

Title page

Title: Cell-based Regeneration of Intervertebral Disc Defects: Review & Concepts

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## ABSTRACT

During the last century low back pain has been emerged as a widespread disease often caused by intervertebral disc degeneration (IDD). IDD in turn is a complex problem, in which a variety of causes play a crucial role. As IDD causes high costs, a corporate interest leads to a number of therapies developed. Today, these therapies focus on the restoration of the IVD function and not only on minimizing the pain caused by this disease.

These approaches are often biological and aim to stimulate the regeneration of the intervertebral disc by injection of activator proteins, biomaterials, different cell types or complex cell-matrix-composites. Furthermore, the genetic engineering of disc cells and the in vitro tissue engineering offer a possibility for curing IDD. This article gives an overview on the concepts mentioned above.

## KEYWORDS

intervertebral disc regeneration

cell transplantation

mesenchymal stem cell

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Low back pain (LBP) is one of the most common musculoskeletal disease [1, 2]; it is estimated that 84% of the population will experience LBP at some point in their lifetime [3, 4], reaching a maximum rate of incidence in the 45- to 64-year-old age group [5, 6]. In addition to direct costs for LBP treatment, indirect costs, such as loss of productivity, are enormous. The causes for LBP are complex and of different origin, but one main reason for LBP is the degeneration of the intervertebral disc.

## IVD BIOLOGY AND DEGENERATION

The intervertebral disc (IVD) is a fibrocartilagineous structure, composed of a central nucleus pulposus (NP) surrounded circumferentially by the annulus fibrosus (AF) (Fig.1). Each component is populated with different cell types and differs in the composition of the extracellular matrix (ECM) produced by the cells. The NP cells are round and lays within a lacunae (chondrocyte-like) while the AF cells are more fibroblastic and elongated. The ECM produced by the NP cells is rich in proteoglycans (PGs), predominantly aggrecan and type-II collagen, whereas the AF cells produce a matrix which is rich in type-I collagen with little PG or type-II collagen [7]. Further on, the IVD is avascular so that nutrient supply is restricted to diffusion.

The degeneration of IVD is multi-factorial and influenced by age and genetic loading [8], biomechanical [9, 10] and environmental factors such as immobilization, trauma, consumption of tobacco [11], diabetes, vascular and infectious diseases [12]. Healthy discs are characterized by a balance between anabolic and catabolic processes which are regulated by anabolic growth factors (e.g. IGF1 [insulin-like growth factor 1], TGF $\beta$ s [transforming growth factors  $\beta$ ], BMPs [bone morphogenetic proteins]) [13] and to some extent by notochordal cells [14] or rather by catabolic enzymes like MMPs (matrix metalloproteases) [15, 16] and ADAMTS (a disintegrin and

metalloprotease with thrombospondin motifs) [17] as well as proinflammatory cytokines (e.g. IL-1 [interleukine-1], TNF- $\alpha$  [tumor necrosis factor  $\alpha$ ]) [18]. In the course of IVD degeneration (IDD), changes in IVD morphology [19] and matrix composition i.e. the loss of proteoglycans and type-II collagen [20] as well as an increase of cell death [21] and a decrease of nutrient diffusion [22] are observed. This is often caused by a metabolic imbalance that means an up-regulation of inflammatory mediators and MMPs [23], the accumulation of regulatory matrix degradation products (e.g. fibronectin fragments) [24, 25] and reactive oxygen species [26] which seem to be responsible for the inhibition of matrix synthesis and repair.

## CELLBASED THERAPY OF DEGENERATED IVD

Traditional treatment to manage IDD and discogenic pain include surgical intervention with total disc excision or minimal invasive procedures [7]. These treatments can reduce the pain, but are not capable of regenerating the discs. Therefore, lots of outcomes including several biological approaches have been focused on restoring the IVD structure and function (Fig. 2).

### *Release of Growth Factors*

The synthesis of matrix components and the proliferation of the IVD cells are influenced by several growth factors and enzymes [13]. Thus the direct injection of activators such as pure protein solution or as a combination with a slow-release matrix is an easy way to stimulate IVD regeneration. Members of the TGF $\beta$  superfamily are potent stimulators of IVD restoration. The addition of recombinant TGF $\beta$  and epidermal growth factor has been demonstrated to increase the

proteoglycan synthesis of canine cells severalfold [27, 28]. Promising results have been shown by the injection of BMP7. After an injection of BMP7 into the discs of rabbits, IVD proteoglycan synthesis increased and the disc height was restored, whereby this effect was stable over eight weeks [29]. Similar results were observed in a rat model [30]. Treatment of disc cells with GDF5 (growth differentiation factor 5) has been shown to stimulate the extracellular matrix synthesis in vitro and restore the disc structure in a rabbit disc-injury model in vivo [31].

Often, the assignability of results from an animal model to patients is problematic. In this case, a pilot study led to similar conclusions for humans. After patient treatment with a mixture of matrix components and growth factors via direct injection, an induction of IVD regeneration over 13 months was seen [32]. This indicates that direct injection of active substances could offer a possibility for IVD regeneration. Nevertheless, the effort of this technique is limited by the presence of viable cells and likely to be suitable for early or moderate stage of IDD. Furthermore, this form of stimulation is only short-term.

### *Gene Therapy*

For a long-term stimulation, it would be more effective to integrate the activator directly into the genome of disc cells (gene therapy). The genetic change can occur in vivo (i.e. direct transfection) or ex vivo (i.e. removal of the cells, transfection in vitro and return of transfected cells into IVD) [33], but due to safety reasons, the ex vivo gene therapy is mostly privileged. The gene of the activator has to be transported into the cell by a vehicle which can be a virus or a nonviral carrier. The nonviral gene transfer is more inefficient [34], that's why viral gene transfer is preferred. Unfortunately the used adenoviruses often cause strong immune reactions in vivo [35, 36]. To minimize these reactions, adeno-associated viral vector are used, which

has already shown positive results for IVD regeneration in vivo [37]. Adeno-mediated therapy of human disc cells with transcription factors (e.g. Sox9 [(sex determining region Y)-box 9]), growth factors (e.g. TGF $\beta$ , BMP2) and anabolic enzymes (e.g. LMP1 [latent membrane protein 1], TIMP [tissue inhibitor of metalloproteinases]) led to some restoration of IVD structure and increased synthesis of proteoglycans and collagen [33, 38, 39]. Moreover, the combination of growth factors showed synergistic effects on the expression of different IVD markers in human disc cells in vitro [40]. Despite promising results, this approach has some limitations. First, up to now little is known about the influence on regulatory pathways caused by unlimited and uncontrolled release of growth factors. Second, for a successful gene transfer healthy autologous disc cells are needed but these are limited in degenerated IVDs. That means it is questionable, if degenerated discs contain enough viable cells and if after transfection these cells are potent enough to express a sufficient amount of growth factor in the degraded IVD of the patient.

### *Tissue Engineering*

For intense degeneration of IVD (grade V) with loss of cell proliferation and disc structure a complete replacement of the IVD will be necessary. Therefore, tissue engineering, that means the cultivation of disc cells on a 3D scaffold, could be a possible approach. Up to now, it is not possible to create a functional IVD in vitro, but the development of several biomaterials imitating properties of IVD and an increasing understanding of disc cell biology mean that tissue engineering of the IVD may soon become reality [41]. There are lots of investigations concerning in vitro cultivation of disc cells on a 3D scaffold ongoing. Similar to the natural conditions several forms of stimulation, like the application of pressure [42-44] and/or other physical forces [45] are used during cultivation. A variety of studies have been described improving the

tissue engineering of AF cells [46-49], NP cells [50, 51] or both cell types [52]. Despite some optimistic results, this technique is still far from clinical use. One major hurdle of current in vitro engineered disc replacement is an insufficient biomechanical behavior, which is not comparable with natural IVDs. Furthermore, the insertion of the engineered tissue generates spacious injuries and the integration of the new tissue into the existent IVD is often inadequate.

### *Cell Transplantation*

Minimally invasive methods of IDD treatment are focused, because of the limitations of in vitro tissue engineering mentioned above. Often these minimally invasive methods use the patient body as a kind of bioreactor for the cultivation of cells. Such a minimal invasive technique to regenerate IDD is the injection of viable cells. It has been shown, that the supplementation with autologous mesenchymal stem cells (MSC) [53-56], NP cells [57, 58] or chondrocytes [59, 60] demonstrates regenerative effects concerning IDD in rabbit models. Moreover, in a clinical pilot study for disc repair with autologous chondrocytes, patients showed stimulated matrix regeneration and a relief of pain after cell transplantation [61]. Although autologous cells have the advantage of causing no immune response, the extraction of appropriate cells in sufficient amounts is difficult. For instance, the density of NP cells in IVD is low and these cells as well as chondrocytes cannot be expanded in monolayer cultures because they lose their phenotype characteristics [62, 63]. Furthermore, additional surgery is needed and potential genetic dispositions are still possible.

The avascular structure of the IVD determines its immune privilege. Even the injection of allogeneic NP cells causes no infiltration of lymphocytes [64]. That is why allogeneic cells in particular MSCs are an attractive source for IVD degeneration. Adult MSC are pluripotent stem cells that have been found in almost every organ in

adulthood [65]. These cells are of high plasticity and have the capacity of multilineage differentiation [66]. In addition, they are accessible in sufficient quantities from bone marrow [67] and fat tissue [68] and comparably easy to expand and manipulate [69] which make them ideal candidates for cell-based IVD regeneration [70]. Furthermore allogeneic MSCs are off-the-shelf available, which means the time span for cell isolation and expansion is omitted. Moreover, the use of allogeneic MSCs eliminates potential genetic dispositions and limited potency dependent on the age of the patient.

Indeed, the injection of pure cell solution led to extensive leakage of these cells through the injection site [71]. It is thought that this leakage is caused by inner disc pressure. Despite promising results in animal models in vitro and in vivo and a pilot study with hematopoietic stem cells [72], an injection of MSC into the disc of humans has never been done. Currently it is still unclear to what extent such a therapy is efficient enough to reduce discogenic pain of LBP patients.

### *Matrix-assisted Cell Transfer*

Compared to pure cell injection, it is possible to inject biomaterials which are ideal to restore the disc volume. Requirements for suitable materials are mechanical stability, biocompatibility and biodegradation, sterilizability and a low viscosity for injection devices. In general, biomaterials are injected as fluids which polymerize in the disc by crosslinking or addition of e.g. agarose [73]. Thereby, it is important that the polymerization is slow enough for injection and fast enough to prevent leakage of the material. To date analyzed biomaterials for IVD regeneration in certain animal models are silicones [74], chitosane [75], aldehyde-linked BSA [76] or components of ECM like hyaluron, fibrin [77], collagen [78] or silk-elastin-copolymers [79, 80]. The sole injection of biomaterials can only restore the disc volume but hardly its function.



Under circumstances functionality can be restored by injection of ECM components. It is known that several ECM components interact with the disc cells and influence their behavior [81]. Unfortunately, in the advanced stage of IDD often no viable cells are left to be stimulated.

Consequently, a better effect could be reached, if biomaterials are used for matrix-assisted cell transfer, injected as a mixture of cells and matrix. In this case, the gel-like matrix prevents the leakage of the cells as shown in a study applying a mixture of fibrin/thrombin and HeLa-cells [71]. Furthermore, the matrix itself could stimulate ECM synthesis and cell proliferation. It is known that a close regulation is available between disc cells and their surrounding matrix [82]. To date, it is not known to what extent the ECM is capable to stimulate cell differentiation as well.

## SUMMARY AND FUTURE PROSPECTS

The intervertebral disc has been shown to be a very unique, highly specialized tissue that undergoes massive alteration during degradation. To fulfill its natural function, the IVD needs a mechanically stable structure with a defined ECM to confer flexibility, as well. IDD caused by diverse circumstances creating a hostile environment, resulting in cell death and concomitant to a variation in matrix composition and finally to an extensive matrix decay. IDD is a global problem connected with enormous health restrictions and high costs.

Therefore, a variety of therapeutically approaches has been developed trying to cure IDD patients. Despite lots of research in this area, an ideal treatment is not available yet. The most promising approach for IVD regeneration seems to be the matrix-assisted cell transfer. Due to of diverse advantages, MSC are thought to be the appropriate cell source for that technique. During the last years, the importance of signaling between matrix and cells has been noticed [83]. Up to now, it is not known

how intense matrix components influence the differentiation or behavior of MSC. Because cells react on alterations in matrices via several surface receptors, it is possible that certain matrix components can induce differentiation of MSC. Although the presumption exists that MSCs can differentiate into disc cells, this has never been demonstrated. One impediment to confirm differentiation into a disc cell is our inability to identify these cells; there are no robust molecular, biochemical or biologic markers known. Up to now, disc cells are treated as chondrocytes although they clearly differ from this cell type [84]. That means that the markers for disc cells are the same as for chondrocytes. These markers like proteoglycans, type II collagen etc. will so far be determined offline via staining or RT-PCR, which points another hurdle of cell-based therapy. There is a need in improvement of real-time observation techniques of cell proliferation and differentiation status as well as matrix production during cultivation in vitro or after application in vivo.

The use of MSC as a cell source led to some open questions, too. It is not known which state of differentiation is needed for cell survival because undifferentiated MSC are not capable to survive in the rough IVD environment [85]. In addition, only differentiated or partly differentiated MSC can produce ECM, which is required for IVD regeneration. Due to the regulation and interaction of matrix and cells is quite complex, our knowledge is not sufficient to estimate all requirements to realize IVD regeneration.

A complete IVD regeneration also needs the restoration of nutrient supply which is supported by the intervertebral endplates [73]. The solution of this problem is as complex as the regeneration of the basic disc structure and function. Finally many questions from different investigation fields have to be answered until IDD can be cured.

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Figure 1

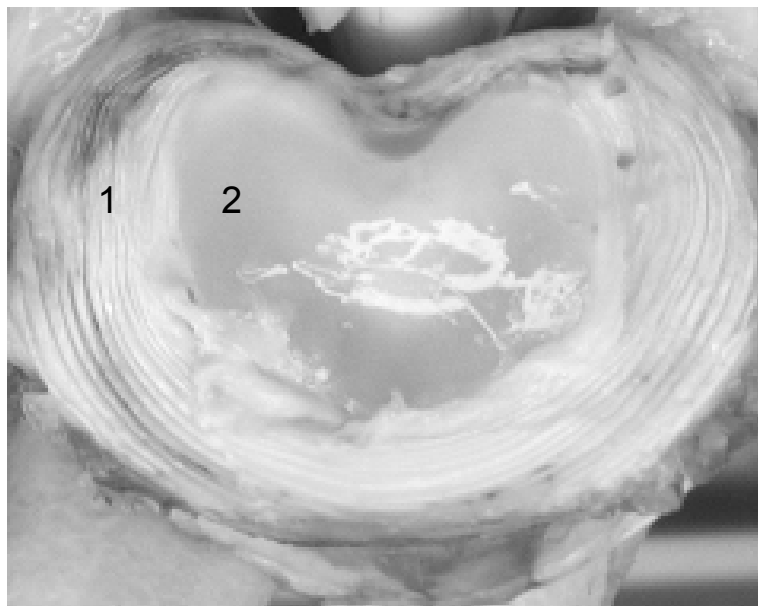
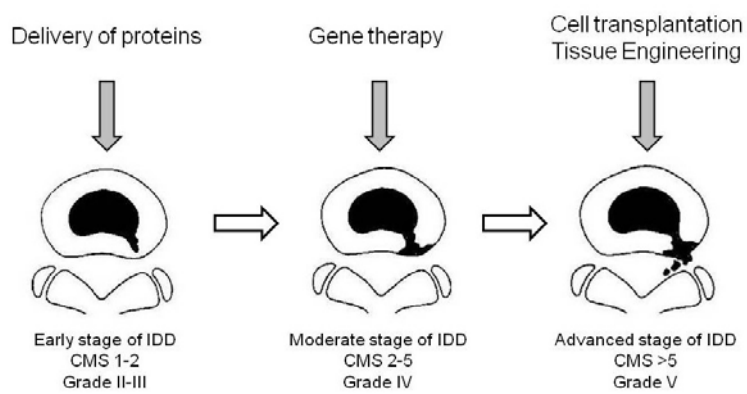


Figure 2





## Legends

1

Intervertebral disc with distinct regions. The outer region (1) is the annulus fibrosus and the inner region (2) the gelatinous nucleus pulposus. The picture was kindly provided by Prof. Dr. Stephanie Gokorsch.

2

Strategies for biological disc repair at different stages of intervertebral disc degeneration (IDD). The classification of IDD via morphological changes (grade) is based on the work of Thompson et al. [86]. The classification of IDD via CMS (composite MRI score) was done by Benneker et al. [87].