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HOT TOPICS IN PAIN AND HEADACHE (N ROSEN, SECTION EDITOR)

Stem Cell Therapies for Treatment of Discogenic Low Back Pain: a Comprehensive Review

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Abstract

Purpose of Review Discogenic low back pain (DLBP) stems from pathology in one or more intervertebral discs identified as the root cause of the pain. It is the most common type of chronic low back pain (LBP), representing 26–42% of attributable cases.

Recent Findings The clinical presentation of DLBP includes increased pain when sitting, coughing, or sneezing, and experiencing relief when standing or ambulating. Dermatomal radiation of pain to the lower extremity and neurological symptoms including numbness, motor weakness, and urinary or fecal incontinence are signs of advanced disease with disc prolapse, nerve root compression, or spinal stenosis. Degenerative disc disease is caused by both a decrease in disc nutrient supply causing decreased oxygen, lowered pH, and lessened ability of the intervertebral disc (IVD) to respond to increased load or injury; moreover, changes in the extracellular matrix composition cause weakening of the tissue and skewing the extracellular matrix's (ECM) harmonious balance between catabolic and anabolic factors for cell turnover in favor of catabolism. Thus, the degeneration of the disc causes a shift from type II to type I collagen expression by NP cells and a decrease in aggrecan synthesis leads to dehydrated matrix cells ultimately with loss of swelling pressure needed for mechanical support. Cell-based therapies such as autologous nucleus pulposus cell re-implantation have in animal models and human trials shown improvements in LBP score, retention of hydration in IVD, and increased disc height. Percutaneously delivered multipotent mesenchymal stem cell (MSC) therapy has been proposed as a potential means to uniquely ameliorate discogenic LBP holistically through three mechanisms: mitigation of primary nociceptive disc pain, slow or reversal of the catabolic metabolism, and restoration of disc tissue. Embryonic stem cells (ESCs) can differentiate into cells of all three germ layers in vitro, but their use is hindered related to ethical concerns, potential for immune rejection after transplantation, disease, and teratoma formation. Another similar approach to treating back pain is transplantation of the nucleus pulposus, which, like stem cell therapy, seeks to address the underlying cause of intervertebral disc degeneration by aiming to reverse the destructive inflammatory process and regenerate the proteoglycans and collagen found in healthy disc tissue.

Summary Preliminary animal models and clinical studies have shown mesenchymal stem cell implantation as a potential therapy for IVD regeneration and ECM restoration via a shift towards favorable anabolic balance and reduction of pain.

Keywords Low back pain · Mesenchymal stem cells · Embryonic stem cells · Degenerative disc disease · Discogenic pain · Nucleus pulposus transplantation

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Introduction

Low back pain (LBP) has 84% lifetime prevalence and is the fifth most common cause for primary care visits in the USA [1–5]. It is estimated that a quarter of US adults have experienced LBP lasting at least 1 day within the last 3 months, with up to 30% annual prevalence of LBP in the USA [6]. The incidence of LBP is growing, likely related to an aging population, higher obesity rates, and other related factors [7].

The prevalence of LBP is equal between men and women, and the onset is characteristically between the ages of 30–50, increasing with age, as intervertebral discs lose fluid and flexibility causing reduced bone strength and muscle elasticity [8]. The prevalence of LBP is higher for those who smoke, are obese, have less education, are dissatisfied with work, have somatization disorder, and those that have anxiety or depression due to higher muscle tension and perception of pain severity [2, 8–15]. Risk factors include heavy lifting, twisting, and physically strenuous work, sedentary work with poor posture or inadequate seated back support. The risk of developing LBP is increased in those with low fitness levels or in those who lack low-impact aerobic exercise, for example, those who exercise extensively on weekends after remaining primarily sedentary during the week [2, 8–15].

Significant heterogeneity exists within symptom severity, ranging from a persistent dull ache to an incapacitating, blinding pain arriving without warning [5]. Etiology of the pain can also vary from acute injury to gradual changes to the spine over time; however, over 85% of patients presenting in primary care settings have non-specific LBP, typically acute in onset [8, 16•, 17•]. In 80–90% of cases, LBP is classified as acute, or self-limited, lasting fewer than 6 weeks with a favorable prognosis [5, 18, 19]. Approximately 5–10% of LBP patients are chronic with symptoms lasting over 3 months [19].

Discogenic Low Back Pain (DLBP)

The lower back includes the five vertebrae in the lumbar region, L1–L5, with cushioning intervertebral discs between each vertebra held in place by ligaments. A majority of LBP is mechanical in nature, most commonly caused by degeneration of the intervertebral discs (IVD) [20]. While IVD degeneration accompanies aging, a subset of the population experiences degenerative disc disease (DDD), a distinct pathologic condition associated with IVD degeneration with a displacement of disc material into the spinal canal, including protrusion, herniation, and sequestration. [20]. Discogenic low back pain (DLBP) is defined as having one or more intervertebral discs identified as the root cause of the pain. DLBP is the most common type of chronic LBP, representing 26–42% of attributable cases [21, 22]. However, 80% of LBP cases cannot be attributed to a particular pathology [21].

DLBP lacks clear diagnostic criteria since a history or physical examination does not reveal disease-defining characteristics. Most patients remain asymptomatic, and the extent of disc degeneration is uncorrelated to symptomatic severity [23]. Clinical presentation of DLBP includes increased pain when sitting, coughing, or sneezing, and relief when standing or ambulation [21, 24]. Dermatomal radiation of pain to the lower extremity and neurological symptoms such as numbness, motor weakness, and urinary or fecal incontinence are signs of advanced disease with disc prolapse, nerve root compression, or spinal stenosis [21]. The diagnostic tests which include centralization phenomenon (CP) as proposed by McKenzie, in which central spinal pain is elicited by lateral movement, and bone vibration tests (BVT), where blunt electric vibrators are applied to spinous processes to provoke the pain, have limited application due to low sensitivity and specificity in differentiating DLBP from other causes of LBP [21, 25, 26].

DLBP can be imaged in several ways. Myelograms and magnetic resonance imaging (MRI) non-invasively exhibit herniated discs [27]. T2-weighted sagittal sequences define a morphologically based grading scale for disc health [28]. MRI can also utilize high-intensity zones to detect annular disc tears, though lacks the ability to detect centrally located fissures [29]. Computerized tomography (CT) reveals disc rupture, and provocation discography can enhance the display of intradiscal pathology on CT, moreover through disc stimulation can identify the pain generating disc [29–31]. Although discography was touted as the gold standard, its value is limited due to its invasive nature and potential complications, including epidural abscesses and acute exacerbation of prolapse [21]. Ultrasound imaging in combination with serum markers such as high sensitivity c reactive protein (hs-CRP) and proteomic fingerprinting may become the non-invasive algorithm of choice to diagnose DLBP in the future [32–34].

Pathophysiology of the painful degenerative disc is only beginning to be understood. As a cartilaginous structure, the IVD lacks vascular support, thus enabling a baseline harsh environment with low pH, low oxygen, and scarce nutrients [29]. Degenerative disc disease is caused by both a decrease in disc nutrient supply causing decreased oxygen, lowered pH, and decreased ability of the IVD to respond to increased load or injury, as well as changes in the extracellular matrix composition, which weakens tissue and skews the extracellular matrix's (ECM) harmonious balance between catabolic and anabolic factors for cell turnover in favor of catabolism [20]. Decreased blood supply in DDD may lead to upregulation of inflammatory cytokines and degradative enzymes [23]. The IVD maintains spinal integrity related to two main components: the central type II collagen and proteoglycan (typically aggrecan)-rich nucleus pulposus (NP) and the peripheral

fibrous, type I collagen-rich annulus fibrosis (AF) [28]. The degeneration of the disc causes a shift from type II to type I collagen expression by NP cells, and a decrease in aggrecan synthesis leads to dehydrated matrix cells ultimately with loss of swelling pressure needed for mechanical support [28].

The disc suffers structural damage to the annulus, which is followed by the development of an inflammatory milieu with changes in the nucleus pulposus microcomposition (fluid content, growth factors, cell composition, loss of proteoglycans and type II collagen) [27, 35–37, 38•]. In some cases, it progresses to the development of vascularized granulation tissue formation, cellular remodeling, increased fibrosis, and increased nerve growth, which may result in the changes of degenerative disc disease [36, 39–41]. In addition, peripheral sensitization of the nociceptive nerve endings by sympathetic afferents occurs [42, 43]. Granulation tissue in the over-innervated annulus irritates the free nerve endings and is further exacerbated by other stimuli such as ischemia, pressure changes, or inflammatory cytokines, in the DLBP pain syndrome [41, 44]. Increased TNF-α expression, increased catabolic cytokines, and lowered aggrecan levels in degenerate tissue stimulate nerve or neurite ingrowth, suggesting a role in innervation [28]. Key elements are the dedifferentiation and change in the cellular growth patterns which disrupt normal repair and are potential targets for future stem cell therapy [23, 29, 45••, 46, 47].

Application of Stem Cell Therapy for the Management of Low Back Pain

Current treatment methods for DLBP include conservative management and surgical fusion, including a myriad of approaches as monotherapies or in combination with little consensus as to the best approach. Pharmacological treatments with NSAIDs, analgesics, lidocaine patches, and muscle relaxants are limited in efficacy, while epidurally administered steroids and local anesthetics and percutaneous heat treatments may improve symptoms though need further investigation [22, 48–50]. Nontraditional treatment including massage and acupuncture are commonly utilized as well. Exercise therapy by the McKenzie method is popular amongst physical therapists [22]. If conservative modalities are ineffective, interventional techniques for pain relief are utilized, which largely can ameliorate pain but lack evidence of a sustained effect. Surgical fusion of the lumbar spine is not without complication [22]. An alternative to surgical fusion is artificial disc replacement, though long-term follow-up research needs to be conducted, as complications may occur years after the procedure [22].

Current surgical methods fail to address the pro-inflammatory milieu of degenerate discs, the loss of matrix anabolism, or the loss of functional native cells and tissue [28]. In principle, surgery eliminates or fixates tissue, altering spine biomechanics providing no opportunity for regeneration. As the pathophysiology of DLBP is better understood, a growing interest in regeneration, to restore the initial disc tissue and metabolic balance of the disc through biological means or cell-based therapies, has emerged. Cell-based therapies such as autologous nucleus pulposus cell re-implantation have in animal models and clinical trials shown statistically significant improvements in LBP score, retention of hydration in IVD, and increased disc height [51, 52]. These mesenchymal progenitor cells are located in the stem cell niche in a quiescent state until activated by specific signals such as trauma to produce daughter cells. However, harvesting enough cells for re-implantation is difficult, as the NP is hypocellular, and NP cells are from degenerate discs themselves, they are reduced with age, catabolic relative to normal cells, making them unsuitable for transplantation where metabolic balance is required [28]. They may, however, be stimulated by stem cells to help aid in the regenerative process.

Stem cells such as olfactory stem cells and chondroprogenitors from murine embryonic stem cells may reverse IVD degeneration [53]. Percutaneously delivered multipotent mesenchymal stem cells (MSC) cell therapy has been most commonly proposed as a potential means to uniquely ameliorate DLBP through three mechanisms: mitigate primary nociceptive disc pain, slow or reverse the catabolic metabolism, and restore disc tissue. Stem cells are multipotent, so they are able to self-renew and differentiate into specific cell types. Embryonic stem cells (ESCs) are able to differentiate into cells of all three germ layers in vitro, but their use is limited due to ethical concerns, potential for immune rejection after transplantation, disease, or teratoma formation, though MSC therapy has no evidence supporting carcinogenesis in vivo or in vitro [29, 54]. MSCs could promote the synthesis of proteoglycans and type II collagen diminished with DDD, thereby reversing or halting the disc degeneration process [23]. MSCs are multipotent immunomodulators and can chemotactically propagate activity in nearby endogenous IVD cells through secretion of anabolic growth factors, assist ECM production, reduce inflammation and catabolism in nearby tissues, adhere to plastic under typical tissue culture conditions, express certain surface markers while excluding expression of other markers, and differentiate into osteoblasts, adipocytes, and chondroblasts in vitro [55–58].

Stem cell therapy is fitting for degenerate IVD cells; as IVDs are cartilaginous and the largest avascular structure in the human body, it is difficult for natural regeneration to occur when metabolic homeostasis is disrupted [29]. NP cells are reliant upon glycolysis and lactic acid removal via diffusion. Degeneration causes calcification of disc end-plates, causing the avascular environment to become even more acidic, hypoxic, and nutrient-deficient. This change may potentially be reversed or mitigated by regenerative cells [28]. The most

significant hurdle to MSC transplantation is the hostile milieu of the degenerative IVD, an acidic environment with high osmolarity. This environment may hinder MSC survival, according to animal studies showing the viability of the transplanted cells up to 6 months [53, 54, 59–61]. A rabbit study and porcine study compared MSC transplantation to NP cell transplantation, and no differences in the efficacy of regeneration were detected [62–65]. A porcine study compared MSCs and committed chondrocytes in the IVD niche to find that cell survival, and ECM formation was superior in the committed chondrocytes, perhaps because they are better suited than MSCs to survive in the avascular IVD environment [62–65]. Further research could illuminate how the degenerating IVD environment impacts viable MSC function.

Recent Advances in Stem Cell Technology

MSC therapy in animal models has shown promise thus far: typical positive outcome measures include increased disc height, higher MRI T2-weighted signal, improved histological grading, and restored ECM content and related gene expression [53, 66]. In animal models, IVD degeneration is induced by trauma, typically puncturing the annulus and aspirating a portion of nucleus pulposus with a needle or degrading ECM with chondroitinase ABC injection. Stress-induced IVD degeneration can occur in humans, but these models are imperfect replicas due to the fact that the animals initially have healthy IVDs unlike the gradual nutrient repletion or progressive degeneration in IVDs of typical aging humans with DDD [53]. Novel ex vivo organ IVD model systems have been tested as well, with promising results for therapies to be tested before clinical implantation [67–69].

Key challenges to stem cell therapy include ensuring that MSCs are able to be procured in large enough numbers, transplanted safely, and differentiated to the correct phenotype. MSCs in basic science research thus far have been derived from bone marrow, adipose tissue, umbilical cord tissue, or more rarely, knee-derived synovial, muscle, and periosteum tissue, all of which are more abundant than embryonic stem cells (ESCs) [54]. Bone marrow–derived MSCs (BM-MSCs) are the most commonly studied and appear the best in differentiation [29]. In obtaining these, bone marrow aspirate is obtained from the posterior iliac crest and then centrifuged to a nucleated cell concentrate [29]. These are easily injected in animal models, but MSC density is lower than ADSCs (adipose-derived MSCs). Umbilical cord MSCs (HUC-MSCs) may be utilized when there is a need for their especially low immunogenicity [29].

Because of the avascular conditions of the NP, the site is immunoprivileged, likely reducing risk of immune reaction by the host upon transplantation of MSCs [54]. Evidence indicates that MSC implantation can cause peripheral osteocyte formation and in MSC implantation for cartilage regeneration, differentiation into chondrocyte phenotypes has shown hypertrophy and ossification. These risks will need to be watched for in IVD studies [28]. Animal studies revealed that a 3:1 ratio between NP and MSC cells is key for differentiation of BM-MSCs to a NP phenotype [54]. MSCs were also able to be differentiated into an AF phenotype, which is useful for sealing annular tears [54].

Several recent studies have been conducted regarding the efficacy of MSCs. An ex vivo bovine study published by Teixeira et al. [70••] in 2018 studied the effect of the IVD microenvironment on BM-MSCs. Bovine NP cell cultures were punctured with a needle to simulate IVD degeneration. Human BM-MSCs were cultured on top and then screened for apoptosis, metabolic activity, migration, inflammatory cytokines in basal and pro-inflammatory conditions, ECM remodeling, and gene expression. Results showed that pro-inflammatory or degenerative IVD conditions did not affect MSC viability but promoted cell migration. No effect was seen on ECM remodeling as measured by type II collagen or aggrecan levels; however, MSCs downregulated levels of bovine pro-inflammatory gene expression, specifically IL-6, IL-8, and TNF-α, through a paracrine effect, which appears promising.

ADSCs also demonstrate an anti-inflammatory effect from bioactive factors, as established by an in vitro rat study by Miguelez-Rivera et al. [71], which investigated the immunomodulation of conditioned medium from ADSC culture in the IVD environment. ADSCs were derived from rat adipose tissue and isolated cells were cultured in monolayer. AF and NP cells were obtained from rats and co-cultured with murine macrophages stimulated with TNF-α to create a pro-inflammatory IVD environment simulating degeneration. The conditioned ADSC medium was added to the AF and NC cells, and levels of pro-inflammatory cytokine expression of IL- 1β, IL-6, IL-17, and TNF-α were measured using flow cytometry and confocal microscopy. Before adding the conditioned ADSC medium, pro-inflammatory cytokine levels were high but were significantly decreased upon adding ADSC medium, indicating that ASCs secrete immunomodulatory bioactive factors. ADSCs were also established as easy to collect, abundant, and proliferating well in vitro.

Wang et al. [72] investigated the hypothesis that since hypoxic conditions hinder BM-MSC differentiation, cell survival, migration, and overall efficacy in IVD transplantation, hypoxic pre-conditioning could improve the therapeutic potential of BM-MSCs by enhancing cell tolerance to subsequent injury. BM-MSCs from rats were treated with cobalt chloride in vitro to simulate hypoxic conditions, causing cell apoptosis and migration. The BM-MSCs were injected in an in vivo rat model of IVD degeneration, which was established with a stab incision to aspirate NP material. Compared with the non-preconditioned group, the cobalt chloride-treated arm resulted

in IVD height significantly increased, and collagen II and aggrecan expression representing ECM generation significantly increased. Apoptosis decreased as measured by activating bcl-2 and inhibiting caspase-3, while migration improved, as exhibited by higher HIF-1α and CXCR4 mRNA expression.

These aforementioned studies demonstrate cross-talk between MSCs and NP cells through paracrine factors; however, Lehmann et al. [73] demonstrated TGF-β to be a MSC paracrine agent mediating ECM generation. Human NP cells and MSCs were co-cultured both directly and indirectly (i.e., intracellular contact through only soluble factors). COLIA1 RNA expression encoding collagen II was increased in both MSCs and NP cells as a result. SOX9 expression encoding chondrocyte differentiation and ACAN encoding aggrecan were increased in MSCs, which indicates that perhaps TGF-β was secreted by NP cells, stimulating TGF-β-sensitive genes in MSCs after a long incubation period. It remains unclear whether TGF-β is the paracrine factor mediating interaction or whether it is a byproduct of a signaling pathway controlled by other factors. Upregulation of SOX9, ACAN, and COLIA1 was diminished once inhibitors of TGF-β receptor I, SB-431542 and SB-525334, were added to the co-culture; the former blocked COLIA1 increase in MSCs while both blocked ACAN and SOX9 expression increase in MSCs. Notably, short incubation time for the co-culture was observed to be not long enough for TGF-β signaling to surpass the effect pro-inflammatory cytokines.

Regarding timing around MSC implantation, Maidhof et al. [74] investigated the timing of degeneration or disease progression MSC therapy to maximize disc regeneration. A rat disc stab IVD model was utilized with MSCs administered in vivo on days 3, 14, and 30 after injury. Infrared imaging was utilized to track the migration 14 days after delivery and showed that cells administered 3 days after injury were retained at the disc site more compared with the other groups. Thus, cell migration into healthy discs from degenerated IVDs after injection appears to be time-dependent, with optimal timing of treatment to be closer to the time degeneration begins.

In addition to the optimal timing of MSC administration, the longevity of implanted MSCs is a critical data point to note for the clinical relevance of stem cell therapy in IVD degeneration. Hang et al. [75] observed the survival time of implanted MSCs in IVDs non-invasively in vivo in a canine model through PET imaging and MRI 3 days, 2 weeks, 3 weeks, and 4 weeks after MSC injection. A canine stab model to remove the NP was conducted in beagles. BM-MSCs extracted from beagles were labeled with magnetic iron oxide nanoparticles (MION), and MION signal was found to not change significantly between 3 days after implantation and 4 weeks after implantation with atelocollagen gel in target IVDs, which was less reliable than PET. PET reporter probe signal was significantly decreased at 3 weeks after implantation. Together, this suggests that MSCs survival and efficacy time after implantation is limited to 3 weeks in canine models, a time frame that cannot be applied clinically to humans without further clinical investigation.

The models so far discussed have been animal models with induced tissue injury, which may not serve as an optimal surrogate for human IVD degeneration occurring gradually over time. Steffen et al. [76] investigated the efficacy of BM-MSCs transplanted in dogs with naturally occurring IVD degeneration. Dogs suffering from naturally occurring degenerative IVD had autologous BM-MSCs isolated, cultured for 3 weeks, and injected. Clinical status, disc height, grading, and volumetry were measured at 1, 5, 6, and 12 months after treatment. Fortunately, implantation was a success with no significant difference in adverse effects between control and treated dogs. There was no difference in the clinical outcome of the dogs displaying signs of IVD regeneration with MSC therapy. Limitations of the study include lack of scaffolds or tests, only observation to ensure that MSCs remained in situ. Also, sample size was limited to six dogs. However, this study tempered the success of previous animal model studies with acute injury–based IVD degeneration models, displaying that animal models are only useful to an extent in showing a specific biological mechanism through carefully controlled conditions. Conducting further clinical studies in naturally occurring, less controlled IVD degeneration situations is imperative to accurately assess the therapeutic potential of MSCs (Table 1).

Clinical Studies

Promising results in human trials are now beginning to investigate the safety and efficacy of stem cell therapy in the treatment of LBP. In a case study of two female patients, autologous bone marrow mesenchymal cells (MSCs) were introduced to the degenerative discs of these patients. No adverse events were reported, and pain relief was noted in both patients. Furthermore, T2-weighted MRI demonstrated increased water content in the treated intervertebral discs at 2-year follow-up, evidence of disc regeneration [77]. In another, 10 patients receiving autologous bone marrow MSCs who were followed for 1 year as part of a pilot study. They found the technique to be safe and feasible, while the clinical results were promising. While MRI did not show a change in disc height for these patients, a significant improvement in disc hydration was noted. Significant pain relief occurred and did so quickly, with 85% of it occurring within the first 3 months post-treatment. Disability and quality of life measures improved alongside the analgesic benefits, which approached 71% of the researchers' optimal efficacy. These measurements proved to compare favorably to other treatment modalities

Table 1 Summary of basic science mechanisms of stem cell therapy

Year, author	Stem cell line/animal model	Results/outcomes measured
2018, Teixeira et al. [70••]	Human BM-MSCs Bovine Ex vivo	Degenerative IVD conditions did not affect MSC or IVD viability (Anx and PI staining < 20%) but promoted cell migration ($p < 0.05$; when IL-1β added) and increased inflammatory cytokine expression. MSC had no effect on ECM production (i.e., collagen II and aggrecan) but lowered inflammation (i.e., IL-6 decreased from 14-fold to 3-fold ($p < 0.05$), IL-8 from 8-fold to 2-fold ($p < 0.05$), and TNF-α expression from 1-fold to 0.3-fold) and cell migration.
2018, Miguelez-Rivera et al. [71]	Autologous rat ADSCs Rat in vitro	ADSC medium lowered inflammation (i.e., IL-1β, IL-6, IL-17, and TNF-α expression in both AF ($p < 0.005$) and NP ($p < 0.05$) cultures), showing ADSCs secrete immunomodulatory bioactive factors.
2018, Wang et al. [72]	Rat BM-MSCs Rat in vivo	Hypoxic pre-treatment of BM-MSCs with $CoCl_2$ enhanced migration (i.e., higher mRNA levels of HIF-1α and CXCR4 ($p < 0.05$)), decreased apoptosis (i.e., lowered bcl-2 and caspase-3 ($p < 0.05$)), increased disc height ($p < 0.05$), increased MSC numbers in the NP and AF regions ($p < 0.05$), and increased ECM protein production (i.e., collagen II and aggrecan ($p < 0.05$)). Hypoxic pre-treatment may enable MSCs to overcome challenging hypoxic conditions in degenerating IVD.
2018, Lehmann et al. [73]	Autologous human BM-MSCs Human in vitro	TGF-β expression increased in co-cultured NP cells and MSCs, concomitant with gene expression for differentiation (SOX9), and ECM proteins aggrecan (ACAN) and collagen II (COLIA1), implicating paracrine TGF-β release from NP cells as involved in cross-talk with MSCs.
2017, Maidhof et al. [74]	Allogeneic rat BM-MSCs Rat in vivo	BM-MSCs administered 3 days after injury or induction of IVD degeneration had much more intensity ($p < 0.01$) in injured discs (20.2) compared with injection after 14 days (10.7) or 30 days (9.9).
2017, Hang et al. [75]	Autologous canine BM-MSCs Canine in vivo	Survival of BM-MSCs was tracked via MRI (MION) and PET. MION at 3 days after injection was not statistically significant for other time points measured (e.g., 2 weeks, 3 weeks) until 4 weeks after implantation (e.g., 751.43 at 4 weeks vs. 243.86 at 3 days ($p < 0.01$)). PET signal significantly decreased 3 weeks after injection compared with 2 weeks after therapeutic MSC administration ($p < 0.001$). In canines, 3 weeks is the survival time for BM-MSCs used in IVD therapy. MRI was less reliable than PET for long-term MSC survival tracking.
2017, Steffen et al. [76]	Autologous canine BM-MSCs Canine in vivo	Feasibility of injecting BM-MSCs into the lumbosacral discs of naturally IVD-degenerative canines was successful—no fluid was expulsed from the IVDs but no explicit tests were conducted to ensure MSCs remained in situ. No adverse events were reported after injection. Both control and treatment dogs improved their pain and disability score from a median of 15 pre-operatively to 21 post-operatively, without a significant relative improvement in the treated group. Disc height and volumetry showed no significant difference pre and post-operatively or between the control and treatment groups.

ADSCs, adipose tissue-derived mesenchymal stem cells; *BM-MSCs*, bone marrow-derived mesenchymal stem cells; *ANxV*, annexin B measuring cell apoptosis/death; *PI*, propidium iodide measuring cell apoptosis/death; *MION*, magnetic iron oxide nanoparticles measuring cell survival

such as spinal fusion or disc replacement while offering the benefit of being less invasive [78].

An open-label pilot study conducted by Pettine et al. observed 26 patients who were referred for surgical consultation but elected to treat their degenerative disc disease with autologous bone marrow MSCs. Half received injections at one symptomatic disc, and half received injections at two adjacent symptomatic discs. There were zero adverse events reported in response to the autologous disc injections throughout the 12-month follow-up period. Validated scales including the Oswestry Disability Index (ODI), visual analog scale (VAS), and modified Pfirrmann scale for MRI were used pre and post-treatment to evaluate efficacy. Results were encouraging, with 21 of 26 patients demonstrating statistically significant improvement in pain scores and impairment, and all subjects reporting a reduction in pain (Table 2). Two patients chose to undergo spinal fusion surgery within 6 months of the stem cell injection. In general, patients receiving higher numbers of stem cells in their injections typically experienced significantly faster and greater reductions in VAS and ODI. The statistical benchmark of 2000 colony forming unit-fibroblast (CFU-F)/mL was determined to be significant, especially in the context of advanced age, in predicting the efficacy of treatment. Patients over 40 receiving less than 2000 CFU-F/mL saw an average ODI and VAS reduction of 33.7% and 29.1%, respectively. Conversely, all other subjects saw average reductions

Table 2 Clinical efficacy of stem cell therapy for the management of chronic low back pain

Year, author	Study design	Results
2010, Yoshikawa et al. [77]	Autologous BM-MSCs *N* = 2 Follow-up: 2 years	Increased water content in discs and improved pain scores in both patients
2011, Orozco et al. [78]	Autologous BM-MSCs *N* = 10 Follow-up: 12 months	No change in disc height, increased disc hydration. Improved pain and quality of life scores. 85% of pain relief occurred in the first 3 months.
2015, Pettine et al. [79] 2016, Pettine et al. [80] 2017, Pettine et al. [81]	Autologous BM-MSCs *N* = 26 Follow-up: 12 months, 2 years, 3 years	1 additional patient elected for surgery. Continued ODI and VAS score improvement between years 2 and 3. Favorable ODI and VAS score improvements when compared with studies analyzing disc replacement or lumbar fusion patients. ODI and VAS scores continued to downtrend at 2-yr follow-up. Continued ODI and VAS score improvement between years 2 and 3. Favorable ODI and VAS score improvements when compared with studies analyzing disc replacement or lumbar fusion patients.
2016, Elabd et al. [82]	Autologous hypoxic-cultured BM-MSCs *N* = 5 Follow-up: 4–6 years	Positive association between a number of stem cells and clinical improvement. MRI showed maintenance or mild disc narrowing. 4 of 5 had reduced disc protrusion.
2017, Centeno et al. [83]	Autologous BM-MSCs *N* = 33 Follow-up: 6 years	3 patients with pain related to the procedure. Mean 60% clinical improvement at 3 years. 20 patients had MRI follow-up, 17 showed a reduction in disc bulge. On average, patients with greater bulge reduction reported less pain.
2017, Kumar et al. [45••]	Autologous AT-MSCs + hyaluronic acid *N* = 10 Follow-up: 12 months	No statistical difference between high and low dose arms. Greater than 50% VAS and ODI at 6 months in 7 patients, 6 patients at 1 year. 1 Patient with improved Pfirmann grade.
2017, Noriega et al. [84]	Allogenic BM-MSCs *N* = 24 Follow-up: 12 months	Study included a sham injection group. Stem cell patients had significant VAS and ODI reductions at 3, 6, 12 months. Effects were insignificant in controls. MRI showed disc degeneration in controls vs improvement in MSC patients.

AT-MSCs, adipose tissue-derived mesenchymal stem cells; *BM-MSCs*, bone marrow-derived mesenchymal stem cells; *ODI*, Oswestry Disability Index; *VAS*, visual analog scale; *SANE*, Single Assessment Numeric Evaluation; *CFU-F*, colony forming unit-fibroblasts (stem cell count proxy)

All studies reported no serious adverse events

of 69.5% and 70.6% at the 1-year mark [79]. Of the two patients who elected for surgery, one had a CFU-F/mL of 1114 while the other had no cell analysis data available. Post-procedure MRIs were taken in 20 of the 26 patients at 12 months. Of the 6 patients not evaluated with MRI, 2 had elected for surgery and the other 4 failed to schedule follow-up MRI. At 1 year, MRI showed an improvement of at least one Pfirrmann grade in 5 of the 10 patients receiving a single-level injection, as rated by a blinded independent radiologist. The same was true of 3 of 10 two-level patients. None of the 20 patients had worsening of their discs according to MRI analysis. Similar associative trends were found between cell counts and MRI improvement, with patients in the > 2000 CFU-F/mL cohort averaging 0.58 grade levels per disc improvement, as opposed to 0.17 per disc in the < 2000 group. Notably, the study design allowed patients with less than a 25% reduction in ODI or VAS after 6 months the option to receive a second injection, an option that two subjects exercised. Both saw significant improvements, with ODI and VAS score reductions from 20 to 2 and 59 to 0 respectively for one patient and 54 to 4 (ODI) and 40 to 0 (VAS) for the other. This study ultimately demonstrated the safety and feasibility of the procedure, as well as evidence suggesting the importance of adequate cell populations and the possibility of successful revisions in patients experiencing inadequate responses. At the 36-month follow-up, only 1 of 6 surgical patients demonstrated improvement from baseline pain and functioning [81]. After 3 years of follow-up, there were no adverse events relating to the procedure other than the progression to surgery in 6 of the 26 patients and reports of self-resolving transient pain in the aspiration and injection sites. In the 21 patients remaining at year 2, ODI and VAS score reductions remained stable and continued to trend down, averaging 19.9 and 27.0, respectively, at 1 year and 18.3 and 22.9 at the end of year 2 [80]. In the 20 non-surgical patients remaining at year 3, ODI and VAS scores averaged 17.5 and 21.9, respectively [81]. Non-surgical patients had an average pre-treatment baseline ODI of 56.7 and an average VAS score of 82.1. Surgical patients were not found to have baseline scores that were statistically different. The association between pain relief and MSC concentration remained consistent, though it should be noted that the difference between those

with greater than 2000 MSCs and those with less was only statistically significant at the 3- and 6-month follow-ups. Changes in ODI and VAS score were found to be significantly different at 3 and 6 months between the surgical and non-surgical patients [81].

In a subsequent study, Pettine et al. compared their results at 2 years with the results from larger studies for similar patients being treated with artificial discs or lumbar fusions. In 519 lumbar fusion patients, the average ODI and VAS scores were improved by 43.3% and 52.7% from baseline. In 944 disc replacements, the corresponding reduction averages were 57% and 63%. Meanwhile, the 2-year reductions in the 21 remaining stem cell patients were 71% for ODI and 70% for VAS [81]. These favorable results are even more encouraging when considering the relative risk and cost reductions seen in stem cell therapy versus surgical alternatives. The BMC injection requires only IV sedation, no hospital stay, and featured zero complications as opposed to a roughly 7% and 2% complication rates for spinal fusions and disc replacements, respectively [81]. Although there are several limitations to this study, most notably the small population, their results suggest stem cell therapy is a good non-surgical option that may carry a lower risk of complication at a lower financial cost.

Another approach besides bone marrow MSCs is the use of adipose tissue-derived mesenchymal stem cells (AT-MSCs). This approach is less invasive and lower risk. In their single arm trial, Kumar et al. followed 10 chronic low back patients for 1 year and measured ODI, VAS, SF-36, and Pfirrmann's scores via MRI after patients had received autologous injections of AT-MSCs combined with hyaluronic acid. No adverse events were observed during the 12-month study period. The study featured a high-cell dose (4×10^7) and a low-cell dose group (2×10^7), but there was no difference in terms of VAS or ODI scores at any of the 1 week or the 1-, 3-, 6-, 9-, or 12-month follow-ups [45••]. Greater than 50% reductions in VAS and ODI were found at 6 months in 7 subjects and in 6 subjects at 1 year. Lumbar X-ray showed no decreased disc height in any patient 12 months out. All patients were initially Pfirrmann grade IV, with one patient improving to grade III at 6 months. Of the 6 patients achieving 50% reductions, 3 demonstrated increased disc water content on MRI. Overall, this new approach was successful, warranting expanded trials.

Thus far, only one study has examined the transplantation of allogeneic stem cells in humans. Noriega et al. randomized 24 patients with lumbar disc degeneration in the setting of chronic back pain to either an allogenic bone marrow intradiscal injection or a sham injection control group. Clinical outcomes were followed up to 1 year using VAS, ODI, and MRI to measure outcomes. No major adverse events were reported, though 8 controls and 3 treatment group patients required brief treatments with NSAIDs [84]. One control and one treatment patient required opioids. For MSC-treated patients, VAS and ODI reductions were significant at 3, 6, and 12 months. In the control group, effects at 3, 6, and 12 months were statistically insignificant, and ODI tended to increase at these time points in controls [84]. MRI results showed continued disc degeneration in controls and improvement in MSC patients via Pfirrman staging. No significant difference was found in the disc height changes of controls versus MSC subjects. The study demonstrated the safety and viability of an allogeneic model of transplantation versus a control group, which was not utilized in any of the other studies to date. Allogenic transplantation does not require the same cell expansion as autologous transplantation, and so advantages to this approach include improved logistics and cost. Given these benefits, more allogenic trials are recommended.

Longer-term safety and feasibility reports have also emerged in recent years. One such study involved five patients with degenerative disc disease who were treated with an autologous injection of hypoxic-cultured bone marrow MSCs and evaluated them for safety and efficacy in a follow-up study conducted 4 to 6 years after stem cell treatment. In this extended time frame, there were no adverse events reported and no evidence of neoplasm or other abnormalities in the treated regions via MRI. In terms of efficacy, a positive association was found between the number of cultured stem cells transplanted and the patients' reported improvement, though the small sample size limited interpretation of these results. MRI measurements of disc height showed maintenance or mild narrowing at follow-up. Additionally, four of the five patients showed a reduction in disc protrusion. All patients in the study reported overall improvement, including improved strength in all patients and improved mobility in all but one. One limitation of this study was that these were subjective self-reported measures in a small, uncontrolled study. Researchers concluded by noting that the safety, feasibility, and patient outcomes are encouraging but that larger double-blind studies are warranted and ought to include validated endpoint measures to demonstrate efficacy [82].

The use of autologous bone marrow MSCs injection for degenerative disc disease with comorbid radicular pain and posterior disc bulge was studied in a 33-subject pilot with long-term follow-up. No infection, tumor, death, or other serious adverse event was reported during the 6 years of post-treatment follow-up. However, three patients did report pain related to the procedure. In all three cases, the pain resolved, and no additional adverse events were reported. Efficacy of treatment was measured with self-reported pain and function scores. Using the modified single assessment numeric evaluation (SANE) rating, mean clinical improvement of 60% was found at 3 years post-treatment [83]. However, in addition to being limited by a lack of a control group, a small sample size, and lack of a standardized post-treatment rehabilitation protocol, the study also suffered from losing patients to follow-up. While 88% of patients provided at least one outcome data point, only 20 of the 33 patients had follow-up MRIs, which

was an important objective measure in the study. Of the 20 who had MRI follow-up, 17 demonstrated a reduction in disc bulge, with an average reduction of 23%. By stratifying the 20 MRI patients based on reduction in disc bulge size, the researchers showed that patients with greater disc bulge change reported significantly less pain at 6 months, while no such link was found between disc bulge and function scores [83].

Nucleus Pulposus Transplantation

Another similar approach to treating back pain is nucleus pulposus (NP) transplantation, which, like stem cell therapy, addresses the underlying cause of intervertebral disc degeneration by reversing the destructive inflammatory process and regenerating the proteoglycans and collagen found in healthy disc tissue. The NP cells are responsible for producing the extracellular matrix of the intervertebral disc but begin to decrease in number beginning in the second decade of life [85••]. Of further complication, the intervertebral disc is largely avascular, which prevents adequate regeneration of damaged nucleus pulposus cells [86]. Transplantation of the NP may overcome this limitation and can restore extracellular matrix composition, particularly the loss of proteoglycans, so that the intervertebral disc morphology can be preserved or restored. This ultimately prevents disc space narrowing and subsequent back pain. To accomplish this, autologous NP cells are harvested from healthy discs and are cultured and activated in vitro before being introduced into the degenerative disc. Other methods include culturing of mesenchymal stromal cells and differentiation into NP-like cells via specific growth factors or other differentiation mediums. The introduction of healthy NP cells allows regeneration of the extracellular matrix to occur, improving the health of the intervertebral disc.

Thus far, results from human and animal studies have been encouraging. It has been demonstrated that the viability of NP cells is augmented via the induction of cell-to-cell contact with autologous mesenchymal stromal cells during cell culture. This method increased the number of viable NP cells and their resultant proteoglycan synthesis as compared with those cultured without stromal cells [86]. Nukaga et al. found that canines treated with NP transplantation had a significant improvement in their preserved disc height with their model of disc degeneration. Similar findings have been reported with studies involving MRI and gross anatomic and histologic examination of the intervertebral discs. Transplanted animals demonstrated discs that were relatively well-preserved and more similar in character to the healthy controls than the degeneration controls who received no transplantation.

More recently, there is interest in creating NP-like cells via umbilical cord mesenchymal stem cells. Benefits to this approach include forgoing the need to remove NP cells from elsewhere in the body, which increases the risk of degeneration at the donor site, as well as providing a way to obtain MSCs non-invasively. Using a differentiation medium composed of a growth factor cocktail or a conditioned medium using rabbit NP cells, researchers successfully differentiated and transplanted NP-like cells from human umbilical MSC precursors into rabbits. Transplanted NPCs improved disc height and demonstrated similar characteristics to the NP of control animals, consistent with successful regeneration within the disc [85••]. Another notable finding was that there was no inflammatory response observed in the transplanted discs, suggesting that the avascularity of the disc reduces the risk of rejection during transplantation.

A clinical study investigated the safety of transplanting activated NP cells via MSC co-culture in humans. Nine patients with grade III disc degeneration were followed for 3 years, during which time no adverse events were reported. All patients showed no further degeneration of their discs, and one patient showed notable improvement of the disc over the 3 years of observation. Furthermore, patients reported improvement in LBP scores. This study demonstrated that NP transplantation may serve a role in the treatment of disc degeneration and that it can be performed safely in human subjects [87].

Conclusion

LBP is a highly prevalent disease with increasing incidence rates. Its burden in direct health costs, days of work missed, mortality, and reduced quality of life are therefore growing as well. Since DLBP accounts for the majority of LBP cases, it harbors significant unmet need in treatment options. Before novel treatments such as stem cell therapies can be improved, the underlying etiology of degenerative disc disease and its differentiation from natural IVD degeneration to cause DLBP must be understood. Preliminary animal models and clinical studies have been conducted to investigate mesenchymal stem cell implantation into the IVD as a potential therapy for IVD regeneration, ECM growth, shift towards favorable anabolic balance, and reduction of pain.

Future directions for MSC trials for IVD regeneration are promising. Useful research may involve animal models with naturally occurring IVD, exploration of prolonged survival of transplanted MSCs, early detection of IVD degeneration for improved timing of MSC implantation, and improved MSC differentiation and survival (e.g., concomitant injection of proteins such as growth factors). Given the promise offered by the field of stem cell therapies, further innovation can be expected in novel stem cell therapies to alleviate DLBP.

Compliance with Ethical Standards

Conflict of Interest Ivan Urits, Alexander Capuco, Medha Sharma, Omar Viswanath, Elyse M. Cornett, and Vwaire Orhurhu declare no conflict of interest. Alan D. Kaye discloses that he is on the Speakers Bureau for Depomed, Inc. and Merck.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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