Review Article

Stem cells in preclinical spine studies

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Abstract

BACKGROUND CONTEXT: The recent identification and characterization of mesenchymal stem cells have introduced a shift in the research focus for future technologies in spinal surgery to achieve spinal fusion and treat degenerative disc disease. Current and past techniques use allograft to replace diseased tissue or rely on host responses to recruit necessary cellular progenitors. Adult stem cells display long-term proliferation, efficient self-renewal, and multipotent differentiation.

PURPOSE: This review will focus on two important applications of stem cells in spinal surgery: spine fusion and the management of degenerative disc disease.

STUDY DESIGN: Review of the literature.

METHODS: Relevant preclinical literature regarding stem cell sources, growth factors, scaffolds, and animal models for both osteogenesis and chondrogenesis will be reviewed, with an emphasis on those studies that focus on spine applications of these technologies.

RESULTS: In both osteogenesis and chondrogenesis, adult stem cells derived from bone marrow or adipose show promise in preclinical studies. Various growth factors and scaffolds have also been shown to enhance the properties and eventual clinical potential of these cells. Although its utility in clinical applications has yet to be proven, gene therapy has also been shown to hold promise in preclinical studies.

CONCLUSIONS: The future of spine surgery is constantly evolving, and the recent advancements in stem cell–based technologies for both spine fusion and the treatment of degenerative disc disease is promising and indicative that stem cells will undoubtedly play a major role clinically. It is likely that these stem cells, growth factors, and scaffolds will play a critical role in the future for replacing diseased tissue in disease processes such as degenerative disc disease and in enhancing host tissue to achieve more reliable spine fusion. © 2014 Elsevier Inc. All rights reserved.

Keywords: Stem cells; Spine fusion; Degenerative disc disease; Scaffold; Growth factors; Intervertebral disc; Bone morphogenetic protein; Adipose derived stem cells

Introduction

The recent identification and characterization of mesenchymal stem cells has introduced a shift in the research focus for future technologies in spinal surgery to achieve spinal fusion and treat degenerative disc disease. Current and past techniques use allograft to replace diseased tissue or rely on host responses to recruit necessary cellular progenitors. Adult stem cells display long-term proliferation, efficient self-renewal, and multipotent differentiation. They also can be genetically modified to secrete growth factors important to tissue healing, thereby functioning as implantable, long-lasting reservoirs for these molecules.

In spinal surgery, stem cells have great potential applicability in numerous areas. This review will focus on two important applications: spine fusion and the management of degenerative disc disease. The treatment of spinal cord injury using stem cells is still experimental and outside the scope of this review. Relevant preclinical literature regarding stem cell sources, growth factors, scaffolds, and animal...
models for both osteogenesis and chondrogenesis will be reviewed, with an emphasis on those studies that focus on spine applications of these technologies.

Osteogenesis/spine fusion

Overview

Spinal fusion has become a universal procedure since its introduction a century ago, with more than 350,000 spinal fusions performed each year in the United States representing a greater than 200% increase over the past decade [1–5]. Despite significant advances in surgical techniques and instrumentation, up to 40% of fusion surgeries result in pseudarthrosis, causing significant morbidity and often a need for reoperation [6–9]. Although failure of spinal fusions is multifactorial, osteoporosis, poor bone biology, smoking, diabetes, and decreased autograft cellularity in older patient populations all likely contribute [10–13]. Sophisticated techniques to improve fusion rates including modern internal fixation have greatly reduced the incidence of symptomatic pseudarthrosis, but a 10% to 15% incidence is still reported in recent literature [14–16]. Additionally, although autograft remains the gold standard for spinal fusions, it has limited availability, often low cellularity, and is associated with up to a 30% donor site morbidity rate [17–22]. These issues have led spine surgeons and researchers to investigate alternatives for achieving fusion and further reducing the pseudarthrosis rate, including the use of mesenchymal stem cells.

For a successful spinal fusion to occur, several vital elements are necessary. They include the presence of the bone-forming cell or its precursor, the appropriate biological signals directing bone synthesis, and a biocompatible scaffold on which the process can occur. The most critical of these components is the bone forming cell (osteoblast) or its precursor, the mesenchymal stem cell (MSC), both of which possess the ability to form bone [23].

In vitro: cells, growth factors, and scaffolds

Cells

Although historical research investigated the use of embryonic stem cells, restrictions and various moral and ethical obligations on their use have led to mesenchymal stem cells as the focus of most stem cell research for spinal fusion. Mesenchymal stem cells have generated considerable interest because of their ability to self-renew and multipotential characteristics. Furthermore, MSCs have been identified in a variety of tissues including bone marrow, muscle, periosteum, and adipose tissue [16,24–28]. Most recent and promising preclinical studies have focused on MSCs from bone marrow and adipose tissue.

Bone marrow is well established as a source of MSCs in laboratory animals and humans, with confirmed differentiation into osteoblasts both in vitro and in vivo. Disadvantages to the use of bone marrow clinically include the painful harvest as already described and its relatively limited supply requiring lengthy culture expansion.

Mesenchymal stem cells from bone marrow were demonstrated to achieve successful spinal fusion more than 10 years ago. In 2001, Cui et al. [29] found that cloned osteoprogenitor cells from bone marrow produced a quicker and more robust posterolateral fusion in an athymic rat model compared with mixed marrow cells. Over the ensuing decade, numerous other studies reinforced these findings. Peterson et al. [30] demonstrated that bone marrow MSCs transfected with bone morphogenic protein-2 (BMP-2) ex vivo successful induced posterolateral spinal fusion in an athymic rat model. In a recent study directly comparing a bone marrow MSC allograft with autograft, Gupta et al. [31] demonstrated similar fusion rates between the two groups in an ovine posterolateral lumbar spine fusion model. Similarly, Miyazaki, et al. [32] compared the effectiveness of human MSCs from bone marrow with adipose-derived MSCs in a posterolateral fusion rat model. The authors found similar fusion rates with both types of MSCs when transfected with BMP-2.

Adipose tissue provides an additional well-researched and promising source of MSCs for spine applications (Fig. 1). Adipose tissue is easily procured from patients and large numbers of MSCs can be obtained from relatively small amounts of adipose tissue, in contrast to bone marrow [33]. Shen et al. confirmed the osteoblastic differentiation of rat adipose–derived MSCs when cultured with growth and differentiation factor-5 (GDF-5). [33,34] Subsequent studies have yielded similar promising results. Hsu et al. [35] found that MSCs from adipose demonstrate potential as cellular delivery vehicles for recombinant proteins such as BMP-2 using an athymic rat posterolateral fusion model. Similarly, Miyazaki et al. [32] demonstrated that BMP-2 producing human adipose–derived MSCs, created using adenoviral gene transfer, induced spinal fusion in athymic rats, demonstrating that adipose-derived MSCs are osteogenic and enhance spinal fusion as effectively as bone marrow MSCs. Gimble et al. [36] reported that syngeneic and allogeneic rat adipose–derived MSCs on a tricalcium phosphate and collagen I scaffold accelerated spinal fusion in a rat model.

Growth factors

The complex process of bone formation can be affected by a variety of biologic signals, including mechanical loads and electromagnetic and chemical factors. The effectiveness of stem cells in promoting spinal fusion is heavily dependent on osteoinductive factors, specifically growth factors that enhance the osteogenic capability of osteoprogenitors such as MSCs [23]. Although many growth factors have been investigated for their role in the osteogenic differentiation of MSCs, only a select few have been researched for their role in inducing spinal fusion with MSCs or through gene therapy with MSCs. Table 1 provides a summary of these growth factors.
Despite recent controversy regarding negative clinical side effects, BMP-2 has been the subject of considerable research for use as an osteoinductive factor with MSCs for spinal fusion. As early as 2003, researchers realized the potential harm in the large doses of BMP required to induce a spinal fusion in humans, which suggested that delivery of such osteoinductive proteins could be improved. Wang et al. [37] used ex vivo adenoviral gene transfer to create BMP-2–producing bone marrow MSCs, which successfully produced an intertransverse fusion in a rat spine model. Peterson et al. [30] reported similar results in their experiments in which human bone marrow MSCs were infected with a BMP-2 containing adenovirus, yielding sufficient bone to fuse the lumbar spine in a rat model. Similar results with ex vivo gene therapy to overproduce BMP-2 were reported by Miyazaki et al. [32], Hsu et al. [35], and Sheyn et al. [38]. Fu et al. [39] also recognized the need to reduce the dose of BMP-2 necessary to achieve fusion. These authors found that combining low doses of BMP-2 with bone marrow MSCs achieved this goal in a rabbit fusion model.

BMP-7, also known as osteogenic protein-1, is another clinically available growth factor that has undergone considerable preclinical evaluation with stem cells. Hidaka et al. [40] assessed the enhancement of spine fusion using bone marrow stem cells modified by an adenovirus vector encoding BMP-7 seeded onto an allograft scaffold in a rat model. The authors found that the addition of adenovirus BMP-7–modified marrow stem cells significantly enhanced allograft spinal fusion. Zhu et al. [41] found that combined gene transfer of BMP-2 and BMP-7 using bone marrow stem cells in a rat model was significantly more effective in inducing osteoblastic differentiation and spine fusion than individual BMP gene transfer. Numerous studies have also been conducted successfully using BMP-7 as an inductive agent without the use of stem cells, which is not the subject of this review [42,43].

As an alternative to BMP-2, GDF-5 has also been studied recently for its use as an osteoinductive agent for stem cells in spine fusion. Shen et al. [34] confirmed that adipose-derived stem cells, when treated with GDF-5, are capable of adhering to a bioengineered scaffold while remaining viable and demonstrating the ability to migrate, proliferate, and subsequently undergo osteogenic differentiation. Similarly, Zeng and coauthors [33,44] proved in two studies that GDF-5 promotes the differentiation of rat adipose–derived stromal cells into osteogenic lineages.

### Scaffolds

The importance of an effective osteoconductive scaffold should not be underestimated. Despite the fact that scaffolds do not contain osteogenic cells or intrinsic biologic signals, an appropriately designed scaffold can provide the framework for the migration and attachment of osteogenic cells and a suitable environment for the synthesis of extracellular matrix [23]. Allograft is currently the most commonly used osteoconductive scaffold and is typically combined with autograft to provide the necessary osteoinductive and osteogenic factors and cells. Unfortunately, the extensive preparation and treatment that allograft is subject to leads to diminished biologic and mechanical properties as well as increased expense, which has led researchers to investigate other scaffolds for preclinical studies of stem cells for spinal fusion.

A scaffold for stem cells should maximize the osteoinductive and osteogenic effects of cells delivered to the site of interest, retain growth factors at that site for optimal time of release, function as an osteoconductive scaffold for bone in-growth with appropriate sized pores for cellular and vascular passage, not compete with or limit bone formation, and limit inflammatory response by biocompatibility [45]. Important scaffold properties to consider include porosity, pore size, geometry, and material. Numerous materials

<table>
<thead>
<tr>
<th>Growth factor</th>
<th>MSC type</th>
<th>Model</th>
<th>References</th>
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<tbody>
<tr>
<td>BMP-2</td>
<td>Adipose, BM</td>
<td>Rat, rabbit</td>
<td>[30,32,35–39]</td>
</tr>
<tr>
<td>BMP-6</td>
<td>Adipose, BM</td>
<td>Mouse, rabbit</td>
<td>[123]</td>
</tr>
<tr>
<td>BMP-7</td>
<td>BM</td>
<td>Rat</td>
<td>[40–43]</td>
</tr>
<tr>
<td>BMP-9</td>
<td>BM</td>
<td>Rat</td>
<td>[124]</td>
</tr>
<tr>
<td>GDF-5</td>
<td>Adipose</td>
<td>In vitro</td>
<td>[34,44]</td>
</tr>
<tr>
<td>FGF</td>
<td>BM</td>
<td>Rabbit</td>
<td>[125]</td>
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</tbody>
</table>

BM, bone marrow; BMP, bone morphogenic protein; FGF, fibroblast growth factor; GDF, growth and differentiation factor; MSC, mesenchymal stem cell.
are currently available for scaffolds, including natural or synthetic polymers, bioactive materials, and osteophilic materials such as ceramics or composites [46].

Type I collagen is a natural polymer and represents the simplest of scaffolds meeting all described criteria. It has a long history of use in surgical applications because of its biocompatibility, ease of degradation, and known interaction with other molecules including proteins. Gabbay et al. [47] found a progressively stimulatory effect on adipose-derived stem cells with regard to osteogenesis when cultured in a three-dimensional collagen I gel compared with a two-dimensional monolayer. Hsu et al. [35] successfully used a type I collagen matrix for their study investigating spinal fusion in an athymic rat model with BMP-2–producing adipose-derived stem cells. Similarly, Miyazaki and coauthors [32] found that both human adipose–derived and bone marrow–derived mesenchymal stem cells on a type I collagen sponge induced spinal fusion. Other natural polymers including matrigel [29] and hydrogels [48] have been investigated. Although these polymers are suitable for carriers of cells and growth factors, they do not possess the mechanical strength of stiffer polymers and thus have been used more for intervertebral disc applications.

Synthetic polymers including polylactic-co-glycolic acid (PLGA), its derivatives, chitosan, and others have been investigated in preclinical studies as well (Fig. 2). PLGA is osteoconductive, is able to deliver osteoinductive growth factors, and has established biocompatibility. Lee et al. [49] and Shen et al. [34] both successfully used PLGA in their studies. Similarly, numerous studies in the past 5 years have established the use of chitosan as an effective and viable scaffold for osteogenic differentiation and delivery of stem cells in preclinical studies [50–52].

Various biomaterials also have served as suitable scaffolds in preclinical studies, the most popular of which are calcium phosphate derivatives including hydroxyapatite (HA) and beta tricalcium phosphate (β-TCP). Both natural and synthetic forms of calcium phosphate have been developed as materials for bone repair and augmentation. They closely resemble the mineral composition, properties, and microarchitecture of human cancellous bone and thus have a high affinity for binding proteins. Commercially available HA is brittle, carries minimal mechanical strength, and is slowly resorbed in vivo, but has still been the subject of several preclinical studies involving stem cells. Chistolini et al. [53] studied porous HA scaffolds as carriers of bone marrow MSCs. The authors found that cell-loaded implants were stronger than cell-free implants with notable bone formation. More recently, Minamide et al. [54] and Seo et al. [55] both reported that bone marrow MSCs on HA scaffolds with and without growth factors induced posterolateral fusion in rat and rabbit models.

Beta tricalcium phosphate is another calcium phosphate biomaterial that serves as a purely osteoconductive scaffold. It has greater solubility than HA and is rapidly resorbed. The resorption rate of β-TCP closely matches the course of normal cancellous bone remodeling, making it an ideal candidate for stem cell–based scaffolds. It has recently been studied in rat and ovine models in preclinical studies for the delivery of both bone marrow– and adipose-derived MSCs for posterolateral spine fusion with uniformly excellent results comparable to that of autograft [31,36,56].

Table 2
Animal models of mesenchymal stem cells for spinal fusion

<table>
<thead>
<tr>
<th>Animal model</th>
<th>Mesenchymal stem cell type</th>
<th>Type of fusion</th>
<th>References</th>
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</thead>
<tbody>
<tr>
<td>Rat</td>
<td>Bone marrow</td>
<td>Posterolateral lumbar spine</td>
<td>[29]</td>
</tr>
<tr>
<td>Rabbit</td>
<td>Bone marrow</td>
<td>Posterolateral and interbody lumbar spine</td>
<td>[39,54,123,125]</td>
</tr>
<tr>
<td>Sheep</td>
<td>Bone marrow</td>
<td>Posterolateral lumbar spine</td>
<td>[31]</td>
</tr>
<tr>
<td>Rhesus monkey</td>
<td>Bone marrow</td>
<td>Interbody lumbar spine</td>
<td>[126]</td>
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Chondrogenesis/intervertebral disc degeneration

Overview

Intervertebral disc (IVD) degeneration is a complex process that is a result of morphologic and molecular changes within the disc itself compounded by progressive structural failure and instability of the vertebral motion segments that surround the affected disc [57,58]. The resultant clinical manifestations of degenerative disc disease have a huge impact on society and the economy, with estimated direct and indirect costs of $50 to $90 billion per year in the United States alone [57,59].

Traditional management of low back pain, which is often attributed to IVD degeneration, has focused on conservative options including lifestyle changes, physical therapy, pain medication, and rehabilitation as well as surgical options that focus on eliminating motion through disc arthroplasty or arthrodesis [60,61]. The clinical results of these procedures are often suboptimal. Both nonsurgical and surgical therapies do not deal with the inherent loss of functional native disc tissue and therefore fail to regenerate or cure the degenerated, painful disc tissue that is the essence of the disease process [62].

The intervertebral disc is a two-part structure composed of the tough annulus fibrosis (AF) on the periphery and the amorphous nucleus pulposus (NP) as the central core [60,63]. The cellular content of the AF and NP differ significantly. Cells in the AF are fibroblast-like with robust collagen fibrils. The extracellular matrix (ECM) in the AF is predominantly composed of type I collagen and contains relatively low amounts of proteoglycan and water [60,64]. Within the NP, cells become more rounded with a chondrocyte-like shape. ECM in the NP is an amorphous arrangement of type II collagen. It has a high proteoglycan concentration with inverse proportions of collagen and proteoglycan compared with the AF [61,64].

In vivo: animal models

Although in vitro studies lay the important foundation for the use of stem cells to achieve spine fusion, the transition to in vivo studies is a key step in the eventual translation of stem cell allografts to clinical use. Various animal models have been used to investigate the application of mesenchymal stem cells in achieving spine fusion. Tables 2 and 3 summarize various animal models for spine fusion using MSCs and gene therapy using MSCs, respectively.

Table 3

<table>
<thead>
<tr>
<th>Animal model</th>
<th>Mesenchymal stem cell type</th>
<th>Type of fusion</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse</td>
<td>Bone marrow, adipose</td>
<td>Posterolateral lumbar spine</td>
<td>[38]</td>
</tr>
<tr>
<td>Rat</td>
<td>Bone marrow, adipose</td>
<td>Posterolateral lumbar spine</td>
<td>[30,32,35,37,40,41,124]</td>
</tr>
<tr>
<td>Rabbit</td>
<td>Bone marrow</td>
<td>Posterolateral and interbody, lumbar spine</td>
<td>[127]</td>
</tr>
<tr>
<td>Pig</td>
<td>Bone marrow</td>
<td>Interbody lumbar spine</td>
<td>[128]</td>
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The relationship between the fluid-like NP and the structural lattice of the AF provides the biomechanical properties necessary for spinal stability [65]. Any disturbance of the NP or AF that compromises this balance leads to disc degeneration. Degeneration is marked by a loss of disc height and blurring of the gross morphologic characteristics of the AF and NP, resulting from the inability of the disc to regenerate, avascularity, and numerous biochemical changes within the disc due to aging [66,67].

Alterations in the cell population of the IVD have been particularly implicated in the pathogenesis of IVD degeneration [68]. Early disc degeneration is characterized by the loss of notochordal cells, which play a key role in maintaining the integrity of the disc and matrix stabilization [69]. Loss of chondrogenic NP cells and their replacement by fibroblast-like cells has also been implicated in the later stages of IVD degeneration [70].

Although considerable research has been dedicated to strategies that boost the ability of native disc cells to synthesize and maintain the ECM, the introduction of exogenous cells such as stem cells to supplement or replenish the declining disc cell population is gaining popularity as a potential treatment for IVD degeneration [68].

In vitro and in vivo: cells, growth factors, scaffolds, and animal models

Cells

Similar to bone regeneration, preclinical studies of stem cells for the IVD focus on MSCs from a variety of sources, including bone marrow [68,71–74], adipose tissue [75–80], muscle tissue [81], synovium [82–84], and even the NP itself (Table 4).

Bone marrow is an excellent source of mesenchymal stem cells for most orthopedic applications including chondrogenic differentiation for the diseased intervertebral disc. Yamamoto et al. [85] investigated the use of bone marrow–derived stromal cells for upregulation of the viability of native nucleus pulposus cells in a direct contact coculture system. The authors found significant upregulation of cell proliferation, DNA synthesis and proteoglycan synthesis in the NP cells which had direct cell-to-cell contact with bone marrow MSCs. Stoyanov et al. [86] compared standard chondrogenic differentiation protocols using transforming growth factor (TGF)-β with a novel technique utilizing hypoxia, GDF-5 and coculture with NP cells. The authors found that both hypoxia and GDF-5 were suitable for directing bone marrow MSCs toward an
intervertebral disc-like phenotype. Allon et al. [72] extended these in vitro findings of successful co-culture to a rat in vivo model. Utilizing a novel bilaminar co-culture pellet of bone marrow MSCs and NP cells, the authors demonstrated prevention of disc degeneration in vivo.

Similar to bone applications of MSCs, adipose derived stem cells have gained significant attention in recent years, given the ease of procurement and larger number of mesenchymal stem cells that can be obtained compared to bone marrow or other sources (Fig. 3). Liang et al. [87] recently conducted an in vitro study of both adolescent and adult adipose-derived stem cells and found that despite the harsh microenvironment of the IVD, adipose derived stem cells are a viable option for IVD regeneration. Choi and co-authors [80] investigated the use of human adipose derived stem cells co-cultured with human nucleus pulposus cells using a porous membrane. The authors found the most ideal culture conditions for chondrogenic differentiation of the ASCs to be ASC pellets cultured with normal NP cells. ASCs cultured in monolayer and the use of human NP cells from degenerative discs had inferior results.

Yang et al. [88] utilized genetic engineering to induce ectopic expression of Sox-9 in human ASCs. The authors found improved chondrogenic differentiation in the engineered ASCs, indicating potential application in the treatment of degenerative disc disease. Lu et al. [89] reported that co-culture of human ASCs and NP cells in a micro-mass culture resulted in differentiation of ASCs into an NP cell-like phenotype. Tapp et al. [78] revealed that either treatment of TGF-β or co-culture with human disc cells could significantly stimulate expression of proteoglycan and type I collagen in 3D-cultured sand rat ADSCs. Gaetani et al. [75] presented data indicating that co-culture of human NP and ASCs improved the quality of the in vitro reconstructed tissue in terms of matrix production and 3D cell organization [62].

Other stem cell sources have been investigated for IVD repair. Vadala et al. [81] investigated the use of adult muscle tissue as a stem cell source. Others have recently investigated the use of synovial cells as a stem cell source for IVD tissue engineering. [82–84] In these in vitro and in vivo studies, the authors found promise with the use of synovial stem cells for their inductive capabilities and their ability to differentiate to a chondrogenic phenotype. Recent literature has also revealed the potential for human annulus fibrosis cells [90] and nucleus pulposus cells [91] to serve as mesenchymal stem cells.

**Growth factors**

Disc cell metabolism is modulated by a variety of growth factors acting in both paracrine and autocrine roles [92,93]. These factors function to increase the synthesis of extracellular matrix components, block their breakdown, or a combination of these roles. Growth factors can be applied in IVD tissue engineering via delivery of the “naked” or “embedded” proteins as well as prolonged supplement by cell-based gene therapy [62,92]. Numerous growth factors have been used for IVD applications (Table 5), including transforming growth factor-β [94,95], insulin-like growth factor-1 [96,97], GDF-5 [98–101], platelet-derived growth factor [96], osteogenic protein-1/BMP-7 [102–104], BMP-2 and 12 [105–107], and fibroblast growth factor-2 [108].

In a 2005 study, Steck, et al. [109] demonstrated the ability of adult bone marrow MSCs to differentiate toward

![Fig. 3. Chondrogenic differentiation of human adipose-derived stem cells for disc regeneration. Cells cultured in basal medium demonstrated no positive staining (A). Safranin-O staining indicated deposition of proteoglycan in cells cultured in chondrogenic medium (B). Supplemen-
ting the chondrogenic medium with transforming growth factor-β (C), and increasing concentrations of growth and differentiation factor-5 (D–F) augmented chondrogenic differentiation.](image-url)
the molecular phenotype of human IVD cells. Using TGF-β mediated generation, the authors found that MSC spheroids preferentially differentiated toward an IVD phenotype rather than articular cartilage, making them an attractive source to obtain IVD-like cells.

Yang et al. [110] strengthened the potential role of TGF-β and MSCs as therapy for IVD degeneration. In their study, bone marrow MSCs with TGF-β were transplanted into degenerative discs of rabbits. The authors found that MSCs can slow the rate at which the degenerative process occurs, possibly due to the inhibition of apoptosis by the MSCs.

Stoyanov and co-authors [86] utilized bone marrow MSCs and compared standard chondrogenic differentiation protocols using TGF-β with hypoxia, GDF-5 and co-culture with bovine NP cells. Their results suggest that hypoxia and GDF-5 may be suitable for directing MSCs toward an IVD-like phenotype.

McCanless et al. [71] investigated the effects of BMP-2 and synthetic peptide B2A on cell proliferation and ECM synthesis by NP-like differentiated bone marrow MSCs. The authors found that B2A induces proliferation, aggrecan synthesis and stabilized collagen accumulation consistent with cells of the young, healthy NP, indicating potential use of B2A in MSC-based NP regeneration therapy.

Feng et al. [100] examined the effects of GDF-5 on chondrogenesis of adipose-derived stem cells (ADSCs) using genetically engineered rat ADSCs in an in vitro pellet culture model. The authors found that GDF-5 is a potent inducer of chondrogenesis in ADSCs, and that ADSCs can be genetically engineered to express pro-chondrogenic growth factors as a promising therapeutic cell source for IVD tissue engineering.

**Scaffolds**

Scaffolds in tissue engineering can help to retain cells in the desired location and provide appropriate mechanical properties and/or biochemical signals. [62] To date, a variety of biomaterials have been used for fabricating scaffolds in both annulus fibrosis (AF) and nucleus pulposus (NP) tissue engineering.

The choice of scaffold is critical as it directly affects the type of tissue that forms. Scaffold fiber diameter and stiffness can influence cell function, proliferation and orientation, all of which can have profound effects on the cell [111]. Numerous biomaterials have been used for scaffolds, including PLGA, poly-d-lactide, chitosan, alginate, fibrin, collagens, gels, calcium polyphosphate, demineralized bone matrix, and many others [61,112–122].

**Conclusions**

The future of spine surgery is constantly evolving, and the recent advancements in stem cell–based technologies for both spine fusion and the treatment of degenerative disc disease are both promising and indicative that stem cells will undoubtedly play a major role clinically. In both osteogenesis and chondrogenesis, adult stem cells derived from bone marrow or adipose show promise in preclinical studies. Various growth factors and scaffolds have also been shown to enhance the properties and eventual clinical potential of these cells. Although its utility in clinical applications has yet to be proven, gene therapy has also been shown to hold promise in preclinical studies. It is likely that these stem cells, growth factors, and scaffolds will play a critical role in the future for replacing diseased tissue in disease processes such as degenerative disc disease and in enhancing host tissue to achieve more reliable spine fusion.

**References**


