*Tissue Engineering Review Series* *J. Cell. Mol. Med. Vol 12, No 6A, 2008 pp. 2205-2216*

***Guest Editor: R. E. Horch***

# Adipose stem cells for intervertebral disc regeneration: current status and concepts for the future

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*Received: November 7, 2007; Accepted: February 12, 2008*



## Abstract

New regenerative treatment strategies are being developed for intervertebral disc degeneration of which the implantation of various cell types is promising. All cell types used so far require *in vitro* expansion prior to clinical use, as these cells are only limited available. Adipose-tissue is an abundant, expendable and easily accessible source of mesenchymal stem cells. The use of these cells therefore eliminates the need for *in vitro* expansion and subsequently one-step regenerative treatment strategies can be developed. Our group envisioned, described and evaluated such a one-step procedure for spinal fusion in the goat model. In this review, we summarize the current status of cell-based treatments for intervertebral disc degeneration and identify the additional research needed before adipose-derived mesenchymal stem cells can be evaluated in a one-step procedure for regenerative treatment of the intervertebral disc. We address the selection of stem cells from the stromal vascular fraction, the specific triggers needed for cell differentiation and potential suitable scaffolds. Although many factors need to be studied in more detail, potential application of a one-step procedure for intervertebral disc regeneration seems realistic.

**Keywords:** intervertebral disc degeneration • mesenchymal stem cells • adipose tissue • regeneration • stem cell selection • scaffold

## Introduction

Disorders of the musculoskeletal system are among the most prevalent and costly medical conditions affecting western societies [1]. Recent advances in cellular biology and material technology, the cornerstones of regenerative medicine, also referred to as reparative medicine or tissue engineering, are beginning to influence the clinical practice of different disciplines including orthopaedic surgery. Regenerative medicine has identified various skeletal tissues as attractive translational skeletal targets, in particular bone, cartilage, meniscus and the intervertebral disc [2, 3]. The identification and characterization of matrix constituents and the increased knowledge about both anabolic and catabolic triggers of musculoskeletal tissues provide important information on possible targets for therapeutic intervention. However, most of these concepts have barely progressed from *in vitro* testing and

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doi:10.1111/j.1582-4934.2008.00291.x

are so detailed that any attempt to summarize them would not do them justice, and is beyond the scope of this review. Therefore, this review will focus on a recently discussed type of biologic therapy: stem cell therapy and its role in intervertebral disc regeneration, in particular the use of adult adipose-derived mesenchymal stem cells.

## Degenerative disc disease and emerging biological treatment approaches

The intervertebral discs tightly connect the vertebrae of the spinal column, providing resistance to compression combined with the permission of limited movements. The outer part of the intervertebral disc (IVD) consists of perpendicularly oriented circumflex lamellae consisting of primarily collagen type I that cross between two vertebral bodies. This is called the annulus fibrosus (AF). These lamellae confine the nucleus pulposus (NP), a gel-like structure consisting of proteoglycans and water, held together by a mainly collagen type II network.

IVD degeneration can be described clinically as a loss of proper stability and mobility, which are the two major roles of the disc. However, the aetiology and pathophysiology of disc degeneration are still largely unknown [4, 5]. From a biomechanical point of view, disc degeneration can be described as a decrease in water content associated with proteoglycan diminution of the nucleus pulposus and inner annulus. This results in flattening of the disc and eventually destruction of the annular structure [6, 7]. Although the cause of IVD degeneration remains unclear, an attempt to define IVD degeneration was recently made as follows: an aberrant, cell-mediated response to progressive structural failure [8].

Degenerative disc disease (DDD) applies to degenerated discs which are also painful [8]. DDD is a highly common musculoskeletal impairment that currently has no identified cause. However, a strong association exists between increasing age and progressive degradation [9, 10]. The traditional view during much of the last century was that DDD was primarily due to physical (over)loading as well as changes associated with the normal aging process. In recent years, however, a dramatic advance has been made in the understanding of risk factors such as age, gender, genetic, environmental, chemical (smoking), and biomechanical influences for disc degeneration, thus changing our traditional views [11–14].

Current treatment options for DDD comprise either pain management or invasive surgical interventions like vertebral interbody fusion or spinal arthroplasty [15]. The expanding comprehension of processes involved in DDD and disc repair, however, present the possibility of developing strategies for restoring disc tissues. The onset of DDD starts with the loss of proteoglycans in the NP and therefore several biologic strategies under investigation aim to restore the proteoglycan level or synthesis within the degenerated IVD. These strategies include the use of natural and recombinant proteins, cytokines or growth factors, gene therapy and cell therapy [16–20].

Growth factors like TGF-β [21–23], BMP-2 [20, 23], BMP-7 (OP-1) [24, 25] or GDF-5 [26, 27] all have shown an anabolic effect on disc cells, characterized by their ability to increase the functional properties of IVD cells, such as production of collagen type II, Sox 9 and aggrecan [28]. Another category of molecules has a similar effect as the growth factors on disc cells, but exerts its effect intracellularly, by controlling one or more aspects of cellular differentiation [20]. Examples of these factors include LMP-1 [29], Sox 9 [30] and SMADs [31, 32]. Anti-inflammatory factors, like TIMP-1 [33] and CPA-926 [34], were shown to reduce degenerative changes by inhibiting naturally present degradative enzymes like MMP-1 or MMP-3 [33]. The above-mentioned categories of biologic agents aim to modify the disc-cell metabolism, while some biologic treatment strategies aim to increase the number of cells in the disc. Mitogenic molecules for disc cells include insulin-like growth factor-1 (IGF-1) and epidermal growth factor (EGF), which were shown to have positive effects on the rate of mitosis and proteoglycan production of disc cells *in vivo* [27, 28]. All of the mentioned factors showed preservation of the architecture of disc tissue and/or increase collagen and proteoglycan synthesis through different mechanisms. However, the success of gene therapy and growth-factor injection depends on a critical mass of cells within the disc. Cell-based treatments do not share this requirement and may therefore be appropriate for a wide range of disease states of degenerative disc disease. Cell therapy is an alternative approach, and the regenerative effects of transplantation of autologous cells, such as nucleus pulposus cells [35, 36], annulus fibrosus cells [37], cartilagenous chondrocytes [38] and mesenchymal stem cells [39–42] into the IVD, have been demonstrated as well. This review focuses on the use of mesenchymal stem cells in intervertebral disc regeneration.

## Stem cell sources

Stem cells are defined as unspecialized cells capable of long-term self-renewal and differentiation into more specialized cells. At the beginning of life, after fertilization of the ovum, a blastocyst is formed containing totipotent cells, which divide and specialize into pluripotent, embryonic stem cells [43]. The pluripotent cells then further specialize into multi-potent stem cells, or progenitor cells, that commit into lineages with tissue-specific functions like mesodermal tissue [43]. Cells capable of producing mesenchymal tissues are referred to as mesenchymal stem cells (MSC) and are capable to differentiate to adipocytic, osteoblastic and chondrocytic lineages under appropriate conditions [44]. MSCs have not only been isolated from embryonic [45] or foetal tissues [46], but also from almost every organ in adulthood [43]. MSCs from

adult tissues provide an attractive, alternative source of cells for tissue engineering, as the use of embryonic stem cells gives rise to ethical controversy. In addition, adult MSCs are relatively easy accessible and can be harvested from tissues like bone marrow, skin, muscle and adipose tissue [2, 44, 47–50]. Currently, bone marrow is the primary used source of adult MSCs, in which one of $10^5$ nucleated cells is an MSC [51]. The low number of cells necessitates *in vitro* culture expansion to obtain sufficient numbers of cells for clinical application [52].

MSCs derived from the stromal vascular fraction (SVF) of adipose tissue were firstly identified by Zuk *et al.* as a source of adult MSCs [49]. SVF is a cell mixture isolated from adipose tissue by collagenase digestion and centrifugal enrichment, harbouring a population of multi-potent MSCs, so-called adipose-derived stem cells (ASCs) [50]. SVF is a pool of various cells, including endothelial cells, smooth muscle cells, pericytes, fibroblasts, mast cells and pre-adipocytes [53, 54]. The incidence of ASCs in adipose tissue is estimated to be about 1 per $10^3$ nucleated cells [50], which is two magnitudes higher than the number of MSCs in bone marrow [51]. Despite the higher frequency and yield of ASCs over bone marrow MSCs, the biological properties of ASCs are not compromised. In culture, ASCs express cell-surface markers similar to those expressed by bone marrow MSCs, including CD105, SH3, Stro-1, CD90 and CD44 [44, 48]. After lineage-specific stimulation, ASCs show multiple-lineage differentiation potentials: they can differentiate into adipogenic, myogenic, chondrogenic, osteogenic, endothelial, cardiomyogenic and potentially neurogenic, phenotypes [48–50]. As interest of clinicians in ASCs increases, several authors have compared ASCs and MSCs in terms of differentiation capacity [55–57]. MSCs from bone marrow are reported to provide a more suitable cell source for osteogenic and chondrogenic differentiation compared to ASCs [55–57], although no significant differences in terms of the multi-lineage differentiation capacity between ASCs and BM-MSCs were found in two other reports [58, 59]. However, MSCs from different sources respond differently to culture conditions: for instance, medium containing dexamethasone is necessary for chondrogenesis in synovium-derived MSCs [60] while the same medium suppresses chondrogenesis in ASCs [61]. Therefore, the development of optimized protocols for the differentiation of MSCs from different tissue sources is crucial for equal comparison of their differentiation capacities. The most important features of adipose tissue as an MSC source are the relative expendability and easy accessibility. Adipose tissue can be obtained in substantial quantities with minimal risk, as liposuction is a common procedure to obtain adipose tissue with zero reported deaths on 66,570 procedures and a serious adverse event rate of 0.68 per 1000 cases [62]. Adipose tissue is also accessible at most sites used for a surgical procedure, neutralizing the need for a separate harvest site and its concomitant morbidity. Thus, ASCs are a promising source of stem cells for tissue engineering, and they have enormous clinical potentials as the principle source for both a one step or a multi-step procedure for tissue regeneration in general.

## Integration of ASC-based regenerative medicine and surgery

The ability to harvest and/or procure high quantities of lineage-specific cells or direct to regeneration-competent progenitor cells towards the proper phenotype is crucial for orthopaedic tissue engineering interventions. As bone marrow derived stem cells must be expanded *in vitro,* current concepts of tissue engineering procedures consist of multi-step procedures, including at least an MSC harvesting step and an MSC re-insertion step after expansion [63, 64]. Based on the current knowledge of tissue engineering technology and ASC technology in particular, we formulated an innovative concept for a one step-procedure for spinal inter-body fusion [65]. A time frame for this procedure is shown in Figure 1. The efficacy of this procedure is based on integration of tissue engineering technology with established surgical interventions performed with off-the-shelf biomaterials (calcium phosphate-based scaffold, bioresorbable polymer cage), and retrieval of sufficient quantities of ASCs harvested with minimal invasive techniques within the scope of a single surgical procedure. Previous research studies focused on the integration of tissue engineering techniques and a posterior lumbar inter-body fusion (PLIF) [66–68], a well-established and widely accepted surgical technique for spinal fusion as a treatment for (severe) intervertebral disc degeneration [15]. ASCs containing SVF were isolated from subcutaneous adipose tissue at the surgical site immediately after skin incision, performed with the digestion and centrifugal enrichment methods as described by Zuk *et al.* [50]. It could be shown that sufficient ASCs in SVF can be retrieved from different areas of the body, enabling various surgical approaches to the spine (*e.g.* anterior, lateral and posterior) [53]. Our group showed the feasibility of short-term *ex vivo* triggering of ASCs in the osteogenic direction performed with biologics [69] and that ASCs acquired bone cell-like responsiveness to loading after osteogenic differentiation [70]. Furthermore, in another study we observed vitality and diffuse, rapid penetration of triggered stem cells on and in a porous calcium phosphate scaffold [65]. Implantation of a bioresorbable cage filled with the triggered stem cell seeded scaffold in a prepared intervertebral disc completes the procedure. Short-term *in vivo* studies in a goat spinal inter-body fusion model showed cellular retention of fluorescently labelled SVF cells at 4 days after implantation and active bone formation by osteoblasts and resorption of scaffold material after 28 days [65].

For mildly degenerated discs, a similar concept might be feasible for ASCs-based transplantation by simple injection in the contained structure of the intervertebral disc (see Fig. 1). It is envisioned that retrieval and procurement of the ASCs (Phase I, see Fig. 1) can be performed in a standardized, similar way for both regenerative as well as fusion techniques, whereas triggering and/or carrier seeding of the cells (Phase II and III, see Fig. 1) must be tailored to the specific aim of the procedure.

However, much is unknown and is currently under investigation with respect to the need of (rapid) selection of ASCs from SVF, the

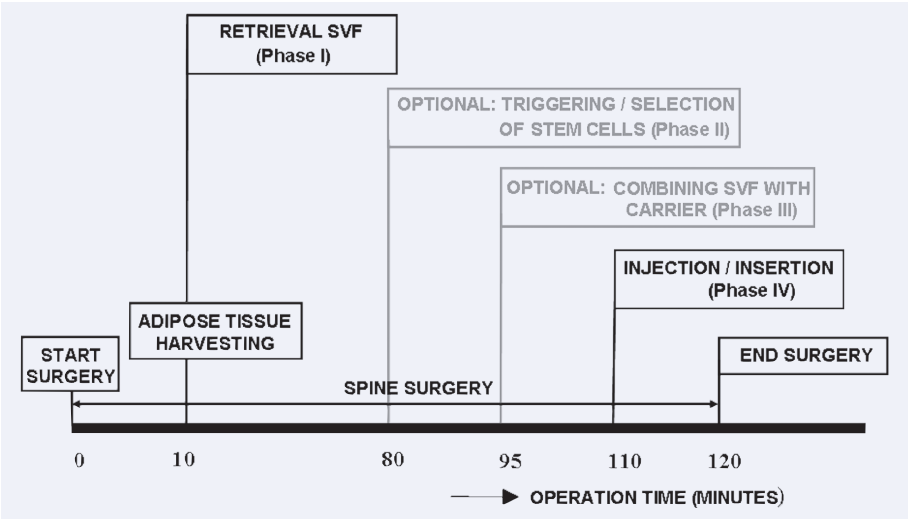


**Fig. 1** Concept of a one-step surgical procedure. The surgery starts with harvesting of the adipose tissue, followed by a split procedure. The surgeon continues the surgery, whereas the tissue engineer isolates the stem cell-containing cell population from the adipose tissue, treat the cells to induce differentiation into the proper phenotype, and seeds the stimulated cells on the scaffold. The surgeon then implants the scaffold containing the stem cells, and finishes the surgery. The whole procedure takes approximately two hours.

need for chondrogenic or NP-cell triggering of the ASCs and the need for carrier materials in the regenerative one-step procedure. Therefore, this review aims to give an overview about current *in vitro* and *in vivo* studies and potentials of MSCs in general in disc regeneration, pointing to ASC-related studies where possible.

## *in vitro* studies

Cells in the nucleus pulposus share several characteristics with articular cartilage chondrocytes, for instance both cell types demonstrate sox9, aggrecan and collagen type II up-regulation [71, 72]. Many studies have shown that adult MSCs can be directed into chondrocytes [73, 74]. The ability to isolate, expand and direct MSCs *in vitro* to particular lineages provides the opportunity to study events associated with differentiation. The specific environmental cues to initiate the proliferation and differentiation of MSCs *in vivo* towards NP cells at present are not fully understood yet. For the purpose of disc regeneration by simple injection of ASCs, it is of particular interest to study the effects of the microenvironment within NP tissue on the differentiation of MSCs, as well as the interaction with scaffold materials potentially involved in disc regeneration.

NP cells and MSCs are likely to interact after injection of MSCs in the intervertebral disc in our envisioned one step-procedure. Co-culture systems, both direct and indirect, have been widely used to investigate the interactions between two different cell types *in vitro*. In the direct system, cells communicate through both cell–cell contacts and paracrine mediators, however, in the indirect system cells communicate only through paracrine mediators. The low density of NP cells in nucleus tissue, which is only about 4000 cells/per $mm^3$ [75], makes direct cell–cell contact between NP cells and ASCs a rare incidence when MSCs are injected into NP tissue. Therefore, the indirect co-culture system is more likely to mimic the *in vivo* situation after injection of ASCs for the NP regeneration. MSCs have been indirectly co-cultured in monolayer with NP cells with contrasting results: Li *et al.* found MSCs differentiating towards the NP-cell-like phenotype [76], but Richardson *et al.* found that direct cell contact was necessary to induce the NP-cell-like phenotype [73]. Regardless of the co-culture system, cell culture configuration is also relevant for chondrogenic differentiation and monolayer culture is not appropriate for chondrogenic differentiation nor mimics the 3D *in vivo* situation [77, 78]. Our group demonstrated that ASCs cultured as micromasses are able to differentiate towards NP-cell-like cells by indirect NP-cell co-culture, as determined with real-time PCR, showing an up-regulation of collagen type II and aggrecan and concomitant down-regulation of osteopontin, collagen type I and PPAR-$\gamma$ (see Fig. 2) [79].

As IVDs consist primarily of extracellular matrix (ECM), injected stem cells are likely to interact with the components of the ECM after injection into the disc. It was shown that ECM plays a critical role in the regulation of stem cell differentiation into different lineages, cell proliferation and cell migration [80–82]. Collagen type II, the predominant collagen in nucleus pulposus ECM [83, 84], was shown to maintain the chondrogenic phenotype [85, 86] and even to induce a chondrogenic phenotype in MSCs [87, 88]. These processes might be influenced by the capacity of chondrocytes to bind to collagen type II through $\alpha_1\beta_1$, $\alpha_2\beta_1$ and $\alpha_{10}\beta_1$ integrins, resulting in the formation of a signalling complex, which plays a role in the differentiation, matrix remodelling and cell survival [89]. To investigate ASC behaviour in a collagen type II environment, our group studied ASCs in collagen type I or II gels, indirectly co-cultured with NP cells. These experiments showed that collagen type II can act in concert with NP cells on chondrogenic differentiation of ASCs [90].

Besides interaction between cells and matrix components of the disc, the interaction with (synthetic) scaffolds might be of interest and is studied at present as well for the purpose of disc regeneration. A general roadmap for designing an optimal scaffold

with respect to survival, proliferation and differentiation of stems cells is currently lacking. Apart from the general requirements such as biocompatibility, recent studies indicate that the material properties of the scaffold may influence the differentiation potential of the seeded stem cells [91, 92]. In the context of osteogenic differentiation, it was suggested that this is due to a selective and material-related adsorption of serum proteins to the tested scaffold materials [93, 94], which directly affects the differentiation potential of the attached cells [93]. Recent advances in basic research on the interaction between stem cells and their physical environment emphasize that the physical properties of the substrate is of utmost importance in the behaviour of stem cells. It has been recently shown that the stiffness of the substrate and the shape that cells adopt on a scaffold can force cells to differentiate to a certain lineage. Most interestingly, it has been shown that these physical stimuli can even overrule the stimulus provided by addition of soluble differentiation factors to the culture medium [95]. This may open new perspectives for the design of scaffold materials with tuned physical properties that facilitate survival, growth and differentiation of stem cells towards disc cells, which ultimately may restore disc function.

Several scaffolds have been investigated to study the interaction between *in vitro* cultured disc cells and the material, including fibrin glue [96], chitosan gel in combination with genipin [97, 98], collagen/ hyaluronate [99], type II collagen-based Atelocollagen® gel [37, 39] and a composite scaffold of polyglycolic acid and alginate/calcium [100, 101]. Recently, the interaction of MSCs with some of these materials was also studied. Performed with a hyaluronan scaffold, it was found that stem cells can survive in the relative hostile environment of the disc [99] and preliminary results suggested that MSCs could differentiate into intervertebral disc cells within an Atelocollagen® scaffold [39].

Currently, major problems still arise when performed with these scaffolds for tissue engineering purposes. A problem with chitosan and collagen/hyaluronan scaffolds is that the proteoglycan content is far lower in comparison to native cartilage. Presumably, the pre-fabricated scaffolds exhibit relatively large pores to allow cell seeding into the scaffolds, so that Glycosaminoglycans (GAG) produced by the cells may not be retained [98] suggesting that *in situ* curable polymers, which entrap both cells and produced ECM molecules, are favourable. In this respect, a trend towards designing micro- or nanoscale dimension scaffolds may provide new perspectives [102].

Within the context of the one-step surgical procedure performed with ASCs, an important issue might be the selection of cells *via* the scaffold material. A prerequisite for a one-step operational procedure is that at least the stem cells within the heterogeneous SVF adhere to a scaffold. In addition, these stem cells should adhere within a short time frame. At present, studies are conducted in our laboratory investigating the adherence of the different cell types within SVF to a bioresorbable polycaprolactone scaffold. Preliminary results indicate that adipose stem cells adhere within less than an hour and that the ASC-like cells preferentially adhere (see Fig. 3). ASCs from the SVF might selectively adhere to micro-particles of caprolactone, which subsequently can be injected into the degenerated disc.

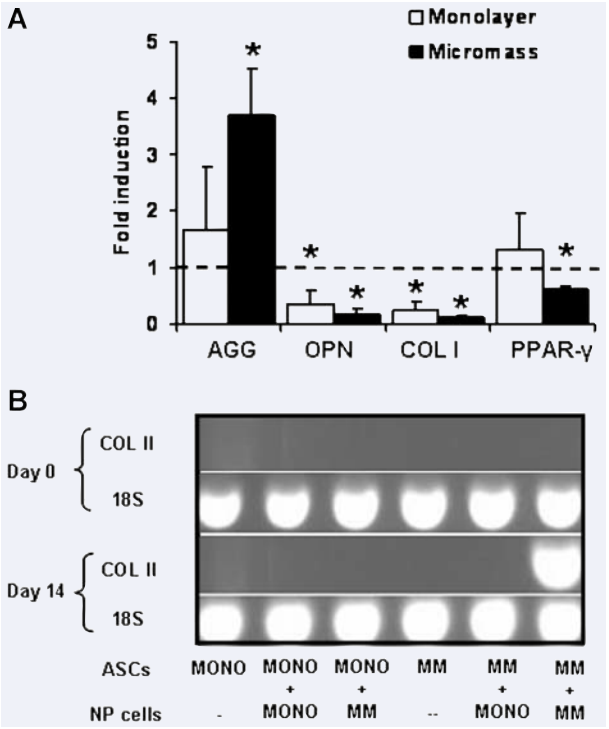


**Fig. 2** Effects of micromass NP cells on the differentiation related gene expression of ASCs in monolayer or micromass. **A**: NP cells only significantly down-regulated the gene expression of osteopontin and type I collagen in monolayer ASCs, but they significantly up-regulated the gene expression of aggrecan and concomitantly down-regulate the gene expression of osteopontin, type I collagen and PPAR-γ in micromass ASCs; the data are expressed as means ± sd, $n$=3; the dash line represents time-point zero. **B**: Gene expression of type II collagen was only induced in the group where both ASCs and NP cells were cultured in micro masses. *: Significant difference ($p$<0.05). NP cells: Nucleus pulposus cells; ASCs: Adipose mesenchymal stem cells. Mono: monolayer, MM: micro mass. AGG: aggrecan, COL II: type II collagen, COL I: type I collagen, PPAR-γ: peroxisome proliferator-activated receptor gamma. (Reprinted from Biochemical and Biophysical Research Communications, vol. 359, Lu ZF, Zandieh Doulabi B, Wuisman PI, Bank RA and Helder MN, Differentiation of adipose stem cells b ynucleus pulposus cells: configuration effects., p. 991–6, 2007 with permission from Elsevier.)

Finally, ASCs will be confronted with the specific hypoxic and acidic environment of the degenerated disc [103, 104]. The influence of hypoxia has been a topic of great interest, because NP cells or chondrocytes grow in a low-oxygen environment. Although there are some contradictory data about the effect of hypoxia on chondrogenic differentiation of MSCs, most studies suggest that hypoxia can promote chondrogenic differentiation [105–107]. The influence of pH on disc cells has been studied less extensively but clearly has a negative effect on the ECM turnover of the NP cells [108].

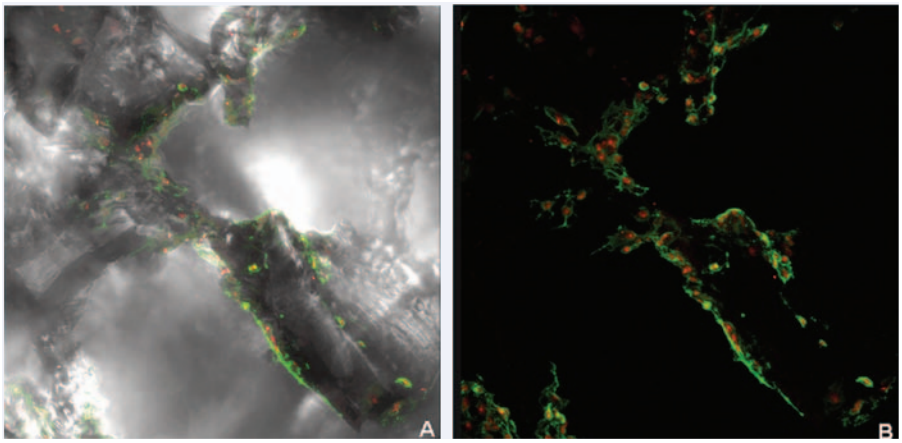

**Fig. 3** Figure A shows a confocal image of SVF cells attaching to the inner pore of a 70:30 Poly(D,L-lactide-co-caprolactone) scaffold. After allowing the heterogeneous mixture of SVF cells to attach to the scaffold for one hour, cells were fixated and stained for CD34 (green). The nuclei of all attached cells were stained with propidium iodide as a counter stain (red). Figure B shows the exact same picture in which the scaffold was not visualized for clarity reasons.

## *in vivo* studies

### Animal models

The complexity of factors involved in regeneration of the intervertebral disc can be studied only partially *in vitro*. Animal models offer the possibility to study the process of degeneration and regeneration over time [109]. Furthermore, *in vivo* studies can be used for a standardized evaluation of biomechanical, histochemical and morphologic characteristics of degenerative processes in the spine [109, 110] and innovative regenerative treatment modalities for disc degeneration can be tested *in vivo* [17, 40]. Several animal models of disc degeneration are currently available [110–114]. However, these animal models, especially small animal models (*e.g.* rat, rabbit), have shortcomings in their comparability to human disc degeneration, in particular with regard to disc geometry and remaining of a certain cell type (notochord cells, see below), even in adult animals [109]. The difference in size between small animal discs and human discs clearly affects the diffusion process, crucial for the oxygenation of disc cells. Larger animal models have been validated as good models of the human disc with respect to biomechanics, geometry, structure and biochemistry, particularly the bovine, ovine and canine models [115–117]. Notochordal cells, however, are present in the intervertebral discs of most of these animals at the age of skeletal maturity, unlike in human beings [118, 119]. Notochordal cells appear to optimize disc matrix synthesis and therefore their presence influences the process of disc degeneration and regeneration [120, 121]. As a natural model for DDD has not been described in a large mammal, our group started to develop a standardized, reproducible DDD model performed with chondroitinase ABC [122]. Most importantly, the animal model must be similar in nature to the human pathologic process that it is intended to mimic. Otherwise, conclusions made from dissimilar animal and human pathologic states may not be clinically appropriate.

### Cells in disc regeneration *in vivo*

Various cell types are currently under investigation for their therapeutic potential for intervertebral disc degeneration. Nucleus pulposus cells were studied in a canine disc degeneration model [35]. Autologous NP cells were isolated, expanded *in vitro* and subsequently returned to an enucleated dog intervertebral disc. The transplanted cells survived, synthesized proteoglycan and disc height was regained [35]. At present, the effect of autologous NP-cell transplantation is being studied in clinical trials as well [123, 124]. Preliminary results after 2 years of follow-up show that reduction of low back pain and prevention of loss of disc height have been achieved with the transplantation treatment [123, 124].

Other strategies for cell-based repair of the nucleus pulposus include the re-insertion of nucleus pulposus [125, 126] or elastic cartilage from the ear [38]. Using different *in vivo* models (rat and rabbit, respectively), in which a disc herniation was induced, the re-insertion of a fresh or cryo-preserved nucleus pulposus was found to prevent the progression of DDD [125, 126]. In another rabbit study, cultured elastic cartilage-derived chondrocytes were injected in a previously reamed nucleus pulposus [38]. After 6 months of follow-up, there was only vital hyaline-like cartilage in the place of the reamed nucleus pulposus and no fibrous tissue. However, for both, autologous disc chondrocytes and elastic cartilage from the ear, an intrusive recovery procedure is required including an *ex vivo* expansion of cells. In case of retrieval of cells from a herniated disc, these cells may be abnormal and only few may be suited for repair.

Few studies have been performed investigating the effect of MSCs on experimentally induced disc degeneration. One group performed several studies in rabbits using a nucleus aspiration model [39–41]. MSCs embedded in a collagen type II gel were injected in the disc [39–41]. MSCs survived over an 8-week period and proteoglycan content was enhanced in the implanted discs [39]. In later studies, implantation of autogenic green fluorescent protein-tagged MSCs also resulted in preservation of annular structure, re-establishing a disc nucleus positive for glycosaminoglycan and keratan

sulfate proteoglycans, as well as partial restoration of disc height and disc hydration [40, 41]. In addition, the authors suggested that the MSCs in the re-established nucleus had differentiated into a chondrocyte-like/nucleus pulposus cell phenotype expressing collagen II, keratan sulfate and chondroitin-4-sulfate [40]. In conclusion, although autogenic MSC implantation could not completely regenerate the disc, it could indeed overcome and counter the degeneration process to some extent. Biological 'triggering' of the MSCs prior to implantation in order to direct differentiation might enhance the possibilities of stem cell therapy [127, 128].

Extending the concept of stem cell therapy further, investigators have exploited the use of allogenic stem cells. This has the added advantage of off-the-shelf availability. Moreover, as the cause of disc degeneration is thought to be multi-factorial, the use of allogenic stem cells could eliminate potential autogenic precipitating factors such as genetic predisposition [11, 129, 130], or the diminished potency of stem cells due to natural aging [131]. In fact, the IVD is suggested to be immune-privileged due to its avascular nature. A study showing that allogenic nucleus pulposus cell transplantation did not elicit lymphocyte infiltration is consistent with this notion [132]. The problem of immune rejection is likely to be even less for allogenic MSCs, since MSCs are capable of escaping allogenic recognition [133, 134]. Allogenic MSC transplantation has been attempted in normal rabbit lumber IVD, with MSCs surviving in the nucleus pulposus for 6 months producing proteoglycan and collagen II, suggesting that allogenic MSCs have similar regeneration potentials as autogenic cells [135]. Allogenic transplantation has also been investigated in normal coccygeal IVD of adult rats [99]. When transplanted in a hyaluronan gel scaffold, bone marrow MSCs survived in the nucleus pulposus over a 4-week period [99]. Thus the potential of allogenic stem cells is worth further investigations using longer timepoints and larger animal models.

## Perspective

Regenerative medicine aims for the replacement, regeneration and remodelling of tissue or the functional enhancement of impaired tissues *in vivo* or to engineer and to grow functional tissue substitutes *in vitro* to implant *in vivo*. For the spine, the ultimate goal is the regeneration of a functional motion segment, consisting of a nucleus pulposus and annulus fibrosis, when the focus is on disc repair. However, DDD is quite complex, involving alteration in nutrition, disturbance in biomechanics, changes in matrix turnover, loss of cells, and in changes and loss of integrity of macrostructures. Such complexities confuse the search for reasonable therapeutic targets. Regenerative medicine building blocks comprise cells, scaffolds and biologics. Biomaterials are designed to promote the organization, growth and differentiation of cells in the process of forming functional tissue by providing structural support, biological containment and chemical clues. Biologics are needed to enhance cell proliferation and differentiation and include growth factors, cytokines and hormones, as well as mechanical signals. Another key element in regenerative medicine is the availability of regeneration competent cells. While cells constitute only 1% of the adult disc tissue by volume, their role in matrix synthesis and metabolic turnover is crucial and therefore a therapeutic strategy could be to replace, regenerate or augment the disc cell population. Despite our imperfect knowledge, several cell-based approaches are in various stages of preclinical and even clinical evaluation [35, 40, 124].

Pre-clinical studies have shown the possibility to direct cells towards the NP-cell-like phenotype for regenerative purposes. When designing *in vitro* or *in vivo* experiments, in our opinion the clinical applicability must be considered. Each culture system has advantages and disadvantages for specific experiments and disc cells behave differently in different systems [136]. The specific questions asked will determine the appropriate experimental model that should be used. Three-dimensional culture systems may be preferable to two-dimensional systems because they promote the retention of the chondrocytic phenotype of NP cells [137] and the induction of NP-cell-like phenotype of co-cultured ASCs (see Fig. 1) [79]. In addition, the microenvironment of the DDD should be considered as degenerated discs have increased levels of proinflammatory cytokines, such as IL-1 and TNF-$\alpha$, as well as a decreased nutrition and low pH and low oxygen tension in the NP [138].

The feasibility of regenerating a degenerated intervertebral disc has been shown by two recent clinical studies in human beings. In one study, fresh frozen composite disc allografts have shown to be an effective treatment for DDD, with good union of the grafts, preservation of motion and stability and without an immune reaction occurring [139]. Another feasible strategy for arresting and reversing DDD is the use of autologous disc chondrocytes as described previously [124]. However, the use of autologous chondrocytes or bone marrow-derived MSCs requires the *ex vivo* expansion of the cells, which is costly, time-consuming and strictly regulated by the FDA, making it an intricate procedure. The use of allogenic progenitor cells would offer a more cost-effective approach. This possibility arises because of claims that MSCs can be successfully allografted [42, 140]. If so, a uniform donor line of these cells could be established and used directly in all suitable patients. Another possibility to circumvent these disadvantages is the use of the one-step procedure, with mesenchymal stem cells obtained from autologous adipose tissue. This concept circumvents these strict and cumbersome regulatory issues by complying with the FDA criteria for minimal manipulation of stem cells [141], thus boosting the feasibility and applicability of stem cell technology in surgical disciplines considerably. Also, clinical costs are reduced if a one-step procedure is available, as the number and duration of hospital admissions may be diminished, as well as the need for expensive stem cell culture facilities. Disease transmission is decreased in a one-step procedure [142], patient discomfort will be diminished as uncomfortable harvesting procedures (BM-MSCs) and successive hospital admissions are not necessary in a one-step procedure performed with ASCs. To further enhance the full potential of ASC disc therapy, future work should be focused on the ways of optimizing the efficacy as well as delineating the biological processes involved. The survival of

transplanted cells can be a limiting factor and therefore the fate of ASCs should be carefully tracked after implantation, with special attention paid to the cell phenotype, induced functions and long-term survival of ASCs. Besides survival and injected cell numbers, biochemical triggering of ASCs, efficient removal or inactivation of degeneration by-products should be considered in future research. ASCs may have to be preconditioned if they are to survive and restore matrix in the harsh environment that is acidic, hypoxic and poor in nutrients of the degenerating disc. Most importantly, the enhancement may simply require 'standard' SVF procurement as SVF of adipose tissue is a mixture of various cells, with varying protein expressions, having the capacity to differentiate into different lineages depending on the involved differentiating-inducing factors and culture conditions. As shown in *in vitro* experiments, the micro-environment of the NP might be a sufficient trigger for ASC to develop into a chondrocyte-like NP cell producing extracellular matrix [73, 79]. At present the impact of this conclusion on cell-based tissue engineering principles of the disc is unknown as, for instance, the use of purified multi-potent SVF with angiogenic potential might also allow better vascularization and tissue growth compared to the unpurified SVF pool. While angiogenesis is favourable in spinal fusion (bone formation), it is not desirable in disc regeneration.

Possibly, survival of the ASCs is not necessarily a prerequisite for a successful regeneration strategy. ASCs might be efficient enough to act as helpers to induce endogenous disc cell proliferation and differentiation, which has not been sufficiently evaluated to date.

## Conclusions

Disc degeneration is a complex issue that involves a myriad of factors and by careful incremental research its mysteries are slowly unravelling. Regenerative medicine concepts have much to offer for orthopaedics in general and disc disorders in particular, aiming to re-establish tissue structural properties. SVF-based treatment concepts for a variety of DDD indications are under development and might be used single or in combination with biologics and scaffold materials, either in a one-step (preferable) or in a multi-step procedure. For clinical application, these concepts should not only be effective, but also safe and affordable, as degenerative disc disease will dramatically increase in the near future posing a large economic burden on the health care system.

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