Review Article

Stem cell therapy in discogenic back pain

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Abstract: Chronic low back pain has both substantial social and economic impacts on patients and healthcare budgets. Adding to the magnitude of the problem is the difficulty in identifying the exact causes of disc degeneration with modern day diagnostic and imaging techniques. With that said, current non-operative and surgical treatment modalities for discogenic low back pain fails to meet the expectations in many patients and hence the challenge. The objective for newly emerging stem cell regenerative therapy is to treat degenerative disc disease (DDD) by restoring the disc's cellularity and modulating the inflammatory response. Appropriate patient selection is crucial for the success of stem cell therapy. Regenerative modalities for discogenic pain currently focus on the use of either primary cells harvested from the intervertebral discs or stem cells from other sources whether autogenic or allogenic. The microenvironment in which stem cells are being cultured has been recognized to play a crucial role in directing or maintaining the production of the desired phenotypes and may enhance their regenerative potential. This has led to a more specific focus on innovating more effective culturing techniques, delivery vehicles and scaffolds for stem cell application. Although stem cell therapy might offer an attractive alternative treatment option, more clinical studies are still needed to establish on the safety and feasibility of such therapy. In this literature review, we aim to present the most recent *in vivo* and *in vitro* studies related to the use of stem cell therapy in the treatment of discogenic low back pain.

Keywords: Stem cell transplantation; intervertebral disc degeneration; low back pain; regenerative medicine; tissue scaffolds

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Introduction

Chronic low back pain affects approximately 632 million people worldwide with prevalence as high as 68% in adults older than 60 years of age (1,2). It has both substantial social and economic impacts on patients and healthcare budgets resulting in the highest economic burden among all musculoskeletal complaints (3). This tremendous impact was portrayed in a prospective multicenter study including 8 industrialized countries which found that patients with chronic spinal conditions have lower quality of life scores when compared with patients with other chronic conditions such as arthritis, chronic lung disease, congestive heart failure, and diabetes (4). Stem cell therapy for degenerative disc disease (DDD) is a relatively novel approach with promising results as an alternative to the conventional surgical and non-operative regimens (5). The purpose of this article is to review the current available evidence and provide a foundation for future research. In this article, we review the literature and present the stem cell regenerative therapies in DDD, different stem cell sources and their delivery mechanisms in the degenerated disc, as well as an overview of the challenges facing the implementation of this technique.

Methods

A comprehensive literature search was performed independently by two authors (AH Barakat, VA Elwell) and the results were collated and de-duplicated. The literature search was conducted on MEDLINE and EMBASE databases from database inception through on January 12th, 2019. The following Mesh terms were used ("Stem Cells"[Mesh]) AND ("Back Pain"[Mesh] OR "Low Back Pain"[Mesh]). Thesaurus terms were adapted for different databases. The search was limited to English language literature with all non-English papers excluded. A total of 286 papers were identified, reviewed and assessed. The studies cited here covers the most recent advances in this rapidly expanding field.

Pathogenesis of discogenic pain

The exact causes of disc degeneration are complex and difficult to pinpoint; they involve aging, genetic predispositions, nutritional factors as obesity, mechanical trauma, smoking and other related comorbidities (6-12).

The adult intervertebral disc (IVD) is an avascular organ that relies on passive diffusion from adjacent endplate vessels for nutrition, resulting in poor inherent healing potential (13,14). The centrally located gelatinous nucleus pulposus (NP) is a hypoxic, hydrophilic proteoglycan-rich structure consisting mainly of type II collagen, while the outer lamellated anulus fibrosus (AF) is a fibrous ring mostly of type I collagen (15-17). The extracellular matrix (ECM) of the disc is formed of water, proteoglycans and matrix proteins. The water content of the disc is dependent on both the proteoglycan and collagen contents, with the proteoglycan providing the swelling pressure through retaining water and the collagen providing the resistance to swelling. The proteoglycan and thus water content of the disc increases on progressing from the outer AF to the NP. Conversely, the collagen content of the disc decreases from the outward inward.

Aging is associated with lower water and proteoglycan content along with high collagen concentrations (18). Histologically, there is progressive loss of demarcation between the NP and AF with loss of the transition zone. This is due to a change in collagen synthesis by the NP cells from collagen II to collagen I with subsequent loss of proteoglycan and dehydration (19). The main proteoglycan of the IVD is aggrecan, it is present in both the NP and the AF and possesses both chondroitin sulfate and keratan sulfate chains bound to a protein core (20). With aging, there is a decrease in the chondroitin sulfate chain length and an increase in keratan sulfate chain lengths. This reciprocal change in chain lengths has been associated with the decreased oxygen supply to the disc with aging. The reason is that, oxidation is a prerequisite for the formation of the glucuronic acid needed for chondroitin sulfate synthesis however it is not for keratan sulfate synthesis. Aggrecan binds to hyaluronan through a link protein to form proteoglycan aggregates responsible for maintaining the swelling pressure. This binding capacity decreases with age leading to decreased aggregates and increased hyaluronan concentration in a degenerating disc (21).

Another constituent of the ECM is the matrix metalloproteinases (MMPs). MMPs play a major role in ECM degradation within the disc with aging as a result of an imbalance in their turnover and activation relative to loss of their inhibitors (22). These MMPs are primarily activated in acidic PH of the degenerated IVDs owing to their decreased nutrition and more anerobic metabolism with accumulation of lactate. This decreased nutrition is mainly due to growth of the disc size and calcification of the end plates.

Subsequent to the diminished disc nutrition, the cell density of the disc decreases with aging (23). During embryonic development, the notochord gives rise to the NP of the disc, whereas the surrounding AF is developed from the mesenchymal tissue. With the aging process, the notochordal cells (NC) in the NP are replaced by cells of mesenchymal origin, which have a chondrocyte and fibroblast-like appearance. This further explains the poor healing potential with aging as the NCs which have the regenerative capacity are depleted (24).

The above-mentioned changes including the decrease in water and proteoglycan aggregate content along with increasing fibrous nature of the NP causes the disc narrowing observed radiographically (25). Loss of the disc's intrinsic hydrostatic pressure owing to its dehydration, affects the disc's resilience to mechanical loading (12). The result is disc space narrowing, bulging and eventually osteophyte formation with end-plate sclerosis. This causes pressure on the nerve roots leading to back pain and eventually weakness and numbness. With the progression of IVD degeneration, the disc tends to be vascularized increasingly starting peripherally via angiogenesis. Vascular ingrowth eventually extends centrally into the NP and is associated with innervation of the disc causing discogenic pain (26).

Another important factor to be considered in the pathogenesis of DDD is biomechanical loading stresses. It is now appreciated that the metabolism of disc cells is enhanced by physiologic intermittent compressive loading which increases production of both proteoglycans and tissue inhibitors of MMPs (27). Genetic factors and familial predisposition are also recognized etiological factors for DDD. An immunogenetic epidemiologic study on 678 patients has strongly implicated both genetic and environmental factors in the etiology of DDD (28). This was supported by further epidemiologic studies which concluded a strong familial predisposition to DDD (29). It has been shown that DDD may be accelerated in some individuals because of genetic polymorphisms in genes such as the aggrecan or the MMPs genes (30). Obesity has been implicated in DDD owing to the biomechanical overloading as well as the metabolic element in these patients. A radiological MRI study on 129 middle-aged men showed a detrimental effect of obesity on DDD (31).

Both non-operative and surgical treatments have failed to meet many patient's expectations in providing a satisfactory means for managing this condition. In a large-scale controlled study on 1,450 patients focusing on the outcome measure [return to work (RTW)], it was revealed that only 26% of patients RTW 2 years after fusion surgery, while 67% of non-operative controls had RTW within 2 years from the date of injury (32).

Initially, recruitment of conservative therapy including the use of nonsteroidal anti-inflammatory drugs (NSAIDs), muscle relaxants, opioids and physiotherapy leads to improvement in most patients. However, for non-responsive patients after exhausting conservative options, lumbar spinal fusion as the standard of surgical treatment can present with significant complications and morbidity (33).

Complications related to spine surgery include deep vein thrombosis, infection, and myocardial infarcts occurring in 6.6% of initial surgeries and in 6.3% of revisions (34,35). Adjacent segment disease and proximal junctional kyphosis are recognized as sequela of spinal fusions by comprising the mechanics of the spine at levels above and below the fusion and ensuing abnormal stresses during spinal motion (36). Other complications include pseudarthrosis and hardware complications. Stem cell-based therapies have advanced significantly over the past decade with numerous clinical trials aiming at providing a non-surgical biologic approach to improve pain and function in DDD. In this approach, the abnormal conditions of the diminished cellularity and altered cell phenotype are the targets for correction.

Patient selection

The objective for stem cell regenerative therapy is to treat DDD by restoring the disc's cellularity and modulating the inflammatory response. Appropriate patient selection is crucial for the success of stem cell therapy. The optimum window for intervention is the following: early stages of degeneration, failure to respond to conservative treatment and prior to the onset of advanced disc height collapse and herniation (37). There is a consensus that the ideal candidates for such regenerative therapies should be limited for patients with moderate chronic back pain and disability as confirmed by functional scores, with affection of single-level disc as proven by radiological Pfirrmann grading of lumbosacral disc degeneration Grade III or IV on MRI (38). Advanced donor age for stem cells and co-morbidities are other factors that needs to be considered as MSCs may exhibit different phenotypic regenerative potentials (39,40).

Cell sources

Regenerative modalities for discogenic pain are currently focusing on the use of either primary cells harvested from the IVD or stem cells from other sources whether autogenic or allogenic.

Primary cells

Studies on primary and native stem cells for DDD have gained interest over the past decade. Several studies have confirmed the presence of NP and AF progenitor cells in IVD tissues possessing multipotential differentiation capabilities (41-44). These progenitor cells are thought to be responsible for regenerative capacity and homeostasis of IVD tissue, while their age-related depletion may be responsible for the loss of this regenerative and reparative capacity of IVDs. Mesenchymal stem cell (MSC) markers have been identified in both NP and AF.

Progenitor niches of importance are the notochord cells (NCs) known as NP progenitor cells (NPPCs). Sakai *et al.* identified NPPCs in the NP tissue via their tunica intima endothelial kinase (Tie2+) and disialoganglioside (GD2+) surface markers (45). Tie2 is a receptor tyrosine kinase receptor expressed in hematopoietic and neural stem cells while GD2 is a plasma membrane marker for bone marrow (BM) and umbilical cord MSCs (46-50). It was found that angiopoietin 1, which is a Tie2 ligand, plays a pivotal role in maintaining the NPPCs and protecting the cells from

apoptosis. This might may lead to future research aiming to develop reliable methods with which to isolate, maintain, and expand these progenitor cells (51).

Regarding the AF progenitor cells, studies have demonstrated that AF-specific progenitor cells were present in both nondegenerative and degenerated IVDs (52). A unique feature of these cells is their potential to differentiate to different cell lineages including adipocytes, chondrocytes, osteoblasts, neural and endothelial cells.

Despite that the feasibility of isolating pure native disc progenitor cells without fibroblasts and macrophages was proven to be challenging, incorporation of IVD tissue-specific progenitors into tissue engineered scaffolds would significantly impact the regeneration potential and efficacy of tissue-engineered IVD constructs.

To overcome this difficulty and in resemblance to the autologous chondrocyte implantation techniques used in degenerated cartilage elsewhere, autologous isolated IVD disc cells were stimulated in conditioned media and re-implanted back into the same degenerated areas from where they were harvested. A canine model demonstrated after 2-year of follow-up, disc persistent cell viability, proliferative capacity, ECM synthetic ability and proteoglycan content (53).

The Euro disc randomized trial is a prospective, randomized, controlled, multicenter study comparing autologous disc chondrocyte transplantation plus discectomy versus discectomy alone in 112 patients (54). At the time of discectomy, autologous disc chondrocytes were sequestered and expanded in culture then reinjected into the disc after 12 weeks. This study demonstrated a clinically significant reduction in low back pain scores in the patients who received autologous disc cell transplantation after discectomy compared with those who had discectomy alone. Furthermore, the MRI of the treatment group revealed 41%-disc hydration when compared to 25% in the adjacent levels that had undergone discectomy without autologous disc chondrocyte transplantation. Mochida *et al.* (55) reported that such treatment has proven safety and efficacy in a 3-year follow-up with no major side effects and with good clinical results.

Owing to the practical and surgical risks in obtaining autologous primary NP tissue from either herniated or adjacent discs, motivation in identifying and characterizing alternative cell sources for disc regeneration has also been pursued (56,57).

Other accessible cell sources with reduced risk for donor site morbidity and relative ease of isolation, such as articular and nasal cartilage, have been investigated *in vitro* and in animal models for NP regeneration (58,59). These cell sources are still in the state of infancy and further research is required.

MSC

MSC transplantation has received considerable attention due to their versatility, and potential for stimulating a healthier host tissue microenvironment by their paracrine effects. MSCs are stem cells that have extensive proliferative capacity and multi-lineage potential *in vitro* and *in vivo* (60). The effects of MSCs in delaying and even reversing the degenerative cascade in IVDs has been well documented in many experimental studies including different animal models prior to being translated for clinical use (61-63). The transplanted cells not only restore the cell population in degenerated IVDs but also were capable of ECM and aggrecan production, leading to an increase in IVD height (64). Multiple tissue sources have been described including BM, adipose tissue, muscle and more recently from umbilical cord (65-68).

BM and adipose derived (AD) MSCs are the most frequently utilized sources for this purpose. However, these are usually associated with procedures for harvesting the cells such as BM aspiration or liposuction. As a result, studies on using MSCs derived from umbilical cord Wharton's jelly have been conducted and may prove to be a potentially favorable alternative (16). A case study on two patients was conducted using human umbilical cord MSCs (HUC-MSCs) with favorable outcomes regarding pain and disability as surveyed by the visual analogue score (VAS) and the Oswestry functional score (69).

BM derived MSCs are of particular interest since they are easily accessible and isolated. Thus, they have been extensively studied as a prime site and working horse for MSC isolation for the purposes of treatment of DDD (70). Intradiscal BM derived allogeneic MSCs are currently being explored in a phase II randomized controlled study (71). Single-level mild degenerated lumbar IVDs were selected. Preliminary data show that a greater number of patients treated with intradiscal MSCs reported ≥50% reduction in low back pain compared with controls at 12 months after injection. Specifically, of the patients treated with intradiscal MSCs, 69% reported this successful outcome, compared with only 33% of control patients. Studies on autologous stem cells derived from BM have shown promise manifested by improvement in clinical outcomes in human trials. In a case series study by Orozco *et al.* (72) autologous BM MSCs

were injected into the NP of 10 patients with chronic back pain and followed up for 1 year resulted in improvement of clinical symptoms with no reported adverse effects. The patients were functionally analyzed using the VAS, Oswestry Disability Index (ODI), and 36-Item Short Form Health Survey (SF-36). MRI measurements of disc height and fluid content were also done. There was a rapid initial improvement in pain and disability at 3 months followed by modest additional improvements at 12 months. Water content increased, but disc height did not recover.

In one prospective study, 33 patients with lower back pain and disc degeneration were treated with culture-expanded, autologous, BM-derived MSCs with follow-up period up to 6 years (73). The study has proven safety with only minor adverse events and significant improvements in pain, function, and overall subjective improvement as evidenced by numeric pain score (NPS), modified single assessment numeric evaluation (SANE) rating and functional rating index (FRI). Measurement of the intervertebral disc posterior dimension has shown that 85% of the patients who were evaluated by MRI demonstrated a reduction in disc bulge size, with an average reduction size of 23% post treatment.

In a pilot study by Pettine *et al.* (74) twenty-six patients with discogenic low back pain had autologous BM-MSCs percutaneous injections. There was a significant improvement in VAS and ODI after the 12-month study period with 8 patients having increased disc heights as evidenced by improvement in their Pfirrmann MRI grading. In another study by Yoshikawa *et al.* (75) similar outcomes were reported after 2-year follow-up.

However, the centrifugation process to obtain autologous stem cells from BM is limited by absence of a high concentration of pure homogeneous MSCs. There is adherence to plastic during the preparation (76,77). One of the emerging separation methods of stem cells other than centrifugation is the cell SELEX (systematic evolution of ligands by exponential enrichment) technique (78). SELEX uses aptamers to selectively capture target cells. Aptamers are modified nucleic acids identified from large nucleic acid libraries by their high affinity to target molecules and specific cells in similarity to antibodies targeting. The concept is still evolving and is now limited by specificity and insufficient collection of high purity stem cells.

Progenitor cells derived from other tissue sources such as adipose tissue have also been shown to possess significant potential for differentiation and tissue forming capabilities (79,80). The ease of harvesting of autologous adipose derived stem cells (AD-MSCs) can be performed as an outpatient setting with yields up to 25,000 MSCs per gram of tissue (81). This has led AD-MSCs to provide a better alternative and candidate for cell therapy and disc regeneration, due to their abundance and ease of isolation. Additionally, they have a lower inherent capacity for endochondral ossification than BM derived MSCs (82).

Furthermore, some studies suggested that AD-MSCs might be a more appropriate cell type than BM-MSCs for IVD regeneration because AD-MSCs could differentiate into cells with a more NP-like phenotype (83). An *in vivo* model of mice with severely degenerated IVDs treated with AD-MSCs intradiscally also found promising positive radiographic findings (84). This study showed survival of the injected AD-MSCs up to 12 weeks after implantation with significant increase of aggrecan tissue levels. Another *in vivo* study on a rabbit model showed that the AD-MSCs injected discs exhibited elevated ECM secretion as well as survival of the implanted cells 10 weeks after implantation (85). A canine model-controlled study showed significantly higher production of aggrecan, collagen type II and increased cell density in AD-MSCs treated discs (86). An *in vitro* Co-culture of AD-MSCs with NP cells in a type II collagen hydrogels caused upregulation of collagen type II and aggrecan gene expression (87).

More recently NCs as nucleus pulposus progenitor cells (NPPCs) for disc regeneration have received considerable interest. NCs are the progenitors of adult NP cells, and their loss in humans during postnatal growth is thought to contribute to the onset of degeneration later in life (88). NCs are crucial since they can generate NP cells and may potentially survive better in the unfavorable microenvironment post transplantation (89). Since NCs are scarcely present in adult human NP tissue and cannot be readily obtained as previously mentioned, most studies have focused on harnessing the therapeutic potential of NC secreted factors by co-culturing with MSCs directing them to a more NP like phenotype (90). This might guide future studies towards the importance of generation of high-quantity and functional NCs from induction of other pluripotent stem cell niches (91).

Recent focus on the isolation of pluripotent stem cells such as induced pluripotent stem cells (iPSCs) or embryonic stem cells (ESCs) could be a potentially promising source for NCs (*Table 1*). ESCs are pluripotent stem cells derived from the inner cell mass of embryonic blastocyst of a donated *in vitro* fertilization (IVF) embryo, while iPSCs are adult cells genetically reprogrammed to be

Table 1 Comparison of different stem cell types and sources used in treatment of discogenic low back pain

Cell type	Cell source	Characteristics
Nucleus pulposus progenitor cells (NPPC) (45,88)	• Primary cells isolated from IVDs	• Difficulty in isolating pure native disc without fibroblasts and macrophages
Annulus fibrosus (AF)-specific progenitor cells (52)		• Possible disc damage during harvest
		• Exhaustion and decreased availability with aging
Autologous intervertebral disc (IVD) cells (53-55)	• Autologous isolated IVD cells stimulated in conditioned media then re-implanted	• Not pure progenitor cells
		• Possible disc damage during harvest
Autologous chondrocytes (58,59)	• From more accessible sites as articular or nasal cartilage	• Differences between chondrocytes and NP cells
		• Mainly *in vitro* studies
Mesenchymal stem cells (MSCs)	• Bone marrow (BM) derived (70-75)	• Need for bone marrow aspiration
		• Absence of a high concentration of pure homogeneous MSCs
		• Higher inherent capacity for endochondral ossification
	• Adipose tissue derived (AD) (79-87)	• Need for adipose tissue liposuction
		• Higher abundance of MSCs
		• Could differentiate into cells with a more NP-like phenotype compared to BM-MSCs
		• Lower inherent capacity for endochondral ossification
	• Umbilical cord Wharton's jelly (16,69)	• More feasible MSC isolation and abundance
		• No need for invasive harvesting of stem cells
		• Ethical issues
Embryonic stem cells (ESCs) (92,93)	• Inner cell mass of the blastocyst of a donated *in vitro* fertilization embryo	• Could be directed to notochord cells
		• Tumorigenesis potential due to pluripotency
		• Ethical issues
		• Allogenic source
Induced Pluripotent stem cells (iPSCs) (89)	• Specialized cells genetically reprogrammed	• Other source for production of notochord cells
		• Tumorigenesis potential due to pluripotency
		• More patient specific (Autogenic)

pluripotent. These cells are different from the multipotent MSCs. Sheikh *et al.* (92) demonstrated that ESC-derived chondroprogenitors could potentially differentiate into NCs in a rabbit model of IVD degeneration. Compared with ESCs, use of iPSCs for disc repair may be more attractive due to patient specificity, less ethical concerns and less immune rejection. Liu *et al.* (89) showed certain effectiveness of using natural NP tissue matrix to direct

NC differentiation of iPSCs. To promote differentiation of MSCs, culturing in NC-conditioned media (NCCM) has been reported to increase secretion of glycosaminoglycans (GAG) and type III collagen and led to the production of a more NP like phenotype (93).

One of the potential challenges in utilizing stem cells is that they are undifferentiated and are being transplanted into a harsh environment consisting of low cellularity, nutrients and acidic conditions (94). All of these factors could detrimentally impact the differentiation potential and viability of the implanted cells (95).

Effect of microenvironment

The microenvironment in which stem cells are being cultured has been recognized to play a crucial role in directing or maintaining the production of the desired phenotypes and may enhance their regenerative potential. Priming stem cells using growth factors or other environmental factors such as altered oxygen or glucose level has been a subject of rigorous research *in vitro* and *in vivo* studies (96-100).

Several studies have shown that exposure to low levels of oxygen enhances MSC differentiation towards NP phenotypes. An *in vitro* study by Kumar *et al.* (101) has demonstrated MSCs enhanced differentiation into NP phenotypes under hypoxic conditions by culturing in a biodegradable polymer hydrogel scaffold. This is consistent with other studies, indicating that differentiation of MSCs depends largely on the local microenvironment (102-104). In one small scale human study, the safety and feasibility of an intra-discal injection of autologous, hypoxic cultured BM derived MSCs in five patients with chronic lower back pain was evaluated (105). The follow-up was up to 6 years and utilized clinical examination, MRI radiological evaluation and quality of life questionnaire as an outcome measures for improvement. All patients reported overall improvement, there was improvement in strength with four out of five patient's leg mobility. At the end of the follow-up period, there were no reported adverse effects or neoplastic transformation by radiological evaluation. The overall results are promising and might pave the way for a larger double-blind, controlled, randomized clinical study with significant number of patients and implementation of validated endpoint measurements as next steps in order to further validate the efficacy of this technique.

Another important factor is glucose levels used in the culture medium of stem cells. An *in vitro* study by Stolzing *et al.* (106) on rat BM-MSCs demonstrated that high glucose concentration has a negative effect on MSC colony formation and phenotypic differentiation. This was supported by other authors who have shown that hypoglycemic conditions on human AD-MSCs have a favorable effect on survival and biological behaviors of these cells (107). An *in vitro* on rat derived BM-MSC showed low glucose enhanced matrix biosynthesis and maintained cell proliferation whereas high osmolarity and low pH conditions reduced biosynthesis and proliferation of the MSCs (108).

High osmolarity have been shown to inhibit DNA damage response and arrest cell proliferation in bovine NP cells. For the MSCs, multiple studies have shown reduced cellular viability and matrix synthesis by disc-like high osmolarity (109-111). PH was also found to be detrimental to stem cell proliferation and ECM expression. An *in vitro* study on rat derived BM-MSC cultured in different PH levels showed that acidic conditions inhibited Aggrecan expression in the ECM as well as a decrease in proliferation and viability and was associated with a change in cell morphology (112).

Differentiation of stem cells is also affected by mechanical stresses depending on the loading pattern and intensity (113,114). It has been found that transition and phenotypic maturation of the resident NCs to NP-like cells, can be significantly enhanced by static compressive loadings or dynamic hydrostatic pressures (115,116). For example, *in vitro* studies have demonstrated that radial compressive loadings promoted the AF-like differentiation in BM-MSC (117), whereas the NP-like differentiation of AD-MSC can be significantly enhanced by dynamic compressions (118).

Despite that this review is mainly focusing on stem cell regenerative therapies, it is worthwhile mentioning the effect of growth factors as part of the microenvironment in enhancing phenotypic differentiation. Recent understanding of the cellular and molecular cascade of IVD homeostasis has engendered the hypothesis of priming the cultured stem cells or direct IVD injection with growth factors such as epidermal growth factor (EGF) and recombinant human growth and differentiation factor-5 (rhGDF-5) to enhance cell proliferation and matrix synthesis (119).

RhGDF-5 is a member of the transforming growth factor-b (TGF-b) superfamily and the bone morphogenetic (BMP) subfamily, and is known to influence the growth and differentiation of various tissues, including the intervertebral disc. Various *in vitro* studies on rhGDF-5

has demonstrated that it has a key role in suppressing ECM degradation by suppression of matrix metalloproteases and in enhancing the proliferation and matrix anabolism through increased production of aggrecan, GAG and collagen type II (120-122). Moreover, it was reported by one study that a combination of rhGDF-5 and hypoxia had a synergistic effect on NP-like differentiation of MSCs (123).

There are challenges in direct application of growth factors causing a sustainable effect on tissue remodeling to be unreliable. For example, the half-lives, interstitial solubility of these factors, the presence of other inhibiting factors and low numbers of viable cells available to be stimulated in already degenerated IVD disks (124). A different approach is to try to control the catabolic pathway leading to IVD degeneration. By antagonizing the transcription factors that activate the proteolytic genes contributing to IVD degeneration such as receptor activator of nuclear factor-k B ligand (RANKL), the degenerative cascade might be halted (125). Upregulation of MMP and ADAMTS expression has been implicated in disc ECM destruction, leading to the cascade of IVD degeneration (126). Exposure of MSCs to inflammatory factors (IL-1b and TNF-a) might negatively modulate the MSC differentiation potential by promoting osteogenic-like mineral deposition, which is not desirable for disk repair (127). These findings might provide alternative future therapeutics depending on administration of specific antagonists of these proteins directly into the disk to prevent pathological proteolysis of disc ECM.

To overcome the temporary effects and the obstacles of such factors when directly applied to IVDs, another strategy has been developed which is gene therapy aiming to silence catabolic or activating anabolic pathways in the degenerated IVDs. Gene therapy has advantages over direct delivery of proteins. For example, possibility of sustained long-term efficacy and maintained endogenous synthesis of growth factors or anti-inflammatory factors (128,129). The desired nucleic acids are being commonly delivered using a viral vector, so that the functional status of recipient cells can be modified. For example, an *in vitro* study has also been established using cultured human IVD cells transfected with adenoviral vectors carrying inhibitor to interleukin-1 (IL-1), an important cytokine in the inflammatory cascade, in degenerate IVD (130). The inhibitory effects on the production of IL-1 was maintained for the 2 weeks period of the study duration. Another alternative for the use of gene transfer is to stimulate the anabolic pathways. Various studies have demonstrated successful maintained regenerative effects after transfection of IVD cells. The transfected cells have shown improved ability of ECM production and collagen II synthesis through utilizing genes responsible for production of BMP-2, LIM mineralization protein-1 (LMP-1), chondroitinase ABC, TIMP (tissue inhibitor of metalloproteinases) and SOX9 (131-133). Nonetheless, despite its potential safety concerns, immunogenicity and tumorigenesis potential for such modality needs further investigations.

Another alternative, other than direct injection of growth factors or their vector mediated delivery through gene therapy, is to simply prime and direct MSCs differentiation during the culturing process, into IVD-like phenotypes with induction by growth differentiation factor 6 (GDF6), bone morphogenic protein-2 (BMP-2) and transforming growth factor-beta (TGF-β) (134). Numerous *in vitro* and animal studies have portrayed that TGF-β signaling in growth plate chondrocytes and inner AF cells to be critical in growth and maintenance of matrix tissue and cellularity of endplate cartilage cells (135-138). It was shown by Clarke *et al.* that both BM-MSCs and AD-MSCs primed with GDF6 have demonstrated through identification of NP marker genes; increased phenotypic differentiation into NP cells with secretion of an ECM that is more proteoglycan rich and consistent with IVD micromechanical properties (100). BMP proteins family plays a pivotal role as increasing proteoglycan synthesis, upregulation of collagen type II mRNA (139). *In vitro* application of BMP-2 in a rat model increased cell proliferation and disc ECM production (140). Rabbit IVDs exposed to injections of BMP-7 also led to restoration of disc height and increased proteoglycan content (141). *Table 2* summarizes the cited studies and shows the effect of different microenvironments on growth and survival of stem cells.

Stem cell delivery

With respect to delivery of cell-based therapies, the traditional working horse has been image-assisted percutaneous injection through the AF. However, trans-annular injection has raised concerns regarding AF damage, leakage from the delivery site inducing osteophyte formation and diminished cell viability due to shear forces caused by small diameter needles (142-145).

To maximize both safety and efficacy of such modality the selection of needle size and the dose to be delivered should be optimized. Small needle diameters can hinder injection of viscous scaffolds as hydrogels while larger diameters might induce AF damage (146,147). *In vivo* animal study using a goat model by Gullbrand *et al.* (148)

Table 2 A summary of the literature review as regards the effect of micro-environment on growth and survival of stem cells

Reference	Type of study	Cell source	Intervention	Outcome
Hudson *et al.* (98)	*In vitro*	hMSCs	Hypoxic (5% O_2) versus normoxic (21% O_2) conditions	Hypoxic expansion of human MSCs enhances 3D maturation of tissue engineered IVDs
Adesida *et al.* (99)	*In vitro*	hBM-MSCs	Hypoxic conditions (3% O_2) versus normoxic conditions (21% O_2)	Hypoxic conditions augment the chondrogenic potential of hBM-MSCs
Clarke *et al.* (100)	*In vitro*	hAD-MSCs and hBM-MSCs	Media supplemented with TGF-β3, GDF5, or GDF6	GDF6 stimulation of AD-MSCs induces differentiation to an NP-like phenotype and results in a more proteoglycan-rich matrix with less stiff composition
Kumar *et al.* (101)	*In vitro*	hMSCs	Hypoxic (2% O_2) chondrogenic media versus normoxic non chondrogenic media	MSCs enhanced differentiation into NP phenotypes with elevated expression levels of aggrecan and collagen II under hypoxic conditions
Elabd *et al.* (105)	Human *in vivo* study	hBM-MSCs	Hypoxic (5% O_2) conditions	MRI radiological evaluation and quality of life questionnaire as an outcome measures showed significant improvement
Stolzing *et al.* (106)	*In vitro*	Rat nonadherent BM-MSCS	Different glucose levels in cultures	Culture in high-glucose-containing medium had a negative effect on colony formation and differentiation
Liang *et al.* (107)	*In vitro*	hAD-MSCs	Age (cells from mature and young male donors), glucose, acidity and osmolarity	Low glucose is a positive factor but high osmolarity and low pH are deleterious factors that affect the survival and biological behaviors of hAD-MSCs. Age did not affect the results
Wuertz *et al.* (108)	*In vitro*	Rat BM-MSCs	Age (cells from mature and young male donors), glucose, acidity, osmolarity and combined conditions	IVD-like glucose conditions stimulated aggrecan and collagen-1 expression. IVD-like osmolarity and pH strongly decreased proliferation and expression of matrix proteins. Osmolarity and pH dominated the effects of glucose
Mavrogonatou and Kletsas (109)	*In vitro*	Bovine-NP cells	High osmolality	
Liang *et al.* (110)	*In vitro*	hAD-MSCs	Low glucose, acidity, high osmolarity, and combined conditions	Low glucose is a positive factor but high osmolarity and low pH are deleterious factors that affect the survival and biological behaviors of AD-MSCs
Tao *et al.* (111)	*In vitro*	Rat NPCs, NP-MSCs and co-culture	High osmolality	High osmolarity inhibited cell viability and decreased the expression of aggrecan and collagen II at the mRNA and protein levels
Wuertz *et al.* (112)	*In vitro*	Rat BM-MSCs	Acidity	Acidity caused an inhibition of aggrecan, and collagen I, as well as a decrease in proliferation and viability and was associated with a change in cell morphology
Purmessur *et al.* (115)	*In vitro*	Porcine NCs	Dynamic pressurization	NP tissue maturation was induced from dynamic hydrostatic pressurization
Yurube *et al.* (116)	*In vitro*	Rat IVD cells	Static pressurization	Static compression-induced disc cell death and degeneration

Table 2 *(continued)*

Table 2 *(continued)*

Referencev	Type of study	Cell source	Intervention	Outcome
See *et al.* (117)	*In vitro*	Rabbit BM-MSCs	Compressive mechanical stimulation	Extensive remodeling and ECM production occurred within the simulated IVD-like assembly
Dai *et al.* (118)	*In vitro*	Rats AD-MSCs	Dynamic compression and NPCs co-culture	Combination of dynamic compression and coculture showed an additive effect on NP-like cell differentiation
Li *et al.* (120)	*In vitro*	Rat NPCs	Priming with rGDF-5	rGDF-5 treatment of disc cells from the GDF-5-deficient mice resulted in a dose-dependent upregulation of the aggrecan and type II collagen genes
Chujo *et al.* (121)	*In vitro* and animal *in vivo* study (rabbit model)	Bovine NP and AF cells, rat IVD model	rhGDF-5	*In vitro*, rhGDF-5 increased the DNA and proteoglycan contents. *In vivo*, the injection of rhGDF-5 improved disc height, MRI scores and histologic grading scores
Stoyanov *et al.* (123)	*In vitro*	hBM-MSCs	TGFß or GDF5 or coculture with bovine NPCs. All groups were incubated at low (2%) or normal (20%) oxygen	Hypoxia and GDF5 led to directing MSCs towards the IVD-like phenotype
Wehling *et al.* (127)	*In vitro*	hBM-MSCs	IL-1beta and TNF alpha	Both IL-1beta and TNF alpha inhibited chondrogenesis in a dose-dependent manner
Steck el al. (134)	*In vitro*	hBM-MSCs	TGF beta-3, dexamethasone, and ascorbate	After TGF beta mediated differentiation, MSC adopted a gene expression profile that resembled native IVD tissue more closely than native joint cartilage
Thompson *et al.* (137)	*In vitro*	Canine IVD disc	ILGF-1, EGF, FGF, or TGF beta-3	TGF beta-3 and EGF elicited greater proliferative responses than FGF; ILGF-1 produced a marginally significant response in the nucleus and no response in the anulus and transition zone
Walsh *et al.* (138)	*In vivo*	Murine IVD disc	GDF-5, TGF beta-3, ILGF-1 or basic FGF	A statistically significant increase in disc height 4 weeks after GDF-5 treatment was measured
Li *et al.* (140)	*In vitro*	Rat AF cells	BMP-2	BMP-2 increases aggrecan and collagen type II mRNA expression. BMP-2 also up-regulates mRNA expression for BMP-7 and TGF beta-3

a 22G needle was found to be safe and feasible with no discernable degenerative changes on MRI or histology after 12 weeks. Since the degenerated IVD provides a very limited nutrient supply, the minimal effective dose of MSCs must be determined to maximize post-implantation survival. Data from larger model (canine, porcine, and ovine) studies showed that a dose of 106 BM-MSCs/per disk had the least number of MSC apoptosis (66).

Current procedures often show backflow of cells through the injection site and a low survival rate of the implanted cells. In an attempt to avoid possible AF damage and to minimize extrusion of the injected material, an alternative delivery route that has been suggested which is through the pedicles (transpedicular approach) (149). Another alternative is the use of a delivery vehicle to act as retainers of injected suspensions. A human study where two patients with IVD degeneration had collagen porous sponges seeded with autologous BM-MSCs implanted through open approach and a collagen sponge was used as a sealant for the surgical hole in operated IVDs. In this study, both patients demonstrated promising results of improved outcome scores and increase in disc hydration as evidenced by MRI evaluation at 2 years after intervention (75). Other biomaterials as delivery vehicles, and in particular hydrogels, have been utilized to overcome retention

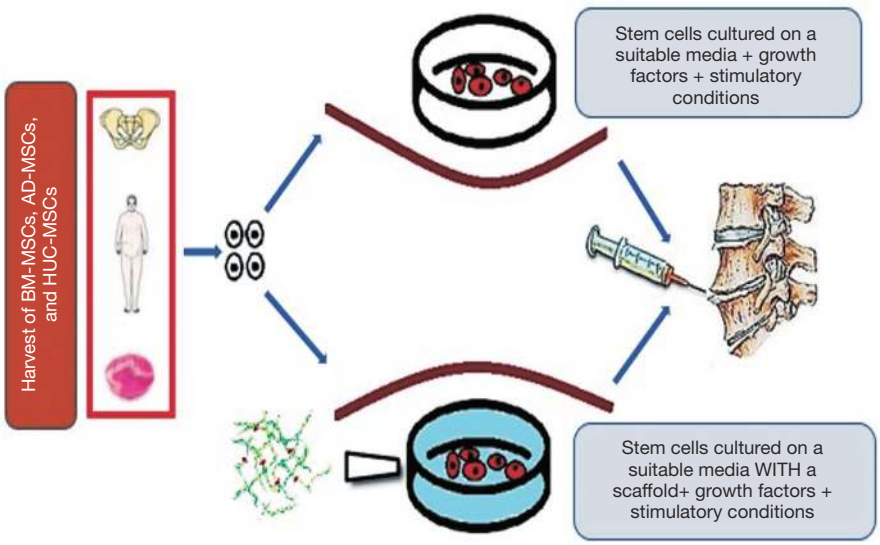

Figure 1 An illustration showing the most common utilized sources for stem cells harvest. After harvesting the stem cells are cultured and primed with growth factors or cultured in a scaffold for additional support of cell survival.

issues and provide additional support for cell survival and phenotype retention (150-155) (*Figure 1*).

Scaffolds

Various scaffolding materials as fibrin, hyaluronan, or atelocollagen have been devised in the past years in effort to maximize efficiency of stem cell delivery into degenerated IVDs (156-158). Ideally, biomaterial scaffolds should withstand physiological loads, possess adequate immune compatibility and ability to maintain the stem cells retained into their construct. A biomaterial scaffold may promote the viability and enhance differentiation of mesenchymal cells in the desired location by providing a three-dimensional (3D) microenvironment.

One unique difficulty in implantation and delivery of stem cells is leakage and inability to retain the implanted cells at site of interest. Injecting a cell suspension into the lumbar discs of rabbits has been shown to result in a 90% loss of the injected cells within the first 30 minutes post injection (159). Another concern is osteophyte formation as a result of ectopic osteoblast differentiation by way of leakage. Thus, application of a scaffolding materials has been strongly recommended to mitigate the risk of leakage and to act as a retainer of the transplanted cells (149). Additionally, animal models have established that autologous BM-MSCs have a relatively short survival time up to 48 weeks after transplantation (66).

Chitosan polysaccharide is a natural component of insect exoskeleton and is both PH and temperature responsive. An *in vitro* study showed chitosan nanoparticles have a potent anti-inflammatory effect on degenerated IVDs by down-regulating mediators as IL-6, IL-8, MMP1 and MMP3 while causing up-regulation of collagen type II and aggrecan production (160). Another *in vitro* study showed that a chitosan scaffold enhanced MSCs phenotypic differentiation to NP like cells with increased production of aggrecans and collagen II (161).

Atelocollagen, an injectable collagen hydrogel, has been studied *in vitro* and provide a biocompatible environment that augmented NP cell function (162-164). *In vivo* implantation of AF cells seeded in atelocollagen scaffolds in rabbit models prevented progression of IVD space narrowing and had viability and proliferative activity (163).

Gelatin is derived from thermo-treated collagen and as collagen, it induces cellular adhesion, proliferation and collagen II expression (165). Culture of AF cells in a gelatin scaffold demonstrated improved cellular adhesion, ECM protein expression and inhibition of MMPs (166). An *in vivo* study showed that transplantation of MSC-gelatin scaffold in punctured rabbit IVD inhibited cellular apoptosis and maintained disc height index (167).

Hyaluronan as a scaffold material was also studied as a potential biomaterial to address difficulties in stem cell

delivery. Subhan *et al*. (168) in a controlled study on a rabbit model have demonstrated significant improvement in radiological and histological outcomes in the cell loaded scaffold group in comparison to other control groups. In another controlled study on an ovine model of damaged IVDs, Cell-free, freeze-dried resorbable polyglycolic acid-hyaluronan implants were implanted after nucleotomy of the IVD. Implantation of this cell-free polymer-based implant induced NPs tissue regeneration and improved disc water content both radiologically and histologically (169).

An *in vivo* sheep model showed increased NPs proteoglycan synthesis in IVDs injected with MSCs combined embedded in a fibrin scaffold (170). After 6 months, biochemical and histological markers showed better disc hydration and cellularity in the discs with scaffolding and MSCs than the discs injected with scaffolding alone. Also, radiological assessment revealed better Pfirmann scores compared to the control group. In a prospective study on 15 patients diagnosed with single-level lumbar spondylosis were treated via a percutaneous transplantation of allogenic juvenile chondrocytes encapsulated in a fibrin matrix (38). Six months follow-up revealed improvement in disc hydration by MRI evaluation and improvement in pain and disability scores with no reported adverse effects. Porous silk scaffolds have been also studied and shown to support AF cell attachment and ECM accumulation (171).

Alginate is another natural polysaccharide polymer derived from algae. Studies on alginate as scaffold showed induced MSCs differentiation to NP-like cells as well as increased ECM protein expressions (172,173). Synthetic polymers have shown promise as a scaffold material. Examples include, Polyethylene glycol (174), Polycaprolactone (175), Polyurethane (176) and Polylactic acid (177).

Restoration of disc height is necessary to ensure functionality of the IVD. Thus, a multifunctional therapeutic modality has been derived. A Recent advance in the field of bioengineering was the combination of such materials to create biphasic scaffolds to engineer the whole IVD by recapitulating the unique structures and functions of both NP and AF (178,179). For instance, a cell loaded biphasic scaffold was fabricated in which silk was used for the AF while fibrin and hyaluronan for the NP thus a whole IVD was generated *in vitro* (180). An *in vivo* animal study, utilized a novel cell loaded biphasic IVD by integrating a freeze-dried, cross-linked porcine bone matrix gelatin for the AF and porcine acellular cartilage for the NP (181).

Another innovative approach to scaffold development that is emerging is based on the inherent ability of cells to form their own matrix, much like that of the destination tissue (182). This approach involves culturing cells to produce ECM that will ultimately serve as the IVD implant with similar structural and compositional features of native tissue (183,184).

Some researchers also paid attention to natural biologic materials such as decellularized matrix from IVD to act as a natural scaffold for implanted cells. An *in vitro* study showed potential for the use of decellularized bovine IVD as a xenogenic scaffold (185). The included studies as regards the used scaffolds and delivery vehicles for stem cells in discogenic low back pain are summarized in *Table 3*.

Table 3 A summary of the literature review as regards the used scaffolds and delivery vehicles for stem cells in discogenic low back pain

Reference	Type of study	Cell source	Scaffold/delivery vehicle	Outcome
Bertram *et al.* (159)	*In vitro* and animal *in vivo* study (Rabbit model)	Rabbit NPCs (cultured in fibrin matrix)	Injectable cell fibrin gel	Up to 50% matrix injected cells remained in the nucleus and transition zone in contrast to a rapid loss of medium-injected cells. Enhanced survival of cells cultured with fibrin matrix
Teixeira *et al.* (160)	*In vitro*	Bovine IVD	Chitosan-Diclofenac nanoparticles	Chitosan-Diclofenac nanoparticles reduces inflammation and also decreases ECM degradation
Richardson *et al.* (161)	*In vitro*	hMSCs	Chitosan-glycerophosphate	Chitosan directs MSCs phenotypic differentiation to more NP like cells

Table 3 *(continued)*

Table 3 *(continued)*

Reference	Type of study	Cell source	Scaffold/delivery vehicle	Outcome
Sato *et al.* (163)	*In vitro*	Rabbit AF cells (cultured in atelocollagen)	Atelocollagen honeycomb-shaped scaffold with a membrane seal	The amount of type II collagen and its mRNA expression, GAG and proteoglycans in the scaffold cultured cells remained at a higher level than in the monolayer cultured cells
Sakai *et al.* (164)	*In vitro*	Human NPCs (cultured in atelocollagen)	Atelocollagen	Results showed that both DNA synthesis and content is significantly greater when cultured in Atelocollagen than in alginate
Yang *et al.* (167)	Animal *in vivo* study (Rabbit model)	Rat IVD MSCs	Pure fibrinous gelatin (PFG)	The transplanted MSCs in PFG inhibited apoptosis and slowed the rate of decrease in disc height index
Subhan *et al.* (168)	Animal *in vivo* study (Rabbit model)	Rabbit BM-MSCs (weren't scaffold cultured but were delivered with hyaluronan hydrogel)	Hyaluronan hydrogel	Better MRI scores for MSCs delivered with the hydrogel. Immunohistochemistry staining for collagen type II and aggrecan staining were also higher
Woiciechowsky *et al.* (169)	Animal *in vivo* study (Ovine model)	Acellular	Polyglycolic acid (PGA)	Histological analysis showed ingrowth of cells with typical chondrocytic morphology, even cell distribution, and ECM rich in proteoglycan
Chang *et al.* (171)	*In vitro*	Bovine AF cells (Cultured in porous silk scaffolds)	porous silk scaffolds and grown in either dynamic or static flow conditions	Dynamic flow conditions and scaffold pore size can affect the formation of engineered AF tissue
Yang *et al.* (172)	*In vivo*	Rat NP and AF cells	Alginate hydrogel seeded with NPCs, Polycaprolactone (PCL) seeded with AF cells	Long term implantation in rats generated highly hydrated soft tissues and well-integrated into the adjacent vertebrae
Zeng *et al.* (173)	*In vivo* and *in vitro*	hAD-MSCs	Polyacrylate microcryogels (PM)	Enhanced cell retention of PMs assisted cell delivery to a load bearing environment of rat IVD
Benz *et al.* (174)	*In vivo* and *in vitro*	Human IVD tissue	Hydrogel composed of chemically activated albumin crosslinked by polyethylene glycol	The expression of cartilage- and disc-specific mRNAs was maintained in hydrogels *in vitro* and *in vivo*
Hu *et al.* (176)	*In vitro*	Rabbit BM-MSCs	Silk fibroin/ polyurethane (SF/PU) composite hydrogel	The composite hydrogel exhibited suitable physical-mechanical properties as prosthetic biomaterial for NP replacement
Kim *et al.* (177)	*In vivo*	Rat NPCs	polylactic-co-glycolic acid	As the pores became smaller, the value of the compressive strength of the scaffold was increased
Choy *et al.* (178)	Mechanical vitro study	Acellular	Biphasic scaffold fabricated with collagen and glycosaminoglycans (GAGs)	Biphasic scaffolds comprised of 10 annulus fibrosus-like lamellae had the best overall mechanical performance among the various designs

Table 3 *(continued)*

Table 3 *(continued)*

Reference	Type of study	Cell source	Scaffold/delivery vehicle	Outcome
Elsaadany *et al.* (179)	Mechanical *in vitro* study	hAD-MSCs	Biphasic mechanically conditioned scaffold encapsulated with hAD-MSCs	Equiaxial loading increased secretion of ECM proteins and gene expression of AF markers compared to unstrained samples
Park *et al.* (180)	*In vitro*	Porcine AF cells (cultured in silk scaffolds)	Silk scaffold (lamellar versus porous)	Histology, biochemical assays, mechanical testing and gene expression indicated that the lamellar scaffold generated results that were more favorable in terms of ECM expression and tissue function than the porous scaffold for AF tissue
Xu *et al.* (186)	Animal *In vivo* study (Rat model)	Rat NP and AF cells (cultured in the biphasic scaffold *in vitro*)	Freeze-dried, cross-linked biphasic scaffold of pig bone matrix gelatin for the outer AF and pig acellular cartilage ECM for the NP	IVD like tissue formed in mice as confirmed by histology after subcutaneous implantation of cell-scaffold constructs
Illien *et al.* (183)	*In vitro*	Human NPC and MSCs (cultured with the scaffold)	Decellularized injectable bovine ECM material	The scaffold maintained native NP tissue structure and composition closest to natural ECM and promoted cellular adaptation of NP cells and MSCs

Current challenges

Stem cell therapy for discogenic low back pain is still in infancy and will need to demonstrate pre-clinical efficacy and safety using *in vitro* and *in vivo* model systems before being translated to wide clinical use. One of the greatest challenges facing *in vivo* animal models is the need to replicate the size and height of the human disc in the pre-clinical trials. For example, lumbar disc height in sheep is about 4 mm compared to about 11 mm in humans (187).

The replication and testing of the regenerative potential of stem cells under conditions which mimics the complex micro-environment in the IVD is another challenge. Not only biochemical conditions but also physiological loading conditions should be replicated to allow reproducible and reliable results after transplantation. The physical environment can be mimicked through culturing in soft 3D scaffolds such as hydrogels and mechanical stresses such as dynamic compressive loads or hydrostatic pressures applied via bioreactors (188-191).

The high cost of clinical trials and ethical concerns regarding the use of embryonic and umbilical stem cells is another obstacle. A recent systematic review showed that most of the literature available on the topic is methodologically poor with small sample sizes, high risk of bias and lack of a control intervention (192). Equally important, current patient reported outcome measure are influenced by significant placebo effects (191). Also ensuring safety and tolerability mandates that uncontrolled differentiation of stem cells to be checked and devising more efficient delivery strategies.

Another challenge is the correct targeting of the painful degenerated discs rather than painless degeneration. Except in cases with nerve root compression or central stenosis, conventional MRI studies do not distinguish effectively between painful and nonpainful degenerating disks (193). Also, to support clinical application and efficacy of stem cell therapy, there is a critical need for new techniques to quantify the therapeutic effects on treated levels.

More recent MRI techniques such as T1ρ and T2 relaxation times, and chemical exchange saturation transfer (CEST) provides a quantitative analysis for disc composition indicating more accurately DDD (194,195). Painful disks are characterized by hypoxia and inflammation which leads to accumulation of certain metabolites such as lactate, alanine, and lipids (196). These metabolites may serve as biomarkers that are detectable by means of MR spectroscopy (197). A recent imaging technique is quantitative sodium MRI which detects sodium levels in

the IVD and thus the hydration status. A recent study suggests that this technique may differentiate discs between asymptomatic and symptomatic patients (198). However, sodium MRI is in limited use because of the requirement for special hardware modifications to the conventional MRI scanner. Fluorine 18 fluorodeoxyglucose positron emission tomography (FDG-PET) has been shown to identify inflamed degenerated discs and may be a beneficial diagnostic tool (199). As previously stated, inflammation is a central feature of painful disks. PET/CT may be useful in selection of patients and localizing the spinal levels.

Invasive provocative discography, which includes disc stimulation and morphological evaluation, is often used to distinguish a painful disc from other potential sources of pain such as facet joint pain. A recent systematic review supported the use of provocative lumbar discography as an accurate diagnostic tool (200).

Conclusions

Novel stem cell regenerative therapy for discogenic low back pain is a promising alternative to the conventional surgical management and other non-operative alternatives. Patient selection as well as accurate localization of pain generators is a pre-requisite for successful effective treatment. The utilized stem cells are broadly either derived from the IVD itself (resident stem cells or primary cells) or derived from other pluripotent sources as BM or adipose tissue. Many challenges face this relatively infant field, ranging from isolation, culture and host delivery. The complex and harsh microenvironment of the degenerated IVD plays a detrimental role in multiplication and survival of the transplanted stem cells. More basic science and clinical studies are needed to establish the clinical efficacy of such treatments.

Limitations

This is a literature review on the current concepts and advances in the field of stem cell regenerative therapy for discogenic back pain. Most of the available studies are animal or *in vitro* studies with relative scarcity of human trials. Nevertheless, a strength of our review is that we conducted a comprehensive search of multiple databases. Independent reviewers performed the search, selected and appraised the included studies.

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None.

Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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