Stem cells sources for intervertebral disc regeneration

Gianluca Vadalà, Fabrizio Russo, Luca Ambrosio, Mattia Loppini, Vincenzo Denaro

Gianluca Vadalà, Fabrizio Russo, Luca Ambrosio, Mattia Loppini, Vincenzo Denaro, Department of Orthopedic and Trauma Surgery, University Campus Bio-Medico of Rome, 00128 Rome, Italy

Author contributions: All authors equally contributed to this paper with conception and design of the manuscript, literature review and analysis, drafting and critical revision and editing and final approval of the final version.

Conflict-of-interest statement: The authors declare no conflicts of interest regarding this manuscript.

Open-Access: This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: http://creativecommons.org/licenses/by-nc/4.0/

Correspondence to: Gianluca Vadalà, MD, PhD, Department of Orthopedic and Trauma Surgery, University Campus Bio-Medico of Rome, Via Alvaro del Portillo 200, Rome 00128, Italy. g.vadalà@unicampus.it
Telephone: +39-06-225419142
Fax: +39-06-225411638

Received: October 30, 2015
Peer-review started: November 4, 2015
First decision: November 30, 2015
Revised: December 18, 2015
Accepted: February 14, 2016
Article in press: February 16, 2016
Published online: May 26, 2016

Abstract

Intervertebral disc regeneration field is rapidly growing since disc disorders represent a major health problem in industrialized countries with very few possible treatments. Indeed, current available therapies are symptomatic, and surgical procedures consist in disc removal and spinal fusion, which is not immune to regardable concerns about possible comorbidities, cost-effectiveness, secondary risks and long-lasting outcomes. This review paper aims to share recent advances in stem cell therapy for the treatment of intervertebral disc degeneration. In literature the potential use of different adult stem cells for intervertebral disc regeneration has already been reported. Bone marrow mesenchymal stromal/stem cells, adipose tissue derived stem cells, synovial stem cells, muscle-derived stem cells, olfactory neural stem cells, induced pluripotent stem cells, hematopoietic stem cells, disc stem cells, and embryonic stem cells have been studied for this purpose either in vitro or in vivo. Moreover, several engineered carriers (e.g., hydrogels), characterized by full biocompatibility and prompt biodegradation, have been designed and combined with different stem cell types in order to optimize the local and controlled delivery of cellular substrates in situ. The paper overviews the literature discussing the current status of our knowledge of the different stem cells types used as a cell-based therapy for disc regeneration.

Key words: Stem cells; Intervertebral disc degeneration; Spine; Tissue engineering; Cell therapy

© The Author(s) 2016. Published by Baishideng Publishing Group Inc. All rights reserved.

Core tip: This review paper aims to share recent advances in stem cell therapy for the treatment of intervertebral disc degeneration. The paper overviews the literature discussing the current status of our knowledge of the different stem cells types used as a cell-based therapy for disc regeneration. Intervertebral disc regeneration field is rapidly growing since disc disorders represent a major health problem in industrialized countries with very few possible treatments. Indeed, current available therapies are symptomatic, and surgical procedures consist in disc removal and spinal fusion.
INTRODUCTION

Low back pain (LBP) is a common musculoskeletal symptom referred by more than 80% of the general population at least once in their life\[^1\]. It results in a relevant social and economic problem, affecting above all the productive population in developed countries. In fact, it presents a maximum rate of incidence in people between the ages of 45 and 64\[^2\] and it is the most frequent cause of disability and loss of days of activity in people under 45 years of age\[^3,4\].

The widespread majority of LBP related to degenerative changes of the intervertebral disc (IVD). The IVD is a complex structure consisting of three specialized tissues: The anulus fibrosus (AF), the nucleus pulposus (NP) and the cartilaginous end-plate (CEP) which coats the adjacent vertebral body.

The AF is a fibro-cartilaginous ring providing the outer part of IVD. It is composed of collagen type I fibers, oriented radially and in opposite directions throughout concentric lamellae\[^5\], associated with an interlamellar matrix consisting of proteoglycans and non-collagenous proteins (such as elastin), in which mesenchymal cells, with a fibroblast-like morphology and phenotype\[^6\], are present. This matrix leads to an efficient interlamellar cohesion\[^7\].

The NP is a less structured gelatinous matrix rich in proteoglycans, mainly aggrecan, and type II collagen fibers randomly oriented. Aggrecan comprises a great number of negatively charged sulfated glycosaminoglycans that attract and imbibe water. The high level of hydration helps to maintain disc height and contributes to the load-bearing ability of the IVD\[^8\]. Small chondrocyte-like cells are scattered within the NP and are responsible for synthesizing and maintaining the matrix\[^9\].

The embryonic human IVD consists in two different structures: The NP, derived from aggregation of notochordal cells within a proteoglycan matrix, forming the gelatinous centre of the disc; the AF, which is derived from the perichondrial mesenchyme, forming organized fibers surrounding the nucleus. During the sixth embryonic month, a mucoid degeneration of notochordal cells takes place in the NP and mesenchymal IVD cells invade the fibrocartilage. However, some notochordal remnants can be found in the IVD up to adulthood\[^10\].

The IVD provides support for vertebrae, shock absorber function and allows movements of flex-extension, lateral bending and rotation. The NP, surrounded by the annulus fibers, resists compressive stress, whereas AF resists primarily tensile, circumferential, longitudinal and torsional stresses\[^10,11\].

Intervertebral disc degeneration (IDD) is an age-related chronic process characterized by a progressive decline of proteoglycans and water content in NP with loss of the disc ability to resist compressive loads\[^12\]. The first symptom of IDD is often LBP, that may lead to disc herniation, degenerative spondylolisthesis, instability and spinal stenosis associated with neurological symptoms such as radiculopathy and/or myelopathy. Current treatments for LBP and IDD range from conservative to surgical procedures\[^13\]. However, these treatment modalities have limited efficacy and do not produce predictable and reliable outcomes. In fact, they target the clinical symptoms instead of the pathophysiology involved in the degenerative process. An effective early treatment for LBP that may prevent, slow down or reverse the degenerative changes of the IVD is the goal of many researchers in the spine field. Exciting advances in tissue engineering have led spine researchers to develop novel regenerative techniques in order to alter the course of IDD and possibly lead to disc repair and recovery of function.

PATHOPHYSIOLOGY OF IDD

Although the increasing interest in biological treatments for IDD, its pathological basis is still not completely understood. The degenerative pathway is related to aging, starting from the second decade of life\[^24\], and to certain genetic profile expressions as well as environmental factors\[^15\]. Moreover, the IVD is the largest avascular tissue in the body, in which nutrition takes place by diffusion through the CEP, maintaining the viability of NP cells\[^16\].

The main structural changes in NP during IDD consist in a progressive reduction of proteoglycan content, first of all aggrecan\[^17\]. Morphological modifications are related to metabolic imbalance between anabolic and catabolic processes, regulated by multiple factors, such as anabolic growth factors (e.g., insulin-like growth factor-1 (IGF1)\[^18\], transforming growth factors β (TGFβ)), bone morphogenetic proteins (BMPs)\[^19\]) and catabolic enzymes [matrix metalloproteinases (MMPs) and a disintegrin and metalloproteinase with thrombospondin motifs (ADAMTS) resulting in changes in NP cells function\[^20\]].

Extracellular matrix changes are associated with alterations of disc cell viability. The progressive reduction of cell density results in the inability of the IVD to revert degenerative changes by producing and maintaining a functional extracellular matrix\[^21\]. The decrease of NP proteoglycan content leads to progressive dehydration of gel-like nuclear matrix, decreasing disc height and altering its load-bearing capacity\[^22\]. The inefficiency of NP to absorb compressive stresses and to transmit forces circumferentially to the AF, leads to deterioration of the lamellar architecture of the AF itself, consisting of internal fissures spreading outward to the periphery\[^23\].
In addition to cracking and fissuring of the AF, disc herniation, subchondral sclerosis, CEP ossification and osteophyte formation[24] may take place. The inherent avascularity, isolation, and low metabolic activity of the IVD may explain its apparent inability for self-repair following injury and degeneration[25].

POTENTIAL BIOLOGICAL TREATMENTS

The therapeutic approach to treat or prevent disc degeneration could consist in recovering the disc ability to synthesize extracellular matrix, rich in proteoglycans, in order to re-establish disc hydration and NP viscoelastic features, thus restoring biomechanical properties of the IVD.

Several genes and growth factors have been found to influence the anabolic and catabolic processes, regulating the extracellular matrix homeostasis within the IVD. In this way recombinant growth factors or gene therapy technologies could be applied to treat the IDD[26,27]. Intradiscal injection of growth factors, such as BMP-7, BMP-2 or IGF-1, has been shown to increase the proteoglycan level within the disc[28-30].

The possibility to synthesize recombinant growth factors and to inject them with a percutaneous approach represents interesting advantages. However, the short half-life of exogenous growth factors has led to increasing interest for gene therapy in the treatment of IDD.

Gene therapy modifies the pattern of gene expression resulting in an in situ sustained production of specific gene products. In literature, numerous anabolic factors (such as TGF-β, BMP-2, BMP-7 or IGF-1), anti-catabolic factors (such as TIMP-1), and gene regulators (such as SOX-9 and LMP-1) have been found able to modulate the metabolic activity of disc cells, increasing proteoglycans disc content[31-34]. However, side effects related to this emerging technology, such as inflammatory reactions of nerve roots and/or dura, have been described[35]. More efficient and safe systems of transfection and transduction may be performed before clinical application of gene therapy[37,36].

According to pathological findings in IVD aging and IDD, characterized by a progressive disc cell loss, cell therapy has been proposed in order to re-establish disc hydration and NP viscoelastic features, thus restoring biomechanical properties of the IVD.

Gene therapy modifies the pattern of gene expression resulting in an in situ sustained production of specific gene products. In literature, numerous anabolic factors (such as TGF-β, BMP-2, BMP-7 or IGF-1), anti-catabolic factors (such as TIMP-1), and gene regulators (such as SOX-9 and LMP-1) have been found able to modulate the metabolic activity of disc cells, increasing proteoglycans disc content[31-34]. However, side effects related to this emerging technology, such as inflammatory reactions of nerve roots and/or dura, have been described[35]. More efficient and safe systems of transfection and transduction may be performed before clinical application of gene therapy[37,36].

According to pathological findings in IVD aging and IDD, characterized by a progressive disc cell loss, cell therapy has been proposed in order to re-establish disc hydration and NP viscoelastic features, thus restoring biomechanical properties of the IVD.

Gene therapy modifies the pattern of gene expression resulting in an in situ sustained production of specific gene products. In literature, numerous anabolic factors (such as TGF-β, BMP-2, BMP-7 or IGF-1), anti-catabolic factors (such as TIMP-1), and gene regulators (such as SOX-9 and LMP-1) have been found able to modulate the metabolic activity of disc cells, increasing proteoglycans disc content[31-34]. However, side effects related to this emerging technology, such as inflammatory reactions of nerve roots and/or dura, have been described[35]. More efficient and safe systems of transfection and transduction may be performed before clinical application of gene therapy[37,36].

According to pathological findings in IVD aging and IDD, characterized by a progressive disc cell loss, cell therapy has been proposed in order to re-establish disc hydration and NP viscoelastic features, thus restoring biomechanical properties of the IVD.

Gene therapy modifies the pattern of gene expression resulting in an in situ sustained production of specific gene products. In literature, numerous anabolic factors (such as TGF-β, BMP-2, BMP-7 or IGF-1), anti-catabolic factors (such as TIMP-1), and gene regulators (such as SOX-9 and LMP-1) have been found able to modulate the metabolic activity of disc cells, increasing proteoglycans disc content[31-34]. However, side effects related to this emerging technology, such as inflammatory reactions of nerve roots and/or dura, have been described[35]. More efficient and safe systems of transfection and transduction may be performed before clinical application of gene therapy[37,36].

According to pathological findings in IVD aging and IDD, characterized by a progressive disc cell loss, cell therapy has been proposed in order to re-establish disc hydration and NP viscoelastic features, thus restoring biomechanical properties of the IVD.

Gene therapy modifies the pattern of gene expression resulting in an in situ sustained production of specific gene products. In literature, numerous anabolic factors (such as TGF-β, BMP-2, BMP-7 or IGF-1), anti-catabolic factors (such as TIMP-1), and gene regulators (such as SOX-9 and LMP-1) have been found able to modulate the metabolic activity of disc cells, increasing proteoglycans disc content[31-34]. However, side effects related to this emerging technology, such as inflammatory reactions of nerve roots and/or dura, have been described[35]. More efficient and safe systems of transfection and transduction may be performed before clinical application of gene therapy[37,36].

According to pathological findings in IVD aging and IDD, characterized by a progressive disc cell loss, cell therapy has been proposed in order to re-establish disc hydration and NP viscoelastic features, thus restoring biomechanical properties of the IVD. In this way recombinant growth factors or gene therapy technologies could be applied to treat the IDD[26,27]. Intradiscal injection of growth factors, such as BMP-7, BMP-2 or IGF-1, has been shown to increase the proteoglycan level within the disc[28-30].

The possibility to synthesize recombinant growth factors and to inject them with a percutaneous approach represents interesting advantages. However, the short half-life of exogenous growth factors has led to increasing interest for gene therapy in the treatment of IDD.

Gene therapy modifies the pattern of gene expression resulting in an in situ sustained production of specific gene products. In literature, numerous anabolic factors (such as TGF-β, BMP-2, BMP-7 or IGF-1), anti-catabolic factors (such as TIMP-1), and gene regulators (such as SOX-9 and LMP-1) have been found able to modulate the metabolic activity of disc cells, increasing proteoglycans disc content[31-34]. However, side effects related to this emerging technology, such as inflammatory reactions of nerve roots and/or dura, have been described[35]. More efficient and safe systems of transfection and transduction may be performed before clinical application of gene therapy[37,36].

According to pathological findings in IVD aging and IDD, characterized by a progressive disc cell loss, cell therapy has been proposed in order to re-establish the disc cell population by introducing exogenous cells. A cell therapy approach can be performed by using different types of differentiated cells, such as NP cells[37,38], AF cells[39], cartilaginous chondrocytes[40] and progenitor cells[41-43]. The autologous disc-derived chondrocyte transplantation (ADTC) is a treatment based on autologous NP cells to replace the tissue loss caused by disc herniation and disc surgery[44]. Although clinical data seems to report back pain improvement and prevention of disc height reduction after the treatment[45], ADTC procedure presents the following limits: (1) it is only applicable when discectomy is required; (2) it is a two-steps procedure, because discectomy and cell transplantation are performed in two different times; and (3) disc cells lose their phenotypic characteristics when expanded in monolayer cell culture[46].

Therefore, stem cell therapy is more attractive due to low harvest site morbidity, ease of ex vivo cell expansion, and favorable modulation of the cell phenotype before or after transplantation. In this review we will discuss about the potential use of different types of stem cells employed for disc repair.

STEM CELLS BASED THERAPY

Stem cells are unspecialized cells characterized by a high proliferation rate. They can reside in a quiescent state, in which they self-renew; during the proliferation process, they perform an asymmetric division producing two daughter populations: One of them constituted by identical stem cells and the second ones formed by progenitor cells committed to a lineage-specific differentiation program[47]. Different types of human stem cells, ranging form embryonic to adult stem cells, have been found. Although embryonic stem (ES) cells are considered to be totipotent, legal and ethical controversies limit their use for clinical application in regenerative medicine[48]. Adult stem cells represent a reservoir of progenitor cells harbored within specialized niches of the adult organism, suggesting the potential for therapeutic application in their host tissues. Adult stem cells have been discovered and characterized in tissues such as bone marrow[49], adipose tissue[50], periosteum[51,52], synovial membrane[33], muscle[54], skin[55], pericytes[56,57], blood[58] and trabecular bone[59,60]. Their function consists in maintenance of the anatomical and functional features of each specialized tissue. Because they are committed to a lineage-specific differentiation pathway, these cells are able to produce a limited range of specialized cells according to the embryonic origin of the tissue itself. The application of adult stem cells in regenerative medicine does not raise any ethical problems, as they can be directly isolated from the patient.

The potential application in IVD regeneration has been described for some types of adult stem cells, including bone marrow-derived mesenchymal stem cells (MSCs)[42,61,62], adipose tissue-derived stem cells (ASCs)[41], muscle-derived stem cells (MdSCs)[43], hematopoietic stem cells, olfactory membrane stem cells and synovial stem cells (Figure 1). Adult stem cell types are committed to differentiate following the lineage of mesenchymal tissues, including bone, cartilage, fat, and muscle[46,50,54,61,62]. Moreover, according to recent findings[44,63], MSCs, such as bone marrow MSCs, ASCs and MdSCs, seem to derive from the perivascular wall[65] of the tissue the stem cells are from. Several therapeutic strategies for IVD regeneration based on stem cells have been described that direct injection into the IVD of undifferentiated or predifferentiated cells (cell therapy). Application of constructs derived by conjunction of stem cells with a viscoelastic hydrogel (tissue engineering strategy); genetic
modification by transfection of target genes followed by injection of transfected stem cells into the IVD (ex vivo gene therapy) \(^{27,27}\) (Figure 2).

Recently, a specific population of progenitor cells has been identified in the degenerated human IVD \(^{68}\). This finding has been confirmed by Blanco et al \(^{69}\), who demonstrated that progenitor stem cells are quite similar to mesenchymal stromal cells derived from bone marrow. Therefore, an alternative approach for treatment consists in recruiting endogenous progenitors to orchestrate IVD repair by administration of suitable drugs/growth factors.

**BONE MARROW MSCS**

In the past few years several *in vitro* studies have been conducted to evaluate the use of MSCs for the treatment of IDD.

The actual capacity of adult human MSCs to differentiate towards NP cells has been one of the first steps in the evaluation of their utilization as a cell source for IVD regeneration. MSCs can differentiate into chondrocyte-like cells phenotypically similar to NP cells in chondrogenic conditions \(^{68,70}\). These methods have been considered a preconditioning system to direct MSCs into NP-like cells before they are implanted into the IVD.

The therapeutic effects of stem cells have been extensively studied *in vitro*. Several studies suggested that the regenerative potential of MSCs may result from MSCs and NP cells interactions that up-regulate extracellular matrix protein synthesis in terms of proteoglycans. Le Visage et al \(^{71}\) cocultured bone marrow MSCs with NP cells at a 50:50 ratio in 3-dimensional pellet culture system for 2 wk showing that, although there was a trend of increase in glycosaminoglycan (GAG) production, the difference was not significant. On the contrary, Sobajima et al \(^{62}\) using a similar pellet coculture system at a different ratio, revealed a synergistic effect between NP cells and MSCs at 75:25 and 50:50 NP:MSC ratio, yielding a significant increase of proteoglycan synthesis rate and GAG content compared with culture of NP cell and MSCs alone. Recently, Svanvik et al \(^{72}\) confirmed that coculture of MSCs with degenerated NP cells increases proteoglycan and collagen-type II production.

In order to delineate the effects, several studies have utilized the coculture model systems to understand whether the interaction between MSCs and IVD cells leads to MSC differentiation to an NP-like phenotype and/or whether MSCs promote regeneration through stimulation of native NP cells. Stem cells undergo differentiation under stimuli that come from the surrounding microenvironment. However, adult stem cells contribute to tissue repair and regeneration by not only differentiating into the phenotypes of the host cells \(^{49}\) but also creating a microenvironment that promotes the local regeneration of endogenous cells \(^{73}\). Richardson et al \(^{74}\) cocultured human MSCs with normal NP cells in monolayer with or without cell-to-cell contact. After 1 wk of culture, fluorescent-labeled MSCs separated by fluorescence-activated cell sorting and gene expression was evaluated. MSCs underwent a change in gene expression profile similar to NP-like cells as demonstrated by an increased expression of aggrecan and collagen type II genes \(^{74}\). However, the low cell density in the NP \(^{75}\) makes direct cell-to-cell contact between MSCs and NP cells a rare event if stem cells are implanted into the disc. Therefore, our group studied the mechanisms of the interaction between human NP cells from degenerating discs and MSCs in 3-dimensional culture, a system that allows
short distance paracrine interactions typical of the NP tissue, hence miming its architecture[76]. Using a double labeling cell system, changes in gene expression profile were analyzed on the MSCs or NP cells populations isolated from the coculture. MSCs acquired a more chondrogenic gene expression profile and influenced mRNA levels within the human NP cells.

Paracrine stimulation in the interaction between MSCs and IVD cells was also assessed in other studies. Yang et al[77] used a noncontact coculture system to elucidate the interaction between NP cells and MSCs mediated by soluble factors. These authors showed that secreted factors in the coculture were able to induce collagen type II expression by uncommitted MSCs when cultured with a higher number of NP cells. Korecki et al[78] studied the effect of conditioned media from notochordal cell cultures on MSCs showing differentiation toward a potentially NP-like phenotype with some characteristics of the developing IVD.

MSCs secrete a variety of cytokines and growth factors that are able to stimulate mitosis and tissue-intrinsic reparative potential of the host cells[79]. Accordingly, Yamamoto et al[80] reported increased cell viability, proliferation and proteoglycan synthesis of rabbit NP cells induced by cell-to-cell contact with MSCs in a coculture monolayer system, supporting a trophic effect. Watanabe et al[81] further confirmed these data by using human cells. The trophic effect only partially reflected in our gene expression study. Although we observed an increase in collagen type II by NP cells after coculture with MSCs, aggrecan gene expression was down-regulated[76]. This data reflected only a modest trophic effect of MSCs on degenerate NP cells. This finding is in agreement with Strassburg et al[82] who investigated differences in the interaction between human MSCs and NP cells from both nondegenerate and degenerate discs during in vitro coculture with direct cell-cell contact. They concluded that, although both nondegenerate and degenerate NP cells are able to stimulate MSCs differentiation to an NP-like phenotype, MSCs were only able to stimulate degenerate NP cells to increase their matrix-associated gene expression to levels comparable to nondegenerate NP cells.

Cell fusion has been found to be potentially responsible for the plasticity and tissue regeneration potential of adult stem cells[83,84]. The possible occurrence of cell fusion in the interaction between male MSCs and female NP cells has been studied by our group[76]. Cell fusion was examined in a pellet coculture system to accentuate cell-to-cell interactions, a necessary condition for inducing cell fusion[83]. Fluorescence in situ hybridization assay for the X and Y chromosomes demonstrated that cell fusion does not occur in the interaction between MSCs and NP cells[76]. This result is in contrast with the data of Strassburg et al[82] who also studied cells fusion in the pellet coculture system. These authors were able to detect binucleated cells among cocultured cells during histologic observation, thus raising the possibility of cell fusion. Therefore, further studies needed to determine if the exposure of NP cells to MSCs leads to spontaneous cell fusion.

Organ culture systems were also used to study the potential effect of MSCs for IVD regeneration. Le Maitre et al[85] transplanted human MSCs in a bovine caudal disc and cultured them in vitro up to 4 wk. These authors showed that MSCs from older individuals differentiate spontaneously into chondrocyte-like NP cells upon insertion into NP tissue. Chen et al[86] studied the effect of porcine MSCs injected in an IVD degeneration model showing a regenerative potential. Therefore the ex vivo degenerative IVD organ culture system has the potential to contribute to a better
comprehension of alternative IVD regeneration strategies.

MSCs engraftment and long survival in the harsh environment of the normal disc tissue has been demonstrated by several in vivo study in small animals. Crevensten et al. have shown that MSCs injected into rat discs with hyaluronic gel as a carrier, maintained viability over 28 d with cell proliferation. Zhang et al. have shown that allogeneic MSCs injected in a healthy disc increased the total proteoglycan content in the NP of rabbit discs. Sobajima et al. evaluated the long-term survival of allogeneic MSCs in healthy rabbit lumbar IVDs. The MSCs, retrovirally transduced with LacZ marker gene, were identified up to 6 mo after transplantation, observing MSCs migration from the NP to the inner AF.

However, in order to determine the efficacy of a stem cell therapy in preventing or delaying the progression of IDD, it is critical to rigorously test stem cell therapy in animal models of IDD. The first efficacy study published in the field is dated back to 2003: Sakai et al. elegantly showed that autologous bone marrow MSCs embedded in a collagen type II based carrier (Atelocollagen®) and injected in an NP aspiration model of IDD enhanced proteoglycan content and hydration. The same authors, using a similar study design, showed that the MSCs injected disc maintained height of 97% and magnetic resonance image (MRI) signal intensity of about 81% in a normal control group discs, while the degenerating disc group that did not received MSCs injection demonstrated a disc height value of about 67% and MRI signal intensity reduction of about 60%. Moreover, Sakai et al. demonstrated that undifferentiated MSCs transplanted into degenerated discs in rabbits proliferated and differentiated into cells expressing some of the major phenotypic characteristics of NP cells, suggesting that these MSCs may have undergone site-dependent differentiation.

Ho et al. studied the influence of the degenerative grade on the therapeutic potential of MSCs. These authors induced IDD in a rabbit model by stabbing and injecting MSCs at 1 or 7 mo. They observed that MSCs appear to be more effective in arresting degeneration at a relatively later stage of disc degeneration.

Jeong et al. used a xenogeneic transplantation model to study the effect of human MSCs injected into the coccygeal rat IVDs 2 wk after a blade stabbing. Disc height and MRI signal intensity of the MSCs transplanted disc increased compared to the degenerated control group at 2 wk after injection.

Yang et al. performed a study on a rabbit model of IDD induced by nucleus aspiration. These authors injected into the IVD a mixture of fibrin glue, TGF-β1 and rabbit MSCs using as a control the carrier alone with the growth factor or the sham. These authors have shown that MSCs injection led to a reduced height loss associated with IDD and an increased quantity of collagen type II content and a decrease in the rate of cell apoptosis.

Hee et al. performed a study on a rabbit model of IDD induced through a compression device. Allogeneic MSCs embedded in Atelocollagen were transplanted into the compressed disc followed by unload or distraction. Controls underwent just distraction or unload period. This animal study showed that the transplanted IVDs performed better with respect to disc height, morphological grading, histological scoring and average dead cell count and that distraction increased the regenerative effect of MSC transplantation.

Allon et al. explored the potential of the use of bilaminar coculture pellets (BCPs) of MSCs in a shell of NP cells for IVD regeneration.

The pellets were transplanted in vivo in a rat tail nucleotomy model of disc degeneration.

BCPs were transplanted in a fibrin sealant (FS) carrier using as a control the FS with a pellet of just MSCs or NP cells, MSCs and NP cells randomly mixed or the FS only; and surgery only. This study showed that the proteoglycan and cytokine levels were not significantly different among groups. The BCP group had higher cell retention, disc height and increased disc grade over time than controls.

Henriksson et al. performed a study using a xenotransplantation model in minipigs. IDD was induced in lumbar IVD by nucleoaspiration and 2 wk later human MSCs were injected in F12 media suspension (cell/med) or with a hydrogel carrier (cell/gel). The animals were sacrificed after 1, 3, or 6 mo. At MRI all injured discs demonstrated degenerative signs with fewer positive changes in the cell/gel group compared with cell/med discs and injured only discs. The authors concluded that transplanted human MSCs survived in the porcine spinal disc up to 6 mo and expressed SOX-9 and Collagen II, thus indicating differentiation. The hydrogel carrier has shown to facilitate the differentiation of transplanted hMSCs.

Recently, MSCs injection has tested in a larger animal model. Hiyama et al. evaluated the effect of MSCs transplantation on the suppression of IDD and preservation of immune privilege in a canine model of IDD. MSCs injection effectively led to the regeneration of degenerated discs and contributed to the maintenance of IVD immune privilege by the differentiation of transplanted MSCs into cells expressing FasL.

Serigano et al. used a canine IDD model to perform a dose-escalation study to assess the optimal number of cells to transplant into the degenerated IVD. Four weeks after nucleoaspiration, autologous MSCs transplanted at 105, 106, or 107 cells per disc. Unoperated and untransplanted disc were used as a control. MSCs-transplantation groups showed preservation of disc height and annular structure compared to the operated control group. The analysis of the survival rate of both transplanted and MSCs as well as NP cells demonstrated the better performance of 106 MSCs, when compared to 105 or 107, producing the best maintenance of the structure of IVDs and best inhibited IVD degeneration.

The goat study conducted by Zhang et al. per-
formed using a disc degeneration model induced by stabbing the disc with a number 15 blade. One month after injury, allogeneic MSCs were injected with a hydrogel into the IVDs. Degenerating IVDs injected with MSCs showed significantly increased proteoglycan content within the disc. However, collagen content, MRI imaging, and histology did not show statistically significant differences between the cell-treated and control IVDs.

Subhan et al.\(^{[100]}\) transplanted allogeneic MSCs embedded in a hyaluronan based hydrogel (HyStem) into degenerate discs by fluoroscopy assisted minimally invasive delivery in a rabbit model. Animals were divided into three groups: Group I treated with MSCs coupled with Hystem, group II injected with Hystem alone and group III was left without any intervention. At 8 wk after transplantation, histological assay and MRI T2 mapping of NP showed higher T2 signal intensity, disc height index and type II collagen and aggrecan content in group I compared to other groups; similar results were reported by Cai et al.\(^{[100]}\) as well.

In a pilot study, Orozco et al.\(^{[102]}\) injected autologous expanded bone marrow MSCs into the nucleus pulposus of 10 patients diagnosed with lumbar disc degeneration. One year follow-up investigated evaluation of back pain, disability and quality of life, whereas disc height and fluid content were assessed through MRI. Patients reported prompt improvement of pain and disability at 3 mo and increased disc water content at 12 mo, even if disc height did not restore.

In a similar study, Yoshikawa et al.\(^{[103]}\) harvested autologous bone marrow MSCs from the ilium of two patients diagnosed with spinal stenosis: Cells cultured in an autogenous serum media and then transplanted percutaneously into the stenosed spinal canal within a collagen sponge graft. At 2 years after surgery, patients reported symptoms improvement, while X-ray, Rontgen kymography and CT showed decreased instability and T2-weighted magnetic resonance indicated high moisture contents.

Overall, MSCs show a great capacity to differentiate towards the NP phenotype, especially when exposed to the chondrogenic molecular signaling within the injured disc, thus potentially restoring its physiological microenvironment and biomechanical properties. MSCs can be readily harvested from multiple sites, even if the procedure itself is not immune to secondary risks. Moreover, MSCs are the only stem cell type that have been transplanted in human disc proving to be safe and being able to reduce LBP in patient affected by IDD.

**ADIPOSE STEM CELLS**

Adipose stem cells (ASCs) are an alternative source of stem cells, instead of bone marrow MSCs, to regenerate the IVD. These cells can easily expand under standard tissue culture conditions and show a pluripotent mesenchymal differentiation capacity. Their therapeutic effect has been tested using coculture system with other adult stem cell types\(^{[104]}\).

Li et al.\(^{[101]}\) evaluated changes in the gene expression pattern of rabbit fat-derived mesenchymal cells when exposed to NP and AF cells in vitro. Authors demonstrated an increase in expression of type II collagen and aggrecan genes from rabbit ASCs cocultured in 3-dimensional alginate beads with NP cells, compared to ASCs cocultured with AF cells and NP cells alone. These data have been also confirmed by Lu et al.\(^{[105]}\) who investigated the ability of ASCs to differentiate when exposed to stimuli secreted by NP cells in vitro. Authors performed a transwell co-culture system of human NP cells and human ASMCs, employing both monolayer and micromass configurations, in order to evaluate the sole effects of soluble signals. Lu et al.\(^{[106]}\) demonstrated that the transwell co-culture of NP cells and ASMCs, both cultured under micromass conditions, induces gene expression of both aggrecan and collagen type II, with concomitant down-regulation of osteopontin, collagen type I and PPAR-\(\alpha\) in ASCs. Moreover, Lu et al.\(^{[106]}\) also evaluated the gene pattern expression of human ASCs cultured in collagen type I or type II hydrogels alone, or cocultured in transwells with micromass human NP cells. They demonstrated that ASCs differentiation along the cartilaginous lineage is characterized by up-regulation of collagen type I\(\alpha\), type I\(\beta\) and aggrecan gene expression and it closely related to cocultures with NP cells and type II hydrogel. Collagen type II represents an appropriate scaffold for the attachment of ASCs and a favorable microenvironment in combination with soluble factors secreted by NP cells inducing the differentiation along cartilage/NP lineage.

Disc regeneration strategies based on adipose stem cells have also evaluated in small and large size animal models. Jeong et al.\(^{[107]}\) investigated the adipose-tissue-derived stromal cell (ADSC) implantation to restore disc in a rat degenerated IVD model. The IVD damaged by needle injection and, after two weeks, injected with ADSCs or saline (as control). At 6 wk after transplantation, authors demonstrated the ability of ADSCs to restore degenerated IVDs, according to reduced disc height loss and restoration of disc signal intensity on MRI. The histological analysis with hematoxylin and eosin staining confirmed a greater IVD restoration in discs transplanted with ADSCs. In addition, positive findings in immunohistochemical staining for collagen type II and aggrecan have also revealed.

Ganey et al.\(^{[108]}\) investigated ADSCs-based cell therapy in degenerated IVD using a dog model obtained by performing a partial nucleotomy on lumbar discs. Six weeks after surgery, authors randomized discs to receive: ADSCs loaded in hyaluronic acid carrier (cells/HA) or HA without cells or nothing. Dogs were killed at 6 mo or at 12 mo. Disc analysis has performed with MRI, radiography, histology and biochemistry. No significant differences between the three different approaches have found in MRI signal intensity and radiographic disc height. However, gene expression of type II colla-
gen and aggrecan demonstrated a statistically significant increase of expression in discs transplanted with ADSCs when compared to discs receiving either the HA only or no treatments. ADSCs are able to provide a regenerative stimulation in the injured IVD. Moreover, the histological analysis showed an abundant extracellular matrix surrounding the cells and cell clustering or clonal within NP. AF fibers were tight and laminated according to the normal IVD morphology. Because of the evidence of injected ADSCs survival, the histology suggested their responsibility for the observed morphology resembling the healthy IVD. Ganey et al.\(^{[108]}\) reported that ADSCs were effective in promoting disc regeneration in an animal injured disc model.

Sun et al.\(^{[109]}\) assessed the influence of ADSCs on NP cells in a compressive load culture: Unphysiological mechanical stimulation was set in order to mimic the stressful conditions leading to IDD. ADSCs protected NP cells from apoptosis through caspase-9 and caspase-3 inhibition, increasing ECM gene expression while diminishing metalloproteinases synthesis inhibiting production of pro-inflammatory factors.

ADSCs have proven to give rise to a chondrogenic lineage and to increase aggrecan and type II collagen synthesis, hence favoring disc regeneration. While they can easily harvested without significant risks, ADSCs actual efficacy has not established yet.

**SYNOVIAL MSCs**

In the last years, synovial MSCs aroused an increasing interest about their application in cell therapy strategies for disc regeneration. They could be a potential source of stem cells because they present a proliferative rate greater than other types of MSCs, such as bone marrow MSCs.\(^{[110]}\) In addition, they show a high chondrogenic potential, demonstrated by the ability to synthesize extracellular matrix after transplantation into articular cartilage defects in a rabbit model.\(^{[111]}\)

Miyamoto et al.\(^{[112]}\) assessed the effect of intradiscal transplantation of synovial MSCs by using an IVD degeneration rabbit model. After allogeneic synovial MSCs transplantation, researchers performed imaging analyses, including X-ray, MRI and histological analysis. Moreover, they performed an in vitro study in order to investigate the interaction between synovial MSCs and NP cells, by producing a co-culture system of human synovial MSCs and rat NP cells. The results showed that synovial MSCs injected into the disc were able to stimulate the remaining NP cells to synthesize type II collagen and to inhibit the expression of matrix degradative enzymes and inflammatory cytokines. These data were confirmed by in vivo findings, showing that IVD height in the MSCs group was higher than disc height in the degeneration group.

Synovial MSCs exhibit a notable proliferative and regenerative potential, which confirmed by pilot studies in articular and disc degenerative models. Further studies needed to support these findings, in order to plan an appropriate therapeutical protocol.

**MDSCS**

MdSCs have shown to reside within skeletal muscles and to be characterized by typical stem cells features, such as self-renewal a multilineage differentiation. Indeed, they are capable of giving rise to other mesodermal cell types, including hematopoietic, osteogenic, chondrogenic, adipogenic and skeletal myogenic cells.\(^{[113]}\)

As Adachi et al.\(^{[114]}\) reported appreciable healing of cartilage defects using muscle-derived cells embedded in collagen gels in a rabbit model, it has been hypothesized that the chondrogenic lineage commitment of MdSCs might therefore provide a prompt source for generating and expanding NP cells as well.

In this regard, our group investigated the role of MdSCs as a source of chondroprogenitor NP cells using an in vitro coculture system. NP cells were isolated from human IVD specimens and then cocultured with MdSCs harvested from the hind limb skeletal muscle of three mice. Proteoglycan synthesis and total GAG content were subsequently analyzed to assess eventual changes in extracellular matrix production, while DNA content measured as an index of cell proliferation. Each of these parameters was significantly increased in the coculture compared to NP cells monoculture, hence suggesting a promising role of MdSCs for disc regeneration.\(^{[115]}\)

MdSCs have been only recently discovered as a novel stem cell population residing within muscles: As mesodermal progenitors, they can differentiate into different cell types, including chondrocytes, thence showing a potential role in disc regeneration. In vivo studies are needed to evaluate the factual MdSCs regenerative potential for disc regeneration cell-based therapy.

**OLFACTORY NEURAL STEM CELLS**

Human olfactory neural stem cells are multipotent stem cells showing the ability to differentiate along both neural lineage, leading to neurons, astrocytes and oligodendrocytes formation, and non-neural lineages.\(^{[116]}\)\(^{[117]}\)

Murrell et al.\(^{[118]}\) investigated the differentiation of olfactory stem cells (OSCs) into NP chondrocyte-like cells both in in vitro and in vivo settings. The in vitro study has performed coculturing OSCs derived from rat olfactory mucosa with rat IVD biopsies. The in vivo study consisted in transplanting genetically engineered OSCs, which were able to express green fluorescent protein, into a rat model of injured IVD, without any pre-differentiation in vitro. Authors showed that olfactory mucosa-derived progenitor cells could induce to differentiate into NP chondrocyte-like cells, as demonstrated by cellular morphology at the microscopy and by expression of proteins suggestive of NP chondrocyte phenotype (collagen Type II - CT2
and aggrecan - CSPG) at the immunochemistry. These findings have been confirmed by both OSCs inducted in vitro with medium conditioned with NP environment and OSCs transplanted into injured rat NP.

OSC can surprisingly differentiate into NP-like chondrocytes when exposed to the injured IVD environment and produce NP matrix constituent elements. However, major concerns related to the invasive approach to harvest OSCs from the olfactory bulb can not be disregarded.

**INDUCED PLURIPOTENT STEM CELLS**

Induced pluripotent stem cells (iPSCs) are somatic cells which have been genetically reprogrammed in order to forcibly express such genes and factors that lead to an embryonic stem cell-like state. Mouse iPSCs were firstly reported in 2006: These cells have been proven to act like pluripotent cells, given that they express stem cells peculiar markers, generate tumours containing cells from all three germ layers and are able to differentiate into different cytotypes when injected in mouse embryos. Human iPSCs, isolated in 2007 for the first time, seem to show similar properties.

Due to their pluriotropy and patient-specificity, human iPSCs have been proposed as a source for generating notochordal cells in order to re-establish disc homeostasis.

Using a mouse model, Chen et al. isolated autologous embryonic fibroblasts which were then epigenetically reprogrammed into iPSCs through a polycistronic lentiviral vector. CD24+ iPSCs subpopulation was further detached using magnetic activated cell sorting and lentiviral vector CD24- obtained similar outcomes.

Liu et al. harvested porcine NP tissue, which was pulverized and added to a culture plate loaded with human iPSCs, in order to induce notochordal cell-like differentiation, which was highlighted by the expression of three notochordal marker genes: Brachyury T, cytokeratin-8 and cytokeratin-18. Most notably, these cells showed the ability to generate NP-like tissue in vitro, which was characterized by NP phenotypic markers such as type II collagen, aggrecan and GAGs.

In spite of their capacity to induce chondrogenic differentiation, iPSCs might potentially lead to tumorigenesis due to their pluriotropy. In addition, genetic engineering reprogramming techniques are characterized by notable costs that might be unlikely borne.

**HEMATOPOIETIC STEM CELLS**

Adult bone marrow includes two different kinds of stem cell populations: Non-hematopoietic stem cells (non-hematopoietic stem cells (HSCs)), including MSCs, which do not express CD34 and HSCs which express CD34. Wei et al. evaluated xenogenic transplantation of human bone marrow cells, both non-HSCs and HSCs, in a rat degenerated disc model in order to find out which population could be used to obtain disc-like cells. The human bone-marrow (CD34+ and CD34−) cells have been injected into rat coccygeal discs, after isolation and labeling with a fluorescent marker. Authors performed histological analysis, immunochemistry and survival rate analysis in all groups at different time points (at 1, 10, 21, and 42 d). They demonstrated that CD34+ cells were able to survive in the NP of host discs until 42 d, whereas CD34− cells detected only up to 21 d. Moreover, only CD34+ cells presented a gene expression pattern similar to chondrocyte cells (positive for Collagen II and SOX-9).

Wei et al. registered data providing evidence that HSCs should not be used to treat IDD, because they are not able to differentiate in chondrocyte-like cells and restore degenerated NP.

The inefficacy of HSCs transplantation in the regenerative cell-based that strategies to treat disc degeneration was also demonstrated by Haufe et al. in a clinical study, in which autologous HSCs derived from pelvic bone marrow were injected into degenerated discs of patients affected by low back pain. This study presents an important methodological bias, because any evidence, both in vitro and in vivo, supporting HSCs transplantation in degenerated disc, has been reported in literature. In fact, authors concluded that HSCs transplantation do not produce any clinical improvement in treated patients.

**DISC STEM CELLS**

To date, several studies have successfully reported the isolation of disc stem cells from the IVD, namely cartilage end plate-derived stem cells (CESCs), annulus fibrosus-derived stem cells (AFSCs) and nucleus pulposus-derived stem cells (NPSCs), according to their localization within the disc.

These cells exhibit typical stem cell markers and are able to differentiate in vitro along the mesenegenic pathway into various cytotypes belonging to osteogenic, chondrogenic and adipogenic lineages. In addition, disc stem cells notably resemble bone marrow MSCs immunophenotype, gene expression profile and self-renewal capacity. It is thought that these cells are remnants of multipotent mesendodermal embryonic cells, while they might, in a smaller proportion, derive from adjacent vertebrae bone marrow.

A real stem cell niche was identified in the pericordium and in the ligament side of the AF: Henriksson et al. proposed that cells from the niche are promptly recruited to NP and AF, along with MSCs from bone marrow, to undergo differentiation in case of tissue injury.

This assumption is further confirmed by the expression of migration and epithelial-mesenchymal transition markers (e.g., SNAI1, SLUG, ITGB1) within the niche...
itself\cite{129}.

However, lack of standardization in both characterization and isolation methodology makes disc stem cells inner potential difficult to be evaluated. Nonetheless, as reported by Sakai \textit{et al.}\cite{130} disc progenitors number seems to decrease progressively with aging and degeneration, thus limiting the possibility to perform autologous re-implantation in older patients. The same study individuated hTIE-2 and GD2 as markers of disc stem cells committed to differentiate into NP progenitor cells. Quantification of these markers might thus correlate with the actual number of progenitor cells present in the disc, in order to assess the extent of disc degeneration.

Wang \textit{et al.}\cite{131} compared human bone marrow MSCs, AFSCs, NPSCs and CECs regenerative properties in a rabbit model of IDD. The abovementioned cells harvested from patients undergoing posterior lumbar interbody fusion and isolated from iliac crest bone marrow and discectomy specimens, respectively. Stem cells were then cultured, expanded and seeded in alginate gel to subsequently injected into rabbit IVDs after NP aspiration. At 6 mo after implantation, animals sacrificed and discs analyzed; morphological evaluation demonstrated that CESCs yielded the highest regenerative potential, followed by NPSCs, BM-MSCs and AFSCs, that showed the lowest potency.

To date, eleven preclinical animal studies investigated outcomes subsequent to IVD tissue or cells transplantation, which demonstrated to delay disc degeneration and, in some models, to favor NP regeneration. However, some of the aforementioned studies reported an increased synthesis of type II collagen while proteoglycan production - and correspondingly disc hydration - was not restored to physiological rates\cite{132}.

Disc stem cells seem to reside within the inner disc niche, as remnants of mesendodermal embryonic stem cells. According to conflicting results, further studies needed to assess their actual usability for disc degeneration cell-based therapy and to establish standardized protocols regarding harvesting techniques, isolation and identification.

**EMBRYONIC STEM CELLS**

Embryonic stem cells (ESCs) represent another possible source of stem cells for disc regeneration, basing on their ability to differentiate along different cell lineages, including notochordal cells.

Notochordal cells are the first cells forming the NP during the embryogenesis of IVD. Moreover, it is also known that the adult NP host chondrocyte-like cells. According to their ability to differentiate along the chondrogenic lineage with opportune culture conditions\cite{133}, they could differentiate into cells able to produce extracellular matrix restoring the inner disc material. Sheikh \textit{et al.}\cite{134} investigated the ESC-derived chondroprogenitors transplantation into a degenerated disc in a rabbit model. Researchers performed a pre-conditioning culture of murine ESCs in order to induce differentiation toward a chondrocyte lineage. In addition, ESC-derived cells have been labeled prior to implantation with a green fluorescent protein. After 8 wk from the implantation, H&E staining, confocal fluorescent microscopy and immunohistochemical analysis have performed on disc samples. Comparing with control non-punctured discs and control degenerate punctured discs, IVDs subdue to implantation of chondroprogenitor cells showed islands of notochordal cells at H&E histological analysis and immunofluorescence staining. These authors demonstrated the proliferation of notochordal-like cells, which are responsible of proteoglycan matrix production, into degenerated IVDs transplanted with ESCs.

However, ESCs show notable tumorigenic properties: They characterized by high telomerase activity (which leads to potentially infinite proliferation) and formation of teratoma. Nonetheless, ESCs handling is surrounded by several ethical issues due to their embryonic provenance, thus making improbable their use in IDD treatment\cite{135}.

**TISSUE ENGINEERING APPROACHES**

The choice of a suitable scaffold for stem cells remains an important issue in the development of this new therapy. Injectable viscoelastic scaffolds are more desirable for IVD tissue engineering to minimize the annular damage and to favor the implantation in a high-pressure structure. Sakai \textit{et al.}\cite{82,89,90} compared cell viability after injection into rabbit NPs of a pure cell suspension compared to a soluble cell-augmented polymer such as fibrin glue that can polymerize in situ, providing the evidence that matrix-assisted cell transfer allows efficient augmentation of IVD. Besides fibrin glue, other scaffolds have been used in IVD tissue engineering such as collagen gels, hyaluronan gel\cite{88}, and genipin cross-linked chitosan\cite{136}. The architectural and mechanical properties of the scaffold are also important. New micro- or nano-scale dimension scaffold as well as new signal release technologies may provide new perspective in IVD stem cell based tissue engineering.

Mercuri \textit{et al.}\cite{137} explored the use of a chemical stabilized elastin-glycosaminoglican-collagen hydrogel as a scaffold capable of resembling NP resilient, mechanical and hydrophilic properties. This material proved to induce chondrogenic differentiation of human-derived adipose tissue stromal cells (hADSCs), resulting into increased aggrecan and type II collagen synthesis \textit{in vitro}. \textit{In vivo} evaluation performed by transplantation of the hydrogel into subdermal pockets of the dorsal mid-line of rats; at 4 wk after injection, the material showed full biocompatibility, cell infiltration and evident remodeling.

Tsyrk \textit{et al.}\cite{138} investigated the use of a collagen-low molecular weight hyaluronic acid semi-interpenetrating network loaded with gelatin microspheres in order to resemble NP main features, such as gel-like consistency,
high hydration rate and appreciable biomechanical strength. Moreover, this fully biocompatible material has proved to easily inject inside the NP and to favor proliferation and chondrogenic differentiation of bone marrow MSCs and nasal chondrocytes, both in vitro and in vivo.

Liu et al\(^{139}\) designed aligned fibrous polyurethane scaffolds using electrospinning to culture rabbit AFSCs in order to perform functional AF replacement. Random fibrous scaffolds were used as a control: Both showed comparable cell attachment and proliferation features, while AFSCs cultured on aligned scaffolds exhibited more natural morphology, higher gene expression activity and increased type I collagen and aggrecan synthesis.

| Table 1  Summary of studies sorted by stem cells types and experimental setting |
|------------------------------------------|------------------------------------------|------------------------------------------|
| Cell type | Ref. | Years | In vitro study | Pre-clinical study | Clinical study |
|------------------------------------------|------------------------------------------|------------------------------------------|
| Bone marrow MSC | Sakai et al\(^{42}\) | 2003 | | | |
| Crevensten et al\(^{40}\) | 2004 | | | | |
| Yamamoto et al\(^{40}\) | 2004 | x | | | |
| Risbud et al\(^{41}\) | 2004 | | x | | |
| Zhang et al\(^{71}\) | 2005 | | | x | |
| Steck et al\(^{64}\) | 2009 | x | | | |
| Sakai et al\(^{80}\) | 2005 | | | x | |
| Sakai et al\(^{80}\) | 2006 | | | x | |
| Richardson et al\(^{62}\) | 2006 | x | | | |
| Le Visage et al\(^{83}\) | 2006 | | | x | |
| Ho et al\(^{60}\) | 2008 | | | x | |
| Vadala et al\(^{82}\) | 2008 | x | | | |
| Hiyama et al\(^{80}\) | 2008 | | | x | |
| Yang et al\(^{71}\) | 2008 | x | | | |
| Sebajima et al\(^{80}\) | 2008 | x | | x | |
| Jeong et al\(^{61}\) | 2009 | x | | | |
| Henrikkson et al\(^{84}\) | 2009 | x | | | |
| Le Maitre et al\(^{86}\) | 2009 | | x | | |
| Chen et al\(^{66}\) | 2009 | | | x | |
| Wei et al\(^{68}\) | 2009 | | | x | |
| Svanvik et al\(^{72}\) | 2010 | x | | | |
| Watanabe et al\(^{80}\) | 2010 | x | | | |
| Yoshikawa et al\(^{108}\) | 2010 | | | | x |
| Hee et al\(^{86}\) | 2010 | | | x | |
| Korecki et al\(^{71}\) | 2010 | x | | | |
| Yang et al\(^{94}\) | 2010 | | | x | |
| Strassburg et al\(^{92}\) | 2010 | x | | | |
| Serigano et al\(^{94}\) | 2010 | | x | | |
| Alton et al\(^{85}\) | 2012 | x | | | |
| Zhang et al\(^{89}\) | 2011 | | | | x |
| Di Martino et al\(^{142}\) | 2012 | | | | x |
| Subhan et al\(^{88}\) | 2014 | x | | | |
| Cai et al\(^{80}\) | 2015 | x | | | |
| Tsaryk et al\(^{134}\) | 2015 | x | | | |
| Orozco et al\(^{110}\) | 2015 | | | | x |
| Vadala et al\(^{94}\) | 2015 | x | | | |
| Lee et al\(^{94}\) | 2000 | | x | | |
| Lu et al\(^{94}\) | 2007 | x | | | |
| Lu et al\(^{94}\) | 2008 | x | | | |
| Jeong et al\(^{71}\) | 2010 | | x | | |
| Ganey et al\(^{94}\) | 2009 | | | x | |
| Mercari et al\(^{133}\) | 2014 | x | | | |
| Vadalà et al\(^{83}\) | 2008 | x | | | |
| Murrell et al\(^{109}\) | 2009 | x | | | |
| Murrell et al\(^{109}\) | 2009 | | x | | |
| Chen et al\(^{132}\) | 2013 | x | | | |
| Liu et al\(^{113}\) | 2014 | x | | | |
| Synovial MSC | Miyamoto et al\(^{113}\) | 2010 | | x | |
| Hematopoietic SC | Haule et al\(^{104}\) | 2006 | | | x |
| Disc stem cells | Liu et al\(^{110}\) | 2011 | x | | |
| Sakai et al\(^{110}\) | 2012 | x | | | |
| Wang et al\(^{101}\) | 2014 | x | | | |
| Liu et al\(^{110}\) | 2015 | x | | | |
| Shi et al\(^{136}\) | 2015 | x | | | |
| Embryonic SC | Sheikh et al\(^{143}\) | 2009 | | | x |
| Wharton’s Jelly SC | Liu et al\(^{110}\) | 2011 | | | |

MSC: Mesenchymal stem cells; SC: Stem cells; iPSCs: Induced pluripotent stem cells.
Peroglio et al. designed a thermoresversible hyaluronan-based hydrogel [(hyaluronan-poly(N-isopropylacrylamide))] to induce human MSCs differentiation into the disc phenotype and to evaluate the effects of preconditioning. Cells conducted in the hydrogel or alginate for 1 wk under hypoxic conditions in a chondropermissive media alone or with TGF-β1 or GDF-5. Then, the cells suspended ex vivo in the gel and supplied to bovine IVDs. The HA-pNIPAM gel led to disc phenotype differentiation more promptly than alginate: Higher GAG/DNA ratio, type II collagen, SOX9 and other markers reported in vitro. In addition, preconditioning seemed to induce a lower degree of differentiation if compared to direct combination of the cells with the gel into the disc environment.

TOWARDS CLINICAL TRIALS

Though many issues remain unresolved, exciting progress certainly has been made toward the realization of stem cell therapy as a potential therapeutic option for the treatment of IDD.

In a systematic review and meta-analysis, Wang et al. assessed the efficacy of cell therapy in IDD treatment in 22 animal controlled trials: Stem cells transplantation was significantly associated with increased disc height index, T2 weighted MRI signal intensity, type II collagen synthesis and diminished degeneration grade. Therefore, these promising results provide a solid basis for testing the effects of cell therapy on humans.

In order to move toward successful human clinical trials, it is critical to rigorously test the long-term effects of stem cell-mediated strategies in animal models of disc degeneration that closely simulate the human condition on disc biology, nutrition and biomechanical functions such as larger animal models or primates. In fact, animal models of IDD are all of relatively short duration, induced in young and previously healthy animal discs rich in notochordal cells, where the effects of the induced degeneration on disc nutrition are unknown.

The patients that could expect to benefit from stem cell-mediated therapy are those with mild or moderate grades of IDD, in whom the structural integrity of the disc remains preserved. Because nutrition supply to many degenerated discs is poor, there is theoretical concern over the added nutritional demands arising from the increased number of metabolic active cells into the disc after transplantation. Therefore, evaluation of the nutrition transport into the IVD, using microelectrodes able to evaluate oxygen or nitrous oxide diffusion, may be useful in order to select the patients that could benefit from the treatment (Table 1).

CONCLUSION

Thanks to the recent research efforts aimed at further developing our knowledge of the biology and biochemistry of the IVD, our understanding of the process of IDD is rapidly growing. While there is still much to learn, some key factors involved in disc breakdown have become evident. Identification of the importance of cell loss within the disc has led to a focus on novel treatments aimed at regenerating the degenerating tissue. With its unique ability to differentiate into different cell types and to secrete a wide range of trophic cytokines, adult stem cell therapy has received considerable interest showing much promise with regard to treating chronic conditions such as IDD. Multiple studies have determined the feasibility of adult stem cell therapy for IDD, and recent studies have demonstrated proof of efficacy of autologous bone marrow MSCs transplantation in reproducible animal models as well as to be safe in human clinical trials. Other stem cells populations are still under evaluation with few proofs of efficacy in animals. Nonetheless, adult stem cell therapy has shown promise in becoming a powerful tool in the future treatment of IDD.

REFERENCES

1. Lively MW. Sports medicine approach to low back pain. South Med J 2002; 95: 642-646 [PMID: 12081221 DOI: 10.1097/00007632-199401001-00013]
2. Weiner DK, Sakamoto S, Perera S, Breuer P. Chronic low back pain in older adults: prevalence, reliability, and validity of physical examination findings. J Am Geriatr Soc 2006; 54: 11-20 [PMID: 16420193 DOI: 10.1111/j.1532-5415.2005.00534.x]
3. Luo X, Pietrobon R, Sun SX, Liu GG, Hey L. Estimates and patterns of direct health care expenditures among individuals with back pain in the United States. Spine (Phila Pa 1976) 2004; 29: 79-86 [PMID: 14699281 DOI: 10.1097/01.BRS.0000105527.13866.0F]
4. Ricci JA, Stewart WF, Choe E, Leotta C, Foley K, Hochberg MC. Back pain exacerbations and lost productive time costs in United States workers. Spine (Phila Pa 1976) 2006; 31: 3052-3060 [PMID: 17173507 DOI: 10.1097/01.BRS.0000208990.13905]
5. Cassidy JJ, Hiltner A, Bauer E. Hierarchical structure of the intervertebral disc. Connect Tissue Res 1989; 23: 75-88 [PMID: 2632144 DOI: 10.3109/030082089013905]
6. Bruehlmann SB, Rattner JB, Matyas JR, Duncan NA. Regional variations in the cellular matrix of the annulus fibrosus of the intervertebral disc. J Anat 2002; 201: 159-171 [PMID: 12220124 DOI: 10.1046/j.1469-7580.2002.00080.x]
7. Pezwicz CA, Robertson PA, Broom ND. The structural basis of interlamellar cohesion in the intervertebral disc wall. J Anat 2006; 208: 317-330 [PMID: 16533315 DOI: 10.1111/j.1469-7580.2006.00536.x]
8. Urban JP, McMullin JF. Swelling pressure of the intervertebral disc: influence of proteoglycan and collagen contents. Biochirnica Biophys Acta 1985; 22: 145-157 [PMID: 3963322]
9. Trout JJ, Buckwalter JA, Moore KC, Landas SK. Ultrastructure of the human intervertebral disc. I. Changes in notochordal cells with age. Tissue Cell 1982; 14: 359-369 [PMID: 7202266 DOI: 10.1016/0040-8168(82)90033-7]
10. Hamzah MD, Soames RW. Human intervertebral disc: structure and function. Anat Rec 1988; 220: 337-356 [PMID: 2398416 DOI: 10.1002/ata.1092200402]
11. Best BA, Guilik F, Setton LA, Zhu W, Saeed-Nejad F, Ratcliffe A, Weidenbaum M, Mow VC. Compressive mechanical properties of the human anulus fibrosus and their relationship to biochemical composition. Spine (Phila Pa 1976) 1994; 19: 212-221 [PMID: 8153833 DOI: 10.1097/00007632-199401001-00017]
12. Boni M, Denaro V. Anatomoclinical correlations in cervical spondylosis. In: Keir P, Weidner A. Cervical spine II. New York:

www.wjgnet.com 196
May 26, 2016 | Volume 8 | Issue 5 |
content in the nucleus pulposus in normal adolescent rabbits. Spine (Phila Pa 1976) 2005; 30: 25-31; discussion 31-32 [PMID: 15626976 DOI: 10.1097/01.brs.0000148002.86656.4d]

Nischida K, Kang JD, Gilbertson LG, Moon SH, Suh JK, Vogt MT, Robbins PD, Evans CJ. Modulation of the biologic activity of the rabbit intervertebral disc by gene therapy: an in vivo study of adenosine-mediated transfer of the human transforming growth factor beta 1 encoding gene. Spine (Phila Pa 1976) 1999; 24: 2419-2425 [PMID: 10623603 DOI: 10.1097/00007632-199912100-00002]

Paul R, Huydon RC, Cheng H, Ishikawa A, Nenadovich N, Jiang W, Zhou L, Breyer B, Feng T, Gupta P, He TC, Phillips FM. Potential use of Sox9 gene therapy for intervertebral degenerative disc disease. Spine (Phila Pa 1976) 2003; 28: 755-763 [PMID: 12698117 DOI: 10.1097/01.BRS.0000058946.64222.92]

Wallach CJ, Sobajima S, Watanabe Y, Kim JS, Georgescu HI, Robbins P, Gilbertson LG, Kang JD. Gene transfer of the catabolic inhibitor TIMP-1 increases measured proteoglycans in cells from degenerated human intervertebral discs. Spine (Phila Pa 1976) 2003; 28: 2331-2337 [PMID: 14560079 DOI: 10.1097/01.BRS.0000146499.94600.85]

Yoon ST, Park JS, Kim KS, Li J, Attallah-Wasif ES, Hutton WC, Boden SD. ISSLS prize winner: LMP-1 upregulates intervertebral disc cell production of proteoglycans and BMPs in vitro and in vivo. Spine (Phila Pa 1976) 2004; 29: 2603-2611 [PMID: 15564908 DOI: 10.1097/01.brs.0000146103.94600.84]

Wallach CJ, Kim JS, Sobajima S, Lattermann C, Oxtner WM, McFadden K, Robbins PD, Gilbertson LG, Kang JD. Safety assessment of intradiscal gene transfer: a pilot study. Spine J 2006; 6: 107-112 [PMID: 16517379 DOI: 10.1016/j.spinee.2005.05.002]

Vadala G, Sowa GA, Smith L, Hubert MG, Levicoff EA, Denaro V, Gilbertson LG, Kang JD. Regulation of transgene expression using an inducible system for improved safety of intervertebral disc gene therapy. Spine (Phila Pa 1976) 2007; 32: 1381-1387 [PMID: 17545904 DOI: 10.1097/BRS.0b013e3181060121]

Ganey T, Libera J, Moos V, Alasevic O, Fritsch KG, Meisel HJ, Hutton WC. Disc chondrocyte transplantation in a canine model: a treatment for degenerated or damaged intervertebral disc. Spine (Phila Pa 1976) 2003; 28: 2609-2620 [PMID: 14652478 DOI: 10.1097/01.BRS.0000079871.63063.78]

Nishimura K, Mochida J. Percutaneous reininsertion of the nucleus pulposus. An experimental study. Spine (Phila Pa 1976) 1998; 23: 1531-1538; discussion 1539 [PMID: 9682309 DOI: 10.1097/00007632-199807150-00006]

Sato M, Asazuma T, Ishihara M, Ishihara M, Kikuchi T, Kikuchi M, Fujikawa K. An experimental study of the regeneration of the intervertebral disc with an allograft of cultured annular fibrous cells using a tissue-engineering method. Spine (Phila Pa 1976) 2003; 28: 548-553 [PMID: 12642760 DOI: 10.1097/01.BRS.0000049099.09102.60]

Goremsk M, Jaksimovic C, Kregar-Velikonja N, Goremsk M, Knezevic M, Jeras M, Pavlovic V, Gor A. Nucleus pulposus repair with cultured autologous elastic cartilage derived chondrocytes. Cell Mol Biol Let 2004; 9: 363-373 [PMID: 15213815]

Li X, Lee JP, Balian G, Greg Anderson D. Modulation of chondrocytic properties of fat-derived mesenchymal cells in cocultures with nucleus pulposus. Connect Tissue Res 2005; 46: 75-82 [PMID: 16019417 DOI: 10.1080/030088050954104]

Sakai D, Mochida J, Yamamoto Y, Nomura T, Okuma M, Nishimura K, Nakai T, Ando K, Hotta T. Transplantation of mesenchymal stem cells embedded in Atelocollagen gel to the intervertebral disc: a potential therapeutic model for disc degeneration. Biomaterials 2003; 24: 3531-3541 [PMID: 12809782 DOI: 10.1016/S0142-9610(03)00222-9]

Vadala G, Sobajima S, Lee YJ, Huard J, Denaro V, Kang JD, Gilbertson LG. In vivo interaction between muscle-derived stem cells and nucleus pulposus cells. Spine J 2008; 8: 804-809 [PMID: 18023623 DOI: 10.1016/j.spinee.2007.07.394]

Meisel JJ, Ganey T, Hutton WC, Libera J, Minkus V, Alasevic O. Clinical experience in cell-based therapeutics: intervention
Factors 2002; 2002; Schmitz-Valverde V, Ganey T, Minkus Y, Hutton WC, Alasevic 2006; 2001; Lorich DG, Kupcha R, Reilly TM, Jones AR, Henriksson HB, Karlsson C, Hagman M, Lindahl 2008; 1143979 [DOI: 10.1016/S0092-8674(01)00409-3]

Wobus AM. Potential of embryonic stem cells. Mol Aspects Med 2002; 21: 149-164 [PMID: 11470141 DOI: 10.1016/S0008-2979(01)00066-1]

Pittenger MF, Mackay AM, Beck SC, Jaiswal RK, Douglas R, Mosca JD, Moorman MA, Simonetti DW, Craig S, Marshall DR. Multilineage potential of adult human mesenchymal stem cells. Science 1999; 284: 143-147 [DOI: 10.1126/science.284.5413.143]

Zuk PA, Zhu M, Mizuno H, Huang J, Futrell JW, Katz AJ, Benhaim P, Lorenz HP, Hedrick MH. Multilineage cells from human adipose tissue: implications for cell-based therapies. Tissue Eng 2001; 7: 211-228 [PMID: 11304456 DOI: 10.1089/ten.2001.0062589]

Nakahara H, Goldberg VM, Caplan AI. Culture-expanded human periosseal-derived cells exhibit osteochondral potential in vivo. J Orthop Res 1991; 9: 465-476 [DOI: 20045973 DOI: 10.1002/jorc.11009402]

De Bari C, Dell’Accio F, Luften P. Human peristemeum-derived cells maintain phenotypic stability and chondrogenic potential throughout expansion regardless of donor age. Arthritis Rheum 2001; 44: 85-95 [PMID: 11212180 DOI: 10.1002/1529-0131(200105)44]

De Bari C, Dell’Accio F, Tyliszanowski P, Luften FP. Multipotent mesenchymal stem cells from adult human synovial membrane. Arthritis Rheum 2001; 44: 1928-1942 [PMID: 11508446 DOI: 10.1002/twrc.1020108544]

Lee JY, Qu-Petersen Z, Cao B, Kimura S, Jankowski R, Cummins J, Olszowska J, Walter A, Kaplan DR, Miller FD. Isolation of multipotent adult stem cells from human adipose tissue: implications for cell-based therapies. J Orthop Res 1999; 17: 95-103 [PMID: 10973997 DOI: 10.1083/jcb.150.5.1085]

Toma JG, Steck E, Fischer J, Lorenz H, Gotterbarm T, Jung M, Richter W. Mesenchymal stem cell differentiation in an experimental cartilage defect: restriction of hypertrophy to bone-close neocartilage. Stem Cells Dev 2009; 18: 969-978 [PMID: 19049440 DOI: 10.1089/scd.2008.0213]

Le Visage C, Kim SW, Tateno K, Sieber AN, Kostouk PJ, Leong KW. Interaction of human mesenchymal stem cells with disc cells: changes in extracellular matrix biosynthesis. Sci Transl Med 2013; 6: 143-155 [PMID: 23658696 DOI: 10.1126/scitranslmed.3006446]

Sobajima S, Vadalà G, Shimera A, Kim JS, Gilberston LG, Kang JD. Feasibility of a stem cell therapy for intervertebral disc degeneration. Spine J 2008; 8: 888-896 [PMID: 18082460 DOI: 10.1016/j.spine.2007.09.011]

Kuroda R, Usas A, Kubo S, Corsi K, Peng H, Rose T, Cummins J, Fu FH, Huard J. Cartilage repair using bone morphogenetic protein 4 and muscle-derived stem cells. Arthritis Rheum 2006; 54: 433-442 [PMID: 16647218 DOI: 10.1002/art.21632]

Saccomi B, Funari A, Michiesi S, Di Cesare S, Piersanti S, Saggio I, Tagliatela F, Ferrari S, Robey PG, Riminucci M, Bianco P. Self-renewing osteoprogenitors in bone marrow sinoviuos can organize a hematopoietic microenviroment. Cell 2007; 131: 324-336 [PMID: 17956733 DOI: 10.1016/j.cell.2007.08.025]

Crisan M, Yap S, Castella L, Chen CW, Corselli M, Park TS, Andriolo G, Sun B, Zheng B, Zhang L, Norotte C, Teng PN, Traas J, Schuger R, Deasy BM, Budyak S, Buhring HJ, Giacobino JP, Lazzari L, Huard J, Paoliti B. A perivascular origin for mesenchymal stem cells in multiple human organs. Cell Stem Cell 2008; 3: 301-313 [PMID: 18786417 DOI: 10.1016/j.stem.2007.07.003]

Caplan AI. All MSCs are pericytes? Cell Stem Cell 2008; 3: 229-230 [PMID: 18786406 DOI: 10.1016/j.stem.2008.08.008]

Hubert MG, Vadalà G, Sowa G, Studer RK, Kang JD. Gene therapy for the treatment of degenerative disk disease. J Am Acad Orthop Surg 2006; 14: 312-319 [PMID: 16524982]

Risbud MV, Guttagapalli A, Tsai TT, Lee JV, Danielson KG, Vaccura AR, Albert TJ, Gazit Z, Gazit D, Shimpiro IM. Evidence for skeletal progenitor cells in the degenerate human intervertebral disc. Spine (Phila Pa 1976) 2007; 32: 2537-2544 [PMID: 17978651 DOI: 10.1097/BRS.0b013e318158dea6]

Blanco JF, Graciani IF, Sanchez-Guijo FM, Muntien S, Hernandez-Campo P, Santamaria C, Carrancio S, Barbado MV, Cruz G, Gutierrez-Cosío S, Herrero C, San Miguel FJ, Brihon JG, del Calizo MC. Isolation and characterization of mesenchymal stromal cells from human degenerated nucleus pulposus: comparison with bone marrow mesenchymal stromal cells from the same subjects. Spine (Phila Pa 1976) 2010; 35: 2259-2265 [PMID: 20662750 DOI: 10.1097/BRS.0b013e318c8b8828]

Steck E, Fischer J, Lorenz H, Gotterbarm T, Jung M, Richter W. Mesenchymal stem cell differentiation in an experimental cartilage defect: restriction of hypertrophy to bone-close neocartilage. Stem Cells Dev 2009; 18: 969-978 [PMID: 19049440 DOI: 10.1089/scd.2008.0213]

Le Visage C, Kim SW, Tateno K, Sieber AN, Kostuik PJ, Leong KW. Interaction of human mesenchymal stem cells with disc cells: changes in extracellular matrix biosynthesis. Sci Transl Med 2013; 6: 143-155 [PMID: 23658696 DOI: 10.1126/scitranslmed.3006446]

Svanvik T, Henriksen BB, Karlsson C, Hagnman M, Lindahl A, Brisyh H. Human disc cells from degenerated disks and mesenchymal stem cells in co-culture result in increased matrix production. Cells Tissues Organs 2010; 191: 2-11 [PMID: 19494482 DOI: 10.1159/000223326]

Hofstetter CP, Schwarz EJ, Hess D, Widenfalk J, El Manira A, Prockop DJ, Olson L. Marrow stromal cells form guiding strands in the injured spinal cord and promote recovery. Proc Natl Acad Sci USA 2002; 99: 2199-2204 [PMID: 11854516 DOI: 10.1073/ pnas.042657899]

Richardson SM, Walker RV, Parker S, Rhodes NP, Hunt JA, Freemont AJ, Hoyland JA. Intervertebral disc cell-mediated mesenchymal stem cell differentiation. Stem Cells 2006; 24: 707-716 [PMID: 16223853 DOI: 10.1634/stemcells.2005-0205]

Maroudas A, Stockwell RA, Nachemson A, Urban J. Factors involved in the nutrition of the human lumbar intervertebral disc: cellularity and diffusion of glucose in vitro. J Anat 1975; 120: 113-130 [PMID: 1184452]

Vadalà G, Studer RK, Sowa G, Spiezia F, Iucu C, Denaro V,
of intervertebral disc injury on mesenchymal stem cell regeneration. Connect Tissue Res 2008; 49: 15-21 [PMID: 18293174 DOI: 10.1080/03008200701818599]

77 Yang SH, Wu CC, Shih SJ, Sun YH, Lin CH. In vitro study on interaction between human nucleus pulposus cells and mesenchymal stem cells through paracrine stimulation. Spine (Phila Pa 1976) 2008; 33: 1508-1514 [PMID: 15245751 DOI: 10.1097/BRS.0b013e3180c6e6df]

78 Korecki CI, Taboas JM, Tuan RS, Iatridis JC. Notochordal cell conditioned medium stimulates mesenchymal stem cell differentiation toward a young nucleus pulposus phenotype. Stem Cell Res Ther 2010; 1: 18 [PMID: 20565707 DOI: 10.1186/s13287-010-0018-8]

79 Caplan AI, Dennis JE. Mesenchymal stem cells as trophic mediators. J Cell Biochem 2006; 98: 1076-1084 [PMID: 16619257 DOI: 10.1002/jcb.20886]

80 Yamamoto Y, Mochida J, Sakai D, Nakai T, Nishimura K, Kawada H, Hotta T. Upregulation of the viability of nucleus pulposus cells by bone marrow-derived stromal cells: significance of direct cell-to-cell contact in coculture system. Spine (Phila Pa 1976) 2004; 29: 1508-1514 [PMID: 15245751 DOI: 10.1097/BRS.0b013e3180c6e6df]

81 Watanabe T, Sakai D, Yamamoto Y, Iwashina T, Serigano K, Tamura F, Mochida J. Human nucleus pulposus cells significantly enhanced biological properties in a coculture system with direct cell-to-cell contact with autologous mesenchymal stem cells. J Orthop Res 2010; 28: 623-630 [PMID: 19953600 DOI: 10.1002/jor.20130]

82 Straussberg S, Richardson SM, Freemont AJ, Hoyland JA. Co-culture induces mesenchymal stem cell differentiation and modulation of the degenerate human nucleus pulposus cell phenotype. Regen Med 2010; 5: 701-711 [PMID: 20868326 DOI: 10.2217/me.10.59]

83 Chen EH, Olson EN. Unveiling the mechanisms of cell-cell fusion. Science 2005; 308: 369-373 [PMID: 15831748 DOI: 10.1126/science.1104799]

84 Terada N, Hamazaki T, Oka M, Hoki M, Mastalerz DM, Nakano Y, Meyer EM, Morel L, Petersen BE, Scott EW. Bone marrow cells adopt the phenotype of other cells by spontaneous cell fusion. Nature 2002; 416: 542-545 [PMID: 11932747 DOI: 10.1038/nature00730]

85 Le Maître CL, Baird P, Freemont AJ, Hoyland JA. An in vitro study investigating the survival and phenotype of mesenchymal stem cells following injection into nucleus pulposus tissue. Arthritis Res Ther 2009; 11: R20 [PMID: 1920770 DOI: 10.1186/ar2611]

86 Chen WH, Liu HY, Le WC, Wu SC, CH, HSiao SH, Wu CH, Chiu WT, Chen BJ, Deng WP. Intervertebral disc regeneration in an ex vivo culture system using mesenchymal stem cells and platelet-rich plasma. Biomaterials 2009; 30: 5523-5533 [PMID: 19646749 DOI: 10.1016/j.biomaterials.2009.07.019]

87 Zhang YG, Guo X, Xu P, Kang LL, Li J. Bone mesenchymal stem cells transplanted into rabbit intervertebral discs can increase proteoglycans. Clin Orthop Relat Res 2005; (430): 219-226 [PMID: 15662327 DOI: 10.1097/01.blo.0000146334.31120.c]

88 Crenvenstn W, Walsh AJ, Ananthakrishnan D, Page P, Wahta GM, Lotz JC, Berven S. Intervertebral disc cell therapy for regeneration: mesenchymal stem cell implantation in rat intervertebral discs. Ann Biomed Eng 2004; 32: 430-443 [PMID: 15905817]

89 Sakai D, Mochida J, Iwashina T, Hiyama A, Omi H, Imai M, Nakai T, Ando K, Hotta T. Regenerative effects of transplanting mesenchymal stem cells embedded in alginate to the degenerated intervertebral disc. Biomaterials 2006; 27: 335-345 [PMID: 16112726 DOI: 10.1016/j.biomaterials.2005.06.038]

90 Sakai D, Mochida J, Iwashina T, Watanabe T, Nakai T, Ando K, Hotta T. Differentiation of mesenchymal stem cells transplanted to a rabbit degenerative disc model: potential and limitations for stem cell therapy in disc regeneration. Spine (Phila Pa 1976) 2005; 30: 2579-2587 [PMID: 16261113 DOI: 10.1097/01.brs.0000184356.28841.c]

91 Ho G, Leung VY, Cheung KM, Chan D. Effect of severity of intervertebral disc injury on mesenchymal stem cell regeneration. Connect Tissue Res 2008; 49: 15-21 [PMID: 18293174 DOI: 10.1080/03008200701818599]

92 Jeong JH, Jin ES, Min JK, Jeon SR, Park CS, Kim HS, Choi KH. Human mesenchymal stem cell implantation into the degenerated coccygeal disc of the rat. Cytochemistry 2009; 59: 55-64 [PMID: 19363763 DOI: 10.1016/j.s0161-009x.2009.01817-x]

93 Yamamoto Y, Hotta T, Lu ZF, Oka M, Hoki M, Mastalerz DM, Nakano Y, Meyer EM, Morel L, Petersen BE, Scott EW. Bone marrow cells adopt the phenotype of other cells by spontaneous cell fusion. Nature 2002; 416: 542-545 [PMID: 11932747 DOI: 10.1038/nature00730]
and differentiation of adipose tissue-derived stem cells. *J Cell Mol Med* 2008; 12: 2812-2822 [PMID: 18266957 DOI: 10.1111/j.1582-4934.2008.00278.x]

107 Jhong JH, Lee JJ, Jin ES, Min JK, Jeon SR, Choi KH. Regeneration of intervertebral discs in a rat disc degeneration model by implanted adipose-tissue-derived stromal cells. *Acta Neurochir* (Wien) 2010; 152: 1771-1777 [PMID: 20571835 DOI: 10.1007/s00701-010-0698-2]

108 Ganev T, Hutton WC, Moseley T, Hedrick M, Meisel HJ. Intervertebral disc repair using adipose tissue-derived stem and regenerative cells: experiments in a canine model. *Spine* (Phila Pa 1976) 2009; 34: 2297-2304 [PMID: 19934809 DOI: 10.1097/BRS.0b013e181a45157]

109 Sun Z, Luo B, Liu ZH, Samartzis D, Liu Z, Gao B, Huang L, Luo ZJ. Adipose-derived stromal cells protect intervertebral discs in compression: implications for stem cell regenerative disc therapy. *Int J Biol Sci* 2015; 11: 133-143 [PMID: 25561896 DOI: 10.7150/ijbs.10598]

110 Nimura A, Muneta T, Koga H, Mochizuki T, Suzuki K, Makino H, Umezawa A, Sekiya I. Increased proliferation of human synovial mesenchymal stem cells with autologous human serum: comparisons with bone marrow mesenchymal stem cells and with fetal bovine serum. *Arthritis Rheum* 2008; 58: 501-510 [PMID: 18240254 DOI: 10.1002/art.23219]

111 Koga H, Muneta T, Nagase T, Nimura A, Ju YJ, Mochizuki T, Sekiya I. Comparison of mesenchymal tissues-derived stem cells for in vivo chondrogenesis: suitable conditions for cell therapy of cartilage defects in rabbit. *Cell Tissue Res* 2008; 333: 207-215 [PMID: 18560897 DOI: 10.1007/s00441-008-0633-5]

112 Miyamoto T, Muneta T, Tabuchi T, Matsumoto K, Saito H, Tsugi K, Sekiya I. Intradiscal transplantation of synovial mesenchymal stem cells prevents intervertebral disc degeneration through suppression of matrix metalloproteinase-related genes in nucleus pulposus cells in rabbits. *Arthritis Rheum Ther* 2010; 12: R206 [PMID: 21054867 DOI: 10.1186/ar3182]

113 Jankowski RJ, Deasy BM, Huard J. Muscle-derived stem cells. *Gene Ther* 2002; 9: 642-647 [PMID: 12032710 DOI: 10.1038/sj.gt.3301719]

114 Adachi N, Sato K, Usas A, Fu FH, Ochi M, Han CW, Niwibzi C, Huard J. Muscle derived, cell based ex vivo gene therapy for treatment of full thickness articular cartilage defects. *J Rheumatol* 2002; 29: 1920-1930 [PMID: 12233887]

115 Liu Y, Rahaman MN, Bal BS. Modulating notochordal differentiation of human induced pluripotent stem cells using natural nucleus pulposus tissue matrix. *PLoS One* 2014; 9: e100885 [PMID: 25062408 DOI: 10.1371/journal.pone.0100885]

116 Roisen FJ, Klueber KM, Lu CL, Hatcher LM, Dozier A, Shields CB, Maguire S. Adult human olfactory stem cells. *Brain Res* 2001; 890: 11-22 [PMID: 11164764 DOI: 10.1016/S0006-8993(00)03016-X]

117 Murrell W, Féron F, Wetzig A, Cameron N, Splatt K, Bellette B, Sanford E, Anderberg L, Cavanagh B, Mackay-Sim A. Identification of cell proliferation zones, progenitor cells and a potential stem cell niche in the intervertebral disc region: a study in four species. *Spine* (Phila Pa 1976) 2009; 34: 2278-2287 [PMID: 19755937 DOI: 10.1097/BRS.0b013e318195aa2d]

118 Wang F, Shi R, Cai F, Wang YT, Wu XT. Stem Cell Approaches to Intervertebral Disc Regeneration: Obstacles from the Disc Microenvironment. *Stem Cells Dev* 2015; 24: 2479-2495 [PMID: 26228642 DOI: 10.1089/scd.2015.0158]

119 Henriksson H, Thormeno M, Karlsson C, Hågg O, Janevik K, Lindahl A, Brödy H. Identification of cell proliferation zones, progenitor cells and a potential stem cell niche in the intervertebral disc region: a study in four species. *Spine* (Phila Pa 1976) 2009; 34: 2278-2287 [PMID: 19755937 DOI: 10.1097/BRS.0b013e318195aa2d]

120 Henriksson HB, Svala E, Sköldebrand E, Lindahl A, Brödy H. Support of concept that migrating progenitor cells from stem cell niches contribute to normal regeneration of the adult mammal intervertebral disc: a descriptive study in the New Zealand white rabbit. *Spine* (Phila Pa 1976) 2012; 37: 722-732 [PMID: 21897341 DOI: 10.1097/BRS.0b013e31821c2127]

121 Sakai D, Nakamura Y, Nakai T, Mishima T, Kato S, Grad S, Alini M, Risbud MV, Chan D, Cheah KS, Yamamura K, Masuda K, Okano H, Ando K, Mochida J. Exhaustion of nucleus pulposus progenitor cells with ageing and degeneration of the intervertebral disc. *Nat Commun* 2012; 3: 1264 [PMID: 23233294 DOI: 10.1038/ncomms2226]

122 Wang H, Zhou Y, Huang B, Liu LT, Liu MH, Wang J, Li CQ, Zhang ZF, Chu TW, Xiong CJ. Utilization of stem cells in alginate for nucleus pulposus tissue engineering. *Tissue Eng Part A* 2014; 20: 908-920 [PMID: 21420374 DOI: 10.1089/ten.tea.2012.0703]

123 Sakai D, Andersen GB. Stem cell therapy for intervertebral disc regeneration: obstacles and solutions. *Nat Rev Rheumatol* 2015; 11: 243-256 [PMID: 25708497 DOI: 10.1038/nrrheum.2015.13]

124 Kawaguchi J, Mee PJ, Smith AG. Osteogenic and chondrogenic differentiation of embryonic stem cells in response to specific growth factors. *Bone* 2005; 36: 758-769 [PMID: 15794925 DOI: 10.1016/j.bone.2004.07.019]

125 Sheikh H, Zachariah K, De La Torre RP, Facek C, Vasquez A, Chaudhry GR, Srinivash D, Perez-Cruz MJ. In vitro intervertebral disc regeneration using stem cell-derived chordoprogenitors. *J Neurosurg Spine* 2009; 10: 265-272 [PMID: 19320588 DOI: 10.3171/2008.12.SPINEO835]

126 Oehme D, Goldschläger T, Rosenfeldt JV, Ghosh P, Jenkins G. The role of stem cell therapies in degenerative lumbar spine disease: a review. *Neurosurgery* 2015; 38: 429-445 [PMID: 25749802 DOI: 10.1093/neuros/nuv021]

127 Mwale F, Iordanova I, Demers CN, Steffen T, Roughley P, Antoniou J. Biological evaluation of chitosan salts cross-linked to genipin as a cell scaffold for disk tissue engineering. *Tissue Eng* 2005; 11: 130-140 [PMID: 15738668 DOI: 10.1089/tv.2005.11.130]

128 Mercuri J, Addington C, Pascal R, Gill S, Simionescu D. Development and initial characterization of a chemically stabilized elastin-glycosaminoglycan-collagen composite shape-memory hydrogel for nucleus pulposus regeneration. *J Biomed Mater Res A* 2014; 102;
Tsaryk R, Gloria A, Russo T, Ansparch L, De Santis R, Ghanati S, Unger RE, Ambrosio L, Kirkpatrick CJ. Collagen-low molecular weight hyaluronic acid semi-interpenetrating network loaded with gelatin microspheres for cell and growth factor delivery for nucleus pulposus regeneration. Acta Biomater 2015; 20: 10-21 [PMID: 25861947 DOI: 10.1016/j.actbio.2015.03.041]

Liu C, Zhu C, Li J, Zhou P, Chen M, Yang H, Li B. The effect of the fibre orientation of electrospun scaffolds on the matrix production of rabbit annulus fibrosus-derived stem cells. Bone Res 2015; 3: 15012 [PMID: 26275539 DOI: 10.1038/boneres.2015.12]

Peroglio M, Eglin D, Benneker LM, Alini M, Grad S. Thermo-reversible hyaluronan-based hydrogel supports in vitro and ex vivo disc-like differentiation of human mesenchymal stem cells. Spine J 2013; 13: 1627-1639 [PMID: 23830827 DOI: 10.1016/j.spinee.2013.05.029]

Vadalà G, De Strobel F, Bernardini M, Denaro L, D’Avella D, Denaro V. The transpedicular approach for the study of intervertebral disc regeneration strategies: in vivo characterization. Eur Spine J 2013; 22 Suppl 6: S972-S978 [PMID: 24105019 DOI: 10.1007/s00586-013-3007-y]

Vadalà G, Russo F, Di Martino A, Denaro V. Intervertebral disc regeneration: from the degenerative cascade to molecular therapy and tissue engineering. J Tissue Eng Regen Med 2015; 9: 679-690 [PMID: 25312973 DOI: 10.1002/term.1719]

Di Martino A, Papapietro N, Lanotte A, Russo F, Vadalà G, Denaro V. Spondylo-discits: standards of current treatment. Curr Med Res Opin 2012; 28: 689-699 [PMID: 22435926 DOI: 10.1185/03007995.2012.678939]

Wang Z, Perez-Terzic CM, Smith J, Mauck WD, Shelkerud RA, Maus TP, Yang TH, Murad MH, Jou S, Terry MJ, Dauffenbach JP, Pingree MJ, Eldridge JS, Mohammed K, Benkhadra K, van Wijnen AJ, Qi W. Efficacy of intervertebral disc regeneration with stem cells – a systematic review and meta-analysis of animal controlled trials. Gene 2015; 564: 1-8 [PMID: 25796605 DOI: 10.1016/j.gene.2015.03.022]

Alini M, Eisenstein SM, Ito K, Little C, Kettler AA, Masada K, Melrose J, Ralphs J, Stokes I, Wilke HJ. Are animal models useful for studying human disc disorders/degeneration? Eur Spine J 2008; 17: 2-19 [PMID: 17632738 DOI: 10.1007/s00586-007-0414-y]

Vadalà G, Russo F, Pattappa G, Schiuma D, Peroglio M, Benneker LM, Grad S, Alini M, Denaro V. The transpedicular approach as an alternative route for intervertebral disc regeneration. Spine (Phila Pa 1976) 2013; 38: E319-E324 [PMID: 23324932 DOI: 10.1097/BRS.0b013e318285bc4a]

Vadalà G, Russo F, Pattappa G, Peroglio M, Stadelmann VA, Roughley P, Grad S, Alini M, Denaro V. A Nucleotomy Model with Intact Annulus Fibrosus to Test Intervertebral Disc Regeneration Strategies. Tissue Eng Part C Methods 2015; 21: 823-833 [PMID: 26035644 DOI: 10.1089/ten.TEC.2015.0086]

Bartels EM, Fairbank JC, Winlove CP, Urban JP. Oxygen and lactate concentrations measured in vivo in the intervertebral discs of patients with scoliosis and back pain. Spine (Phila Pa 1976) 1998; 23: 1-7; discussion 8 [PMID: 9460145 DOI: 10.1097/00007632-199801010-00001]

Urban MR, Fairbank JC, Etherington PJ, Loh FRCA L, Winlove CP, Urban JP. Electrochemical measurement of transport into scoliotic intervertebral discs in vivo using nitrous oxide as a tracer. Spine (Phila Pa 1976) 2001; 26: 984-990 [PMID: 11317725 DOI: 10.1097/00007632-200104150-00028]

P- Reviewer: Deng WP, Gantenbein-Ritter B S- Editor: Qiu S L- Editor: A E- Editor: Wu HL
