



# Intervertebral disc regeneration: From cell therapy to the development of novel bioinspired endogenous repair strategies<sup>☆</sup>

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## ABSTRACT

Low back pain (LBP), frequently associated with intervertebral disc (IVD) degeneration, is a major public health concern. LBP is currently managed by pharmacological treatments and, if unsuccessful, by invasive surgical procedures, which do not counteract the degenerative process.

Considering that IVD cell depletion is critical in the degenerative process, the supplementation of IVD with reparative cells, associated or not with biomaterials, has been contemplated. Recently, the discovery of reparative stem/progenitor cells in the IVD has led to increased interest in the potential of endogenous repair strategies. Recruitment of these cells by specific signals might constitute an alternative strategy to cell transplantation. Here, we review the status of cell-based therapies for treating IVD degeneration and emphasize the current concept of endogenous repair as well as future perspectives. This review also highlights the challenges of the mobilization/differentiation of reparative progenitor cells through the delivery of biologics factors to stimulate IVD regeneration.

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## 1. Introduction

Low back pain (LBP) is a major public health concern. Approximately 650 million people are currently affected in the world and its socio-economic cost is increasing with population aging [1–3]. In the United States of America, the annual cost of chronic LBP exceeds \$30 billion, which is in excess of the combined cost of stroke, respiratory infection, diabetes, coronary artery disease, and rheumatoid disease [4–6]. Moreover, LBP is the second most frequent cause for hospital visits and the leading cause of years lived with disability (the prevalence of a disorder multiplied by the short- or long-term loss of health associated with that disability) [7].

LBP is frequently associated (~40%) with intervertebral disc degenerative disease (DDD) and is commonly named discogenic lumbalgia [8]. Intervertebral discs (IVD) are fibrocartilaginous tissues connecting the vertebral bodies. IVD are important to spinal function as they provide stability between vertebrae while permitting motion. The central part of the IVD, the *Nucleus pulposus* (NP), forms the hydrogel-like core of IVD. NP is primarily composed of proteoglycans and type II collagen fibers and its elastic function distributes hydraulic pressure in all directions within each IVD. NP is surrounded by the peripheral *Annulus fibrosus* (AF). The structure of the AF is characterized by concentrically arranged lamellae composed of type I and type II collagen fibers, as well as elastin fibers, which help withstand compressive forces and hold the NP in place during compression. Finally, NP and AF are sandwiched by the cartilaginous endplates (CEP). The CEP is a hyaline cartilaginous tissue that joins IVD with the adjacent bony vertebrae, ultimately providing intervertebral joints with functional continuity. DDD commonly involves changes in the IVD morphology as a result of the qualitative and quantitative alteration of the extracellular matrix (ECM) composition and a loss of resident NP cells. It is well established that a young and healthy NP tissue contains both nucleopulocytes (NPCy; formerly named chondrocyte-like cells), mostly involved in ECM synthesis, and notochordal cells (NTC), which are known to play a pivotal role in IVD development, growth, and homeostasis (Fig. 1). The decrease in resident NP cell number is likely to be one of the initiating events of IVD degeneration. As a consequence, the balance between anabolic and catabolic processes in ECM synthesis is disrupted, which ultimately leads to the onset of a degenerative process associated with biomechanical modifications and discogenic lumbalgia [9].

Currently, discogenic lumbalgia is managed by pharmacological treatments and, if unsuccessful, by invasive surgical procedures (spine fusion or arthroplasty) as a last resort. The clinical success rates following spine fusion is generally reported to be between 50% and 70% [10–12]. An additional morbidity associated with spine fusion is the development of adjacent level degeneration that is often associated with additional surgery [13–16]. Moreover, the cost of spine fusion surgery is substantial and includes additional costs associated with the long recovery time, and possibly from permanent impairments. Regarding arthroplasty (total disc replacement, TDR), a meta-analysis of randomized controlled trials showed a similar safety and efficacy for TDR compared with spine fusion at 2-year follow-up with TDR superior in improving physical function, decreasing pain, and shortening the duration of hospitalization [17]. Nevertheless, an earlier systematic review suggested that the spine surgery community should be prudent in

adopting arthroplasty on a large scale because complications may occur after some years [18].

Faced with the limitations of these treatments, and considering the recent knowledge on the mechanisms underlying the pathophysiology of DDD, notably the critical role of resident NP cell depletion in IVD degeneration, regenerative medicine offers new hope for the treatment of DDD [19–21].

Approaches based on NP supplementation (cell therapy) with functional cells, associated or not with biomaterials, were first developed approximately twenty years ago and now offer a potential solution for the prevention of DDD. Various pre-clinical studies have been carried out and have partially confirmed the proof of concept of such a regenerative cellular approach [22–25]. In parallel, the effectiveness of cell therapy in human pilot studies has also been evaluated [26].

Recently, the discovery of cells exhibiting stemness properties and residing in a multitude of tissues/organs has substantiated theories about the presence of populations of reparative cells in developed organisms and has led to a growing interest in the development of endogenous repair strategies [27]. Interestingly, such stem/progenitor cells have been recently discovered in the IVD vicinity and within the NP [28–36]. Nevertheless, their role in regenerative or repair endogenous processes remains poorly understood. It is however tempting to speculate that they could constitute a reservoir of reparative cells potentially able to reverse or slow down DDD [28–36].

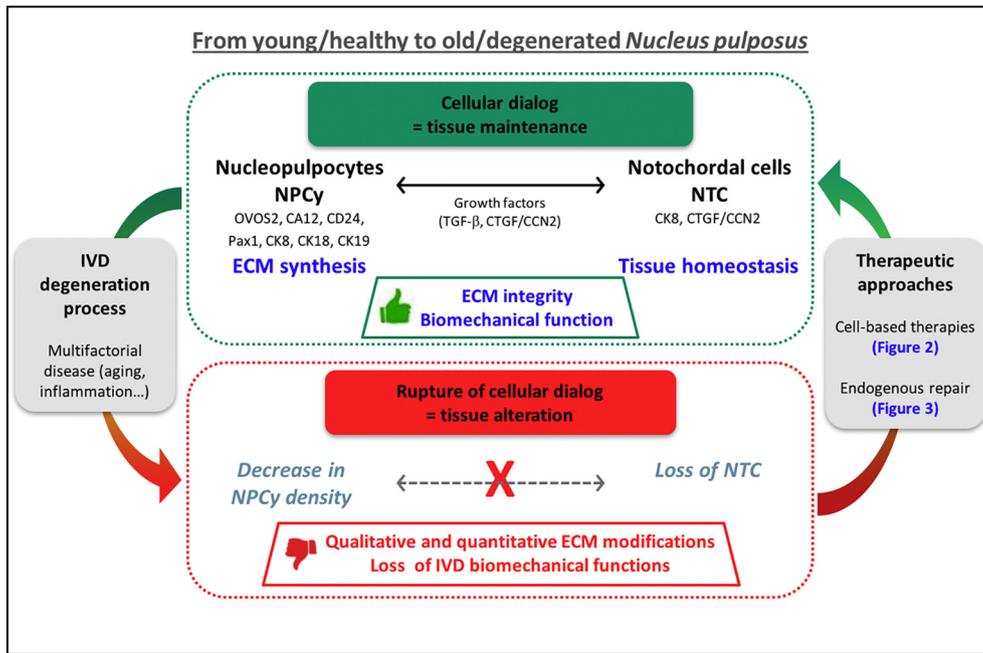
A bioinspired strategy could involve the activation of this reservoir of reparative cells, localized in specific anatomical niches, by specific signals such as those arising from injury. Upon activation, these cells would divide and produce daughter cells, which would then differentiate into the required cell type to repair or regenerate the damaged tissue. In this context, the recruitment of endogenous reparative cells might constitute an alternative strategy to exogenous cell transplantation. This bioinspired endogenous repair strategy could provide new therapeutic options for the stimulation and regeneration of the IVD microenvironment. Such strategies would be technically less complex and less costly than approaches that require substantial *in vitro* cell manipulation and transplantation. Nevertheless, such regenerative strategies would have several difficulties that would need to be overcome, such as the capacity to attract and stimulate reparative cells *in situ* as well as the maintenance of their efficacy over a long period of time.

This review aims firstly to review the research conducted in the past twenty years using conventional approaches based on exogenous cell transplantation, and will then focus on the new concept of regenerative medicine based on endogenous repair. The review will also discuss the lessons learned about the IVD pathophysiology and document how we can unlock the potential of novel, bioinspired, endogenous approaches.

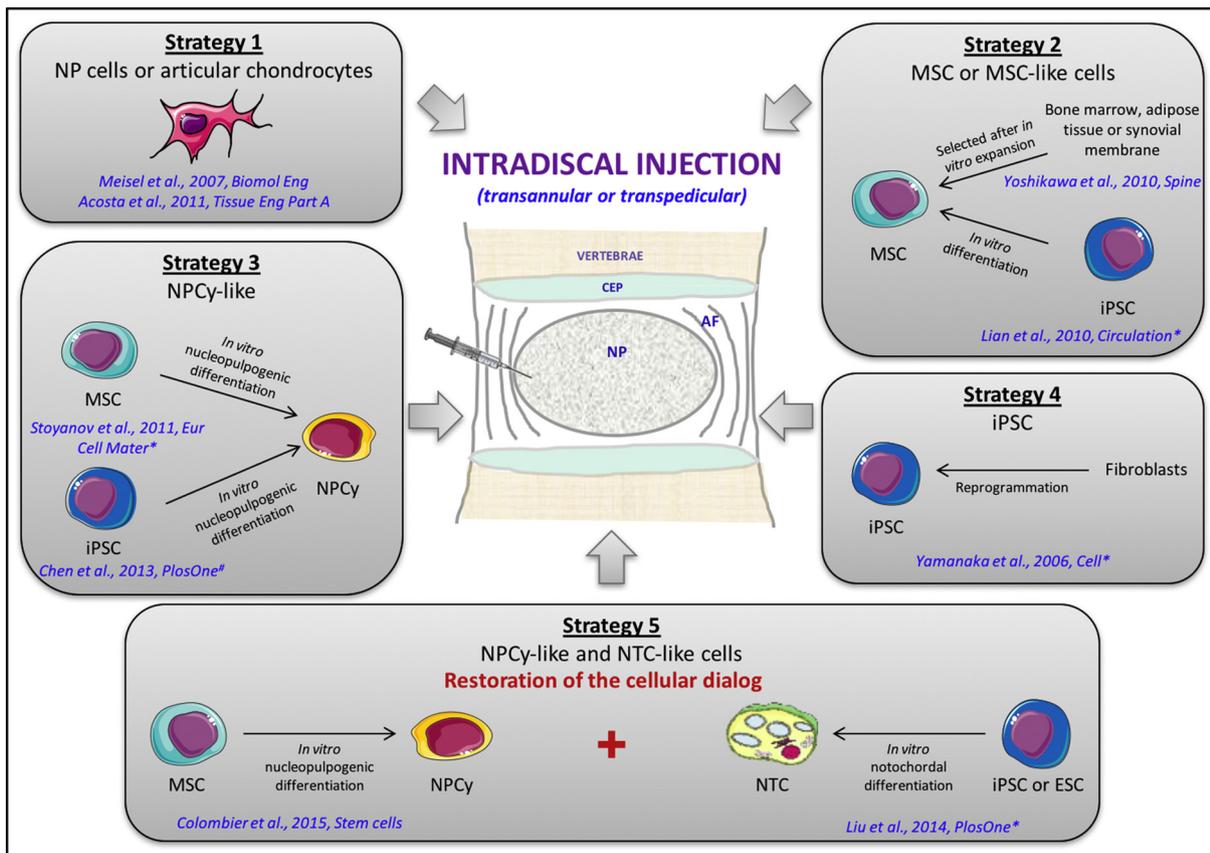
## 2. Cell-based therapies: state of the art and future developments

### 2.1. Cell sources

IVD cell-based therapies for treating DDD, particularly NP supplementation by direct intradiscal injection, have gathered considerable attention over the past two decades. The major cell-based approaches are summarized in Fig. 2 and categorized according to cell type and source



**Fig. 1.** Pivotal role of the cellular dialog during intervertebral disc (IVD) degenerative process. *Nucleus pulposus* changes in cell and extracellular matrix (ECM) composition are both associated with aging and degeneration. In healthy NP, a cellular dialog is established between Nucleopulpyocytes (NPCy) and Notochordal cells (NTC), allowing tissue homeostasis. During aging/degeneration, the loss of NTC has been speculated to initiate the onset of tissue degeneration. The rupture of the cellular dialog induces a decrease of NPCy density and an imbalance of the ECM synthesis associated to biomechanical function alterations. OVOS2: Ovostatin 2; CA12: Carbonic Anhydrase 12; CD24: cluster of differentiation 24 or heat stable antigen (HSA); Pax1: paired box gene 1; TGF- $\beta$ : Transforming Growth Factor- $\beta$ ; CTGF/CCN2: Connective Tissue Growth Factor; CK: Cytokeratin; IVD: intervertebral disc; ECM: extracellular matrix.



**Fig. 2.** Cell-based strategies for IVD regeneration. For each strategy described in text (Part 2), the first human experimental paper is cited. \* indicates that no IVD pre-clinical nor clinical data is available for that strategy. # indicates that this strategy has been described in mouse. NP: *Nucleus pulposus*; AF: *Annulus fibrosus*; CEP: cartilage endplate; NP cells: NP tissue-resident cells; MSC: mesenchymal stromal cells; iPSC: induced pluripotent stem cells; NPCy: nucleopulpyocytes; NTC: notochordal cells; IVD: intervertebral disc.

**Table 1**

Cell types of potential interest for application in IVD cell-based therapy. NP: *Nucleus pulposus*; NPCy: nucleopulpyocyte; NTC: notochordal cell; MSC: mesenchymal stromal cell; iPSC: induced pluripotent stem cell; ESC: embryonic stem cell.

Abbreviation	Description	Comments
NP cells	NP tissue-resident cells	Cell characteristics and composition vary during IVD growth and aging
NPCy	Nucleopulpyocytes	Present in juvenile and mature NP tissue
NTC	Notochordal cells	Direct progenitor of NP cells only present in juvenile NP
–	Chondrocytes	From juvenile articular cartilage tissue
MSC	Mesenchymal stromal cells	Multipotent cells derived from bone marrow, adipose tissue or synovial membrane
iPSC	Induced pluripotent stem cells	Reprogramming of somatic cells
ESC	Embryonic stem cells	Pluripotent cells derived from early-stage preimplantation embryo
NPCy-like		<i>In vitro</i> differentiation
MSC-like		<i>In vitro</i> differentiation
NTC-like		<i>In vitro</i> differentiation

(Table 1). All these approaches aim to (i) restore the altered ECM by supplementing the NP with cells already differentiated into NPCy-like cells *in vitro* or able to undergo such a differentiation *in situ* once transplanted into the NP or to (ii) use additional anti-inflammatory or trophic effects from injected cells (mesenchymal stromal cells notably).

Among cell sources of interest for IVD cell-based therapies, native NP cells [37,38] and articular chondrocytes [39] (Fig. 2, strategy 1) from autologous origin have been promptly abandoned because of their low availability and proliferation capacity, as well as their tendency for de-differentiation when cultured *in vitro*. Moreover, other intrinsic limitations, such as a degenerated phenotype in advanced stages of IVD degeneration and donor site morbidity have also been reported [37,38]. Allogenic option with NP cells and articular chondrocytes available from cell banks could offer new opportunities to eliminate previous mentioned limitations.

As an alternative, several studies reported the use of mesenchymal stromal cells (MSC). MSC are a heterogeneous population of multipotent cells capable of differentiating along the chondrogenic, osteogenic, and adipogenic lineages but not the hematopoietic lineage. In recent years, tremendous research efforts and funding have been devoted worldwide toward investigating the efficacy of the use of MSC to treat IVD degeneration in large part based on their intrinsic ability of differentiation but also on their immunomodulatory and anti-inflammatory properties (Fig. 2, strategy 2) [40]. Although not yet evaluated at the pre-clinical or clinical level, another strategy has been proposed based on IVD injection of pre-differentiated MSC (Fig. 2, strategy 3). Indeed, recent data demonstrated the ability of MSC to differentiate into mature NPCy-like cells when exposed *in vitro* to a specific cocktail of growth factors (such as GDF5 or GDF6) [41–43].

With the discovery of induced pluripotent stem cells (iPSC) by Takahashi and Yamanaka in 2006 [44], an inexhaustible cell resource has been offered to scientists that is similar to embryonic stem cells in term of pluripotency but with fewer ethical issues. Various strategies could be proposed based on the intradiscal injection of iPSC (Fig. 2, strategy 4). However, to date, only limited investigation have been performed on the use of iPSC for IVD application, but the use of human iPSC may overcome many of the obstacles encountered with adult MSC such as limited proliferation or replicative senescence following *in vitro* expansion. Alternatively, large populations of cells phenotypically and functionally resembling adult MSC could be obtained through the controlled differentiation of iPSC into MSC-like

cells (Fig. 2, strategy 2) [45,46]. However, since there is evidence that MSC-like cells derived from different iPSC lines exhibit variability in their differentiation capacity, further characterization of iPSC-derived MSC-like cells is still needed [47,48]. Currently, the clinical experience with iPSC is limited while their rapid growth and safety remains a matter of debate.

All of these strategies are based on a partial view of the cellular environment of the NP. Indeed, it is well established that NTC, which are known to play a pivotal role in IVD development and growth, coexist with resident NPCy in the young and healthy NP. NTC originate from the embryonic axial structure called the notochord. Lineage tracing experiments using a notochord-specific CRE-inducible mouse model have established that NTC are the precursor cells that give rise to the NP [49,50]. The NTC phenotype undergoes pronounced changes throughout embryogenesis and in the developing foetus as the IVD is formed. It is hypothesized that these modifications continue in the post-natal period during NP maturation and lead to substantial changes in the morphology of the NTC toward the predominantly functional NPCy phenotype observed in adult IVD. It is commonly accepted that NTC disappearance from the NP at skeletal maturity coincides with the first signs of IVD degeneration in humans, but not in small animals such as rabbits or rats, or non-chondrodystrophic dogs, where NTC persist throughout the animals' lifetime. Recent studies clearly demonstrate the instrumental role of the cellular dialog established between NTC and NPCy [19,51] in the maintenance of IVD homeostasis (Fig. 1). The growth factors Connective Tissue Growth Factor (CTGF/CCN2) and TGF- $\beta$  are secreted by NTC and stimulate ECM synthesis by NPCy, and are thus involved in NP homeostasis [52–55]. These studies undoubtedly established that the CTGF-mediated NTC/NPCy dialog is widely involved in controlling maintenance of the NP niche during aging. These studies also raised the possibility that the restoration of the cellular dialog between NTC and NPCy could be exploited to support NP regeneration. In this context, an innovative approach could consist of the intradiscal injection of a combination of NPCy and NTC to mimic the cellular composition of the native and healthy NP (Fig. 2, strategy 5). Given the early specification of the notochordal fate in the hierarchy of lineages, pluripotent stem cells, either embryonic stem cells (ESC) or iPSC, would have to be used for the generation of NTC-like cells. Although mimicking the critical steps occurring during embryonic development remains a challenge for stem cell biologists, this strategy could enable the differentiation of iPSC into NTC-like cells. Studies in mice have established that the onset of the specific NTC fate is controlled by the transcription factors FoxA2 and Brachyury (T). Both transcription factors are required for the expression of the notochordal transcription factor Noto, which plays a pivotal role in the maintenance of notochordal identity in mouse [56,57]. In humans, the expression and the role of NOTO in notochord formation has not yet been elucidated [58–60]. To date, very few studies have reported the differentiation of mouse or human pluripotent stem cells toward the notochord lineage. Embryoid body (EB) aggregation of human ESC followed by the activation of TGF- $\beta$  and Wnt pathways has resulted in the induction of early notochord-related gene expression [61]. In addition, a differentiation protocol of mouse ESC, based on the activation of TGF- $\beta$  and FGF pathways simultaneously, with the inhibition of endogenous retinoic acid, BMP (bone morphogenic protein), and Wnt pathways, resulted in the formation of a discrete population of NTC-like cells [62]. Another study showed that the spontaneous differentiation of mouse iPSC-derived EB generated CD24+ cells expressing significant levels of notochord-related transcripts (*Foxa2*, *Noto* and *Shh*) [57]. Interestingly, when cultured in a laminin-rich culture system supplemented with notochord conditioned media (NCCM), these cells were able to differentiate into NPCy-like cells expressing characteristic NP markers (integrin  $\alpha 6$  subunit, cytokeratin 5/8, and vimentin) and produce a matrix rich in type II collagen, proteoglycans, and laminin 511 [57]. A more recent study reported the possibility of directing human iPSC

notochordal differentiation by supplementing the media with ECM extracted from juvenile porcine NP tissue [63]. Differentiated cells showed the characteristics of mature NTC found in the juvenile NP (expressing *Brachyury*, *cytokeratin-8 (CK-8)*, and *cytokeratin-18 (CK-18)* genes, which were absent in the undifferentiated iPSCs). When differentiated further with the addition of TGF $\beta$ -3 factor, these cells synthesized ECM of a high glycosaminoglycan/collagen ratio resembling that of native NP tissue, thereby suggesting that they may adopt a NPCy phenotype. Clearly, a better understanding of the transcription factors required to direct the differentiation of stem cells into the NTC and NPCy lineages, together with effective culture methodologies for high cell viability, expandability, and sorting, will advance the use of iPSC in cellular therapy for disc repair and regeneration.

## 2.2. Pre-clinical studies and lessons learned

Over the past 20 years, about forty pre-clinical studies have been carried out that have partially confirmed the proof of concept of cell-based approaches. Two meta-analyses [22,23] and two reviews [24,25] present a comprehensive comparison of these pre-clinical results. The authors identified studies focused on cell-based therapies, associated [64–84] or not [37,39,65,85–107] with biomaterials. All these pre-clinical studies consisted of the intra-discal injection of cells obtained from NP tissue or chondrocytes (Fig. 2, Strategy 1) or undifferentiated MSC (Fig. 2, Strategy 2). However, a critical comparison of the data from these pre-clinical studies is complicated by the disparity in (i) the animal models of DDD used (spontaneous or experimentally-induced, small or large animals, persistence of NTC in adulthood); and (ii) materials and methods (cell source, number of injected cells, injection route, association or not with various biomaterials, endpoints of the study). A large majority of studies used MSC (mainly from bone marrow, adipose tissue, and synovial origin), and association of the cells with biomaterials was not the general rule. In general, these studies report that treatment of an injured/degenerated IVD with cells or cell/biomaterial constructs induces significant regeneration of the IVD. However, so far, none of these treatments have been able to induce an *ad-integrum* restoration of degenerated IVD in terms of disc height index, MRI T2 signal intensity or biomechanical properties. Interestingly, treatments with cell/biomaterial constructs have consistently showed better results.

While it is well acknowledged that the intradiscal injection of MSC has beneficial effects on DDD, the underlying mechanisms are still not fully understood.

To date, two cell-mediated mechanisms that may account for the therapeutic efficacy of MSC have been proposed. These mechanisms are not exclusive and may potentially be synergistic. The first one is based on the ability of MSC to differentiate into NPCy-like cells after intradiscal implantation in rabbit, bovine, and porcine models [40,96,108,109]. This mechanism (albeit it has yet to be elucidated) relies on the fact that the IVD tissue itself could promote the differentiation of MSC into a NPCy-like phenotype and induce the cells to synthesize new and appropriate ECM. However, further long-term studies are needed with detailed analysis of the newly formed tissue by transplanted MSC. Currently, the viability of MSC during longitudinal follow-up is controversial. Several studies have reported that MSC are undetectable as they rapidly die after implantation [39,110], whereas other reports have indicated the presence of cells from 1 to 24 weeks after their implantation [37,65,95,96,108,109]. Interestingly, most of the studies associating biomaterials to cells showed an improved cell viability and differentiation. These results highlight the supportive role of biomaterials in providing a favourable microenvironment for cells [65,96,111,112].

The second mechanism that may account for the pre-clinical efficacy of MSC in DDD is related to the cells' capacity to secrete paracrine

or trophic biological factors able to stimulate resident IVD cells and ECM synthesis. In a variety of applications, the capacity of MSC to secrete growth factors, chemokines, and others biological factors, including ECM components and anti-inflammatory or immuno-modulatory agents, has been demonstrated [113–118]. In addition, a few studies, based on the co-culture of MSC and cells from NP tissue before injection, have recently confirmed the cells' paracrine effect, with the secretion of many biological factors able to stimulate ECM synthesis [97,119–121]. In conclusion, the combination of differentiation and paracrine mechanisms could likely explain the pre-clinical efficacy of MSC-based therapy. The trophic/anti-inflammatory/immunomodulatory properties of MSC could be responsible for the discogenic pain relief reported in the early stages of therapy [122], while MSC differentiation and, as a consequence, their ability to produce NP-related ECM, could explain MSC-based therapy's long-term efficacy [122]. Injection of NP cells after their *in vitro* activation with autologous MSC confirmed the safety and efficacy of this strategy in human after 3 years [123]. Nevertheless, the absence of pre-clinical experiments with *in vitro* differentiated cells derived from MSC or iPS (strategies 3–5) makes it very difficult to critically assess the clinical relevance of such a concept.

The role of biomaterials could be pivotal to providing transplanted cells with a supportive environment. However, the development of specific biomaterials for IVD application remains a real challenge and a variety of scaffolds, based on collagen, alginate, and fibrin, to list a few, have been developed to support cell engraftment and NP formation [124–127]. An ideal cell carrier should: (i) demonstrate injectability; (ii) provide no deleterious effect on cells (cytocompatibility); (iii) provide an appropriate response from host (biocompatibility); (iv) provide structural support for cells to reside and allow the *de novo* synthesis of ECM as well as metabolic exchanges (biofunctionality); (v) contribute to the mechanical properties of NP; and (vi) provide a tunable physical environment to allow remodeling in response to tissue dynamic processes such as wound healing (biodegradability) [128,129]. The ideal biomaterial has yet to be identified, but the hydrogel family exhibits intrinsic properties that are particularly interesting from the IVD perspective. Indeed, hydrogels are made of 90%–95% water, a proportion similar to the ECM of healthy IVD, although the exact nature and parameters of hydrogels are not clearly settled [130]. Additional investigations have to be performed in the next few years but there is no doubt that the IVD's deleterious environment (hypoxia, acidic pH, osmotic pressure) is a parameter that must be taken into account in the design of the optimal hydrogel for cell transplantation. Interestingly, an alternative approach involving the systemic injection of BM-MSC was proposed to limit the influence of the harsh environment on cell survival. Recent studies demonstrated a beneficial effect on *in situ* IVD regeneration in an animal model but further studies are needed to confirm this data [98,131].

## 2.3. Clinical studies and lessons learned

In parallel to pre-clinical studies, several human studies have been performed in the past 10 years [26,132–137]. On the one hand, and as for pre-clinical studies, the first source of cells tested for IVD cell-based therapies were NP cells originating from herniated discs. However, due to the associated limitations (low availability and herniated NP with cells exhibiting a senescent phenotype) the use of NP cells has remained marginal [37]. On the other hand, undifferentiated autologous BM-MSC have been more widely investigated and generally reported to provide significant benefits in terms of pain relief (70%–75% at 12 months) and increased IVD hydration [26,132–134,136,137], although IVD height was usually not restored. Moreover, physical examination and patient quality of life were improved after 3 [137] and up to 6 years post injection [135]. Results should however be read with some caution as the number of patients in these pilot studies was limited (2 in

[132], 5 in [135], 10 in [26], 15 in [136], and 26 in [133,134,137]) and the results were obtained from non-controlled and non-randomized clinical trials. In 2016, the results of the first randomized controlled clinical trial including 24 patients using allogeneic MSC for IVD were published [138], which confirmed the feasibility and the safety of intra-discal injection as well as a significant decrease in pain. Thus cells from an allogeneic source could constitute a real opportunity. Indeed, contrary to autologous cells, off-the-shelf allogeneic cells allow the performance of a one-step surgery to treat the patient and their use is logistically more convenient than autologous cells. In addition, allogeneic cells with high and reproducible repair capacity could be obtained from healthy young donors, in contrast to autologous MSC generally obtained from older patients or patients with co-morbidities.

Additional clinical trials are in progress using autologous BM-MSC for cell therapy (NCT01643681, NCT02097862, NCT02338271, NCT02097862, NCT01640457, and NCT01771471 as retrieved from [clinicaltrials.gov](http://clinicaltrials.gov) on September 22, 2017) or allogeneic MSC from different origin (bone marrow and adipose tissue) (NCT02412735, NCT01860417, NCT01290367). RESPINE, a European, multi-centre, randomized controlled Phase IIb clinical trial, has recently been initiated. The main objective of the RESPINE trial is to define the efficacy of allogeneic undifferentiated BM-MSC therapy (*versus* placebo injection) in 112 patients with LBP. RESPINE also aims to provide new knowledge on the immune response and safety associated with intra-discal allogeneic cell injection.

Few results from clinical studies using BM-MSC associated with a biomaterial have been reported. One study (NCT01290367), consisting in the injection of low- or high-dose MSC combined with a hyaluronic acid hydrogel, was recently completed but data is not yet available (September 2017). In parallel, two randomized studies are currently recruiting patients to compare the efficacy and safety of a single injection of MSC alone or combined with hyaluronic acid hydrogel (using BM-MSC in NCT02412735 and adipose MSC in NCT02338271).

The objective of all these studies is to assess the efficacy of the injection of undifferentiated MSC into the IVD. An alternative approach could consist of the injection of differentiated MSC (Fig. 2, strategy 3) but such a strategy has not yet been tested in pre-clinical trials. As mentioned earlier, the differentiation of MSC into NPCy-like cells has only been described very recently. This differentiation is based not only on chondrogenic markers (type II collagen and aggrecan) but also on the definition of NP-specific markers (PAX1, OVOS2, CD24, and KRT8, 18, and 19) [41–43], whose relevance is still debated [139,140]. Among the factors capable of driving the differentiation of MSC toward NPCy-like cells, GDF5 and 6 (Growth Differentiation Factor 5 and 6 or Bone Morphogenetic Protein 14 and 13, respectively) are key growth factors that, in synergy with TGF- $\beta$ , are capable of inducing the production of proteoglycans and type II collagen. The pivotal role of GDF5 in the process of IVD degeneration [141] and regeneration [142] is well documented. Interestingly, clinical trials are in progress to demonstrate the safety and efficacy of the intradiscal injection of GDF-5 (NCT01124006, NCT00813813, NCT01158924, NCT01182337, documented on [clinicaltrials.org](http://clinicaltrials.org)).

In addition, to the classical use of bone-marrow or adipose-derived MSC, it has also been reported the use of bone marrow concentrate (BMC) for the treatment of discogenic LBP. Bone marrow concentrate, that does not involve cell expansion *in vitro*, have thus been successfully used in clinical studies [133,134]. In these studies, the intradiscal percutaneous injection of BMC has led to improve painful symptom [133,134] and radiography parameters [134]. Due to the limited number of studies but considering these encouraging clinical results, BMC likely deserves to be further analyzed. To date, only one study in 2013 has reported the *in vitro* differentiation of iPSC into NPCy-like cells [57], and no pre-clinical or clinical study involving the use of undifferentiated or differentiated iPSC has yet to be performed in IVD.

## 2.4. Cell-based therapies issues and safety concerns

Despite the demonstration of the proof of concept and the benefits of cell injection for IVD regeneration reported in pre- and clinical studies, many hurdles must still be overcome.

First of all, the effects of the harsh environment of the degenerated IVD on MSC survival, phenotype, and function remains a poorly understood issue that deserves to be further investigated. Indeed, healthy IVD is characterized by a very specific environment that associates avascularity, hypoxia, high osmolarity, acidic pH, and low diffusion of anabolic products and waste with mechanical constraints [143–146]. Such a harsh environment (even more marked in degenerated IVD) may be considered to be highly hostile for transplanted cells. In particular, current evidence suggests that while hypoxia and mechanical constraints may be beneficial for cell differentiation, high osmolarity and low pH may be deleterious to MSC survival and function [41,147,148].

Furthermore, safety issues regarding the use of MSC or iPSC in regenerative treatments are of crucial importance. Indeed, various complications, including tumor formation and immune reactions, have been illustrated with the use of stem cells for other medical conditions [149]. While MSC have a more limited differentiation potential than pluripotent stem cells, tumor formation is a significant possible risk and MSC transplantation in the human body was met with scepticism at first [150–152]. Another undesirable side effect of MSC-cell based therapy in IVD could be the formation of ectopic bone. Indeed, in a rabbit model, the observation of ectopic ossification in close vicinity to the MSC-injected IVD was described [110].

Issues in relation to the immunogenic effect of allogeneic cells, as opposed to autologous cells, are widely documented. Due to its avascular nature, the NP is considered as an immune privileged tissue. This immune-privileged status of IVD may greatly contribute to a reduced risk of graft-*versus*-host reaction upon the implantation of allogeneic cells [95,153], but measures of precaution should nevertheless be taken. Currently, a large number of trials are being performed worldwide with allogeneic cells, and no significant immune side effects have been reported following single injections to date. For example, in a recent meta-analysis of 87 patients with lupus erythematosus, no transplantation-related adverse events were found after a 4-year follow up [154]. Similarly, no transplantation-related adverse events occurred in MSC-treated patients with breast cancer [155], ankylosing spondylitis [156], graft *versus* host disease [157], and other autoimmune diseases [158]. The immune-privileged status and immunosuppressive properties of MSC result from both their lack of major histocompatibility class II antigen expression and absence of secretion of T helper type 2 cytokines. A randomized dose comparison study of allogeneic *versus* autologous MSC delivered by transcatheter injection [159] showed that injection of allogeneic MSC did not stimulate significant donor-specific allo-immune reactions. In IVD, HLA matching showed no influence on the efficacy of allogeneic mesenchymal stromal cell therapies for DDD. The study's authors hypothesized that the downregulation of the host immune response by the transplanted MSCs and effective sequestration of these cells inside the articular cavity of the IVD induced a weak immune response [160]. Despite these promising safety results with single injection treatments, it is important to keep in mind that repeated injections of allogeneic MSC might result in sensitization [161]. The creation of stem cell banks containing HLA-matched MSC is thus a realistic strategy that could be used to address the concerns related to graft rejection. It has recently been estimated [162] that a reduced number of stem cell lines with very well conserved homozygous HLA haplotypes would provide cells for the vast majority of patients.

An important step for the implementation of cell-therapy in humans is the determination of the optimal dose range of cells for maximum

benefit, as well as the optimal timing for the injection of cells into IVD. Although differences in efficiency were clearly evidenced in a canine IVD degeneration model treated with several concentrations of autologous MSC [90], the question of the optimal dose range of cells is rarely investigated in published studies. Currently, there is no consensus regarding the optimal cell number to be injected per IVD, with values ranging from  $10^4$  to  $25 \cdot 10^6$ , and no consistent data defines how cell number affects the regenerative capability of MSC treatment. Regarding the definition of the optimal timing for the delivery of cells into the IVD, limited investigations in animal models have been performed which suggested that injection at an early stage of IVD degeneration had greater chances of counteracting the ECM alteration [163]. It is also important to consider that the transposition of data obtained from animal studies to human application is particularly precarious.

Finally, concerted efforts in basic research are needed to advance our understanding of the effective mechanism underlying the IVD-regenerative potential of MSC. This issue will have tremendous impacts on the choice of cell source and the design of innovative regenerative strategies. Indeed, if MSC have the potency to differentiate into IVD cells, the paradigm of cell therapy will be reinforced. In contrast, if the regenerative effect is a consequence of a paracrine effect and related to the specific MSC secretome, the identification of key factors able to stimulate the ECM regeneration will be the ultimate goal. The identification of these key factors could open up the development of new strategies based on their *in situ* injection to promote IVD self-repair.

For all these reasons, the safety of MSC injections for IVD regeneration needs to be systematically investigated.

### 2.5. Technical, technological, and regulatory hurdles

In addition to cellular concerns, cell-based therapies exhibit several technical, technological, and regulatory issues.

Technical difficulties have to be taken into account, notably the optimal route to specifically inject into the NP. Indeed, it is well reported that *in situ* injection can have an eventual degenerative effect on the IVD. The lateral surgical approach to the NP through the AF is preferentially used in most pre- and clinical studies, as well as in animal models of IVD degeneration [24]. However, it is important to note that this surgical route, based on needle puncture of the AF, leads to further degeneration and an increased risk of disc herniation [24]. In addition, a prospective human study has convincingly reported that the injection of a contrast agent (discography) through the AF to observe NP integrity led to accelerated degeneration [164]. Finally, backflow of the injected material through the AF injury is an additional problem. Thus, the lack of validated approaches for injection within the NP without the risk of associated IVD injury is a major obstacle to the development of regenerative strategies for DDD. Thus, alternative routes need to be investigated; among them, the transpedicular approach (TPA), recently described in the ovine model, is promising [165,166]. This procedure consists in targeting the NP after the introduction of a wire in the caudal vertebra through the pedicle and the inferior CEP toward the NP. Since its description in 1987, the TPA procedure has commonly been used to achieve osteosynthesis [167] and cementoplasty [168,169]. Partial data are available on the consequences of the TPA on the vertebra and rostral endplate, which seem to be particularly exposed by this route, and recent data confirm that some technical difficulties are encountered [170]. Risk of encroachment into the spinal canal during TPA [165] and leakage of injected biomaterials related to the high swelling pressure of IVD [165,166] are poorly documented but severe possibilities in clinical transposition. To decrease cell leakage, association with a biomaterial is a particularly interesting option, with one study reporting a decrease of output cell numbers from 90% to 50% in IVD therapy [171].

In parallel to technical hurdles, cell-based therapies exhibit technological difficulties. Regardless of the use of autologous or allogeneic regenerative cells, the implementation of good manufacturing practice (GMP), labor-intensive, and high-cost *in vitro* cell manipulation procedures are concerns that will need to be faced. The use of allogeneic cells as ready-to-use “off the shelf” treatments is less costly and has advantages compared to autologous cells. Contrary to the use of allogeneic cells, which only need a one-step surgical procedure for the patient (single hospitalization), the use of autologous cells requires two successive steps with (i) cell harvesting, transport, and *in vitro* expansion followed by (ii) injection in the degenerated IVD several weeks later, in a second hospitalization of the patient. Nevertheless, the burden of the quality procedure required for allogeneic cells compared with autologous cells has to be taken into account. Quality requirements are more stringent in terms of viral, bacterial, and fungal analysis, as well as HLA analysis. Currently, it is very difficult to estimate the real cost of cell-based strategies and medico-economic studies will certainly be required to persuade the authorities of their relevance.

The role that iPSC could play in innovative IVD cell-based therapies remains hypothetical. One disadvantage of their use is that iPSC production is time consuming and costly due to the extensive quality controls performed owing to the elevated risk of random mutations occurring during the iPSC generation, which could possibly lead to the formation of tumours [172]. Allogeneic iPSC banks, like the CiRA Center (Kyoto, Japan) or Ingestem (Paris, France), constitute a promising strategy to support the clinical development of iPSC for a large number of applications. Nevertheless, the number of available allogeneic cell lines is limited at this time and only a small fraction of the world population could potentially be treated [173]. Moreover, the regulation and funding of these cell banks are not clearly defined and should be clarified in the near future to avoid potential ethical problems.

Finally, difficulties in regulatory approval regarding the status of Advanced Therapy Medicinal Product (ATMP) according to the Directive 1394/2007CE was clearly evoked by scientific communities [174,175]. Currently, few ATMPs are approved due to limited results from the small number of clinical trials available.

As a whole, cell-based therapies have opened new perspectives for clinicians and patients suffering from LBP. While clinical proof of concept studies show promising results, many questions have to be answered before the complicated transposition to humans can be envisioned. The lessons learned include the realization of the difficulty of defining a gold-standard strategy. Among the novel treatment opportunities that could potentially bypass previously reported issues for exogenous cell transplantation, the discovery of stem/progenitor cells residing in a multitude of tissues/organs (intestine, bone marrow, brain, skin, muscle, knee joint, tendon, and heart) [176–183] has substantiated theories about our capacity to use these reparative cells and develop endogenous repair strategies, notably within the IVD.

### 3. From exogenous cell transplantation to bioinspired endogenous repair strategies

Although NPCy residing in the adult NP are considered to be the terminal differentiation product of NTC, numerous studies in the literature have highlighted the heterogeneity of the NP cell population [184]. This complexity becomes greater with aging and at early stages of degeneration, raising the idea that progenitor cells other than the NTC may contribute to the heterogeneous nature of NP cells in the adult IVD. These findings have prompted the search for endogenous stem/progenitor cells that could be involved in the maintenance of a healthy IVD. Cells with stemness properties and, as such, candidates for IVD progenitors, have recently been discovered in the CEP, AF, and NP (Table 2). The presence of these cells could offer an opportunity to counteract the

**Table 2**

Identification of stem/progenitor cells of the intervertebral disc (IVD).

AF: *Annulus fibrosus*; AFo: AF border to ligament zone; CEP: cartilage endplates; NP: *Nucleus pulposus*; P: perichondrium region; Brachyury: Notochordal cell marker; CD system: cluster of differentiation used as cell surface markers in immunophenotyping; CD105, CD90, CD73 and CD166: MSC markers; CD45: pan-hematopoietic marker; CD34: hematopoietic stem cell marker; CD133/1 (prominin-1 or AC133): membrane glycoprotein expressed by many types of progenitor cells; CD4 and CD8: T-cell antigens; CD14: pan-monocytic antigen; CD19: pan-B-cell marker; C-KIT (CD117): primitive marker of bone marrow stem cells; Delta4: Notch1 ligand; GD2: disialoganglioside 2; HLA-DR: human leukocyte antigens D Related, hematopoietic stem cells marker; IVD: intervertebral disc; Jagged 1: Notch 1 ligand; Ki67: cell cycling protein expressed in proliferating cells; Nanog: homeobox protein Nanog; Nestin: neuroectodermal stem cell marker; Notch-1: Notch homolog 1, cell fate determinant, progenitor marker; NSE: neuron-specific enolase; NTR: low affinity nerve growth factor receptor; Oct-4: octamer-binding transcription factor-4, multipotency marker; Sox-2: SRY-related HMG-box-2; SSEA-4: stage-specific embryonic antigen-4; Stro-1: stromal cell antigen-1, MSC marker; Tie2: tyrosine kinase receptor 2.

Donor tissues Anatomical region/species/status of IVD	Type of stem cells	Expression of stem cell, progenitor and proliferation markers	References
<b>Peripheral IVD stem/progenitors cells</b>			
Human, rabbit, rat, pig/IVD/healthy and degenerated	IVD progenitors and stem cells niches in the P and AFo regions	Delta4+, Jagged1+, Notch-1+, STRO-1+, C-kit low, Ki67 low	Henriksson et al., 2009 and 2012 Spine [195]
Rat/EP Perichondrium/Healthy	MSC-like cells derived from IVD stem cell niches	CD29+, CD44+, CD90+, CD11b-, CD19-, CD34-, CD45-	Shi et al., 2015, Eur Spine J [214]
<b>Resident IVD stem/progenitors cells</b>			
Human, rat/NP or AF/healthy and moderately degenerated	Skeletal progenitors resembling BM-MSc	CD49+, CD63+, CD73+, CD90+, CD105+, CD133/1+, CD166+, p75NTR+, CD34-	Risbud et al., 2007, Spine [28]
Human/NP/moderately to severely degenerated	NP-MSc resembling BM-MSc	CD73+, CD90+, CD105+, CD106+, CD166+, CD14-, CD19-, CD24-, CD34-, CD45-, HLA-DR-	Blanco et al., 2010, Spine [27]
Human, mouse/NP/healthy and degenerated	NP progenitor cells	CD44+, CD271+, Flt1+, GD2+, Tie2+, CD24-	Sakai et al., 2012, Nat Commun [208]
Human/NP/healthy and degenerated	NP-MSc	CD73+, CD90+, CD105+, CD34-, CD45-, HLA-DR-	Jia et al., 2017, Exp Ther Med [206]
Human/NP/degenerated	NP-derived stem cells with BM-MSc characteristics	Tie2+, GD2+, Nanog+, Oct-4+, Sox-2+	Li et al., 2017, BMC Musculoskeletal Disorders [201]
Canine/NP/healthy non-chondrodystrophic	NP progenitor cells	CD133+, Ki67+, Nanog+, NCAM+, Nestin+, Oct3/4+, Sox2+, Brachyury-	Erwin et al., 2013, Spine [215]
Rhesus macaque/NP or AF/healthy	IVD-progenitor cells with MSC characteristics	CD44+, CD90+, CD146+, CD166+, HLA-DR+, Notch-1+, CD271-, CD106-, CD29-, CD270-	Huang et al., 2013, Biomaterials [216]
Mini-pig/NP/healthy and induced degeneration	NP-derived multipotent cells	CD29+, CD44+, CD90+	Mizrahi et al., 2013, Spine J [205]
Human/AF/healthy, juvenile	AF-derived multipotent cells	CD29+, CD49+, CD51+, CD73+, CD90+, CD105+, CD166+, CD184+, Nestin+, NSE+, Stro-1+	Feng et al., 2010 J Bone Joint Surg Am [217]
Rabbit/AF/healthy	AF-derived stem cells with MSC characteristics	CD29+, CD44+, CD166+, Oct4+, SSEA-4+, CD4-, CD8-, CD14-	Liu et al., 2014, PLoS One [29]
Human/AF/moderate to advanced degeneration	AF progenitor cells	CD14+, CD29+, CD44+, CD73+, CD90+, CD105+, STRO-1+, CD34-, CD45-	Gruber et al., 2016, J Orthop Res [218]
Human/CEP/degenerated	CEP-derived stem cells resembling BM-MSc	CD44+, CD73+, CD90+, CD105+, CD133/1+, CD166+, Nanog+, Oct4 +, Sox2+, STRO-1+, CD14-, CD19-, CD34-, CD45-, HLA-DR-	Liu et al., 2011, PLoS One [197]
Human/IVD/degenerated	IVD-derived mesenchymal progenitor cells	CD90+, CD105+, NOTCH1+, Oct3/4+, STRO-1+	Brisby et al., 2013, Stem Cells Dev [26]
Human/IVD/degenerated	IVD progenitor cells with MSC characteristics	CD73+, CD90+, CD105+, CK-8+, CK-19+, Notch-1+	Turner et al., 2014, Eur Spine J [191]

drawbacks related to exogenous cell-based therapy and lead to the proposal of innovative endogenous repair strategies. These progenitor cells would constitute a reservoir of reparative cells that could alleviate many technical, technological, and regulatory hurdles encountered when using exogenous cells. In this context, a new paradigm for DDD treatment could consist in the mobilization and activation of endogenous progenitor cells to orchestrate repair processes able to reverse or slow down IVD degeneration. However, while such a strategy has already been exploited in other tissues, many challenges have to be addressed before IVD endogenous repair can be considered as a feasible regenerative therapy.

### 3.1. Peripheral and resident intervertebral disc (IVD) reparative cells

Recent studies have highlighted the presence of stem/progenitor cells in intervertebral niches found at the vicinity and within the IVD. Peripheral niche of stem cells

A Nature insight review [185] defined a niche: “Stem-cell populations are established in ‘niches’ specific anatomic locations that regulate how they participate in tissue generation, maintenance and repair. The niche saves stem cells from depletion.” Recently, it has been reported

that stem cell niches can be found at the vicinity of the IVD, notably in the perichondrium (P) region adjacent to the epiphyseal plate and in the AF border to ligament zone (AFo) [28,186]. *In vivo* BrdU labelling studies in the rabbit model documented the localization of stem/progenitor cells. In addition, the detection of proliferative and progenitors markers were evidenced in healthy IVD tissues in humans, rabbits, rats, and pigs. Also, the analysis of human degenerative IVD demonstrated the presence of these stem/progenitor cells (Table 2) and suggested that stem cells recruited from P and AFo regions give rise to progenitors that contribute to the maintenance of the cellular organization within the IVD.

In the rat model, following *in vitro* expansion, cells from these IVD potential stem cells niches highly resemble BM-MSc in terms of immunophenotype, gene expression profile, and proliferation capacity [187]. Their ability to differentiate into osteogenic, chondrogenic, and adipogenic lineages was also demonstrated, confirming their multipotency [187,188]. Interestingly, they showed greater chondrogenesis and osteogenesis potency compared with BM-MSc isolated from the same individual. *In vivo* experiments with implantation in rabbit IVD revealed that cells isolated from the CEP induced the highest NP regenerative effect, as compared to BM-MSc [187].

In the rabbit model, immunohistochemistry analyses of the chondrogenic marker GDF5 in parallel with the migration markers SNAI1 (SNAIL homolog 1) and SLUG (SNAIL homolog 2) and cell adhesion marker ITGB1 (Integrin  $\beta$ 1) suggested that stem cells recruited from P and AFo regions can migrate toward AF and NP to contribute to normal growth and regeneration of the IVD. Thus, these findings and *in vitro* assays in the rabbit and human models support the existence of a cellular migration route within the IVD [189]. Interestingly, cellular migration was also identified in other cartilaginous tissues such as the knee joint [190]. However, robust experimental evidence must be generated to confirm the origin of the cells and their migration from the niche regions to the IVD area and to precisely define the regulatory signaling pathways involved. In particular, the implication of chemokines in the cell migration process should be investigated, as they are known to have a pivotal role in cell attraction, mobilization, and invasion potency [191].

### 3.1.1. Resident IVD progenitor cells

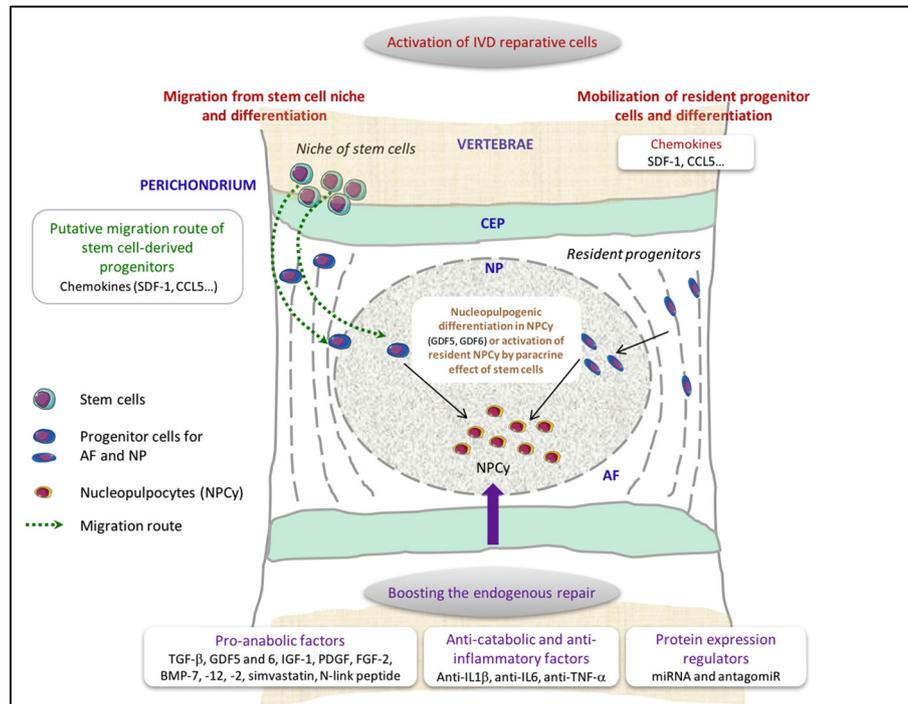
In parallel to the identification of stem cells in the vicinity of the IVD, progenitor cells have been successfully isolated using stem cell markers from the NP, AF, and CEP [29–32,34,182,188,192–198] (Table 2). These progenitors were derived from healthy or moderate-to-severely degenerated IVD from humans as well as other mammalian species. In most studies, the cells selected after *in vitro* expansion were found to express classical stem cell markers, and in particular BM-MSC markers. These progenitors were able to differentiate into the three classical lineages. However, the absence or inferior adipogenic differentiation was reported for progenitor cells isolated from degenerated IVD in some studies [30,193].

Sakai et al. identified a population of progenitor cells with self-renewal potential within the mouse and human NP compartment expressing angiopoietin-1 receptor (Tie2) and disialoganglioside 2

(GD2), two specific cell-surface markers [34]. Isolated cells were evaluated for clonogenicity *in vitro* and for multipotency and self-renewal ability *in vivo*. The study's results highlighted that progenitor cells sequentially switch expression of specific cell-surface markers, from Tie2 + GD2+ to Tie2- GD2+ and CD24+ cells, as they differentiate into mature NP cells and lose proliferative capacity. Results also highlighted that angiopoietin-1, a ligand of Tie2, is involved in the survival of NP cells. Another study used the co-expression of GD2+ and Tie2+ for the cell sorting of NP-derived stem cells from DDD patients and compared their differentiation abilities with BM-MSC isolated from the same individuals [193]. These NP-derived stem cells showed similar cell colony-forming ability and proliferation capacity to those of BM-MSC but were superior in terms of chondrogenic differentiation, with higher protein levels of collagen II $\alpha$ 1 and aggrecan. As GD2+ and Tie2+ markers appear to define a specific precursor cell subpopulation within the NP area, they will certainly have an impact on future regenerative strategies.

### 3.1.2. IVD stem/progenitor cells: pros and cons

Together, these studies support the existence of resident progenitor cell populations in the IVD or vicinity (P and AFo regions) and opens up new perspectives for the development of DDD therapeutic treatments. Indeed, it seems reasonable to speculate that the stem/progenitor cells from IVD niches could be activated by specific signals such as those arising from injury or after the injection of biological factors [29]. Once activated, they could divide to give rise to daughter cells able to migrate to the site of injury and ultimately differentiate into the required cell type to repair the damaged tissue (Fig. 3). The selection of the best source, *i.e.* peripheral or internal reservoirs, for endogenous repair strategy is thus a serious consideration. Some important issues remain to be clarified in relation to IVD reparative cells (particularly the mechanism



**Fig. 3.** Bioinspired endogenous repair strategies. *Upper section:* this strategy consists in the recruitment of stem cells by migration from peripheral niches to the NP, followed by induction of their nucleopulpopogenic differentiation. The second one consists in the activation of resident NP progenitor cells and their nucleopulpopogenic differentiation. *Lower section:* numerous factors play a crucial role in IVD physiopathology and could be of interest to boost IVD repair. Three categories can be defined: biological factors with pro-anabolic effect; biological factors with anti-catabolic and anti-inflammatory effects; nucleic acid and particularly interfering RNA (siRNA and miRNA) with a large panel of biological effects. NP: *Nucleus pulposus*; AF: *Annulus fibrosus*; CEP: *Cartilage endplate*; NPCy: *nucleopulpopocytes*; IVD: *intervertebral disc*; SDF-1: *Stromal cell-Derived Factor-1*; CCL5: *CC Chemokine Ligand-5*; GDF5 and 6: *Growth Differentiation Factor 5 and 6*; TGF- $\beta$ : *Transforming Growth Factor- $\beta$* ; IGF-1: *Insulin Growth Factor-1*; PDGF: *Platelet Derived Growth Factor*; BMP-7, 12 and 2: *Bone Morphogenetic Protein-7, 12 and 2*; IL-1 $\beta$ : *Interleukin-1 $\beta$* ; TNF- $\alpha$ : *Tumor Necrosis Factor- $\alpha$* ; FGF-2: *Fibroblast Growth Factor-2*.

by which they participate in the maintenance of healthy IVD) and will be discussed below.

While these stem/progenitor cell populations exhibit properties resembling those of MSC, differences have been described in terms of their immunophenotypes and gene expression profiles.

One study reported that rabbit AF-derived stem cells lost their “stemness” (as assessed by the colony formation assay, cell proliferation rate, and stem cell markers nucleostemin, Oct-4, SSEA-4, and Stro-1) faster than NP-derived stem cells [199].

In addition, some disparities in the osteogenic and chondrogenic differentiation potentials were observed between progenitors isolated from distinct IVD areas [186,198]. Further investigations are needed to consolidate these results, not only with respect to their chondrogenic but also their nucleopulpopogenic differentiation ability. To our knowledge, only one study has addressed the nucleopulpopogenic differentiation ability of NP-derived multipotent cells isolated from porcine discs [196]. This study has assessed the effect of IVD degeneration on cells residing in the NP and clearly showed that degenerative NP cells compared to healthy NP cells exhibit a significantly higher rate of proliferation. Future research should address this important issue and investigate the nucleopulpopogenic differentiation potency of IVD progenitors based on the expression of NPCy-related markers (OVOS2, PAX1, CD24) [41–43] and the ability to synthesize a NP-like ECM.

The effect of age and the stage of degeneration on progenitor cells residing in the IVD are not fully understood. Studies in humans and rabbits have reported a decreased ability of colony formation and chemotactic migration and a reduced expression of stemness-related genes in IVD progenitors derived from degenerative compared with non-degenerative tissues [192,200]. Remarkably, the Tie2+ GD2+ population of progenitor cells identified within the human NP decreases with both age and degeneration [34].

These differences could be attributed to the accumulation of repeated stress stimuli in degenerated IVD. Alteration of the ECM and changes in mechanical properties with the secretion of cytokines in degenerated IVD could influence the local environment of the progenitor cells and could explain these differences [201]. To anticipate future hurdles to the development of endogenous repair strategies, it is particularly important to demonstrate efficacy in the early stages of IVD degeneration when progenitor cells retain their repair capacity.

While the inhospitable IVD environment can significantly alter the viability and function of injected exogenous MSC, endogenous progenitor cells from NP have been shown to be more able to withstand the harsh IVD environment in hypoxia [202], hyperosmotic [203], and acidic culture [204] conditions. These data reinforce the relevance of using endogenous progenitor cells as reparative cells for IVD degeneration.

Unlike progenitor cells found in the IVD, stem cells located at the vicinity of IVD require an initial stage of migration, for which a mechanism is partially elucidated. Further studies are expected to precisely define the migration route of these cells and identify the biological factors able to regulate this process [189,205]. Accordingly, the presence of progenitor cells in the NP could be an opportunity since no migration is needed in this case. Nevertheless, the pivotal biological factors required for the stimulation of these progenitor cells are not yet characterized, as discussed below.

### 3.2. Activation of IVD reparative cells: a new paradigm and future challenge

Endogenous repair is a promising therapeutic approach for the treatment of IVD degeneration and two strategies can be proposed (Fig. 3). The first one consists of the recruitment of stem cells by migration from peripheral niches to the NP, followed by (i) their nucleopulpopogenic differentiation or (ii) the stimulation of resident NP cells by paracrine effect of stem cells recruited by induction of their nucleopulpopogenic differentiation. The second one consists of

the activation of resident NP progenitor cells and their nucleopulpopogenic differentiation. These innovative approaches are exciting but several challenges have to be overcome to enable their development into successful therapeutics. These challenges are: (i) how to attract stem/progenitor cells; (ii) how to differentiate these reparative cells toward NP cells; (iii) how to locally and sequentially release biological factors in a controlled manner to facilitate the recruitment and differentiation of reparative cells; and (iv) how to boost endogenous repair strategies.

#### 3.2.1. Recruitment of quiescent reparative cells

The first challenge consists in defining the modalities to attract reparative stem cells from the peripheral niches as well as induce the activation of progenitor cells. It is well established that MSC from exogenous origin express chemotactic receptors in response to cytokines, which facilitate engraftment to sites of injury [206–209]. Moreover, it was demonstrated that exogenous MSC have the capacity to migrate into the NP/AF tissues of degenerated IVD through the CEP in a bovine *ex vivo* model [210]. The use of biological factors with chemo-attractive properties seems particularly relevant. Thus, cytokines of the chemokine family have been considered with interest since they are involved in cell recruitment, mobilization, and homing. Notably, studies on stem cell migration have identified CCL5 (also named Regulated on Activation, Normal T cell Expressed and Secreted, or RANTES) as a chemokine involved in the recruitment and mobilization of stem cells in different tissues [211,212], including the IVD [33]. Other studies have demonstrated the implication of SDF-1 (Stromal cell-derived factor 1, also named CXCL12) in stem cell attraction in many tissues such as myocardium, periodontal ligament [213–216], and IVD [217]. Various chemokines, such as CXCL16 [218], CCL2, CCL7, and CXCL8 have also been identified [219] in healthy or degenerated IVDs. The expression of SDF-1 and its receptor CXCR4 was shown to be upregulated in degenerated IVD, notably in CEP and NP progenitors [220,221]. SDF-1 has also been shown to increase MSC attraction into a nucleotomized IVD model [217]. Interestingly, the secretion of a high concentration of CCL5 is induced by culturing NP cells in degenerative conditions, *i.e.* by *in vitro* stimulation with IL-1 $\beta$  and TNF- $\alpha$  [33,222]. In parallel, CCL5 was identified by proteomic assays in human degenerated IVD as well as in herniated and scoliotic IVD [223,224].

To our knowledge, only one study has analyzed the migration capacity of peripheral and NP progenitors. Interestingly, stem cells from CEP showed a higher migration ability and invasion potency, whereas NP progenitor cells showed low migration ability and almost no invasion potency [221]. This data is crucial to confirm that cell migration does happen in spite of the avascular nature of the tissue. The migration route of progenitor cells into the IVD was explored by following the proliferation marker (BrdU) as well as migration and adhesion markers (SNAI1, SLUG, ITGB1) [28,225].

In conclusion, the relationship between chemokine release and cell migration patterns, as well as the temporally regulated action of such chemokines remains to be characterized. Nevertheless, the studies carried out to date highlight the potential role of chemokines in IVD regeneration and support their relevance in the development of strategies to attract reparative progenitor cells and stimulate endogenous repair.

#### 3.2.2. Differentiation of recruited reparative cells

Once progenitor cells are mobilized and recruited, another challenge consists in controlling their activation and differentiation into phenotypically relevant cells able to secrete a functional ECM and support the regenerative process.

The harsh environment found in the degenerated IVD could drastically alter progenitor cells' differentiation capacity. Interestingly, and of translatable relevance, endogenous progenitors have shown a specific adaptability to this harsh microenvironment [202,204].

In this context, the stimulation of the nucleopulpopogenic differentiation of recruited reparative progenitor cells should be considered (notably those arising from the NP, AF, CEP, and IVD vicinity). Albeit conceptually simple, this strategy raises some major issues, especially taking into account the low number of nucleopulpopogenic growth factors that have been identified (as stated in Section 2.3). GDF5 and GDF6 have been convincingly shown to trigger the *in vitro* differentiation of MSC into NPCy [42]. The enrichment of a chondrogenic culture medium with GDF5 or GDF6 [42] has thus led to the expression and synthesis of specific nucleopulpopogenic markers, nowadays associated with the nucleopulpopogenic differentiation of MSC [41,43,140,226–228]. Interestingly, *in vivo* experiments also reported the formation of a NP-like tissue following the subcutaneous injection of differentiated MSC associated to a biomaterial after GDF5 treatment in *nude* mice [43]. In light of these data, it makes sense to assume that locally released GDF5 or GDF6 could induce the differentiation of recruited progenitor cells.

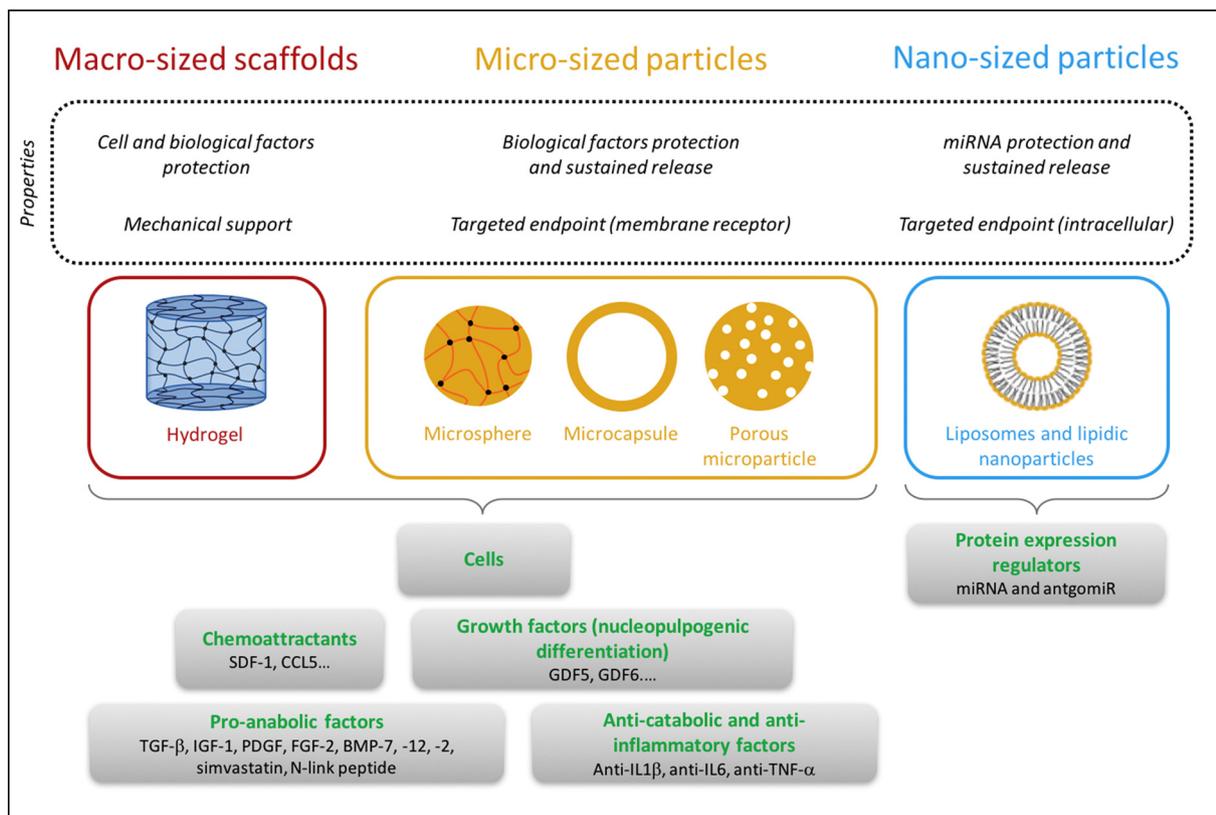
### 3.2.3. Controlled released of biological factors

Biological factors have to be injected into IVD in order to drive the endogenous process that will lead to IVD regeneration. Unfortunately, the *in vivo* half-life of such biological factors remains short after direct injection, and iterative intradiscal injections will likely be required to reach significant efficacy, thereby increasing the risk of DDD [229] as well as dose-dependent adverse effects. Delivery platforms must be adapted to the intrinsic properties of these biological factors (molecular weight, hydrophilicity) in addition to their endpoint targets (cell membrane receptor or intracellular), taking into account their own biocompatibility and injectability (Fig. 4). Clear differences exist between the molecular weights of chemokines (approximately 8 kDa) and those of growth factors (approximately 25 kDa). Moreover, chemokines and

growth factors have to bind to cell membrane receptors to induce their biological effects. In this context, the development of macro-, micro- and nanosized platforms will be a particularly promising way to vectorize these biological factors (see review [230]).

Macrosized platforms consisting of natural, hemi-synthetic, and synthetic biomaterials in the form of hydrogels are of major significance for IVD application due to their similarity with the ECM of the NP (highly hydrated with 95% water), their biocompatibility, their injectability properties, and their capacity to retain release factors. Moreover, hydrogels provide mechanical support and a 3D microenvironment adapted for the survival, differentiation, and proliferation of reparative cells (see review [130]). These hydrogels can also be designed as microparticles with varying biological factor release profiles.

Microparticulate platforms could be particularly adapted to chemokines and growth factors since they provide a highly specific surface allowing for a high loading content and a prolonged release [231]. Currently, many controlled delivery systems of microparticulate size have been developed and are commercially available to improve the local delivery of biological factors and thereby increase their efficiency [232–234]. Among them, polymer-based spherical microcarriers have been thoroughly investigated [231]. Different polymers can be used to produce microcarriers, but among them, naturally-derived polysaccharides exhibit several advantages for IVD applications [231]: (i) their repeating structure of osidic units will ensure their controlled enzymatic degradation; (ii) their structural similarities with glycosaminoglycans will allow their incorporation within the NP extracellular matrix; (iii) their cyto- and bio-compatibility have been largely documented; (iv) their ability to release biological factors has been demonstrated, notably with CCL5 [235] and GDF5 [236]; (v) the possibility exists to finely tune the release kinetics; (vi) the absence of organic solvents required for



**Fig. 4.** Macro-, micro-, and nano-sized systems developed for cell-based therapy and bioinspired endogenous approach. SDF-1: Stromal cell- Derived Factor-1; CCL5: CC Chemokine Ligand-5, GDF5 and 6: Growth Differentiation Factor 5 and 6; TGF-b: Transforming Growth Factor-b; IGF-1: Insulin Growth Factor-1; PDGF: Platelet Derived Growth Factor; BMP-7, 12 and 2: Bone Morphogenetic Protein-7, 12 and 2; IL-1b: Interleukin-1b; TNF-a: Tumor Necrosis Factor-a; FGF-2: Fibroblast Growth Factor-2.

their production [237,238]. Thus, microcarriers could represent an attractive platform for the delivery of biological factors such as chemokines and growth factors. One can envisage the design of microcarriers with different release kinetics; namely, the fast release of chemokines to trigger the recruitment of the reparative cells, followed by a sustained release of growth factors to stimulate the nucleopulpopogenic differentiation of the recruited progenitors.

Nanosized platforms could be also particularly interesting for the *in situ* delivery of chemokines and growth factors (as well as siRNA and miRNA, as described in the section dedicated to RNA interference). Currently, many materials are used to design nanoparticles for biomedical applications (polymeric nanoparticles, liposomes, lipid nanocapsules, mesoporous silica nanoparticles, ...) [230]. In the context of DDD, polymeric nanoparticles have already been developed to encapsulate or immobilize growth factors onto the surface of nanoparticles [239]. In parallel, self-organized spherical liposomes (80–300 nm in diameter) contain at least one lipid bilayer surrounding an aqueous core. Thus, hydrophobic and hydrophilic molecules can be encapsulated within the lipid bilayer or the aqueous core, respectively. Liposomes were particularly studied as gene and siRNA carriers, especially cationic liposomes (see the part dedicated to RNA interference). To date, use of liposomes for tackling IVD degeneration has not yet been reported. Mesoporous silica nanoparticles could be also a promising nanosized platform to deliver biological factors. Indeed, mesoporous colloidal silica exhibit several advantages such as cytocompatibility, a high specific surface area, and ease of functionalization [240]. Additionally, the size, shape, surface pattern, porosity, and pore size of silica nanoparticles are easily tunable.

### 3.2.4. Boosting the endogenous repair process

With respect to the numerous factors playing a crucial role in IVD physiopathology, many biological factors could be associated to GDF5 and GDF6 for the activation and stimulation of reparative cells (Fig. 3). Three categories can be defined: (i) biological factors with pro-anabolic effect; (ii) biological factors with anti-catabolic and anti-inflammatory effects; (iii) nucleic acid, and particularly interfering RNA (siRNA and miRNA), with a large panel of biological effects.

#### • Pro-anabolic biological factors

Among the anabolic factors that have been identified as pivotal elements in IVD growth and homeostasis, this review is focused on those with therapeutic potential. Among them, TGF- $\beta$  is considered to be a potent factor capable of stimulating aggrecan synthesis by NP cells. TGF- $\beta$  maintains the expression level of CCN2 [241] and is also able to counteract the effects of pro-inflammatory cytokines (IL-1, TNF- $\alpha$ ) that classically down-regulate CCN2 expression [242]. Interestingly, a synergistic effect of TGF- $\beta$  associated to GDF5 was recently demonstrated to induce the nucleopulpopogenic differentiation of MSC [43].

Other biological factors with pro-anabolic effects and anti-apoptotic activity toward NP cells have been identified. Indeed, insulin-like growth factor (IGF-1) [243,244], platelet-derived growth factors (PDGFs) [243,245], and fibroblast growth factor (FGF-2) [246] are able to stimulate the *in vitro* proliferation of human and mammalian NP cells and inhibit their apoptosis.

Other members of the BMP family, including GDF5 and GDF6, are also highlighted to play a pivotal role in IVD physiopathology and to enhance ECM synthesis. BMP7 (also named OP-1, osteogenic protein 1) [247–249], BMP12 [250], and BMP2 [250,251] induce an increase in proteoglycan synthesis and the proliferation of NP cells.

Despite their promising properties, the potential adverse effects of BMPs also have to be taken into account, particularly in the case of BMP2, which has been reported to cause unwanted bone formation

[252]. One group published that intradiscal bolus injection of BMP-7 showed no regenerative effects as well as an extensive extradiscal bone formation in a spontaneous canine IVD degeneration model, although the data is questionable [253]. Conversely, the upregulation of BMP-2 by simvastatin injection reverses IVD degeneration with an increase of type II collagen and aggrecan expression [254].

Besides full-length growth factors, the use of synthetic peptides, such as Link-N, as a mediator of NP repair may also be an interesting option, notably because of their reduced cost. N-link has been shown to stimulate the synthesis of ECM components (type II collagen and aggrecan expression) in a rabbit IVD model [255] and an *ex vivo* organ culture model [256]. Interestingly, NP injection of Link-N peptide and exogenous MSC in a bovine model supports the concept that biological repair of IVD is feasible in the early stage of degeneration [257].

#### • Anti-catabolic and anti-inflammatory biological factors

During IVD degeneration, it is well established that matrix metalloproteinases (MMP), disintegrin and metalloproteinase with thrombospondin motifs (ADAMTS), and tissue inhibitor of metalloproteinase (TIMP) affect the overall ECM turnover, resulting in a net loss of proteoglycans [258]. In parallel, tumor necrosis factor alpha (TNF- $\alpha$ ), interleukin-1 (IL-1), interleukin-6 (IL-6), nitric oxide (NO), and prostaglandin E2 (PGE2) have the ability to enhance the catabolic activity of MMP3, MMP12, and ADAMTS-4, and suppress proteoglycan and collagen production. Consequently, anti-TNF- $\alpha$  treatment (monoclonal antibody infliximab, REMICADE®) has been proposed and was shown to significantly decrease the *in vitro* expression of IL-1 $\beta$  and IL-6 in NP cells [259]. Interestingly, the injection of human IVD cells overexpressing IL-1 receptor antagonist in human IVD degenerative explants led to a decrease of IL-1 proteolytic activity, thus demonstrating their anti-catabolic potential [260]. In addition, the IVD injection of monoclonal anti-IL-6 receptors, such as atezolizumab (TECENTRIQ®) or tocilizumab (ACTEMRA®), decreased inflammation-related pain in patients with LBP [261]. From our perspective, the local injection into the IVD of these monoclonal antibodies could theoretically minimize their potential systemic adverse. Before their agreement to treat the IVD degeneration, a rigorous analysis of benefit/risk from the FDA (Food and Drug Administration) or the EMA (European Medicines Agency) will be nevertheless needed.

#### • RNA interference (RNAi): small interfering RNA (siRNA) and micro RNA (miRNA)

In 2006, the Nobel Prize in Physiology or Medicine was awarded jointly to Andrew Fire and Craig Mello, a mere eight years after they published their breakthrough discovery of RNA interference (RNAi) [262]. From then on, the regulatory potential of RNAi to inhibit gene expression by neutralizing targeted mRNA was described and, nowadays, RNAi is being considered as a possible explanation of degenerative processes as well as a novel therapeutic tool.

The biological process for neutralizing mRNA molecules involves siRNA (small interfering RNA) and miRNA (microRNA). Both are processed inside the cell by the enzyme Dicer and are incorporated into the RNA-induced silencing complex (RISC). siRNA is considered an exogenous double-stranded RNA taken up by cells. It enters the cells *via* vectors and typically binds perfectly to its mRNA target. On the other hand, miRNA is single stranded and comes from endogenous non-coding RNA that is found within the introns of larger RNA molecules. In contrast to siRNA, miRNA can inhibit the translation of many different mRNA sequences because its pairing is imperfect. Although RNAi molecules represent only 1%–3% of human genome, they are able to regulate

approximately 30% of the protein-coding genes in humans. Consequently, it is well established that miRNA dysregulation is a key player in many diseases, e.g. cancers [263], degenerative diseases [264], and cardiovascular diseases [265], as well as in IVD degeneration, and thus could be envisioned as a new tool and/or target for therapeutic strategies [266]. Currently, there are 449 clinical studies investigating miRNAs worldwide ([ClinicalTrials.gov](http://ClinicalTrials.gov)) and covering a large panel of diseases. Some forms of miRNA dysregulation have emerged as key players in IVD degeneration due to their triggering of multiple pathological processes, including apoptosis, ECM homeostasis (anabolism and catabolism), cell proliferation, cell migration, and the inflammatory response (see [267] for review and Table 3 with updated data).

In this context, strategies that consist in restoring dysregulated miRNA by using targeted mimics and inhibitors (also named antagomirs) have substantial merit and could be a promising biological approach for mitigating or reversing IVD degeneration. Despite the huge progress in miRNA basic science, many challenges are still ahead; among them, the off-target effects of therapeutic miRNAs remain a real concern. Moreover, considering that the majority of miRNAs have multiple targets, the identification of all miRNA targets implicated in IVD degeneration will be an important prerequisite. The safety of miRNA as a therapeutic approach also needs to be taken into account. Indeed, the incorporation of miRNA in the RISC complex is random in mammalian cells, thus any alteration could affect the activity of other miRNAs and lead to misregulation with potential adverse effects, notably tumorigenesis. Consequently, as there is clear overlap between the key genes that control pluripotency, differentiation, proliferation, apoptosis, and other biological processes, miRNA strategies for the purposes of IVD repair and regeneration must be meticulously designed.

Another major challenge will be the *in situ* vectorization of these miRNA to permit their delivery into the cell for their subsequent regulation of target genes. The direct injection of miRNAs and antagomiRNAs could be a simple method but the effect would typically be short-lived due to the degradation of oligonucleotides by endonucleases. Moreover, miRNAs induce their effects on target genes after transmembrane import and translocation to the nucleus. The improvement of *in vivo* delivery systems will be necessary for the future clinical application of miRNAs; indeed, nanoparticulate platforms could be particularly adapted for miRNA vectorization, allowing for their transfer and release in the cell cytoplasm [268]. Notably, lipid nanoparticles are interesting potential delivery systems because of their biocompatibility, their capacity to release miRNA within the cell, and their well-controlled formulation and characterization [269]. Currently, nanoparticles are considered as an optimal delivery system for miRNA due to their inherent properties (small size, stability, and biocompatibility) [270]. Among their associated drawbacks, the removal of nanoparticles by the reticulo-endothelial system following systemic injection has been highlighted, but this is unlikely to be a problem in the perspective of IVD injection. While the efficacy of nanoparticles to deliver miRNAs to target cells was clearly demonstrated in several applications, notably in treatment of cancer using systemic administration [271], data regarding IVD local injection is required to confirm the suitability of miRNA-nanoparticles strategies for IVD regeneration.

In summary, the combination of anabolic, anti-catabolic, and anti-inflammatory biological factors, as well as miRNAs, with GDF5 and GDF6 could provide new opportunities for the prevention and treatment of DDD. However, additional experiments are needed to assess the safety of this cocktail of biological factors and to demonstrate their potential synergistic effects.

#### 4. Conclusion

IVD cell-based therapies and endogenous repair strategies have the common goal of helping clinicians manage patients suffering from discogenic LBP. It should be stressed that intradiscal injection of exogenous cells has shown attractive results albeit to a lower extent than

**Table 3**

miRNAs closely associated with the development of IVD degeneration and candidates for bioinspired innovative approach.

GDF-5: Growth differentiation factor-5; IL-1 $\beta$ : Interleukin-1 $\beta$ ; NP: *Nucleus pulposus*; IDD: Intervertebral disc disease; ATG7: Ubiquitin-like modifier-activating enzyme; MMP13: Matrix Metalloproteinase 13; ERK1/2: Extracellular signal-regulated kinases; ACAN: aggrecan; ADAMTS5: A disintegrin and metalloproteinase with thrombospondin motifs; TNF- $\alpha$ : Tumor necrosis factor- $\alpha$ ; LPS: lipopolysaccharide; TRAF6: TNF receptor associated factor; IVD: Intervertebral disc; PTEN: phosphatase and TENsin homolog; GAS1: Growth Arrest Specific 1; AKT: serine-threonine protein kinase; MAP3K9: Mitogen-Activated Protein Kinase Kinase Kinase 9; FADD: Fas-Associated protein with Death Domain; BCL2: B-cell lymphoma 2; SDF-1: Stromal Derived Factor-1.

miRNA	Target and biological activity	References
miR7	Identification of GDF5 as a target of miR7. Downregulation of miR7 prevents NP cell detrimental catabolic changes in response to IL-1 $\beta$ .	Liu et al., 2016, Biomed Pharmacother [286]
miR34a	miR34a downregulation prevents IL-1 $\beta$ -induced extracellular matrix degradation in human NP by increasing GDF5 expression.	Liu et al., 2016, Exp Biol Med [287]
miR98	Downregulation of miR98 promotes IDD through the IL-6/STAT3 signaling pathway.	Ji et al., 2016, JBMR [288]
miR210	miR210 inhibits autophagy via silencing of ATG7, leading to increased Col II and aggrecan degradation in human degenerated NP cells.	Zhang et al., 2017, Biomed Pharmacother [289]
miR27b	Downregulation of miR27b involved in loss of type II collagen by targeting MMP13 in Human NP cells.	Li et al., 2016, Spine [290]
miR133a	Dysregulation of miR-133a mediates loss of type II collagen by targeting MMP9 in Human NP cells.	Xu et al., 2016, Spine [291]
miR193a-3p	miR193a-3p promotes type II collagen expression in NP cells. Identification of MMP14 as a target of miR193a-3p. Downregulation of miR193a-3p induces the expression of MMP14, which promotes loss of type II collagen.	Ji et al., 2016, J Mol Med [292]
miR93	miR93 down-regulated in degenerative NP tissues. Overexpression of miR-93 stimulates type II collagen expression in NP cells. Identification of MMP3 as a target of miR-93.	Jing and Jiang, 2015, Cell Prolif [293]
miR155	Identification of ERK1/2 as a target protein regulated by miR155. Inhibition of miR155 decreases the expressions of type II collagen and glycosaminoglycan.	Ye et al., 2016, Dis Markers [294]
miR377	PKC $\epsilon$ signaling upregulates aggrecan as part of a PKC $\epsilon$ /ERK/CREB/AP-1-dependent transcriptional program that includes concurrent upregulation of ACAN and hsa-miR-377. Downregulation of the miR-377 targets ADAMTS5.	Tsirimoniaki et al., 2013, PLoS One [295]
miR494	miR494 is a regulator of Human NP cells apoptosis induced by TNF- $\alpha$ via the regulation of JunD.	Wang et al., 2015, Biochimie [296]
miR194, 515	Inflammatory cytokines stimulated miR194 and 515. Inhibition of microRNA194 and 515 rescued CHSY-1/2/3 expressions and chondroitin sulfate deposition.	Hu et al., 2017, Oncotarget [297]
miR146	Transfection of miR-146a decreases IL-1 induced mRNA levels of inflammatory genes and catabolic proteases in NP cells. miR146a suppresses IL-1 induced protein levels of MMP and aggrecanases.	Gu et al., 2015, Gene [298]
miR146a	Overexpression of miR146a decreased the levels of pro-inflammatory cytokines in LPS-stimulated NP cells. TRAF6 and NF- $\kappa$ B were downregulated by miR-146a overexpression.	Be et al., 2017, Med Sci Monit [299]
miR10b	miR10b levels associated with IVD degeneration grade and downregulation of	Yu et al., 2013, PLoS One [300]

Table 3 (continued)

miRNA	Target and biological activity	References
	HOXD10. Overexpression of miR-10b stimulates cell proliferation with concomitant translational inhibition of HOXD10. Aberrant miR10b upregulation in IVD degeneration contributes to abnormal NP cell proliferation.	
miR21	miR21 upregulated in degenerative NP. Identification of PTEN as a target of miR-21. miR21 overexpression stimulates cell proliferation and AKT signaling pathway activation.	Liu et al., 2014, Int J Mol Sci [301]
miR184	Expression of miR184 is positively correlated with IVD degeneration grade. Identification of GAS1 as a direct target gene of miR184 in NP cells. miR184 overexpression induces AKT phosphorylation.	Li et al., 2016, World Neurosurgery [302]
miR15a	miR15a up-regulated in degenerative NP tissues. Identification of MAP3K9 (activator of MAPK kinase) as a target of miR-15a Overexpression of miR-15a promotes NP cells proliferation and induced apoptosis.	Cai et al., 2017, Biomed Pharmacother [303]
miR143	miR143 expression significantly increased, while that of BCL2 decreased in degenerative IVD. Identification of BCL2 as a target gene of miR143. miR143 overexpression enhances apoptosis of NP cells.	Zhao et al., 2017, Gene [304]
miR155	Transfection of miR155 results in repression of FADD and caspase-3. Fas-mediated apoptosis increases when antagonizing miR155 and decreased when using pre-miR-155 in human NP cells.	Wang et al., 2011, J Pathol [305]
miR23a	Modulation of miR23a changes the migratory potential of hematopoietic progenitor cells in co-culture experiments. TGFβ1 mediates its inhibitory effect on SDF-1 levels by upregulation of miR23a.	Arabianian et al., 2014, Haematologica [306]
miR886-3p	Transfection of miR886-3p into stromal cells down-regulates the expression of SDF-1.	Pillai et al., 2010, PLoS One [307]

initially expected. Many obstacles have been identified, particularly the lack of understanding of IVD pathophysiology. The clinical expansion of cell therapy is also limited by regulatory and technological problems as well as economic hurdles. Exogenous cell-based therapies and endogenous repair should not be opposed and lessons learned from the former will be particularly instrumental for the development of the latter.

Endogenous repair could overcome some of the obstacles to IVD cell-based therapies. There are, however, many critical issues to be solved before a validated approach can be transposed to the clinic. *In vitro*, the effects of biological factors (growth factors, chemokines, etc.) are well controlled; though controlling the *in vivo* effects of these various biological factors and the resulting inappropriate and potentially dramatic reactions will be a major challenge. The anticipation associated with the discovery of progenitor cells should not mask the difficulties that biomaterial experts will face in guaranteeing delivery of the right dose, at the right time, and in the right place, and biologists will face in confirming the target and efficacy of potential treatments.

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