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Stem Cell-Mediated Intervertebral Disc Regeneration

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1. Introduction

Currently, degenerative disk disease (DDD) and the subsequent chronic lower back pain that results from it represent a significant source of morbidity and mortality worldwide. The available treatment modalities such as pain therapy and surgical interventions aim to provide symptomatic relief; however, they do not address the underlying pathophysiology of DDD. The disease also has high societal health care costs (Chan et al., 2006; Cassinelli et al, 2001). Many modalities exist for symptomatic treatment of this condition, including bed rest, massage, stretching, strengthening exercises, physical therapy, epidural injections and other pain management therapies, and spinal surgery. Most conservative therapies are

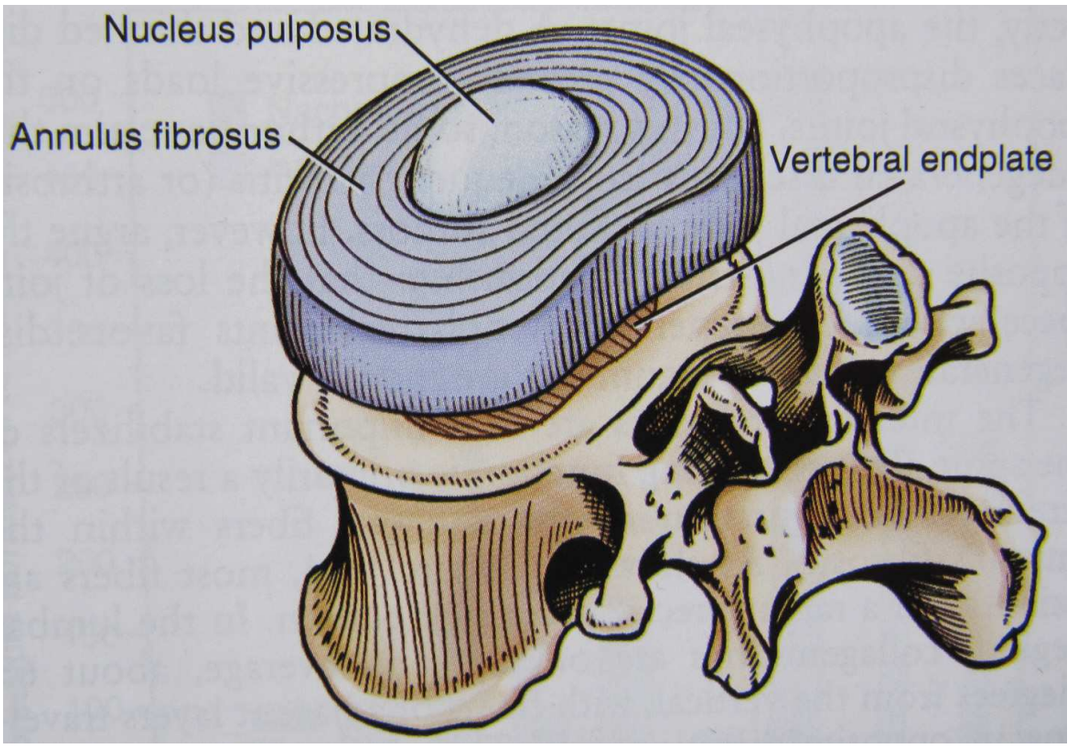


Fig. 1. Anatomy of the spine with the compartmentalization of the IVD.

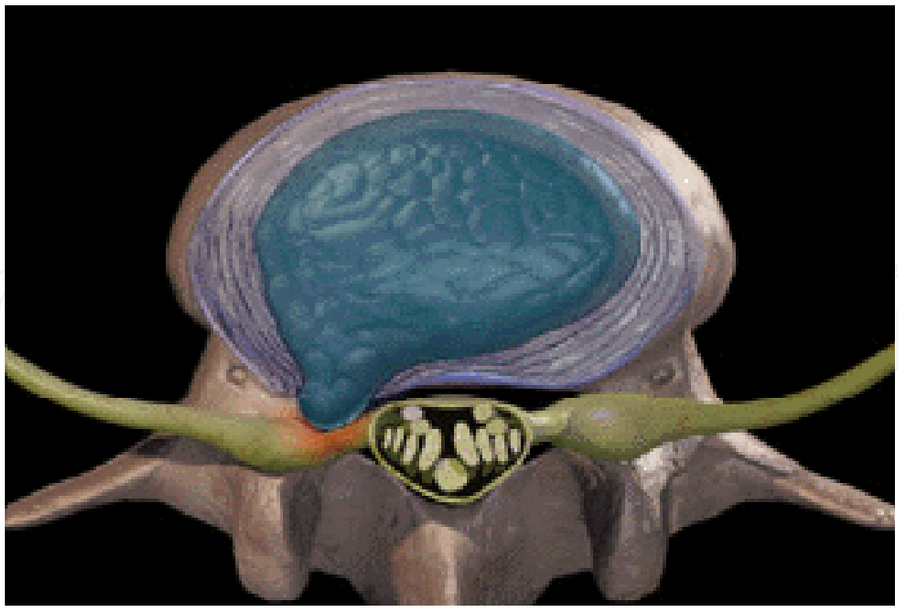


Fig. 2. Axial slice model of the intervertebral disc with an image of a disc herniation

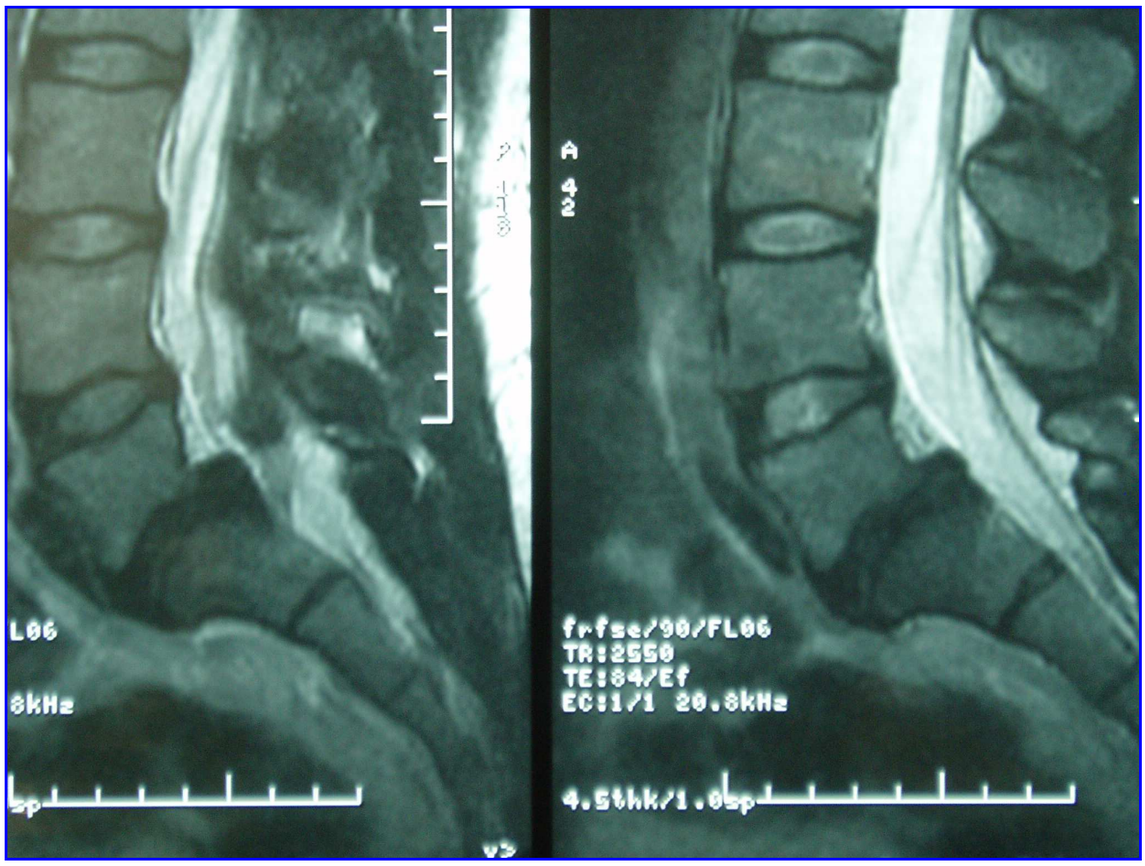


Fig. 3. Sagittal T2-weighted MRI showing degeneration and loss of T2 signal in the L5-S1 IVD

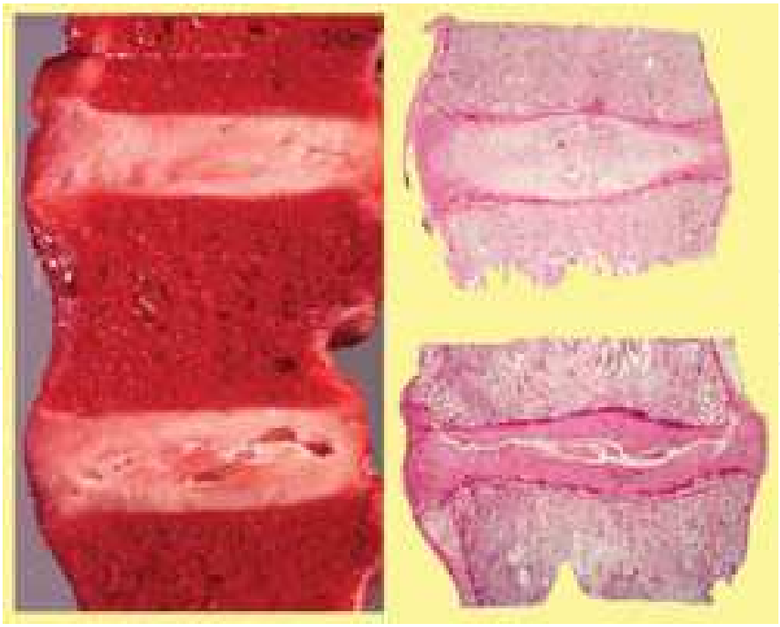


Fig. 4. Postmortem specimen and hematoxylin and eosin staining showing multilevel degeneration of the IVDs with fissuring of the actual disc structure

attempted before surgery with the intent to spare patients the possible complications associated with surgical intervention. However, these conservative measures and even surgery itself with its associated risks only address the symptoms with no impact on the disease process in the disc itself. Recent research has given further insight into the pathogenesis of DDD, which has borne out a renewed interest in biologic therapies centered on the nucleus pulposus (NP) and the annulus fibrosus and the potential of stem cells to reverse the disease process at a histological and cellular level. In this chapter, we will systemically review the current literature and the most salient studies regarding biologic therapies in the regeneration of the intervertebral disc (IVD). We go on to describe the direction this field is heading in and the future potential of the therapies being developed using ESCs.

2. Basic science laboratory studies

Before examining the utility of stem cells in human and animal models, it is important to review several of the basic science benchtop laboratory studies that have provided the rationale for in-vivo testable treatments and hypotheses. These studies examined factors influencing both mesenchymal and embryonic stem cell proliferation and differentiation towards a NP-like phenotype. We will examine how these studies have provided valuable information regarding multiple factors that can stimulate embryonic stem cells (ESCs) and mesenchymal stem cells (MSCs) towards a chondrocytic lineage, as well as factors that can inhibit this differentiation in basic in-vitro models.

2.1 Genetic studies

DDD is a condition that rises from a combination of a genetic predisposition (Chan et al., 2006) along with environmental modifiers (Stokes & Iatridis, 2004). Several causes of age-

related degeneration of the IVD include loss of biomechanical support by surrounding muscular and ligamentous structures, uneven force loading as the aging spine deforms while trying to compensate for these changes, cell senescence, loss of viable progenitor cells, accumulation of degraded matrix molecules, and fatigue failure of both the disc matrix and surrounding annulus fibrosus. Correlations have been made between DDD and collagen, aggrecan, and matrix metalloproteinase polymorphisms coding for structural proteins (Ala-Kokko, 2002).

2.2 Factors influencing stem cell proliferation

In order to further study how these cells would interact in various factor environments, it became crucial to more fully characterize these cells. This point is very important with regard to stem cell research because it is essential to characterize and identify what factors provide the best type of environment to stimulate ESCs and MSCs to differentiate toward a chondrocytic-type cell lineage.

2.2.1 Mesenchymal Stem Cells

Transforming growth factor-β3 (TGF-β3) is one factor that has been shown in multiple studies (Steck et al., 2005; Risbud et al., 2004; Shen, 2009) to stimulate cells to differentiate into chondrocytes. Several studies have shown that after TGF-β3 stimulation, MSCs turned positive for collagen type II protein and expressed a large panel of genes characteristic for chondrocytes, such as aggrecan, decorin, fibromodulin, and cartilage oligomeric matrix protein (Steck et al., 2005; Risbud et al., 2004). Shen et al. have shown that bone morphogenic protein-2 (BMP-2) can help to enhance TGF-β3-mediated chondrogenesis in MSCs (Shen, 2009). The combination of BMP-2 and TGF-β3 in alginate culture was found to be superior to the standard differentiation method using TGF-β3 alone as evinced by increased mRNA expression of aggrecan, type II collagen, Sox-9, BMP-2, and BMP-7, all of which are chondrocyte markers. This effect was even more pronounced when TGF-β3 and rhBMP-2 were both added (Kuh et al., 2008). This synergistic effect was consistently found in the study, providing further support as to an as yet unknown pathway towards chondrocytic differentiation.

2.2.2 Embryonic Stem Cells

Hoben et al performed a similar characterization study using human ESCs (Hoben et al., 2009). Growth factors were studied with a coculture method for 3 weeks and evaluated for collagen and glycosaminoglycan (GAG) synthesis. The growth factors studied were TGF-β3, BMP-2, BMP-4, BMP-6, and sonic hedgehog protein. The investigators found that the combination of BMP-4 and TGF-β3 within the fibrochondrocyte coculture led to an increase in cell proliferation and GAG production compared to either treatment alone. Koay et al had similar results with BMP-2 and TGF-β3 leading human ESCs down a differentiation path that produced an end product with high type I collagen content (Koay et al., 2007). However, they also found that human ESCs treated with TGF-β3 followed by TGF-β1 and IGF-1 produced constructs with no collagen I, showing that different growth factor application in different temporal sequences can have a marked impact on end-product composition and biomechanical properties. The importance of temporal sequences cannot

be understated with regard to stem cell development and has important implications pertaining to harvesting and large-scale production of these cells for future potential therapeutic uses.

2.3 Stem cell growth in the native IVD microenvironment

Several groups have conducted well-designed in-vitro studies that have gone one step beyond identifying environmental factors that affect differentiation of stem cells into NP-like cells, and have actually studied how these factors may correlate to the current in-vivo microenvironment of the IVD. This was done in order to obtain a clear picture of what would happen if these stem cells were implanted into these native biological conditions. Culturing under IVD-like glucose conditions (1.0 mg/mL glucose) stimulated aggrecan and collagen I expression and deposition. IVD-like osmolarity (485 mOsm) and pH (pH = 6.8) conditions, on the other hand, strongly decreased proliferation and expression of matrix proteins. Combining these conditions resulted in decreased proliferation and gene expression of matrix proteins, demonstrating that, in this case, osmolarity and pH play a larger impact in inhibiting differentiation than glucose does in stimulating it (Wuertz et al., 2008).

Another study by the same group showed that acidity caused an inhibition of aggrecan and collagen I expression, as well as a decrease in proliferation and cell viability. This demonstrates that pH may be the major limitation for stem cell-based IVD repair (Wuertz, 2009). This also illustrates the importance of early intervention and the role of predifferentiation when planning to use stem cells for reparative treatments. However, some studies have shown that implantation of stem cells at a later stage in the DDD process may result in a greater increase in disc height when compared to implantation at an earlier stage (Ho et al., 2008). This finding highlights the importance of studies involving stem cell-based intervertebral disc regeneration being carefully controlled in the context of stage of disc degeneration. Again, this point highlights the importance of temporal sequence when examining therapeutics with stem cells. Additionally, inflammatory processes have been shown to inhibit the chondrogenic differentiation of stem cells, whereas hypoxic conditions exert beneficial effects on chondrogenesis and phenotype stability of transplanted stem cells (Felka et al., 2009).

2.4 Optimizing conditions to promote proliferation

There is currently an avid interest in using our accumulated data and knowledge of the factors influencing stem cell proliferation and the exact conditions in the native IVD microenvironment to optimize the chances for stem cell proliferation.

Multiple studies have investigated culturing MSCs with NP cells in a co-culture system, allowing for cell-to-cell contact (Yang et al., 2009; Le Maitre et al., 2009; Vadalà et al., 2009; Richardson et al., 2006; Richardson et al., 2008). This contact has been shown to stimulate these MSCs to differentiate toward a chondrocytic lineage, therefore removing the need for pre-differentiation in-vitro (Watanabe et al., 2010; Svanvik et al., 2010; Niu et al., 2009; Wei et al., 2009; Tao et al., 2008; Le Visage et al., 2006; Richardson et al., 2006). This was evidenced by mRNA expression levels of Type II collagen and aggrecan being elevated in co-cultured cells and cells undergoing morphological changes to form three-dimensional micromasses

expressing collagen-2, aggrecan, and Sox-9 at RNA and protein levels after 14 days of co-culture. These changes were unique and not detected in the samples of stem cells cultured alone (Svanvik et al., 2010; Niu et al., 2009; Wei et al., 2009). Furthermore, MSCs from older individuals differentiate spontaneously into chondrocyte-like NP cells upon insertion into NP tissue in-vitro, and thus may not require additional stimulation to induce differentiation. This is a key finding, as such a strategy would minimize the level of external manipulation required prior to insertion of these cells into the patient, thus simplifying the treatment strategy and reducing costs (Le Maitre et al., 2009).

Adipose-Derived Stem Cells (ADSCs) have also been shown to be able to differentiate into NP cells in multiple in-vitro studies (Xie et al., 2009; Tapp et al., 2008; Lu et al., 2007; Lu et al., 2008; Li et al., 2005). Soluble factors released by NP cells direct chondrogenic differentiation of ADSCs in collagen hydrogels, and combination with a nucleus-mimicking collagen type II microenvironment enhances differentiation towards a more pronounced cartilaginous lineage (Lu et al., 2007; Lu et al., 2008).

Studies using annulus fibrosus cells isolated from nondegenerated intervertebral discs have shown that these cells have the capability of differentiating into adipocytes, osteoblasts, chondrocytes, neurons, and endothelial cells in-vitro. These cells may also be induced to become more plastic, allowing them to differentiate along more mesenchymal lineages (Li et al., 2005; Feng et al., 2010; Saraiya et al., 2010). However, when annulus cells are differentiated into a chondrocyte micromass, it was not as rounded or compact as that which occurs with stem cells induced into chondrocyte differentiation (Saraiya et al., 2010). TGF-β stimulation of fetal cells cultured in high cell density led to the production of aggrecan, type I and II collagens and variable levels of type X collagen, although fetal cells had lower adipogenic and osteogenic differentiation capacity than MSCs and variability in matrix synthesis was observed between specific donors (Quintin et al., 2009; Quintin et al., 2010).

3. Animal studies

Many studies using stem cells for disc regeneration have been performed in a wide array of animal models with promising results. Two recent studies were conducted utilizing ADSCs in a murine (Jeong et al., 2010) and a canine model (Ganey et al., 2009). Staining in both studies demonstrated increased Type II collagen and aggrecan in the transplantation group. Additionally, at 6 weeks after transplantation, discs exhibited a restoration of disc hydration and MRI T2 signal intensity and more closely resembled the healthy controls as evidenced by matrix translucency, compartmentalization of the annulus, and increased cell density within the nucleus pulposus. Discs also showed a significantly smaller reduction in disc height when compared with controls.

Multiple studies have shown that MSCs are able to proliferate and survive inside the IVD, with assessments being made as far out as six months post-transplant (Tan et al., 2009; Jeong et al., 2009; Henriksson et al., 2009; Sobajima et al., 2008; Zhang et al., 2005; Crevensten et al., 2004). Additionally, these cells have been proven to differentiate into cells expressing chondrocytic phenotypes, as evidenced by positive immunostaining of collagen type II, aggrecan, and other markers (Henriksson et al., 2009; Yang et al., 2010; Wei et al., 2009; Sakai et al., 2005). Cells were also shown to exhibit NP phenotypic

markers (Sakai et al., 2005). The injected discs had a central NP-like region which had a close similarity to the normal biconvex structure of the IVD and contained viable chondrocytes forming a matrix like that of the normal disc (Sakai et al., 2003; Revell et al., 2007). Omlor et al. studied the practical phenomenon of transplanted stem cell loss through the actual annular puncture which was used to not only simulate disc damage and herniation but also to inject the stem cells themselves. They made a logical conclusion that IVD regeneration strategies should increasingly focus on annulus reconstruction in order to reduce implant loss due to annular failure (Omlor et al., 2010). Most studies focusing on this point are still ongoing.

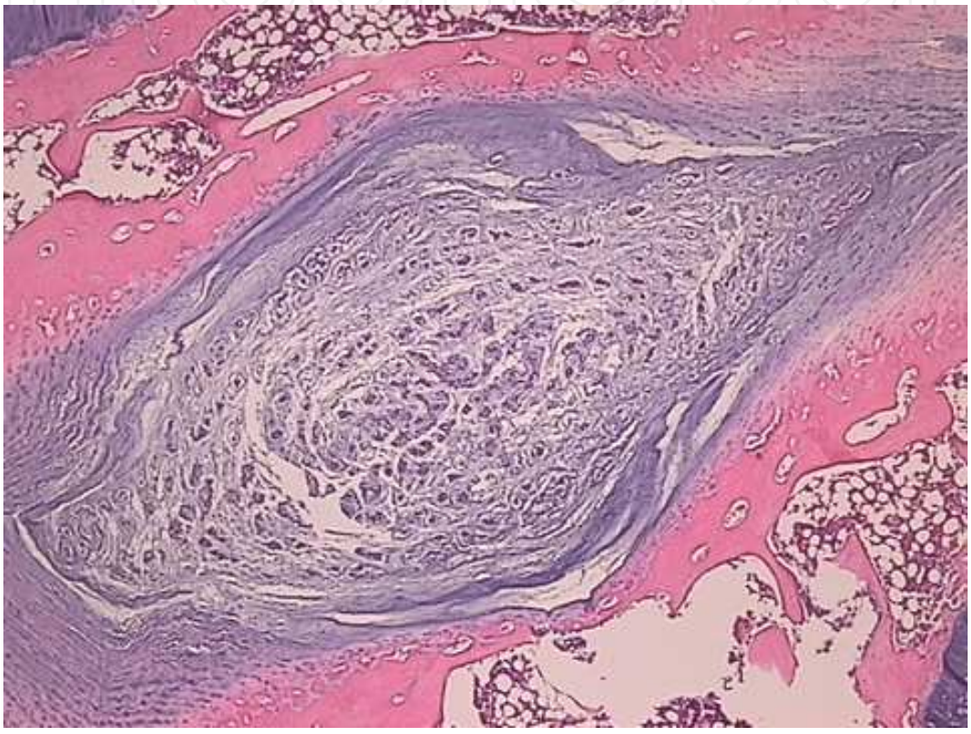


Fig. 5. Hematoxylin and eosin staining of the rabbit IVD, showing healthy notochordal cell rests

Several xenotransplant studies involving ESCs have been conducted with promising results. Jeong et al have shown that rats receiving human ESCs showed relative restoration of the inner annulus structure compared to a control group (Jeong et al., 2010). This finding may help to address the concern of loss of implanted material through the needle puncture.

Many of the stem cells in these studies were xenografted from other species and the recipient animals were not treated with immunosuppressive agents. In spite of this, there was a lack of immune response suggesting an unrecognized immune-privileged site within the intervertebral disc space (Wei et al., 2009; Sheikh et al., 2009). On top of this, there has been some study with MSC showing that transplantation contributes to this immunosuppressive phenomenon by the differentiation of these cells into cells expressing FasL, which has been shown to be an immunosuppressive factor (Hiyama et al., 2008).

Studies	Model	Intervention	Results
Jeong et al.	Rat model	The first coccygeal disc segments of Sprague-Dawley rat were left undamaged as controls, and other two segments were damaged by needle injection. Two weeks later, stem cells or saline were injected into each of the two damaged segments.	At 6 weeks after transplantation, the experimental group showed a significantly smaller reduction in disc height than the saline-injected group and exhibited a restoration of MRI signal intensity. Hematoxylin and eosin staining revealed a greater restoration of the inner annulus structure. There was also increased collagen type II and aggrecan.
Ganey et al.	Canine model	3 discs that had undergone partial nucleotomy were randomized to receive: (1) stem cells in hyaluronic acid carrier (Cells/HA); (2) HA only; or (3) No Intervention.	Disc levels receiving stem cells more closely resembled the healthy controls as evidenced in matrix translucency, compartmentalization of the annulus, and in cell density within the nucleus pulposus. Matrix analysis showed increased Type-II collagen and aggrecan.
Hiyama et al.	Canine model	4 weeks after nucleotomy, MSCs were transplanted into the degeneration-induced discs. The animals were followed for 12 weeks when radiological, histological, biochemical, immunohistochemical, and RT-PCR analyses were performed.	MSC transplantation effectively led to the regeneration of degenerated discs. GFP-positive MSCs detected in the NP region 8 weeks after transplantation expressed FasL protein.
Sobajima et al.	Rabbit model	MSCs were isolated New Zealand White rabbits, retrovirally transduced with the lacZ marker gene, and injected into the nucleus pulposus of the L2-3, L3-4, and L4-5 lumbar discs of 12 other NZW rabbits. Rabbits each were sacrificed at 3, 6, 12, or 24 weeks after cell implantation, and staining	MSCs were detected in histological sections of rabbit discs up to 24 weeks after transplant with engraftment into the inner annulus fibrosus.

Studies	Model	Intervention	Results
		was done to assess cell survival and localization.	
Henriksson et al.	Porcine model	Three lumbar discs in each of 9 were damaged with needle puncture. 2 weeks later human MSCs were injected The animals were sacrificed after 1, 3, or 6 months. Disc appearance was visualized by MRI. Immunohistochemistry was used to detect human MSCs.	All injured discs demonstrated degenerative signs on MRI. Immunostaining for Aggrecan and Collagen type II expression were observed in NP after 3 and 6 months. mRNA expression of Collagen IIA, Collagen IIB, Versican, Collagen 1A, Aggrecan, and SOX9 were detected at 3 and 6 months by real-time PCR.
Sheikh et al.	Rabbit model	16 New Zealand white rabbits underwent needle puncture of the disc with MRIs before and after injection with ESCs expressing green fluorescent protein. At 8 weeks post-ESC implantation, the animals were killed and the intervertebral discs were harvested and analyzed using H & E staining and immunohistochemical analysis.	MRI confirmed intervertebral disc degeneration at needle-punctured segments. Postmortem H & E histological analysis of Group A discs (no intervention) showed mature chondrocytes and no notochordal cells. Group B discs (needle puncture only) displayed an intact annulus fibrosus and generalized disorganization within the NP. Group C discs showed islands of notochordal cell growth (injection of ESCs).
Sakai et al.	Rabbit model	Stem cells labeled with green fluorescent protein, were transplanted into mature rabbits. Consecutive counts of transplanted cells in the nucleus area were performed for 48 weeks with immunohistochemical and proteoglycan content analyses along with PCR detection of mRNA expression of Type I and II collagen, aggrecan and versican.	Cells that were positive for green fluorescent protein were observed in the nucleus pulposus of cell-transplanted rabbit discs 2 weeks after transplantation. GFP-positive cells were positive for Type II collagen, keratan sulfate, chondroitin sulfate, and aggrecan.

Studies	Model	Intervention	Results
Bendtsen et al.	Porcine model	DDD was induced in 15 minipigs. After 12 weeks, the animals underwent percutaneous intradiscal injection of stem cells. MRI was performed before treatment and at 24 weeks.	Stem cell treated animal had increased T2 signal in the disc along with increased relative vertebral blood flow.
Omlor et al.	Porcine model	6 minipigs underwent matrix based cell transfer after partial nucleotomy of lumbar IVDs. Segments were analyzed for retained volume of labeling particles	There was a 90% loss of the implant material under in vivo conditions when the annulus was not reconstructed.

Table 1. Animal Studies

Our group recently reported seminal work with regard to ESC implantation in a rabbit model (Sheikh et al., 2009). This study used a needle puncture model with appropriate controls to simulate disc injury. The effects of implanted murine ESCs were measured at 8 weeks using imaging, histological, and immunohistochemical analyses. In-vivo new notochordal cell populations were seen in ESC-injected discs, providing convincing evidence for stem-cell mediated regeneration of the IVD. Another study established the utility of stem cells implanted at 12 weeks post-injury in regenerating the IVD and maintaining perfusion to the endplate and subchondral bone in a porcine model (Bendtsen et al., 2010). Sobajima et al used a rabbit model to show that IVD cells harvested 48 weeks post-implantation revealed a restoration of both glycoprotein content and matrix characteristics (Sobajima et al., 2008). These analyses all provide further evidence that ESC transplantation does have strong potential for clinical use in regenerating the IVD and reversing the cascade of degeneration that occurs with time.

4. Human studies

To date, there have been only two studies where stem cells were injected into the IVD in humans to stimulate regeneration of the disc. Yoshikawa et al percutaneously grafted MSCs into degenerated IVDs in two women aged 67 and 70 years. After two years, both individuals had alleviation of symptoms and radiographic changes that included improvement of vacuum phenomenon on X-ray and increased signal intensity of IVDs on T2-weighted MRI (Yoshikawa et al., 2010). Another study involved intradiscal injection of hematopoietic stem cells into ten patients that had confirmed disc pain and these patients' pain was assessed at 6-month and 12-month intervals. In contrast to previous study, none of these individuals had any relief of symptoms (Haufe et al., 2006). These trials suggest that stem cells have the potential to relieve symptoms of DDD and restore normal IVD anatomy; however, more human studies are needed to truly establish this. To date, there have been no human ESC implantation studies into the IVD in humans. Further study is needed to verify safety before such work is undertaken.

Studies	Subjects	Intervention	Results	Study Critique
Yoshikawa et al.	2 patients	Percutaneous stem cell grafting	Clinical symptoms improved; increased T2 signal in the disc space on MRI	Few patients
Haufe et al.	10 patients	Percutaneous stem cell grafting	No clinical symptom relief	No imaging conducted

Table 2. Human Studies

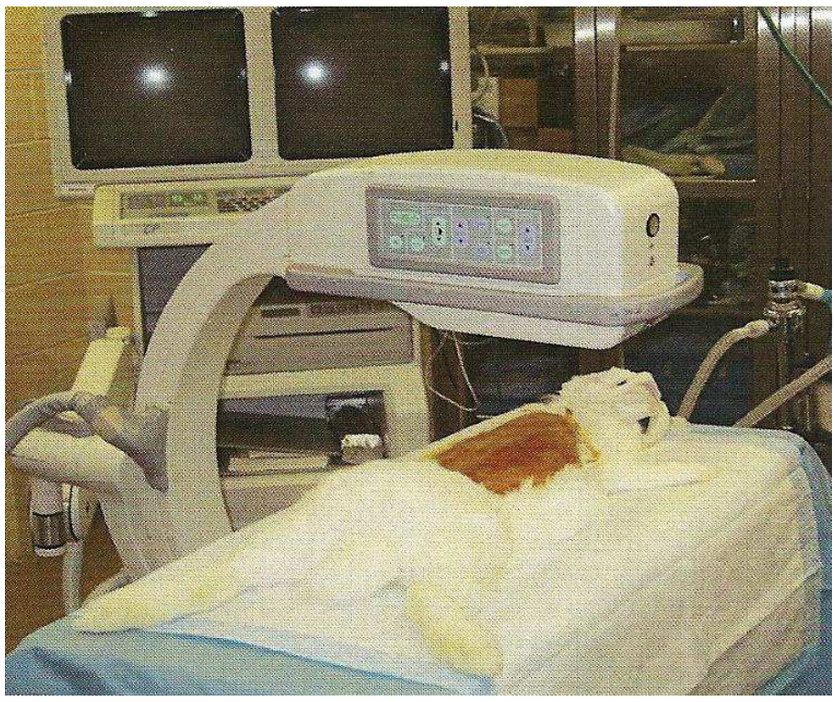
5. Future potential of ESCs

Although many laboratory and animal studies have been performed utilizing stem cells for the purposes of cell characterization and inducing chondrocyte formation, much further study is needed before human trials are undertaken on a larger scale. Several studies have already showcased the ability of ESCs to differentiate towards a chondrocytic lineage in-vitro and also to improve DDD in in-vivo animal and human trials, using a combination of imaging and histological analyses. Several benchtop lab studies have been performed to show that ESCs can be successfully stimulated to differentiate into chondrocyte-like cells (Hoben et al., 2009; Fecek et al., 2008; Hegert et al., 2002; Kawaguchi et al., 2005; zur Nieden et al., 2005; Kramer et al., 2000). Similar to the case with MSCs, different factors affect this process in ESCs, such as TGF-β3, BMP-2, and BMP-4 (Hegert et al., 2002; Kawaguchi et al., 2005; zur Nieden et al., 2005; Kramer et al., 2000; Sakai et al., 2005). Biological scaffolds seeded with chondrocytic cells derived from ESCs, when implanted in mice have been shown to generate cartilage tissue in-vivo (Kramer et al., 2000). Injection of ESCs in a DDD-induced rabbit model led to viable notochordal-type cells within the discs (Sheikh et al., 2009). These animal studies demonstrate the ability of ESCs to differentiate into a chondrocytic lineage in-vitro and in-vivo.

Our group is currently developing chondroprogenitor stem cell lines that can restore the functional capability of the IVD (Sheikh et al., 2009). Our rationale stemmed from the idea that currently there is no biologic therapy for repairing a degenerated IVD and that ESCs have a potential to fill this role based on their regenerative potential. Studies have shown that ESCs can be induced to differentiate into specific cell lineages by using selective culture media and growth environments (Kawaguchi et al., 2005).

Relying on the significant strides made by these basic science groups with regard to cell and factor characterization, our lab proceeded for further refine these methods and develop a protocol for both stem cell differentiation along a chondrocytic lineage and also for examining the utility of transplantation of these cells in a rabbit model of DDD. We initially developed a novel percutaneous animal model of disc degeneration using New Zealand white rabbits (Figure 1) and used this model to explore the possibility of ESC implantation for both structural regeneration and for the growth and continued presence of notochordal stem cells in the disc space (Sheikh et al., 2009).

Previous research transplanting MSCs into degenerated rabbit discs has shown consistent biochemical and radiographic (MRI) evidence of IVD restoration (Sakai et al., 2005). Human



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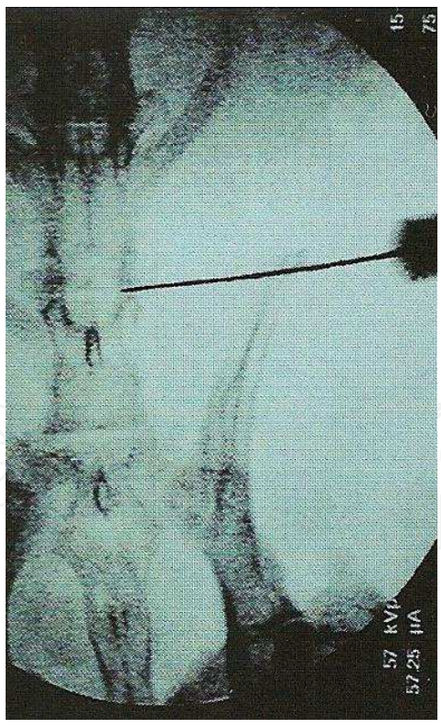
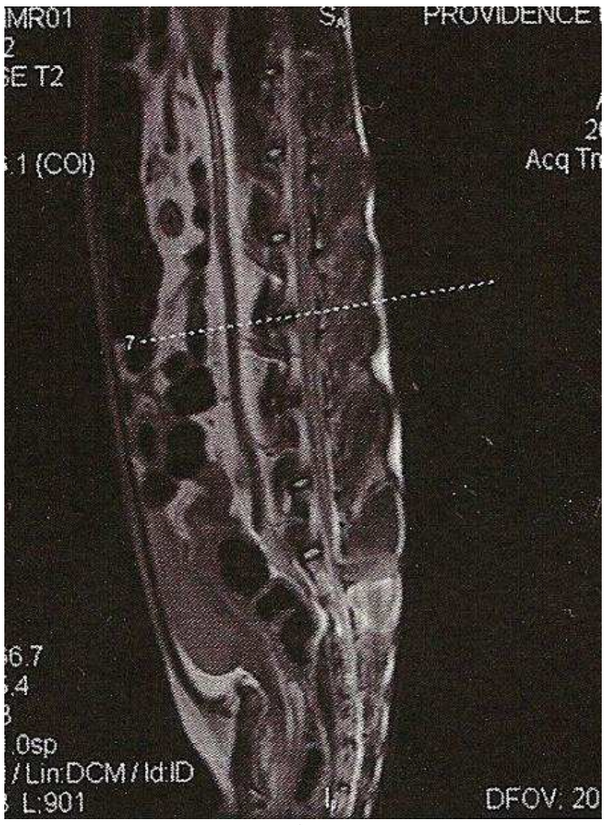


B.

Fig. 6. Photographs of our group's rabbit model for IVD degeneration. The rabbit is positioned prone, its back is shaved and prepared for surgery (A), with a corresponding fluoroscopic view (B).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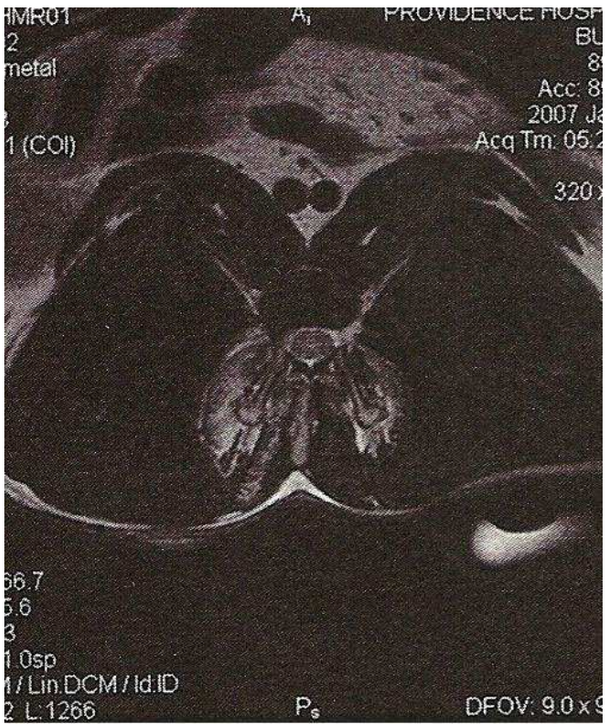
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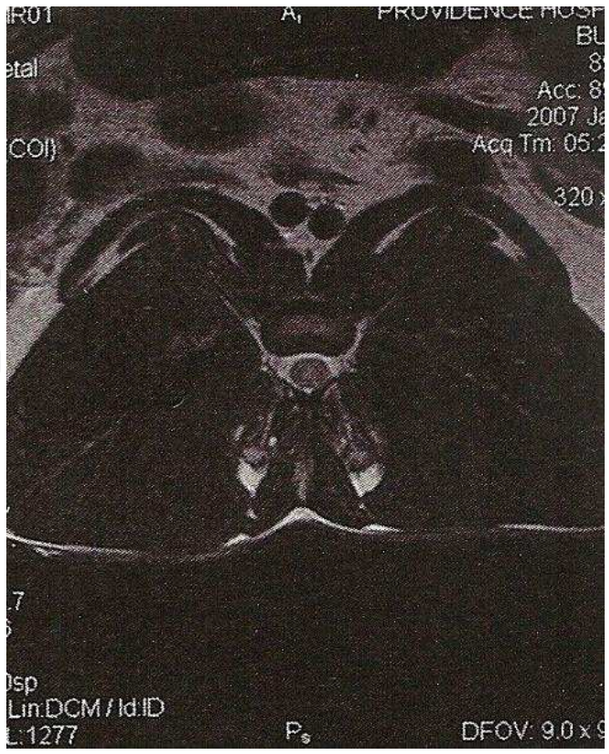


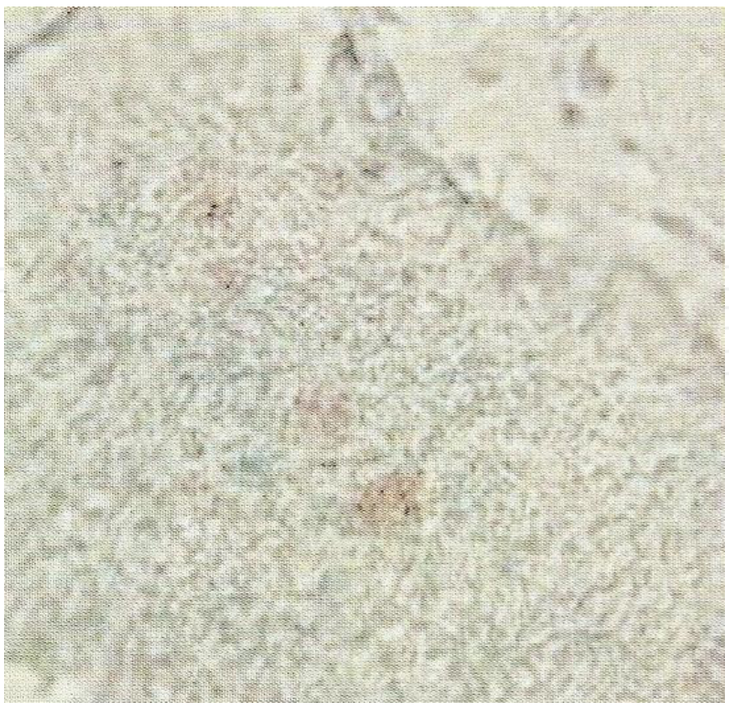
C

Fig. 7. Sagittal T2-weighted MRI of the rabbit spine (A), with a corresponding axial view at the level of the induced disc degeneration (B) and at a separate normal control level (C).

MSCs have also been investigated for their bone-forming capabilities with good results (Jaiswal et al., 1997). Stem cells are already being used in therapeutic applications with placement of cells directly at the site of intended spinal fusion during open surgical procedures.

Our lab has developed chondroprogenitor cells lines that can restore the functional capacity of the IVD, with these cells differentiating into chondrocytes. Using our novel percutaneous model of disc degeneration in a rabbit model, we obtained MRIs preoperatively and at 2, 4, and 8 weeks postoperatively (Figure 2). Before implantation, ESCs were cultured with cis-retinoic acid, TGF-beta, ascorbic acid, and insulin-like growth factor to induce differentiation along a chondrocyte lineage. After MRI confirmation of disc degeneration, the discs were then injected with murine ESCs that were labeled with mutant green fluoroscent protein (GFP). At 8 weeks post-implantation, IVDs were harvested and analyzed with hematoxylin and eosin staining along with immunohistochemical analyses (Figure 3).

Three groups were analyzed: group A consisted of control animals with nonpunctured discs; group B consisted of control animals with experimentally punctured discs; and group C consisted of animals with experimentally punctured discs that were subsequently implanted with ESCs. Gel electrophoresis was used to analyze ESCs for cartilaginous tissue formation. MRI confirmed IVD degeneration after needle puncture starting at 2 weeks postoperatively. Postmortem histological analysis of group A IVDs showed chondrocytes, but no notochordal cells. Group B disc displayed intact annulus fibrosus but disorganized



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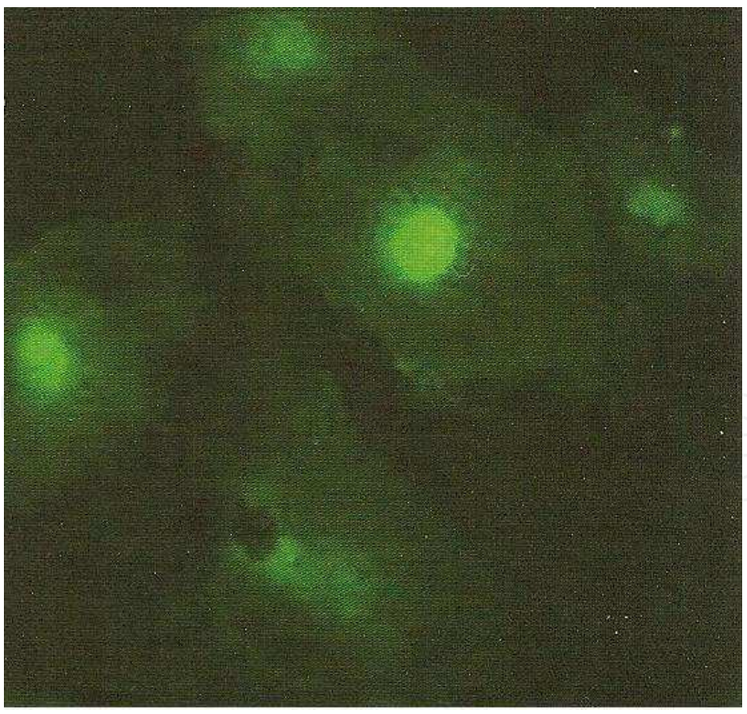

B.

Fig. 8. Photomicrographs of tissue obtained preimplanation for histological analysis of ESCs grown in-vitro with Alcian blue staining showing 86% viability (A) and high power magnification showed adequate GFP cell labeling (B).

fibrous tissue in the NP. Group C discs showed new notochordal cell growth, indicating survival and proper differentiation of the injected ESCs. Fluorescent microscopic analysis was positive in group C tissue, confirming the viability of GFP-labeled ESCs within the injected IVD. In addition, the notochordal cells in group C stained positive for cytokeratin and vimentin, providing further evidence of their chondrocyte origin. There was no inflammatory response in group C discs, indicating no cell-mediated immune response.

Our study provides a novel, reproducible model for the study of disc degeneration. New notochordal cell populations were seen in discs injected with ESCs. The lack of an immune response to xenograft-implanted mouse stem cells in an immune-competent rabbit suggests an immunoprivileged site within the IVD. Although preliminary, this study highlights the possible use of stem cells to promote IVD regeneration. Further ongoing studies are in the process of fully elucidating the processes involved with ESC differentiation along chondrogenic cell lines and how they may be used for new disc formation in the future. These studies will provide a good deal of evidence with regard to the future potential of ESCs for use in restoring the IVD in humans.

6. Summary

DDD is a high-morbidity condition with many modalities of treatment including surgery and more conservative measures such as pain injections, which only provide symptomatic treatment. No therapy has been developed that targets DDD at the cellular level. Recently, many biologic therapies have emerged that may be able to restore the NP and the normal cellular structure of the IVD. This restoration may in turn alleviate the symptoms of DDD through restoration of foraminal height, removing the compression of nerves. In-vitro studies have been performed to identify what cells are capable of differentiating towards a chondrocytic lineage and to best define parameters and factors that influence this differentiation. Multiple laboratory studies have been performed showing that MSCs, ADSCs, fetal cartilaginous cells, and annulus fibrosus cells all have the ability to differentiate towards a chondrocytic pathway. Factors that can induce these cells to differentiate toward a chondrocytic lineage have been identified and include TGF-β3 and BMP-2, which have a synergistic effect when used together. Other factors that may be beneficial include hypoxia, IVD-like glucose conditions (1.0 mg/mL glucose), and cell-to-cell contact with NP cells; the latter negating the need for other soluble factors (i.e. TGF-β3). A major limiting factor may be the acidic pH (6.8) of the IVD, one that may be especially important as acidic pH levels are typical of increasingly degenerated discs. These studies yielded encouraging results with cells in the IVD being positive for markers of chondrocytic differentiation such as collagen type II and aggrecan. Additionally, cells exhibited NP phenotypic markers and had a close similarity to the normal biconvex structure of the NP. In-vitro studies have clearly established that ESCs are capable of differentiating into a chondrocytic lineage and have delineated some of the factors that affect this. The optimal microenvironment needs to be more accurately characterized at this time.

Animal studies of cell implantation have been performed in DDD-induction models. Weeks after injury, stem cells have been implanted and outcomes followed. These outcomes which

have included radiographic analyses along with histological and immunohistochemical analyses have provided preliminary data that stem cell therapies are a viable option with regard to IVD regeneration (Sheikh et al., 2009). Human studies have further provided some preliminary evidence that stem cell therapy may be of clinical value (Haufe et al., 2006). The use of ESCs in regenerating IVD shows exciting new possibilities and further studies are needed in humans to establish its efficacy.

ESC-based regeneration of the human IVD is still in its infancy. Much progress has been made regarding laboratory research identifying the correct factors and microenvironment, and initial results from animal studies using stem cells remain promising. ESCs may be useful for repairing DDD as evidenced by their ability to differentiate into a chondrocytic lineage and yield notochordal-type cells in DDD models. ESCs need to be further studied and characterized with respect to safety, and larger human trials with appropriate clinical outcomes such as pain and disability reduction are needed to definitively establish its clinical efficacy.

7. Conclusions

The last half-century has seen an exponential rate of progress with regard to elucidating the mechanisms of degeneration of the IVD and how targeted therapies can help to alleviate this common condition. These studies have provided us with an improved understanding of the IVD and how it behaves under typical biomechanical forces and loads experienced in in-vivo conditions. Novel therapies are being studied, including stem cells with their potential regenerative capabilities in the spine. The development and action of these stem cells can be further modified through gene therapy and microenvironment manipulation. Immunologic markers are being used for more efficient targeting of these cells. With enhanced cell delivery and an improved understanding of the cell differentiation process, true regeneration of the IVD and surrounding supportive structures of the spine will become a reality that can be applied to treat patients with this common, debilitating condition.

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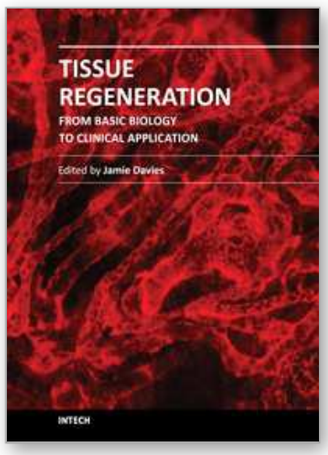

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