# Cell-based therapies for the regeneration of the intervertebral disc: promises and challenges

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## ABSTRACT

Intervertebral disc (IVD) degeneration (IDD) has been yet inextricably associated to the manifestation of low back pain, a major cause of disability with a vast socioeconomic impact worldwide. IDD treatment has been challenging given that IDD is characterized by a constellation of changes, major among them being the reduction in cell number and the modification of the cellular phenotype and function, ultimately contributing to tissue structural breakdown. As alternative options to the conservative and surgical approaches that only target IDD symptoms, injection of bioactive substances, gene therapy or cell transplantation have been attempted with some encouraging results even though no complete restoration of the injured tissue has been achieved thus far. In this short review we discuss the effect of the particular IVD environment (a combination of nutrients' and oxygen deprivation, mechanical and oxidative stress, high osmolality and acidic pH) on several parameters of the physiology of the resident or implanted cells that should be taken under consideration for a successful regenerative intervention. The role of cells' senescence in IVD physiology is also discussed as a putative novel therapeutic target for IDD. Deep understanding of the molecular alterations underlying IVD cells' responses could lead to more effective IDD treatment modalities.

**KEY WORDS: intervertebral disc, low back pain, cell-based therapy, gene therapy, senescence**

### 1. Therapeutic strategies for the treatment of intervertebral disc degeneration

Intervertebral disc (IVD) degeneration (IDD) with a yet established incrimination in the aetiology of chronic low back pain (LBP) [1, 2] represents the leading cause of disability, activity limitation and loss of productivity in the adult population in Greece [3] and worldwide [4, 5].

IVDs, charged to play the role of suspension for the spine, intervene between vertebrae, with direct adjacency to the superior and inferior cartilage endplates. They consist of an outer layer of concen-

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trically arranged fibrous lamellae (containing cells similar to fibroblasts) and a gelatinous core (with chondrocyte-like cells), namely annulus fibrosus (AF) and nucleus pulposus (NP), respectively [2]. In addition, native IVD stem/progenitor cells, expressing a set of mesenchymal stem cells' surface markers, have been isolated from human degenerated discs [6]. The IVD is mostly extracellular matrix (ECM) characterized by a rigid AF collagenous network that encapsulates a well-hydrated NP proteoglycan (mainly aggrecan) matrix [2]. The negatively-charged IVD ECM and the diurnal compressive load-driven water loss due to posture and other activities constantly expose IVD cells to extreme variations in extracellular osmolality [7, 8]. In addition, the avascular nature of the tissue leads to oxygen deprivation, nutrients' deficiency, acidic pH and accumulation of IVD cells' metabolic byproducts and oxidative stress [7, 9]. As a consequence of this harsh microenvironment, a very low number of cells are embedded in the IVD ECM [2, 7, 10], with a pivotal role though in maintaining disc homeostasis, since they are the producers of ECM molecules, as well as of the ECM-degrading enzymes [e.g., matrix metalloproteinases (MMPs) and a disintegrin and metalloproteinase with thrombospondin motifs (ADAMTSs)].

IVD degenerative changes concern the number, phenotype and secretome of IVD cells, the accumulation of inflammatory mediators and the disorganization of the ECM [11, 12], characterized by depletion, cross-linking and oxidation of collagen and lower aggrecan content, which all lead to greater stiffness and progressive dehydration [12-14]. Furthermore, cell number is reducing due to apoptosis at the same time that cell clusters are appearing possibly due to the degradation of the surrounding restrictive ECM. IVD ECM structural breakdown ultimately allows disc herniation and nerve intrusion that lead to LBP. Current IDD treatments such as administration of analgesics, non-steroidal anti-inflammatory drugs and opioids, exercise, physiotherapy and spinal manipulation for rehabilitation mostly target symptoms' alleviation without addressing the causes of the disease [12, 15]. On the other hand, invasive disc and spinal surgical procedures (discectomy, spinal fusion or arthroplasty) stand as the last recourse as they are high-cost and in many instances non-effective or even risky for post-operative complications [12, 16, 17].

In an attempt to override the limitations of the hitherto employed therapeutic strategies against IDD, injection of bioactive substances, genetic interventions or cell transplantation could serve as promising alternative options [12, 15]. One of the first approaches was based on the injection of growth factors in the degenerated disc, since these molecules induce not only disc cell proliferation and survival, but also the local production of ECM constituents by the cells [18, 19]. Indeed, disc cells secrete growth factors to which they respond with the activation of pivotal signalling pathways leading to cell proliferation [20-22]. Some of the growth factors that have been investigated in animal models against experimentally induced IDD include TGF-β, IGF-I, basic fibroblast growth factor (bFGF) and various bone morphogenetic proteins (BMPs), with BMP-14 or growth and differentiation factor-5 (GDF-5) [23, 24], while natural mixtures of multiple growth factors, such as platelet-rich plasma (PRP) have been also proposed for such use [25, 26]. Among the disadvantages of this approach are its high cost, the *in vivo* proteolysis of growth factors and the possible adverse effects due to enhanced angiogenesis in the IVD. Still their use *in vivo* could be possible in conjunction with appropriate biomaterials offering the capability of controlled release [17]. Unfortunately, the injection of growth factors (e.g., GDF-5 and BMP-7) and other bioactive substances (e.g., the IL-6 receptor antibody tocilizumab and the TNFα selective inhibitor Etanercept) had no conclusive results in most cases so far [15, 17].

Gene therapy - that is the *in vivo* or *ex vivo* genetic manipulation of cells aiming at the modification of the deduced encoded products at the RNA and protein level - can be carried out using viral or non-viral vectors. Furthermore, genetic engineering techniques employed for gene therapy could be RNA interference or the recently discovered state-of the-art clustered regularly interspaced short palindromic repeats (CRISPR) [27]. TGF-β1, TGF-β3, connective tissue growth factor (CTGF), BMP-2, BMP-7, IGF-I,

latent membrane protein (LMP)-1, SRY-box transcription factor (SOX)-9 and tissue inhibitor of metalloproteinases (TIMP)-1 delivery resulted in significant anabolic effects and increased ECM deposition [27, 28]. Despite these auspicious findings, skepticism remains regarding the usage of viral vectors in clinical applications in humans due to the existing risk of insertional mutagenesis and immunogenicity [29-31]. On the other hand, miR-29a, miR193a-3p, miR93, miR146, mR146a have shown ECM-promoting or anti-inflammatory properties [17, 27]. Small interfering RNA (siRNA)-mediated knockdown has been used to target Fas ligand, ADAMTS-5, caspase-3 and mTOR *in vitro* and/or *in vivo* [27, 28]. CRISPR genome and epigenome editing have been also endeavored with some positive results [32, 33]. Non-viral gene therapy methods seem to be safer, but still have the disadvantage of lower transfection efficacies compared to viral vector methods [34].

**2. Challenges for a successful IDD cell-based therapy**

As mentioned earlier, one of the initiating events of IVD degeneration seems to be the decline in the resident IVD, and especially NP, cell number, which disrupts the balance between anabolic and catabolic processes in ECM synthesis. Taking this into account, punctual NP supplementation by direct transannular or transpedicular intradiscal injection with functional cells - owning themselves or stimulating in the resident cells a desired ECM-restoring and/or anti-inflammatory phenotype - can offer a potential solution for preventing or delaying IDD. Available cell sources for IVD cell-based therapies are autologous and allogeneic NP cells or articular chondrocytes; mesenchymal stromal cells (MSCs) able to both replenish the number of NP cells and to stimulate NP reconstruction; induced pluripotent stem cells (iPSCs) [17, 35]. Although autologous NP cells would be the ideal foolproof selection, followed by articular chondrocytes, their low availability and proliferative potential or already acquired catabolic phenotype along with their high prevalence for de-differentiation when cultured *in vitro* have rendered them challenging or sometimes unsuitable candidates for cell therapy. For that reason, the requirement for alternative options, such as NP and chondrocytic cells of allogeneic origin or MSCs and iPSCs, has emerged. Adult stem cells may contribute to IVD regeneration either by their differentiation into NP-like cells or by acting as feeders that induce the up-regulation of ECM synthesis by their native NP counterparts [36]. IVD progenitor cells also hold prospects for their potential use in IDD treatment [6, 12]. It is intelligible that in favor of using cells of allogeneic origin is that the patient is only subjected to one-step surgery, but the risk of stimulating an immunogenic effect always exists. Then again, the use of MSCs or iPSCs involves the peril of tumor formation [17]. As already mentioned above for growth factors, the use of biomaterials seems to be necessary for cells' delivery in the disc, as well. These include hydrogels based on proteins (e.g. collagen) or polysaccharides (e.g. alginate) [37, 38], composite systems, such as a collagen hydrogel supplemented with chondroitin sulfate [39], hydrogels cross-linked or in the form of microparticles and natural materials [40]. The first clinical trials based in the use of autologous or allogeneic MSCs resulted in pain relief. Clinical studies using discogenic cells, autologous disc chondrocytes or MSCs combined with biomaterials have been also conducted [14, 17]. Still, there is no until now strong evidence to support the preference of anyone of the cell sources.

An important step for the refinement of IVD cell therapy is the determination of the optimal timing and expedient precise cell number for intradiscal delivery (accounting for the putative cell leakage during injection at the delivery site and/or the cytotoxicity ensuing from the shear forces applied by the needle or from the harsh conditions of the final destination) in order to achieve maximal benefit. It is, for instance, important to apply the treatment when the grade of degeneration is still low, prior to the launching of an advanced and irreversible IDD to expect a possible successful regenerative effect. In addition, given that implanted cells (irrespective of the source) not only need to be able to survive but also to be functional and to produce ECM of the desired quality, it is essential to consider the hostile local IVD microenvironment, which worsens with the progression of degeneration [35].

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*IVD cells' responses to inflammatory cytokines*

Inflammatory mediators including interleukins (ILs) and TNFα have been shown to be expressed in the human NP and what is more their expression along with the expression of their receptors increases with age and in symptomatic and degenerated discs [41, 42]. ILs and TNFα have been reported to exert a catabolic/anti-anabolic effect in the IVD [43] [41]. We have shown that TNFα up-regulates MMP-3 expression in bovine NP cells, which is attenuated by the presence of glucosamine [44].

*IVD cells' responses to mechanical stress*

Mechanical loading is indissolubly connected with IVD homeostasis [45]. We have shown that cyclic tensile stress stimulates the expression of the pro-inflammatory genes, cyclooxygenase-2 (COX-2), IL-6, and IL-8 in AF IVD cells, mediated by members of the MAPK superfamily [46]. Moreover, changes in type II collagen expression and altered proteoglycan synthesis have been reported as a response to the application of mechanical loads and hydrostatic pressure [45].

*IVD cells' osmo-regulatory response*

High osmolality raises a torrent of biochemical events in NP IVD cells, as shown by our whole-genome array analysis, revealing the simultaneous transcriptional change of >200 genes [47]. We have shown that this stress is genotoxic and has an anti-proliferative effect on NP cells [48, 49]. In addition, high osmolality restrained the mitogenic effect of platelet-derived growth factor (PDGF) or IGF-I via ERK and Akt activation [50]. This strict control of hyperosmolality on the proliferation of NP IVD cells is retained even after the administration of glucosamine, shown to result in an increase in the glycosaminoglycan content [51]. Regarding ECM components, it has been reported that aggrecan and collagen type II were up-regulated, while collagen type I expression was inhibited by high osmolality in human IVD cells [45].

*IVD cells' responses to oxidative stress*

The presence of oxidative stress in the IVD has been established *in vivo* [9, 52-54]. We have shown that oxidative stress activated survival and stress signalling pathways in human NP cells, while it proved to be genotoxic, triggering the activation of the DNA repair response [55]. Oxidative stress-induced NF-κB activation has been also shown in the human NP *in vivo* [42].

Moreover, we have shown that a combination of all IVD conditions (i.e. low glucose, hypoxia, high osmolality and absence of serum) is anti-proliferative for IVD cells [56] and it has been reported that a concurrent exposure to low glucose, acidic pH and hypo-osmolality down-regulates the expression of ECM components and up-regulates the expression of MMPs [45, 57].

**3. IVD cells' senescence**

A key step for the elucidation of IDD-related modifications in the IVD tissue microenvironment was the discovery of senescent cells in IVDs *in vivo*, first reported by Roberts et al. [10, 58] and later verified by other groups [59, 60]. There are two types of cellular senescence: the "replicative senescence" attributed to telomere attrition arising from the consecutive replications of the cells and the "stress-induced premature senescence" (SIPS) manifested as the result of several genotoxic stresses encountered by the cells [10, 13]. Given the restraining physicochemical conditions of the IVD microenvironment [61], senescence in the IVD is most probably stress-induced rather than replicative [10]. Beyond their enlarged and irregular shape and their inability for proliferation, senescent cells are characterized by a catabolic and pro-inflammatory phenotype namely the "senescence-associated secretory phenotype" (SASP) (consisting of soluble inflammatory mediators, proteolytic enzymes or growth factors and insoluble ECM components) [13, 62, 63] that may contribute to the IDD-associated tissue remodelling. We have shown that senescent human NP cells up-regulated MMPs and ADAMTSs and down-regulated aggrecan, biglycan, decorin and versican [55, 64]. MMP-1 has been also shown to be up-regulated in line with the degree of the deformity in an experimentally induced scoliotic deformity rat model [65]. This senescence-induced catabolic phenotype of the IVD cells has been confirmed using several means of se-

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nescence induction, as well as in a progeria mouse model *in vivo* [13]. Most importantly, we recently demonstrated that the IVD cells' senescent phenotype is maintained when cells are cultured under the actual conditions they face *in vivo* (hyperosmolality, low oxygen and glucose concentration and serum starvation), which supports their possible implication in IDD [56].

The implication of senescent cells in age-related diseases and the improvement of tissue homeostasis by their elimination have been recently experimentally supported by using the p16-3MR transgenic mouse model in which the $p16^{INK4a}$-positive senescent cells can be removed by ganciclovir [66]. Reducing the number of senescent cells in aged mice increased IVD proteoglycan matrix content, thus improving the histological features of the disc [67] and indicating that cellular senescence could be a therapeutic objective for IDD. However, the above-mentioned approach cannot be applied to humans. A recently developed alternative is the use of new class of drugs that can selectively kill senescent cells (senolytics) or reverse the inflammatory phenotype of senescent cells (senomorphics). Senolytics activate the apoptotic machinery in senescent cells. Interestingly, the combination of the first senolytics discovered, i.e. the well-known anticancer drug Dasatinib and the natural flavonoid Quercetin led to an increase of proteoglycans in the NP of prematurely aged transgenic animals [68], while the MDM2 inhibitor RG-7112 and the natural anti-oxidant and anti-inflammatory compound o-Vanillin express senotherapeutic properties in IVD cells and an *ex vivo* model [69, 70]. The above indicate a novel, non-invasive, approach for preventing or treating IDD and LBP.

## 4. Conclusion

Based on the above, it becomes unambiguous that IVD microenvironment is a parameter that must be taken into account in the design of cell-based therapies. The heretofore carried out pre-clinical and clinical trials using NP cells, chondrocytes or MSCs had already some encouraging results [14, 17]. Better survival in the disc environment and improvement of the clinical success for patients could be achieved by preconditioning of exogenous cells prior to implantation (e.g. under hypoxic and acidic conditions and with culture medium enriched with growth factors), CRISPR-mediated knockout (e.g. of cytokine receptors to reduce inflammatory responses or of cell cycle regulators to delay senescence) and knockin (e.g. of ECM components) or co-administration of senotherapeutics [35, 71]. Thus, more efficacious therapeutic options could be developed in the future, involving the joint application of appropriate cell sources, targeted genetic manipulations, bio-active substances and bio-compatible scaffolds.

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***Conflict of interest***

*The authors declare no conflicts of interest.*

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