

Stem cell horizons in intervertebral disc degeneration

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Abstract: Intervertebral disc degeneration remains a pervasive and intractable disease arising from a combination of aging and stress on the back and spine. The growing field of regenerative medicine brings the promise of stem cells in the treatment of disc disease. Scientists and physicians hope to employ stem cells not only to stop, but also reverse degeneration. However, there are many important outstanding issues, including the hostile avascular, apoptotic physiological environment of the intervertebral disc, and the difficulty of obtaining mesenchymal stem cells, and directing them towards chondrocytic differentiation and integration within the nucleus pulposus of the disc. Given the recent advances in minimally invasive spine surgery, and developing body of work on stem cell manipulation and transplantation, stem cells are uniquely poised to bring about large-scale improvements in treatment and outcomes for degenerative disc disease. In this review we will first discuss the cellular and molecular factors influencing degeneration, and then examine the efficacy and difficulties of stem cell transplantation.

Keywords: intervertebral disc degeneration, stem cells, disc disease, mesenchymal stem cells, stem cell transplantation

Introduction

Two anatomically distinct regions comprise the cartilaginous intervertebral discs (IVD) of the human spine. Concurrently, the nucleus pulposus and annulus fibrosus provide both fluid and viscoelastic support within the IVD. First, the central nucleus pulposus (NP) occupies the internal structure of disc. It is filled with collagen type II extracellular matrix (ECM) and hydrophilic proteoglycans. The unique extracellular matrix of the NP provides “shock absorbing” capacity to the IVD derived from the water content of its components. The NP is enveloped by the second component of the IVD, the annulus fibrosus (AF). The AF is mainly collagen type I, and forms a fibrotic circumferential boundary to the more liquid NP (Paesold et al 2007). Consequently, the AF functions to gird the viscoelastic NP and provide structural integrity and resistance to its extrusion when compressive forces are applied to the NP.

The cellular composition of the IVD forms three separate sections. The concentric lamellae of collagen I fibers of the AF surround the NP. The NP contains two unique types of cells: a population of primitive notochordal cells, and a population of chondrocytic cells. The former are most likely the vestige of an embryonic notochord cell that directed the development of the IVD and spine. They disappear in humans after approximately 10 years of age. This may be due to differentiation into chondrocytic cells or apoptosis. Both regions of the IVD are bound above and below by endplates of cartilage. Some experts believe that these cells play a role in the IVD niche that provides for successful mesenchymal stem cell (MSC) differentiation into the cells of the NP (Hunter et al 2003).

Mechanisms of disc degeneration

To attain the goal of cellular regeneration of the IVD, the nature of cellular degeneration within the IVD must be determined. Most accepted cellular mechanism for IVD

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degeneration focuses on the NP because of its importance in maintaining a healthy and functioning IVD (Figure 1). Specifically, the ECM of the NP fails to maintain homeostasis for adequate collagen and proteoglycan synthesis (Sive et al 2002). The first step in understanding this phenomenon begins with identifying the molecular phenotype of the NP cells. No marker currently exists to distinguish these cells from common hyaline cartilage cells, since both have similar ECM macromolecules. Furthermore, even if stem cells could be instructed to differentiate into hyaline cartilage, they would still lack the essential fluid properties of the NP, and would fail to recreate normal function of the IVD. Also of tantamount importance is being able to separate the different cells. This could be accomplished first by elucidating the ratio of proteoglycans and collagen within the NP (Mwale et al 2004). Eventually, both the repopulation of NP cells and the concomitant reproduction of adequate ECM, with a normal proteoglycan/collagen ratio, must be realized for stem cell regeneration of the IVD to be considered.

Among the culprits to be targeted when considering IVD degeneration are the issues of diffusion of nutrients,

cell viability, proteoglycan synthesis, and disruptions in collagen production leading to decreased proteoglycan and collagen II in the IVD disc matrix. Molecular mediators such as degrading enzymes, inflammatory mediators, and growth factors are involved in all the above processes.

Matrix metalloproteinases (MMPs) are the best characterized matrix degrading enzyme, and are major players in IVD degeneration. MMPs degrade various collagens, but their activity is post-translationally inhibited by the binding of inhibitor of matrix metalloproteinases (TIMPs). The contribution of these enzymes to IVD degeneration most likely results from an imbalance of normal ECM collagen degradation by MMPs and MMP inhibition by TIMPs (Le Maitre et al 2004). Proteoglycan and cathepsins degrading enzymes, such as aggrecanase-1 which targets aggrecan, have also been suspects in IVD degeneration.

Pain manifesting from IVD degeneration may be linked to participating molecular inflammatory mediators. Though heterogeneous models have generally been used to investigate cytokines, they clearly play a role. Some interleukins identified may even recruit MMPs. In the same way, growth

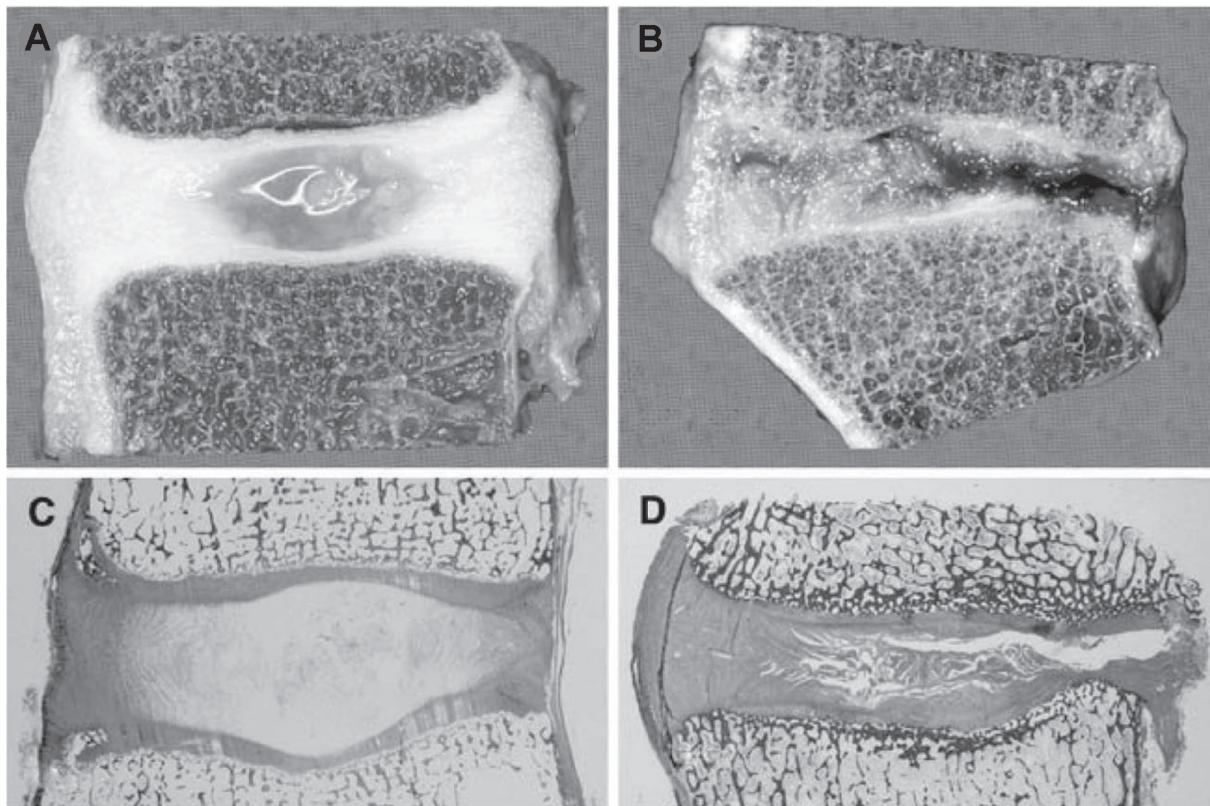


Figure 1 Degenerative changes of the intervertebral disc. Comparison of young and healthy (a, c) and severely degenerated (b, d) discs illustrates that alterations are observed in all anatomical regions of the disc and are obvious on macroscopical (a, b) and histological (c, d) level. Copyright © 2007. Reproduced with permission from Paesold G, Nerlich A, Boos N. 2007. Biological treatment strategies for disc degeneration: potentials and shortcomings. *Eur Spine J*, 16:447–68.

factors involved in IVD degeneration, such as transforming growth factor beta (TGF- β), insulin growth factor-1 (IGF-1), and fibroblast growth factor (FGF), have also been examined. These factors function as mitogens that increase rates of mitosis and proliferation of essential IVD components. For instance, TGF- β induces increased proteoglycan production and inhibits MMPs (leading to less ECM degradation) in disc cells (Pattison et al 2001). Bone morphogenic proteins (BMPs) produce similar effects as growth factors but are considered morphogenic since they are highly chondrogenic. However, though their function has been delineated, no clear correlation has been seen between factor levels and IVD degeneration. Effects on degeneration varied widely in the presence of different factor levels, although some of this confusion is probably attributable towards the heterogeneous assortment of investigations, some of which included extruded discs. Definitive studies remain outstanding, but many hypothesize that increased growth factor expression is a likely physiological response to disc herniation that encourages disc repair.

Transcription factors have also been implicated in degeneration since ECM turnover is also under the direction of genetic regulation. SMADs and latent membrane protein-1 (LMP-1) are intracellular regulators that stimulate proteoglycan and collagen II synthesis by upregulating BMP-2 and BMP-7 (Yoon et al 2004). Sox9 is another factor that promotes collagen II expression within the disc by increasing collagen II mRNA transcription (Li et al 2004).

The molecular pathways involved in IVD degeneration are not all the natural consequences of stress and aging, but can also be influenced by genetics. Gene polymorphisms for ECM proteins, like aggrecan, have been associated with disc and early multilevel degeneration (Doege et al 1997). A polymorphism in cartilage intermediate layer protein (CLIP) has also been recently correlated with susceptibility to disc degeneration (Seki et al 2005). Because of the limited innervations and blood supply of the intervertebral discs, the pathology of their degeneration hinges upon the interaction between a wide array of environmental, genetic, and molecular factors. Therefore, the therapeutic strategy will have to be a concerted approach that addresses all these issues.

The aforementioned mechanisms for molecular and cellular degeneration are important components of the clinical presentation of disc degeneration. However, it should be noted that not every patient that has degenerative disc disease has back or leg pain (Jensen et al 1994). This underscores the fact that the mechanisms of pain are not well understood. The degenerated IVD is associated with progressive

desiccation, loss of biomechanical properties, loss of disc height, and in some cases disc herniations (Haefeli et al 2006). These entities must be clinically appreciated because stem cell mediated IVD repair would not obviate the need to remove a herniated disc compressive a spinal root. Current experience and knowledge focus on stem cell mediated IVD repair with the objective of returning the disc ECM to its pre-morbid state and allowing for the imbibition of water and subsequent return of disc height. This may or may not be associated with pain relief, but currently offers the best cellular strategy for disc repair. In fact, a cellular strategy could be used for multiple aims, not just disc repair, but also the delivery of anti-inflammatory agents to help with the ultimate clinical objective- reduction of pain.

MSCs and the IVD

Originally discovered 30 years ago, mesenchymal stem cells (MSCs) are the elusive vehicle for cell therapy in the IVD (Friedenstein et al 1976). They are valued for their multipotency, or ability to differentiate into cell types of mesenchymal origin (fat, bone, cartilage, etc.), and down the necessary cell lineage for regeneration or replacement of degenerated disc cells (Prockop et al 2003). There are two main strategies for acquisition of these desired somatic stem cells: through manipulation of embryonic stem cells (ESCs), or through extraction of MSCs from the fat or bone marrow of the patient.

ESCs are extracted from the inner cell mass of blastocyst stage embryos. The promise of ESCs lies in their plasticity and immortality. These cells are pluripotent, that is, through specialized culture techniques, ESCs can be induced to differentiate into cells of all three germ layers (endoderm, mesoderm, and ectoderm). Indeed, the plasticity of these cells is much greater than any one group of somatic stem cells such as bone marrow derived MSCs. Furthermore, once in culture, ESC lines can be maintained indefinitely, providing an everlasting source of cells for implantation. There is still much to uncover about how to derive chondrocytes or even MSCs from human ESC lines, and the danger of teratoma formation is always present with ESCs (Trounson 2002). Instead of direct MSCs differentiation, recent investigations in the field point towards neural crest stem cells (generated from ESCs) as a source of MSCs, presenting an alternate possibility of a reliable and efficient method of MSCs derivation from human ESCs (Lee et al 2007).

Even though ESCs are uniquely versatile, adult MSCs remain the ideal candidate for IVD repair. Readily extracted from adipose tissue or bone marrow, they offer

an autologous source of cells with low risk of infection and immunogenicity. In addition, there is a long history of *in vitro* manipulation and clinical *in vivo* investigations for orthopedic trials with these cells (Horwitz et al 2002). Despite the fact that true MSC yield from bone marrow aspirate is less than 0.01%, their proliferative capacity of makes this a sufficient amount for MSC or MSC derived chondrocyte mediated IVD regeneration (Pittenger et al 1999). Adipose tissue is also an attractive substitute source of MSCs because of the relative ease in procuring fat over bone marrow. However, some studies have found that the gene expression profiles of bone marrow derived MSCs match that of native cartilage more closely when differentiating into chondrocytes (Winter et al 2003). Bone marrow and adipose derived MSCs also have differing properties when evaluated for cell surface markers (De Ugarte et al 2003; Huang et al 2005). Thus, until adipose MSCs are more thoroughly investigated and understood, bone marrow MSCs remain the most efficacious option for stem cell IVD therapy.

Preclinical studies of MSC transplantation exist with disc degeneration typically induced with a needle puncture method, and subsequent evaluation of degeneration before and after MSC transplantation. Though this does not accurately represent the complex disease and degeneration process that normally occurs in humans, it is successful in demonstrating the regenerative capabilities of MSCs in the presence of IVD desiccation

(Leung et al 2006). Sakai and colleagues (2006) used a rabbit model of nucleus aspiration to induce degeneration. They injected MSCs embedded in an atelocollagen matrix where they persisted over a month, amplifying the proteoglycan content of targeted discs. Implantation of autogenic MSCs was shown to preserve annular structure, reestablish disc nuclei positive for glycosaminoglycan and keratin sulfate proteoglycans, and partially restore disc height and hydration in similar studies (Leung et al 2006).

MSC derivation

The limiting factor for exploiting stem cells for therapeutic use is obtaining well characterized cells for transplantation. Directing the appropriate differentiation of MSCs (and ESCs) is a complex molecular and cellular puzzle that is contingent upon not only the inherent properties of cells, but also the environment in which they are cultured.

The soluble factors TGF- β and BMP are necessary components of culture media used to induce *in vitro* chondrogenic differentiation of MSCs. In fact, careful use of soluble factors in media can lead to chondrogenesis with a genetic profile more analogous to IVD tissue than articular cartilage (Figure 2) (Steck et al 2005). Another method of alter MSC microenvironment to trigger chondrogenic differentiation involves co-culturing with different cell populations to take advantage of cell-cell contact and molecular signal activation.

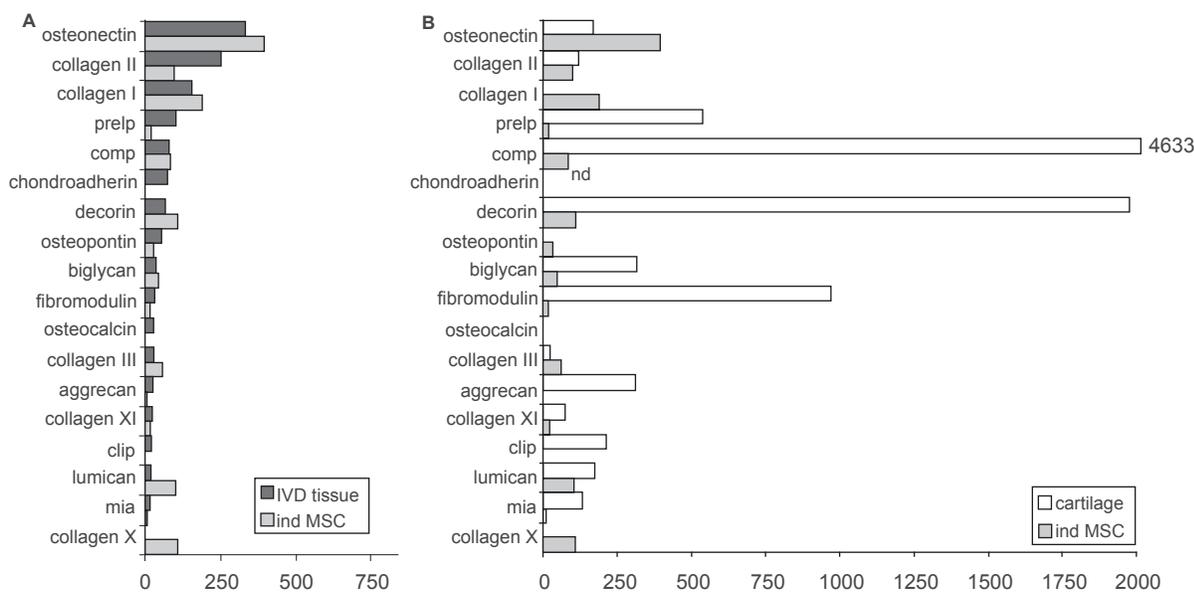


Figure 2 Quantitative analysis of gene expression levels of selected genes (signal intensity above 15% in IVD tissue, except collagen type X, which was negative). Spheroid cultures of MSCs 2 weeks after TGF β -mediated induction ($n = 7$) were compared with IVD tissue ($n = 6$) **A**) and articular cartilage tissue ($n = 5$) **B**). The signal intensities were normalized to the gene expression levels of the housekeeping genes on each filter. The medians of independent experiments are shown and expressed as relative values in percent of the housekeeping genes. Copyright © 2005. Reproduced with permission from Steck E, Bertram H, Abel R, et al 2005. Induction of intervertebral disc-like cells from adult mesenchymal stem cells. *Stem Cells*, 23:403–11.

Utilizing the autocrine and paracrine factors secreted by one cell type leads to the activation of cell surface receptors on MSCs. Experiments culturing human NP cells and MSCs found that differentiation was reliant on cell-cell contact by looking at gene expression of Sox9, type II collagen, and aggrecan (Figure 3) (Richardson et al 2006).

The three dimensional properties of the culture system have also shown to exercise substantial influence on the process of cell fate determination. MSCs are pelleted down into a dense micromass before addition of soluble factors to recreate the *in vivo* state that leads to cartilage formation. This structure helps direct the chondrogenic cascade of MSC differentiation from micromass into cartilage. Mesenchymal condensation allows for extracellular signaling molecules such as Wnt glycoproteins and N-cadherin to form cadherin and connexin adhesion complexes for the beginning stages of ECM formation. Cartilage then begins to form on this three dimensional scaffold. Plating density of MSCs prior to soluble factor addition also influences the efficiency of differentiation (Figure 4). This is because plating density can change the cell morphology; specifically, wider spindle shaped cells corresponding with denser MSCs plating. (Figure 5). Wider cells also have an increased propensity to differentiate after exposure to soluble factors *in vitro* (Sekiya et al 2002). In this way, employing density dependent culturing techniques can produce cartilage formation from MSCs *in vitro*, and increase

the efficiency of MSC differentiation. Another technique described to direct MSCs toward the desired cell fate has been with co-culture systems, where the differentiated cell types provide the autocrine factors to increase the amount of nucleus pulposus cells *in vitro* (Yamamoto et al 2004). Clearly, there are many microenvironmental considerations to be made when designing an optimal *in vitro* MSC culturing system (Le Visage et al 2006). Exposure to a particular microenvironment may result in physiological variations that can be genetically perpetuated to daughter cells, epigenetically conditioning them to a particular cell fate (Gregory et al 2005).

MSC implantation to IVD and its options

Stem cell therapy can easily be adapted in the clinical setting (with or without MHC typing) once an appropriate delivery “package” of cells and molecular adjuncts is devised. The objective, with our present understanding, would be to restore disc height by decreasing the desiccation of degenerated discs. Although, disc degeneration is associated with back pain, it is clearly not the only contributor. As such, stem cell mediated disc repair would aim to remedy on component of a complex disease entity. Knowledge and experience gained, along with increasing understanding of multifactorial mechanisms of back pain would lay the foundation for further advances. With that noted, the delivery “package”

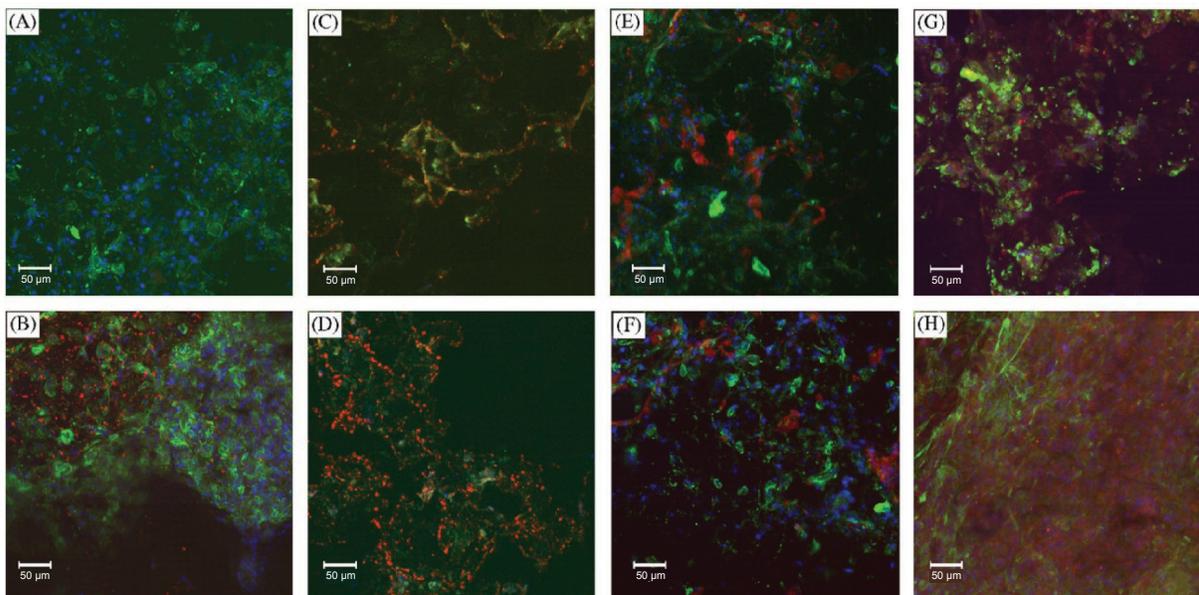


Figure 3 Confocal microscopy images showing deposition of matrix proteins on PLLA scaffolds. Blue is Hoechst of nuclei, green in F-actin and red in specific stain: **A)** MSC+SM 1 week type II collagen; **B)** MSC+SOX-9+CM 1 week type II collagen; **C)** MSC+SM 4 weeks type II collagen; **D)** MSC+SOX-9+CM 4 weeks type II collagen; **E)** MSC+SM 1 week type I collagen; **F)** MSC+SOX-9+CM 1 week type I collagen; **G)** MSC+SM 1 week aggrecan; and **H)** MSC+SOX-9+CM 1 week aggrecan. Copyright © 2006. Reproduced with permission from Richardson S, Curran J, Chen R, et al 2006. The differentiation of bone marrow mesenchymal stem cells into chondrocyte-like cells on poly-L-lactic acid (PLLA) scaffolds. *Biomaterials*, 27:4069–78.

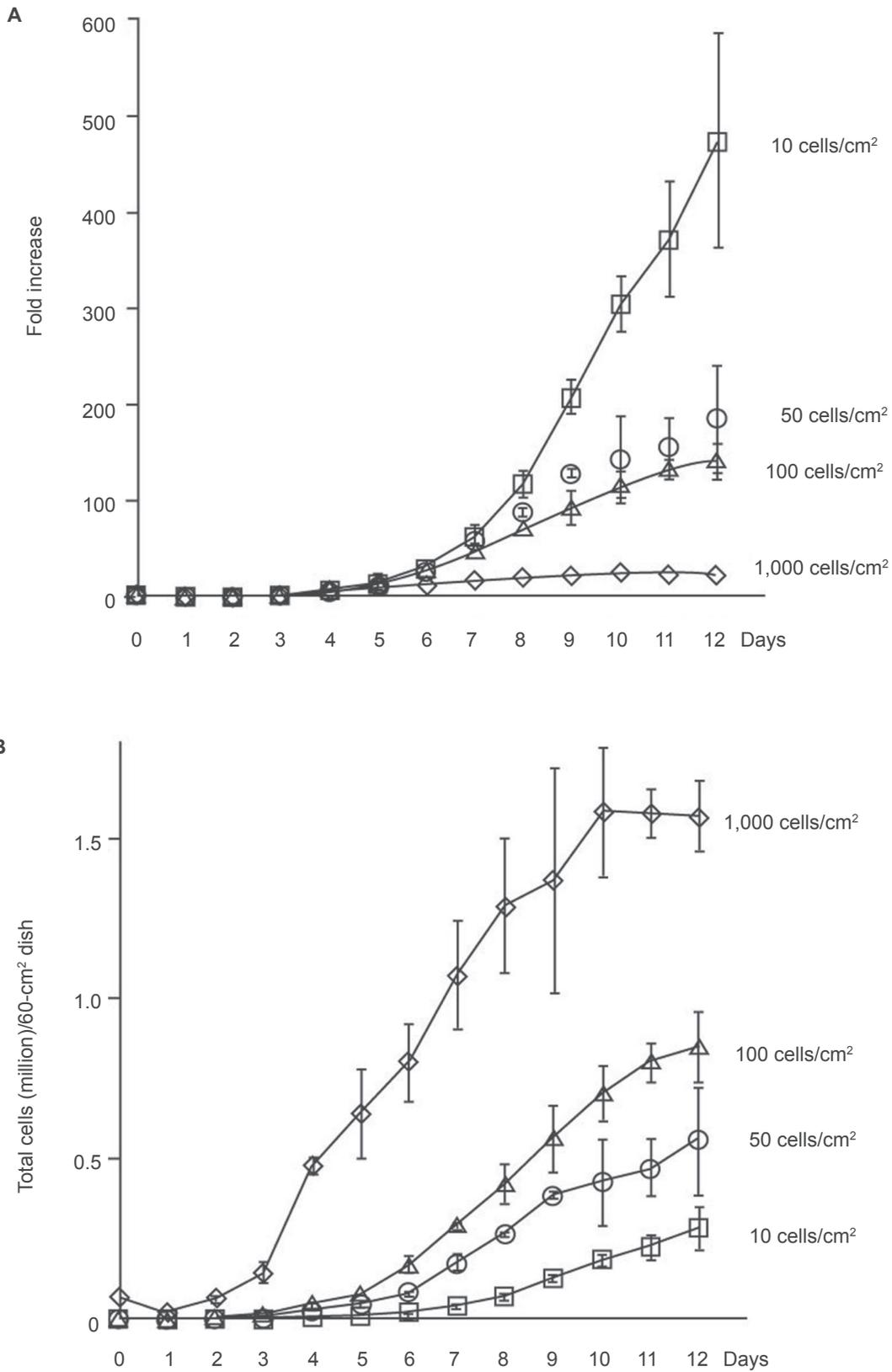


Figure 4 Relation between initial plating density and expansion of MSCs. Passage 3 MSCs (donor 89L) were plated on 60 cm² dishes at 10, 50, 100, and 1,000 cells/cm². The cells were harvested and counted at 1 to 12 days. Fold increase **A**) and total cell numbers per 60 cm² dish **B**) are shown. Data are expressed as mean ± standard deviation (n = 3). Copyright © 2002. Reproduced with permission from Sekiya I, Larson B, Smith J, et al 2002. Expansion of human adult stem cells from bone marrow stroma: conditions that maximize the yields of early progenitors and evaluate their quality. *Stem Cells*, 20:530–41.

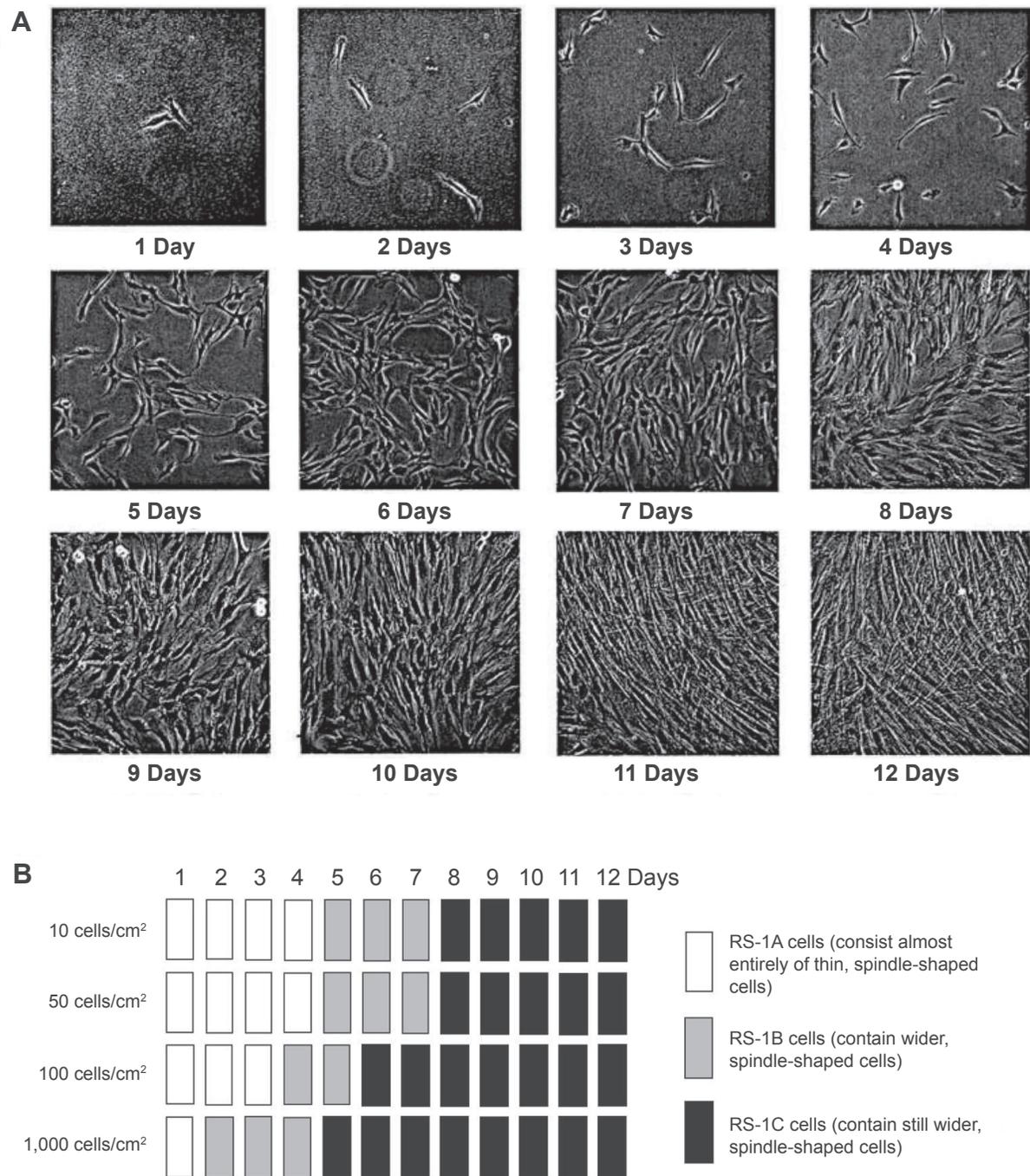


Figure 5 Initial cell density and time in culture affect cell morphology. Passage 3 MSCs were plated at 10, 50, 100, and 1,000 cells/cm² at 1 to 12 days. **A)** Representative pictures of MSCs plated at an initial cell density of 50 cells/cm² at 1 to 12 days. **B)** Schematic diagram of MSC culture morphologies at four different initial cell densities from 1 to 12 days. Copyright © 2002. Reproduced with permission from Sekiya I, Larson B, Smith J, et al 2002. Expansion of human adult stem cells from bone marrow stroma: conditions that maximize the yields of early progenitors and evaluate their quality. *Stem Cells*, 20:530–41.

would not only consist of growth factors for effective MSC engraftment, but could also include various biodegradable gels or grafts to provide initial biomechanical support and a structure for biological reconstruction of more severely degenerated discs. Allogenic stem cells could be implanted in minimally degenerated discs via percutaneous techniques.

If disc degeneration was more severe, stem cells could be transplanted with minimally invasive surgical and radiological techniques through a true lateral retroperitoneal approached guided by discogram. Employing this minimally invasive approach would also allow for co-implantation of a biodegradable interbody graft, that would be replaced by the

engrafted stem cells. Ultimately, the best clinical intervention would incorporate a personalized strategy for each patient and the degree and type of disc degeneration identified. The tools for this strategy would include, but not limited to, cell transplants, biodegradable implants, and internal fixation as needed.

A large number of NP like cells will need to be generated for proficient IVD repair. The NP cell type is not well characterized, but NP cells identified by their production of the requisite ECM components can suffice. Though autologous bone marrow derived MSCs are the ideal candidates for therapy, allogenic MSCs could serve as a more economical substitute. MSCs lack HLA class II receptors and sibling donors should not generate an immune response in recipients (Yang 2007). A cocktail of MSCs or differentiated NP cells would have to include molecules that promote the survival and engraftment of transplanted cells, and be rigorously tested in large animal models before clinical trials are undertaken. It will also be imperative to develop approved protocols of MSC culture and differentiation with strict quality controls to ensure safety before application in humans. Once the science and methods of MSC manipulation are better elucidated, IVD regeneration with stem cell therapy could be a viable treatment option for patients. In fact, osteogenesis imperfecta is already being treated effectively with MSCs and presents a model of the potential of MSCs for regenerative medicine. Developing this therapy to the unique cartilaginous tissue of the IVD could be the safest, most efficient application of stem cell biology to disease processes treated by surgeons.

Disclosure

The authors report no conflicts of interest in this work.

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