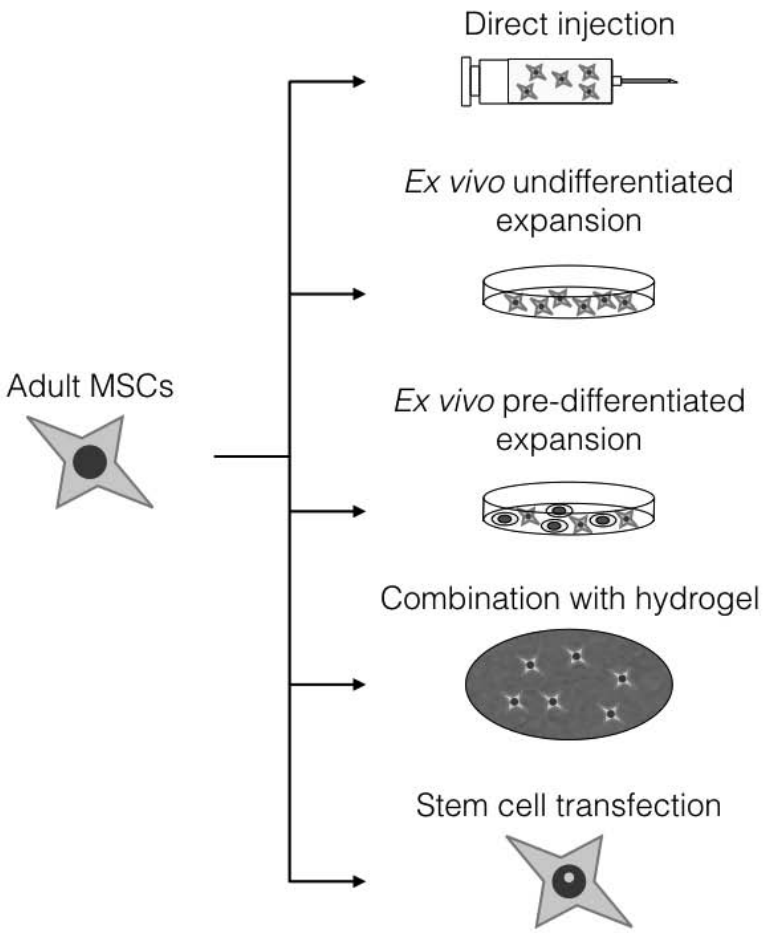

FIG. 1. *Different approaches adopted in the stem cell-based therapy of IDD. Once isolated, MSCs can be either directly intraoperatively injected into the injured disc, expanded ex vivo as undifferentiated cells or in coculture with other cytotypes or growth factors, combined with engineered biocompatible hydrogel scaffolds or transfected with target genes (from top to bottom).*

factors (EGF, IGF-1, TGF-α etc.) and decreased expression of pro-inflammatory cytokines (namely IL-1α, IL-1β, TNF-α and IL-6) when compared with monocultures (18).

Efficacy evidences in preclinical models

The IVD is characterized by avascularity, hypoxia, low pH, hyperosmolarity and low glucose content. All these features make it a harsh environment that may exhaust its intrinsic potential for self-repair and antagonize the engraftment of exogenously delivered stem cells. MSCs long term survival after disc injection was reported by several studies. Among those studies, our group assessed MSCs presence in the lumbar IVD of a healthy rabbit until 6 months after transplantation (16).

In order to evaluate the role of stem cell therapy for driving IVD regeneration, it is crucial to test regenerative approaches on animal models that may realistically resemble the human condition. Sakai *et al.* were the first ones to report that bone marrow-derived MSCs embedded in a type II collagen-based carrier and injected in a rabbit model of IDD led to increased proteoglycan content, disc hydration and height, which were maintained at 6 months after MRI evaluation (9).

Since then, significant advances in animal controlled trials based on stem cell therapy have been made. A systematic review and meta-analysis showed that 22 studies reported statistically

significant enhancements in disc height, MRI T2 signal intensity, type II collagen synthesis and decreased degenerative alterations by histological evaluation (19). These results are characterized by statistical heterogeneity due to different animal types (small versus large ones), cell types (NP cells, MSCs etc.) and injectable carrier (aqueous solution versus biocompatible scaffolds) (20).

A recent study from Maidhof *et al*. showed that not only cell type and delivery should be considered, but also planning a suitable timing for transplantation may influence the clinical outcomes. They administrated MSCs at 3, 14 or 30 days post injury and then assessed the effect of timing on the biochemistry and biomechanics of the disc; cells delivered at 3 days led to a significantly improvement on cell retention, compressive biomechanical properties and matrix reconstitution, while cells administered later did not yield comparable result, thus showing that cell therapy may be decisive if delivered at an early stage of degeneration (21).

A preclinical study performed by our group showed a potential side effect of stem cell-based therapy. MSC transplanted in a stab model of IDD in rabbit led to osteophytes formation due to MSC leakage from the injection side (22). In order to overcome the problem of AF damage and cell leakage, our group introduced a new method to deliver cells and hydrogels into the NP via the endplate with a small tunnel that can be repaired after delivery. This could be a promising regenerative approach to treat latest stages of IDD (23).

Clinical translation

The first phase I clinical study has been published by Orozco *et al*. who studied the transplantation of expanded autologous bone barrow MSCs in the NP of ten patients suffering from lumbar disc degeneration with intact AF. At 1 year after surgery, safety has been shown and improvement of pain and disability index was observed (24).

Elabd et al. reported that autologous bone marrow-derived MSCs cultured in hypoxic conditions and then injected in the discs of five patients led to an overall quality of life improvement at 4-6 years after surgery (25).

In a randomized controlled trial using allogeneic MSC, Noriega et al. treated 24 patients with LBP diagnosed with IDD divided into 2 groups. The test group received an intradiscal injection of 25×10^6 MSCs per segment. The control group received a sham infiltration of paravertebral musculature with the anesthetic. This preliminary phase II study showed clinical efficacy in terms of significant pain reduction in the treatment group compared to control. Moreover, IDD, quantified by Pfirrmann grading, improved in the MSC-treated patients and worsened in the controls (13). Although encouraging, these studies were including a small number of patients.

CONCLUSION

Significant advances towards the knowledge and the comprehension of the IVD biology and biochemistry, as well as regarding IDD pathophysiology, have been made so far. The progressive cell loss that characterizes disc aging and degeneration, together with the apparent inability of NP residing cells to self-repair intrinsic damage, have raised the possibility to replenish the native tissue with adult stem cells transplantation. This approach has repeatedly shown to help restore the disc microenvironment, thus leading to tissue regeneration. However, more effort is needed in order to assess efficacy of adult stem cell therapy for treating IDD in large controlled clinical studies. MSCs transplantation has been recognized to be promising and safe in both animal studies and clinical trials, which may make this approach a powerful tool for treating IDD in the near future.

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