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# Innovative strategies for intervertebral disc regenerative medicine: from cell therapies to multiscale delivery systems

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1 **1. Abstract**

2 As our understanding of the physiopathology of intervertebral disc (IVD) degeneration has improved,  
3 novel therapeutic strategies have emerged, based on the local injection of cells, bioactive molecules, and  
4 nucleic acids. However, with regard to the harsh environment constituted by degenerated IVDs, protecting  
5 biologics from in situ degradation while allowing their long-term delivery is a major challenge. Yet, the  
6 design of the optimal approach for IVD regeneration is still under debate and only a few papers provide a  
7 critical assessment of IVD-specific carriers for local and sustained delivery of biologics. In this review, we  
8 highlight the IVD-relevant polymers as well as their design as macro-, micro-, and nano-sized particles to  
9 promote endogenous repair. Finally, we illustrate how multiscale systems, combining in situ-forming  
10 hydrogels with ready-to-use particles, might drive IVD regenerative medicine strategies towards  
11 innovation.

12

13 **2. Outlook section**

- 14
- 15 • Intervertebral disc (IVD) regenerative medicine could benefit from the emergence of cell,  
16 molecule-, gene- and siRNA-based strategies.
  - 17 • Design of IVD-specific carriers to protect biologics from in situ degradation, while allowing their  
18 local and sustained delivery, is a major challenge.
  - 19 • A critical assessment of IVD-relevant natural and synthetic polymers, and their design as macro-,  
20 micro-, and nano-sized particles is provided.
  - 21 • Multiscale systems, combining in situ forming hydrogels with ready-to-use particles, are  
22 introduced as new opportunities to stimulate IVD regeneration.

### 23 **3. Introduction**

24 Low back pain (LBP) is a common health condition and it is now well established that over 80% of adults  
25 will experience LBP during their lifetime. LBP has become the second most frequent cause of hospital  
26 visits and the leading cause of years lived with disability (Maher et al., 2017; Vos et al., 2016). Thanks to  
27 a better understanding of the etiology of LBP, the involvement of intervertebral disc (IVD) degeneration,  
28 referred to as disc degenerative disease (DDD) (Vos et al., 2016), was identified.

29 Current treatments of LBP include nonpharmacological, pharmacological, or surgical approaches, all  
30 aimed at controlling pain. For early degenerative stages, pharmacotherapies are mainly used (Abdel  
31 Shaheed et al., 2016) in association with rehabilitation or education of the patient (Latimer et al., 2004).  
32 For more advanced cases, surgical approaches are considered if conservative methods fail. Spine fusion  
33 aims to directly remove the IVD and immobilize the adjacent vertebrae. However, this intervention  
34 reduces spine mobility and neighboring segments can be affected by an accelerated degenerative process  
35 (Baliga et al., 2015). Arthroplasty, with *Nucleus pulposus* or IVD total replacement, aims at conserving  
36 the spine motion notably at the affected levels. These interventions, although generally clinically effective  
37 in alleviating pain (Galbusera et al., 2008), are very invasive and often include a long postoperative  
38 recovery and a non negligible risk of complications (Baliga et al., 2015).

39 Recent progress in our understanding of IVD physiopathology has led to new approaches for IVD  
40 regenerative medicine. In view of the decreasing number of cells in degenerating IVD, cell-based  
41 therapies were studied with growing interest. However, owing to the restrictive regulatory framework and  
42 the difficult clinical translatability of cell-based therapies, the use of biological factors targeting IVD  
43 degenerative processes was also contemplated. More recently, the role of gene mutations or microRNA  
44 dysregulation was identified at the onset of DDD, leading to the development of a third therapeutic  
45 approach based on the delivery of genes or small interfering RNAs (siRNA). In this context, this review  
46 will first briefly give recent insights into the pathophysiological mechanisms of DDD and then focus on  
47 describing cell-, molecule-, gene-, and siRNA-based strategies for tackling DDD as well as the scaffolding  
48 biomaterials used for their respective delivery.

49

50

### 51 **4. IVD degeneration physiopathology**

#### 52 **4.1. IVD structure**

53 IVDs are fibro-cartilaginous tissues located between the vertebrae that are avascular and non-innervated  
54 (Whatley and Wen, 2012). They act as shock absorbers by distributing the mechanical loads along the  
55 spine and facilitate trunk mobility (Whatley and Wen, 2012; Zhou et al., 2014). IVDs are generally  
56 presented as a tripartite anatomical entity composed of the following parts (Figure 1).

57 **The *Nucleus pulposus* (NP)** is the inner part and the most hydrated region of the IVD (Fontana et al.,  
58 2015). NP cell density in human is the lowest of the IVD (Maroudas et al., 1975). These cells can be  
59 classified into two types: notochordal cells and nucleopulpyocytes (Colombier et al., 2014). Human adult  
60 NP cells produce a highly hydrated extracellular matrix (ECM), rich in proteoglycans (mainly aggrecan)  
61 and type II collagen.

62 **The *Annulus fibrosus* (AF)** is the outer part of the IVD. It can be divided into the outer AF containing a  
63 oriented lamellar array of densely packed collagen fibers, while the inner AF is less dense and less  
64 organized (Raj, 2008; Whatley and Wen, 2012). AF cells have a fibroblastic morphology (Sakai and Grad,  
65 2015). The AF is composed of water, types I and II collagen fibers, proteoglycans, and other proteins  
66 (Roughley, 2004). The AF retains the pressurized NP and allows for disc compression/decompression  
67 (Fontana et al., 2015).

68 **The endplates (EPs)**, composed of hyaline cartilage at the top and bottom of the vertebral bodies,  
69 interface the IVDs and the adjacent vertebrae. EPs cells are chondrocytes that synthesize an ECM rich in  
70 proteoglycans, types I and II collagens, and water. Mechanically, EPs support significant loads during  
71 daily-living activities and distribute the intradiscal pressures onto the adjacent vertebrae. They represent  
72 the main route for glucose and oxygen to reach the disc cells through the vertebral capillaries and for  
73 waste products to be eliminated from the disc (Fontana et al., 2015; Lotz et al., 2013).

74

#### 96 **4.2. Extracellular matrix anabolism/catabolism homeostasis**

97 DDD has a complex and multifactorial etiology. Genetics, environmental causes, mechanical factors,  
98 aging, as well as systemic and toxic factors (Elmasry et al., 2015; Hadjipavlou et al., 2008) were identified  
99 as risk factors. Interestingly, recent data highlighted that the concomitant presence of notochordal cells  
100 and nucleopulpocytes was a pivotal factor for IVD ECM homeostasis (Erwin et al., 2006). Indeed, these  
101 two cell types interact via synthesized molecules and the early disappearance of notochordal cells is  
102 associated with a downregulation of nucleopulpocyte survival. This decrease in nucleopulpocyte density  
103 leads to a misbalance in the ECM anabolism/catabolism ratio that culminates in the alteration of the  
104 mechanical properties of IVDs (Erwin et al., 2011). In the advanced stages of IVD degeneration,  
105 proteoglycans, hydration, and the type-II/type-I collagen ratio are decreased (Russo et al., 2016; W.-J.  
106 Wang et al., 2015), leading to a dehydrated and fibrotic NP with a solid-like mechanical behavior (Russo  
107 et al., 2016). Additionally, the AF structure is disorganized and EPs calcify (Fontana et al., 2015; W.-J.  
108 Wang et al., 2015). As the degeneration worsens, innervation and vascularization of the disc occur  
109 (Whatley and Wen, 2012), leading to discogenic pain (Alkhatib et al., 2014).

110 From a molecular point of view, IVD degeneration is associated with an upregulation of pro-inflammatory  
111 cytokines such as interleukin-1 $\beta$  (IL-1 $\beta$ ) and tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) (Sakai and Grad, 2015).  
112 These two molecules trigger the over-expression of matrix metalloproteinases (MMPs) notably MMP-1, -  
113 2, -3, -7, -8, and -13. These MMPs are known for their proteolytic action toward collagen and  
114 proteoglycans (Kepler et al., 2013b; W.-J. Wang et al., 2015). Similarly to MMPs, there is a marked  
115 increase in the a disintegrin and metalloprotease with thrombospondin motifs (ADAMTS) 4 and 5.  
116 ADAMTS 4 and 5 act as aggrecanases, thereby contributing to worsening ECM degradation (Molinos et  
117 al., 2015; W.-J. Wang et al., 2015). As a pro-inflammatory cytokine, IL-1 $\beta$  up-regulates the expression of  
118 vascular endothelial growth factor (VEGF), nerve growth factor (NGF), and brain-derived neurotrophic  
119 factor (BDNF), thereby triggering the neo-vascularization and neo-innervation of IVD that ultimately  
120 result in inflammation and pain (Alkhatib et al., 2014; Whatley and Wen, 2012). Finally, in the presence  
121 of TNF- $\alpha$  or IL-1 $\beta$ , NP cells tend to secrete chemo-attractant molecules including (C-C motif) ligand  
122 5/regulated on activation of normal T cells expressed and secreted (CCL5/RANTES) or chemokine (C-X-  
123 C motif) ligand 6 (CXCL6) (Grad et al., 2016), thus increasing the migration of mesenchymal stem cells  
124 (MSCs) (Pattappa et al., 2014). This increased secretion of CCL5 was correlated with painful degenerated  
125 IVDs (Kepler et al., 2013a). In addition, stromal cell-derived factor-1 (SDF-1) was over-expressed in  
126 degenerated IVDs and involved in NP cell apoptosis (Z. Liu et al., 2016). Interestingly, SDF-1 also  
127 demonstrated a chemo-attracting effect toward MSC migration in an ex vivo IVD culture model (Pereira  
128 et al., 2014).

129 In contrast to these catabolic factors, anabolic factors stimulate the biological cascade involved in  
130 regenerative processes. Among them, Transforming Growth Factor- $\beta$  (TGF- $\beta$ ) is known to play a pivotal  
131 role in collagen and proteoglycan synthesis and in ECM homeostasis (S. Wang et al., 2015). TGF- $\beta$  is also  
132 one of the most potent inhibitors of TNF- $\alpha$ -induced upregulation of MMPs as well as of aggrecan and  
133 type-II collagen degradation (Yang et al., 2015). In parallel, Bone Morphogenic Proteins (BMPs) are  
134 known to increase proteoglycan and type-II collagen synthesis as well as cell proliferation and  
135 differentiation (S. Wang et al., 2015). Among them, Growth and Differentiation Factor-5 (GDF-5 or  
136 BMP-14) plays a crucial role during musculoskeletal development and postnatal IVD maintenance (Feng

137 et al., 2015). GDF-5 gene mutations notably lead to disc abnormalities in mice (Li et al., 2004). GDF-5  
138 also upregulates the proliferation and secretory activities of NP and AF cells (Chujo et al., 2006) and  
139 modulates MMPs, IL-1 $\beta$ , or TNF- $\alpha$  expression levels (Enochson et al., 2014; Gruber et al., 2014a).  
140 Altogether, these data highlight the pivotal role of GDF-5 in IVD matrix homeostasis.

141 The ECM homeostasis of IVDs and the consequences associated with a homeostasis rupture are illustrated  
142 in Figure 2.

143

## 144 **5. From physiopathology to IVD specific biotherapies**

145 Regarding the multifactorial nature of DDD, three main therapeutic strategies were considered in the past  
146 few years: cell-, molecular-, and nucleic acid-based therapies (Figure 3).

### 147 **5.1. Cell-based therapies**

148 A converging body of evidence suggested that IVD degeneration originates from the early disappearance  
149 of IVD resident cells. Supplementation of the IVD with regenerative cells thus naturally emerged as a  
150 strategy. Repopulating a degenerated IVD with healthy cells aims at either (i) restoring tissue homeostasis  
151 by transplanting cells able to secrete a functional ECM or (ii) tackling in situ inflammation by  
152 transplanting stem cells with immune-suppressive and anti-inflammatory properties. To achieve these  
153 ambitious and clinically relevant objectives, various cells sources were considered. Logically, NP cells  
154 harvested from surgically removed IVDs were the first to be used, with promising results in a canine study  
155 and in human clinical trials (Meisel et al., 2007). However, their further use was dramatically hampered  
156 by the low cell number harvested (Sakai and Grad, 2015). In parallel, pluripotent stem cells such as  
157 embryonic stem cells could be of great interest in view of their theoretical potential to differentiate into  
158 NP cells. Nevertheless, legal and ethical controversies limit the use of these cells for clinical application.  
159 Induced pluripotent stem (iPS) cells were also considered for various applications in regenerative  
160 medicine (Hirshi et al., 2014) and their suitability for IVD repair is being studied (Chen et al., 2013) but  
161 further studies are needed before their potential can be judged. Last but not least, adipose- (ASCs) or bone  
162 marrow-derived (MSCs) mesenchymal stem cells in their undifferentiated state are likely the most widely  
163 used in the DDD context. In addition to providing us with promising results (see next section), the  
164 extensive use of MSCs raised the question of their allogeneic or autologous origin (Richardson et al.,  
165 2016). Indeed, MSCs are considered hypo-immunogenic and immune-evasive cells able to be transplanted  
166 across major histocompatibility barriers (Ankrum et al., 2014), making the use of allogeneic MSCs a  
167 credible alternative to autologous ones.

168 ASCs are considered particularly advantageous because of their abundance in fat tissue, their accessibility,  
169 and their more potent immuno-modulatory effects in comparison with MSC isolated from bone marrow  
170 (Li et al., 2015). Of interest, human ASCs exhibit a nucleopulpogenic potential when committed with  
171 TGF- $\beta$ 1 and GDF-5 (Colombier et al., 2016). When implanted in mice, these cells also demonstrated their  
172 ability to secrete GAGs and type II collagen, consistently with native NP cell capacities (Colombier et al.,  
173 2016). In rabbits, undifferentiated MSCs injected in the NP inhibited disc degeneration by reducing MMP-  
174 2, MMP-3, and MMP-13 expression and by promoting type-II collagen synthesis, resulting in the  
175 maintenance of the IVD structure (Miyamoto et al., 2010). The beneficial outcomes of injecting  
176 undifferentiated MSCs were further demonstrated in other animal models, such as rat, mouse, dog, or pig  
177 models (Henriksson et al., 2009; Hiyama et al., 2008; Jeong et al., 2009; Marfia et al., 2014; Richardson et  
178 al., 2016). In these studies, IVD degeneration was significantly slowed down, disc height and MRI signal  
179 intensity improved, and nucleopulpogenic ECM neo-synthesis was observed. In humans, the intradiscal  
180 injection of MSCs could lower pain and disability (Orozco et al., 2011). This relief was associated with  
181 the anti-inflammatory (Maria et al., 2016; Sakai and Grad, 2015) and pro-anabolic properties of MSCs  
182 (Shabbir et al., 2009). Several ongoing clinical trials are conducted worldwide to determine the optimal  
183 cell source (autologous or allogeneic) to be used in DDD (Sakai and Andersson, 2015).

184 Despite their global promise, the intradiscal injection of “naked” MSCs remains hampered by the harsh  
185 environmental condition the cells experience when injected into a degenerated IVD (low oxygen tension,  
186 low nutrient level, metabolic product accumulation, acidic pH, high osmolarity, and high intradiscal  
187 pressure). These stringent conditions are responsible for the poor survival rate and altered activity of the  
188 injected cells. To overcome this hurdle, tissue engineers proposed to use cytoprotective 3D scaffolds able  
189 to create a biomimetic environment that could support cell survival. These scaffolds could additionally  
190 promote cell retention at the injection site thereby limiting the risk of off-target effects related to cell  
191 effusion. These scaffolds will be detailed in the last section of this review.

192

## 193 **5.2. Molecular therapies**

194 More recently, and facing the numerous limitations of cell transplantation, an alternative to cell-based  
195 therapies emerged. It consists in exploiting the ability of chemoattractant molecules to mobilize and  
196 recruit endogenous progenitor cells at the degenerated site (Illien-Jünger et al., 2012). This strategy, based  
197 on the capability of the endogenous body to self-regenerate, could free clinicians from the restrictive  
198 regulatory framework and cost ineffectiveness of cell transplantation. With respect to the myriad of factors  
199 playing a crucial role in IVD physiopathology, many chemoattractant, pro-anabolic, and anti-catabolic  
200 molecules were contemplated for the stimulation of resident cells or the attraction/recruitment of  
201 endogenous progenitors.

202

203 One key factor for improving the endogenous repair of IVDs via cell attraction/recruitment is the presence  
204 of endogenous progenitors with stem cell properties in the IVD (Risbud et al., 2007). These IVD-derived  
205 progenitor cells (either from NP or EPs) are very similar to MSCs isolated from the bone marrow of the  
206 same patient (Blanco et al., 2010). Interestingly, analyses of the migration capacity of progenitors located  
207 in the NP, AF, and EP indicate that EP progenitors have the highest migration and invasion potency (S.  
208 Liu et al., 2016). To favor cell migration in degenerative IVDs, chemokines were studied since, as  
209 chemotactic cytokines, they are involved in cell mobilization, attraction, and homing. Regarding IVDs, the  
210 three main chemokines expressed are SDF-1, CCL5, and CXCL16, both in healthy or degenerated IVDs.  
211 Interestingly, the expression of SDF-1 and CXCR4 was upregulated in degenerated IVDs, notably in  
212 MSCs isolated from the EPs (S. Liu et al., 2016; Zhang et al., 2014). SDF-1 also increased MSC attraction  
213 in a nucleotomized disc model (Pereira et al., 2014). The implication of SDF-1 in stem cell attraction was  
214 also demonstrated in many tissues including myocardium or periodontal ligament (Kaku et al., 2017;  
215 Purcell et al., 2012). Recently, IVD cells stimulated with IL-1 $\beta$  and TNF- $\alpha$  were found to secrete a higher  
216 concentration of CCL5 compared with IVD cells cultured under physiological conditions (Gruber et al.,  
217 2014b). The presence of CCL5 in degenerated IVDs as well as herniated and scoliotic discs was further  
218 demonstrated by proteomic assays in other in vitro studies in humans (Kawaguchi et al., 2002; Kepler et  
219 al., 2013a). Finally, in AF cells, CXCL16 is up-regulated by IL-1 $\beta$ , suggesting a role for the  
220 CXCL16/CXCR6 system (Gruber et al., 2016).

221 Altogether, these data highlight the role of chemokines in the degenerative process of IVDs and strengthen  
222 their relevance in developing new strategies to attract progenitor cells and stimulate endogenous repair  
223 (Illien-Jünger et al., 2012).

224

225 In line with our understanding of DDD, anabolic factors can contribute to IVD regeneration. A large panel  
226 of anabolic factors was contemplated for IVD regeneration. Here, we mainly focus on the factors that  
227 demonstrated promising results in several reports.

228 TGF- $\beta$  contributes toward maintaining the expression levels of connective tissue growth factor (CCN2),  
229 one of the most potent factors able to stimulate aggrecan synthesis, in NP cells (Tran et al., 2014). In  
230 addition, CCN2 suppressed the inductive effect of IL-1 $\beta$  on catabolic genes (Tran et al., 2014).



231 Considering that pro-inflammatory cytokines decreased CCN2 expression, this makes TGF- $\beta$  an anabolic  
232 factor able to counteract the effects of pro-inflammatory cytokines (Bedore et al., 2014). TGF- $\beta$  was also  
233 used in association with dexamethasone to stimulate the proliferation of degenerated human NP cells and  
234 exhibit an anti-catabolic gene expression profile (Liang et al., 2013).

235 GDF-5 is a major factor involved in early IVD development, and mutations of its related gene lead to  
236 severe structural impairment (Feng et al., 2015). Noteworthy, GDF-5 is down-regulated by IL-1 $\beta$  and  
237 TNF- $\alpha$ . In vitro, GDF-5 favors cell proliferation and stimulates the synthesis of proteoglycan and type II  
238 collagen, thus demonstrating its anabolic activity (Chujo et al., 2006). GDF-5 was used to differentiate  
239 human ASCs into nucleopulpcytes, in association with TGF- $\beta$ 1 (Colombier et al., 2016). Interestingly,  
240 intradiscal injection of GDF-5 in rats and rabbits was shown to increase disc height. Consequently, clinical  
241 trials were conducted to evaluate the safety and efficacy of intradiscal injection of GDF-5 in patients with  
242 DDD (NCT01158924, NCT00813813, NCT01182337, and NCT01124006 on clinicaltrials.gov). As far as  
243 we know, no peer-reviewed publication is currently available. While a modest improvement in clinical  
244 parameters was observed, some adverse events were also reported suggesting that despite the relevance of  
245 GDF-5 in DDD, its direct intradiscal injection may not be the most suitable route.

246 Other anabolic factors were investigated and their anti-apoptotic activity toward NP cells was evidenced.  
247 Indeed, aside from promoting angiogenesis, it is now clear that in IVD, vascular endothelial growth factor  
248 (VEGF) also prevents NP cell apoptosis (Tran et al., 2010). Additionally, insulin-like growth factor (IGF-  
249 1), platelet-derived growth factors (PDGFs), epidermal growth factor (EGF), and fibroblast growth factor  
250 (FGF-2) stimulate cell proliferation and inhibit cell apoptosis in human and animal cells (Vasiliadis and  
251 Pneumaticos, 2014). Together with the aforementioned CCN2 (Tran et al., 2014), these factors  
252 demonstrated their anabolic activity.

253 Regarding the role of anabolic factors in DDD treatment, the use of platelet-rich plasma (PRP), which  
254 contains a myriad of multifunctional growth factors, was recently proposed. Two clinical trials suggested  
255 the efficacy of PRP in DDD (Kristin et al., 2017; Tuakli-Wosornu et al., 2016). Further double-blind and  
256 controlled clinical trials are now required to confirm these first results.

257

258 Recent studies highlighted the key role of both TNF- $\alpha$  and interleukins in inflammation and further IVD  
259 degeneration, notably via the up regulation of MMPs and aggrecanases.

260 Thus, anti-TNF- $\alpha$  treatments (infliximab) were proposed and showed a significant decreased expression of  
261 IL-1 $\beta$  and IL-6 in NP cells (Walter et al., 2015), thereby demonstrating their anti-catabolic activity. In  
262 addition, the local administration of anti-IL-6 receptor, such as atlizumab or tocilizumab, lowered  
263 inflammation-related pain in humans (Sainoh et al., 2016). Interestingly, human IVD cells overexpressing  
264 IL-1 receptor antagonist were able to lower an IL-1-dependent increase of MMPs when injected into IVD  
265 degenerative explants, demonstrating their anti-catabolic potential (Le Maitre et al., 2007). In parallel,  
266 because NF- $\kappa$ B increases IVD degeneration via the upregulation of pro-inflammatory cytokines (Fontana  
267 et al., 2015), studies examined its inhibition. Indeed, human NP cell exposure to NF- $\kappa$ B inhibitor led to  
268 significantly decreased IL-1 $\beta$ -dependent MMPs and ADAMTS upregulation (Orita et al., 2013; Zhongyi  
269 et al., 2015). In addition, inhibition of other cytokines such as BDNF, NGF, VEGF, or TNF- $\alpha$  was studied  
270 and showed promising results in the context of inflammation-associated DDD (Orita et al., 2011; Walter et  
271 al., 2015; Wu et al., 2011)

272 In parallel to the previously described IVD degenerative processes, aging-associated accumulation of  
273 oxidative damage is of great interest. Collagen is subject to glycoxidation reactions that form advanced-  
274 glycation end-products (AGEs). The accumulation of these AGEs molecules contributes to IVD  
275 degeneration by increasing TNF- $\alpha$ , MMPs, and aggrecanases expression, and to a greater extent AGEs  
276 participate in the alteration of structural and mechanical properties of the ECM (Colombini et al., 2008).  
277 Interestingly, administration of an anti-inflammatory drug (pentosan-polysulfate) associated with anti-

278 AGE molecules (pyridoxamine) alleviated NP degeneration by lowering MMPs and ADAMTS production  
279 in diabetic mice (Illien-Junger et al., 2013).

280

281 With the discovery of molecules involved in IVD degenerative processes, new therapeutic approaches  
282 were proposed, either through stimulating anabolism or inhibiting catabolism. Despite encouraging  
283 results, the short half-life of such molecules does not allow for long-term activity, and they may not be  
284 sufficient to reactivate the physiological IVD machinery. Whether embedding these molecules in  
285 biomaterials to allow their in situ sustained and controlled delivery, while protecting them from  
286 proteolytic degradation, is a good option will be discussed in the next section.

287

### 288 **5.3. Gene and interfering RNA therapies**

289 An alternative to biotherapies may be the genetic modification of resident cells through gene transfer, for  
290 which two strategies can be envisioned: (i) The vector containing the gene can be introduced in the body  
291 (direct or in vivo gene therapy) or (ii) target cells can be harvested, genetically modified in vitro, and re-  
292 implanted (indirect or ex vivo gene therapy) (Goldspiel et al., 1993). Considering the decreased number of  
293 cells in degenerative IVD, direct gene therapy could be of interest for early degeneration, while the  
294 indirect approach seems more suitable for more severe cases. Two carriers were used for gene delivery:  
295 non-viral and viral vectors. Non-viral gene therapy refers to the use of naked DNA or cationic compounds  
296 (liposomes, polyplexes, lipopolyplexes) (Daraee et al., 2014; Rezaee et al., 2016). Such gene carriers help  
297 avoid the risks of mutagenicity, viral infections, and viral immunogenicity (Mintzer and Simanek, 2009).  
298 However, their application in IVD degeneration treatment remains limited. Regarding viral vectors, while  
299 retroviruses are the most commonly used, they have a more potent action on cells with a high dividing rate  
300 making them poorly adapted for NP-cell-mediated IVD gene therapy (Miller et al., 1990). Many authors  
301 thus chose adenoviruses despite their potential infectious and immunogenic risk owing to their higher  
302 transduction efficiency notably in quiescent or slowly dividing cells. Therapeutic genes encoding for anti-  
303 inflammatory agents or MMPs inhibitors (e.g., TIMP-1) were considered as they could drastically reduce  
304 the expression of MMPs involved in ECM degradation (W.-J. Wang et al., 2015). It was found that the use  
305 of adenoviral vectors coding for BMP-2 and TIMP-1 on human degenerated disc cells increased  
306 proteoglycan synthesis (Wallach et al., 2003). Adenoviruses were also used to deliver genes coding for  
307 growth factors such as GDF-5 (X.-W. Luo et al., 2016) or TGF- $\beta$  (Nishida et al., 1999) and they were  
308 shown to efficiently stimulated anabolism.

309 In parallel to classic gene therapies, there is growing interest in the use of siRNAs. SiRNAs are short non-  
310 coding sequences of 20–25 nucleotides that control gene expression by binding to their target mRNA and  
311 causing the repression of RNA translation or degradation (Bartel, 2004). Many miRNAs deregulations  
312 emerged as key players in IVD degeneration by triggering NP cell apoptosis, ECM degradation, or NP cell  
313 proliferation and inflammatory response (see (Zhou et al., 2017) for review) and can thus be envisioned as  
314 new therapeutic targets.

315 Gene- and RNA interfering-based therapies seem to be particularly interesting for restoring the  
316 anabolic/catabolic balance in IVDs. However, as NP cell density drastically decreases during  
317 degeneration, the effectiveness of such approaches needs to be clarified. Finally, adenoviruses or siRNAs  
318 are not integrative tools and do not lead to extended gene expression. In addition, as siRNAs need to be  
319 protected from endonuclease degradation, the use of specifically designed biomaterial-based systems, as  
320 mentioned previously for peptidic molecules, could be a reasonable option.

321

322 As stated above, the broadening of our understanding of IVD degenerative processes led to new  
323 therapeutic strategies based on the local administration of cells, molecules, genes, or siRNA and several  
324 clinical trials assessing these three main therapeutic strategies have been reported (Table 1). As of

325 November 2017, eight clinical trials have been published using autologous or allogenic cells, four trials  
326 have reported the injection of anabolic factor GDF-5 and only one trial is based on PRP. To the best of our  
327 knowledge, there is no ongoing clinical trial based on gene or RNA interfering therapies for IVD  
328 regeneration. Despite encouraging results, these therapies need further improvement with regard to the  
329 harsh environment in which these biologics are injected. Thus, biomaterials were considered so as to  
330 improve cell survival and protect molecules and nucleic acids from degradation. These biomaterials could  
331 additionally allow the biologics to be delivered in a spatiotemporally controlled manner thereby promoting  
332 long-term IVD regeneration.

333

## 334 **6. Engineering biomaterials for IVD delivery**

335 Successful delivery of fragile biologics into IVDs is highly challenging, mostly because of their short in  
336 vivo half-life. With the aim of targeting this poorly vascularized tissue, delivery routes are limited and  
337 thus minimally invasive direct injection received attention. While there are reviews extensively describing  
338 pharmaceutical innovations, including self-setting hydrogels, microspheres, and liposomes, for the oral  
339 (Diab et al., 2017), the intra-vitreous (Bochet and Fattal, 2012), and the transdermal (Naves et al., 2017)  
340 routes, only few papers are available providing a critical assessment of IVD-specific approaches for local  
341 and sustained delivery. Here, we highlight the rational selection of natural or synthetic polymers, we  
342 exemplify the design of delivery systems based on macro-, micro-, and nano- sized properties, and we  
343 illustrate how multiscale combined systems might help direct IVD regenerative medicine strategies.

344

### 345 **6.1. Natural and synthetic polymers**

346 One key parameter in the design of delivery systems is the selection of the constitutive polymer, since the  
347 chemical functionalities of a polymer affect all aspects of its performance, including encapsulation  
348 efficiency as well as polymer degradation rate and release (Mitragotri et al., 2014). As such, recent  
349 advances in delivery systems are based on polymers tailored for a specific tissue and engineered to exert  
350 distinct biological functions.

351 **Among natural polymers**, collagen, hyaluronic acid (HA), alginate, and chitosan were extensively  
352 studied (Sionkowska, 2011). Extracted from natural sources or obtained by bacterial fermentation, these  
353 macromolecules offer various advantages such as large-scale production, low toxicity, and similarity to  
354 ECM components.

355 Collagen, a major component of healthy IVD ECM, exhibits biodegradability, weak antigenicity, and  
356 superior biocompatibility compared with other natural proteins. The widespread biomedical use of  
357 collagen is based on its ability to be shaped into films, sponges, and micro- or nanoparticles (Liu et al.,  
358 2013). Gelatin, derived from collagen hydrolysis, is biodegradable in a physiological environment, with  
359 the formation of harmless metabolic products. In degenerated IVD, injectable gelatin microspheres  
360 impregnated with PRP reduced NP cells apoptosis and slowed down degeneration in adult rabbits  
361 (Sawamura et al., 2009). HA, another leading constituent of the NP ECM, was also studied as a scaffold  
362 for tissue engineering (Sionkowska et al., 2016), but less so for IVD strategies.

363 Although they cannot be found in the human body, alginate, a cell wall polysaccharide constituent of  
364 brown algae, and chitosan, a polysaccharide synthesized by crustaceans, were used for multiple  
365 biomedical applications (Bernkop-Schnürch and Dünnhaupt, 2012). Alginate microparticles were  
366 designed for the in vitro delivery of PRP in the treatment of degenerated IVDs (Sawamura et al., 2009);  
367 however, their ionic gelation-dependent low stability in the body might constitute a drawback. Similarly,  
368 cationic chitosan was considered for IVD regenerative applications, as a cell or molecule carrier (Cheng et  
369 al., 2010; Roughley et al., 2006).

370 **Synthetic polymers** are obtained through reproducible industrial processes, with tunable physicochemical  
371 and mechanical properties. The most commonly used polymers for drug delivery systems are poly(lactic-  
372 co-glycolic acid) (PLGA) and poly- $\epsilon$ -caprolactone (PCL) (Kondiah et al., 2016).

373 PLGA microspheres were widely used for the release of TGF- $\beta$ 3 or anti-inflammatory molecules  
374 (Bhardwaj et al., 2007; Liang et al., 2013). They were used for recombinant human GDF-5 delivery in  
375 vivo in a rat model and increased the synthesis of aggrecan and type II collagen (Yan et al., 2013). PLGA  
376 microspheres were also used for the sustained delivery of IL-1 $\beta$  receptor antagonist in a 3D culture model,  
377 and this treatment effectively attenuated the IL-1 $\beta$ -mediated inflammatory response of the 3D cultured NP  
378 cells (Gorth et al., 2012).

379 PCL, a semi-crystalline and hydrophobic polymer, is currently used in FDA-approved sutures for surgery  
380 and in commercially available contraceptive devices (Wei et al., 2009). PCL is often associated with  
381 hydrophilic polymers, either natural or synthetic ones (e.g., PEG, PAA or pNIPAAm), to form di- or  
382 triblock amphiphilic copolymers (Fu et al., 2014; Li and Tan, 2015). Moreover, PCL was extensively used  
383 for IVD tissue engineering, especially for AF repair (Wismer et al., 2014). PCL-based or PCL-coated  
384 microparticles were successfully designed for the sustained delivery of bioactive molecules, although not  
385 for IVD applications (Jung et al., 2015; Z. Luo et al., 2016).

386 In the context of IVD regenerative medicine, the usefulness of these natural and synthetic polymers is  
387 broadened further by our ability to create systems with multiple shapes and sizes that deliver drugs,  
388 cytokines, growth factors, or stem cells in a controlled manner. They are reviewed in the following  
389 sections.

390

## 391 **6.2. *In situ* forming hydrogels as macro-scaled delivery systems**

392 Hydrogels, networks of hydrophilic polymers capable of absorbing large amounts of water, are of  
393 particular interest for highly hydrated NP and AF tissue regeneration. Besides water, they provide  
394 mechanical support and a 3D microenvironment adapted for cell survival and proliferation. Attention was  
395 focused on the development of stimuli-responsive polymer solutions that could be injected in a minimally  
396 invasive manner prior to *in situ* solidifying, ensuring a perfect fit to the desired tissue. The *in situ*  
397 formation of hydrogels occurs through chemical covalent bonds or physical bonds (electrostatic or  
398 hydrophobic interactions).

399 **Among chemical crosslinking**, photo-induced crosslinking requires the production of radical initiating  
400 species and a precursor solution of a photoreactive polymer, usually a polyethylene glycol (PEG) acrylate  
401 or methacrylate derivative (Francisco et al., 2014; Li et al., 2014). While the use of photo-crosslinked  
402 polymers allows for time-controlled gelation, the limited access of the light apparatus to the target tissue  
403 weakens this strategy for IVD regeneration. Click chemistry, Schiff's base chemistry, Michael-type  
404 reactions, and enzymatic, protein, or pH modification-based crosslinking approaches might be better  
405 suited for IVD *in situ* gelation (Blanquer et al., 2014). These processes are generally compatible with cell  
406 or bioactive molecule loading as they often take place in mild conditions (37°C, physiological pH, short  
407 period). In the early 2000s, a silanized cellulose-based hydrogel, in which crosslinking is triggered by pH,  
408 was developed (Bourges et al., 2002). The importance of this hydrogel has been demonstrated for the  
409 regeneration of articular cartilage (Vinatier et al., 2009) and myocardium (Mathieu et al., 2012) and it is  
410 currently studied for IVD regeneration (Henry et al., 2017).

411 **Physically crosslinked** hydrogels are formed via non-covalent interactions in response to an external  
412 stimulus (pH, temperature, molecule level, light, pressure, electric current) (Qiu and Park, 2012). Thermo-  
413 responsive polymers spontaneously undergo a sol-gel transition when exposed to physiological  
414 temperature after *in vivo* injection. Recombinant polypeptides [e.g., elastin-like polypeptide (Nettles et al.,  
415 2010)], naturally derived polysaccharides [e.g., chitosan (Cheng et al., 2013)], and synthetic polymers  
416 [e.g., block copolymers of PEG and PLGA (Tran et al., 2012), poly (N-isopropylacrylamide) (p-

417 NIPAAAM) (Willems et al., 2015)] are the most commonly reported thermo-responsive polymers for IVD  
418 regeneration.

419 **In situ-forming hydrogels** exhibit many advantages for IVD treatment. They provide an aqueous  
420 environment that can rehydrate the degenerated microenvironment, they protect biologics, and they can  
421 restore, at least partially, the mechanical properties of IVDs. The permeability of hydrogels facilitates  
422 good transport of nutrients to encapsulated cells. Nevertheless, hydrogels can present some restrictions  
423 due to, for example, their weak mechanical properties. One of the main drawbacks of in situ-forming bulk  
424 hydrogels is the difficulty to tune their release characteristics. Indeed, while the diffusion of entrapped  
425 molecules can be slightly modified by adjusting the polymer network mesh, exchanges with the targeted  
426 microenvironment are limited to the interface hydrogel/tissue, usually in the order of a few cm<sup>2</sup>/g. By  
427 comparison, micro- and nano-scale delivery systems can develop areas greater than 1,000 m<sup>2</sup>/g as  
428 measured by N<sub>2</sub> adsorption–desorption experiments.

429

### 430 **6.3. Micro-scale delivery systems**

431 In parallel to stimuli-responsive polymer solutions ensuring a perfect fit to the IVD defect following in  
432 situ gelation, injectable micro-sized particles, with diameters varying from 20 μm to 200 μm, have release  
433 kinetics depending on their size and shape. Their release properties can be modified by tuning parameters  
434 such as the size, chemical composition, crosslinking degree, or molecular weight of the polymer (Balmert  
435 and Little, 2012). Moreover, chemical modification of the microparticle surface can allow for covalent  
436 coupling of factors such as antibodies and receptors. Microparticles are able to deliver biological factors  
437 over a long period while protecting them from inactivation (Wenk et al., 2009).

438 Many inorganic and polymer-based microparticles can be prepared by simple or double emulsion  
439 methods, in which a solution of the material is emulsified in water, followed by the formation of particles  
440 during the evaporation of the organic solvent. A recent modified protocol combines a crosslinking process  
441 and the emulsion step in an oil phase (Phromsopha and Baimark, 2014). Other production methods are  
442 spray drying, gelation, coacervation, electrospray, supercritical fluid mixing, and microfluidic technique.  
443 While their main advantage over classic emulsion is the narrower particle size distribution, the yield is  
444 often disappointing.

445 Recently, GDF-5-loaded PLGA microspheres, with an in vitro sustained delivery for 42 days, were  
446 injected into degenerated rat IVDs. Restoration of the disc height to almost 90% of the non-punctured disc  
447 was achieved (Yan et al., 2013). Moreover, the injection significantly increased aggrecan and type II  
448 collagen mRNA expression, whereas type I collagen mRNA expression was significantly decreased,  
449 suggesting that a micro-sized carrier could be of interest for IVD regeneration (Yan et al., 2013).

### 450 **6.4. Nano-scale delivery systems**

451 Alongside microparticles, many materials were used to design nanoparticles for biomedical applications,  
452 as nanocarriers are easily taken up by cells (Wilczewska et al., 2012). Polymeric nanoparticles, liposomes,  
453 dendrimers, polymers, silicon or carbon materials, and magnetic nanoparticles are examples of nano-  
454 carriers used as drug delivery systems notably for cancer (Sun et al., 2014) or bone regeneration (van Rijt  
455 and Habibovic, 2017). In this section, we highlight the particles that are feasible for use in the context of  
456 DDD.

457 **Polymeric nanoparticles:** Bioactive molecules can be either encapsulated or immobilized onto the  
458 surface of the nanoparticles, then released by desorption, diffusion, or nanoparticle erosion. Thus,  
459 degradable nanocarriers are widely used in biomedical applications as they can be hydrolyzed in situ  
460 (Kohane et al., 2006) and often considered to be biocompatible with tissues and cells, since they do not  
461 induce pro-inflammatory or immune reactions (des Rieux et al., 2006). An upregulation of aggrecan and  
462 type II collagen was achieved by using chitosan/Poly-(c-glutamic acid) nanoparticles loaded with the anti-

463 inflammatory drug diclofenac. These nanoparticles were injected into a needle-punctured bovine IVD  
464 cultured in vitro with IL-1 $\beta$ . Additionally, down-regulation of IL-6, IL-8, MMP1, and MMP3 was noted  
465 (Teixeira et al., 2016).

466 **Self-organized spherical liposomes**, usually 80–300 nm in diameter, contain at least one lipid bilayer  
467 surrounding an aqueous core (Daraee et al., 2014). They are thus able to encapsulate both hydrophobic  
468 and hydrophilic molecules within the lipid bilayer or the aqueous core, respectively. Liposomes were first  
469 used to improve the solubility and therapeutic index of chemotherapeutic agents, by allowing targeted  
470 delivery and enhanced tissue penetration. Since liposomes interact with cells by adsorption, fusion,  
471 endocytosis, or lipid transfer, they were studied as gene and siRNA carriers, especially cationic liposomes  
472 (Majzoub et al., 2016). The release kinetics can be tuned by the liposome composition, pH, osmotic  
473 gradient, incorporation of temperature- or pH-sensitive lipids (dos Santos Giuberti et al., 2011), and the  
474 surrounding environment. Liposomes were thus used for drug delivery in various applications such as in  
475 anti-cancer or anti-inflammation therapies or to reach specific organs (lungs, liver, spleen, brain).  
476 However, to date, their use for tackling IVD degeneration has not been reported. Interestingly, lipid  
477 nanocapsules were successfully designed for nucleic acid delivery (Huynh et al., 2009) and their  
478 application in siRNA delivery for IVD regeneration could be of interest.

479 **Dendrimers** are branched polymers whose toxicity is mainly related to functional groups presented at the  
480 surface layer and can be lowered by functionalization, e.g., by PEGylation (Zeng et al., 2016). This  
481 functionalization improves the surface activity of dendrimers as well as their biological and  
482 physicochemical properties. Drugs are entrapped in the internal structure of the dendrimer, adsorbed or  
483 chemically bonded onto the dendrimer surface according to its properties (sensitivity to temperature or  
484 pH, toxicity, solubility). One of the most commonly used in biomedical applications is poly-(amino  
485 amide; PAMAM-) as it was proven to be non-toxic and safe (Chauhan et al., 2009) and its capacity for  
486 gene or drug delivery was extensively explored (Zhao et al., 2011). Indeed, PAMAM dendrimers can  
487 modulate cytokine and chemokine release (Seelbach et al., 2015) and exhibit anti-inflammatory behavior  
488 by inhibiting proinflammatory cytokine secretion (Bosch, 2011). Thereby, dendrimers demonstrated their  
489 suitability in rheumatoid arthritis and musculoskeletal diseases and their application in IVD regeneration  
490 could be envisioned.

491 **Carbon nanotubes** are the most commonly used carbon nanoparticles and were widely studied for drug  
492 delivery in biomedical applications (Beg et al., 2011). They are characterized by a single or multiwall  
493 graphene-based tube with a high specific surface area and possibly excellent electronic and thermal  
494 conductivity (Beg et al., 2011). They have exceptional mechanical properties and are thus of interest as  
495 fillers in composite materials (Harrison and Atala, 2007). Toxicity due to their dimensions and released  
496 impurities was reported, but their biocompatibility can be enhanced with chemical surface engraftment of  
497 PEG, PAMAM, or albumin (Harrison and Atala, 2007). Molecules are encapsulated inside carbon  
498 nanotubes or chemically adsorbed onto their surface. Carbon nanotubes improve the mechanical properties  
499 of hydrogels (Dong et al., 2013) and can be of potential interest for IVD regenerative medicine.

500 **Mesoporous silica nanoparticles** are a widely studied biomaterial for their drug delivery capacity in  
501 particular for cancer therapies (Yang and Yu, 2016) or nerve growth (Sun et al., 2016). Indeed,  
502 mesoporous colloidal silica exhibit several advantages such as cytocompatibility, a high specific surface  
503 area, and ease of functionalization (Moritz and Geszke-Moritz, 2015). Additionally, the size, shape,  
504 surface pattern, porosity, and pore size of silica nanoparticles are easily tunable (Hoffmann et al., 2006).  
505 In the biomedical field, the best known mesoporous silica nanoparticles are MCM-41 and SBA-15. Drug  
506 loading into mesoporous silica might occur via chemical or physical adsorption, and release is usually  
507 controlled by diffusion throughout the mesopores. The potential of these particles was demonstrated for  
508 biomedical applications (Vallet-Regi and Balas, 2008) and more recently for the IVD (Henry et al., 2017).

509 **6.5. Multiscale systems for complex regenerative strategies**

510 Over the past few years, composite materials, composed of at least two distinct components, drew  
511 attention in biomedical engineering since they possess additional properties that each constituent alone  
512 does not exhibit. They were widely studied for bone regeneration, and successful clinical trials were  
513 recently reviewed (Jahan and Tabrizian, 2016).

514 Regarding specific IVD applications, we would like to direct the reader's attention to the multiscale  
515 systems illustrated in Figure 4 that emphasize the integration of technologies such as in situ-forming  
516 hydrogels with ready-to-use micro- or nanoparticles. The assembly of specific functionalities obtained by  
517 combining a bulk matrix, to restore hydration and mechanical properties, with a long-term reservoir of  
518 cells or molecules, to provide biological cues, could constitute a breakthrough in IVD regenerative  
519 therapies. The main outcome of this review is to highlight the crucial role of biomaterials in IVD  
520 regenerative medicine strategies. With regard to the aforementioned device needs, we would like to stress  
521 that biomaterials are considered the driving force of the IVD regenerative medicine train (Figure 5).  
522 Biomaterials are essential for the design and development of hydrogels capable of restoring load-bearing  
523 function while supporting cells until an appropriate ECM is synthesized. Biomaterials are also essential  
524 for engineering efficient and robust delivery devices that can be tailored to specific tissues.

525 For example, we recently reported on a multiscale system where an Si-HPMC hydrogel supplemented  
526 with rod-shaped silica nanofibers provided sustained release of TGF- $\beta$ 1 and GDF-5, compared with the  
527 plain hydrogel, while maintaining their in vitro biological activity (Henry et al., 2017). Recently, TGF- $\beta$ 3-  
528 loaded PLGA nanoparticles dispersed in a dextran/gelatin hydrogel, although not injectable, increased the  
529 gene expression of both aggrecan and type II collagen of MSCs in vitro (Gan et al., 2016). We believe that  
530 promising engineering technologies such as interpenetrating network-strengthened hydrogels (Gan et al.,  
531 2017), self-assembling nanofibrous peptide hydrogels (Wan et al., 2016), and programmed  
532 sequential bioactive factor delivery to target multiple repair steps (Bayer et al., 2017) could lead to  
533 significant advances in IVD regenerative strategies. Finally, an ambitious strategy would aim at delivering  
534 chemo-attractants from micro- or nanoparticles to attract progenitor cells toward a hydrogel-based  
535 microenvironment that would promote endogenous repair.

536

537

538

## 539 **7. Conclusion**

540 IVD degeneration is a major health concern and today, thanks to recent discoveries, the physiopathology  
541 of this disease is better understood. Building on this deeper knowledge, various biotherapies were  
542 proposed in the past few years, including cell-based therapies, molecular therapies, and nucleic acid-based  
543 therapies. These therapies aim at locally injecting biologics including cells, proteins, or DNA as  
544 mentioned earlier. Even if promising results have been obtained in vitro, the harsh environment of  
545 degenerated IVDs is a major concern that has hampered the clinical translatability of such concepts. The  
546 main outcome of this review is to highlight the essential role of biomaterials in strategies for IVD  
547 regenerative medicine. Indeed, injectable in situ crosslinking hydrogels could restore IVD height and  
548 hydration and would be particularly suited for IVD regenerative strategies. Additionally, hydrogels can  
549 improve cell viability and protect bioactive molecules from in situ degradation. Finally, hydrogels can be  
550 used as scaffolds, supplemented or not with micro- or nanoparticles. These so-formed multiscale systems  
551 would allow for a sustained delivery of the biologics promoting long-term IVD regenerative processes.

552 The design of the optimal system for IVD regeneration is still under debate as disc degeneration is a  
553 multifactorial disease. Cell-based therapies were widely considered in view of the major decrease in cell  
554 numbers that is observed with degeneration. However, the regulatory framework and the costs associated  
555 with these strategies may restrict their use. We believe that an effective approach would target the early  
556 degenerative processes and involve multiscale biomaterials allowing molecules to be sustainably delivered  
557 in situ and enabling a prolonged stimulation of endogenous repair.

558

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564

## 565 **9. Figures legends**

566

### 567 **Figure 1: Healthy intervertebral disc (IVD) local vascularization, innervation, and internal organization**

568 A. Located between vertebrae, the IVD is composed of two vertebral endplates, an *Annulus fibrosus*  
569 (purple) and a *Nucleus pulposus* (NP, blue). Since the IVD is non-innervated and non-vascularized,  
570 oxygen and glucose supply as well as metabolite excretion occur by diffusion through the vertebral  
571 endplates. This particular property induces specific conditions of osmolarity and pH, leading the IVD  
572 resident cells to adapt their metabolism. B. In AF tissue, fibroblast-like cells are organized parallel to  
573 collagen concentric lamellae. In NP tissue, large vacuolated notochordal cells and nucleopulpoocytes  
574 coexist and ensure homeostasis of the extracellular matrix.

575

### 576 **Figure 2: NP homeostasis rupture leads to IVD degeneration**

577 In a healthy IVD, regulation of anabolic and catabolic processes (i.e., homeostasis) enables the tissue to be  
578 hydrated and maintains its mechanical properties. Under various factors such as genetics, aging,  
579 environmental causes, or mechanical stress, the cellular dialogue underlying the extracellular matrix  
580 homeostasis is disrupted. This rupture causes lower anabolism and increased catabolism, ultimately



581 leading to ECM degradation, tissue inflammation, vascularization, fibrosis, and nerve ingrowth.  
582 Altogether, these phenomena are the key players in IVD degeneration.

583

584 Figure 3: Current IVD regenerative medicine is based on three approaches

585 **Cell-based therapies.** Considering the pivotal role of the decreased cell number in IVD degeneration,  
586 cell-based therapies have been increasingly considered. Autologous or allogeneic cells have been  
587 proposed and stem cells have been the subject of major investigations. Stem cells have been mainly  
588 isolated from adipose tissue or bone marrow and more recently pluripotent stem cells have been  
589 contemplated. It is also largely debated whether the optimal cells for IVD repair should be differentiated  
590 or undifferentiated. **Molecular therapies.** The broadened understanding of the phenomena involved  
591 during IVD degeneration has made it possible to consider molecular therapies via the direct injection of  
592 various factors to promote anabolism, to manage inflammation, or to attract regenerative cells. **Nucleic**  
593 **acid-based therapies.** Recent approaches target DNA and miRNA sequences and mostly aim to  
594 downplay catabolic factor expression. Finally, combined approaches such as genetically modified cells  
595 that could contribute to decreasing MMP local expression and activity could potentiate the efficacy of  
596 these therapies.

597

598 Figure 4: Multiscale systems associate in situ-forming scaffolds with ready-to-use particles

599 In the context of IVD regenerative medicine, we propose to combine in situ-forming systems with ready-  
600 to-use micro- or nanoparticles, thereby forming multiscale systems. We hypothesize that injectable  
601 polymers that undergo local gel formation will restore IVD hydration and mechanical properties, while  
602 micro- or nano-sized carriers loaded with cells or molecules will provide supportive cues for regenerative  
603 processes.

604

605 Figure 5: Biomaterials: the driving force of the IVD regenerative medicine train

606 Biomaterials are essential for the design and development of delivery systems for IVD regenerative  
607 medicine. They can protect and deliver nucleic acids, bioactive molecules, or cells for a long period and  
608 may thus contribute to regenerating the degraded ECM of IVDs.

609

610

## 611 **10. References**

612 Abdel Shaheed, C., Maher, C.G., Williams, K.A., Day, R., McLachlan, A.J., 2016. Efficacy,  
613 Tolerability, and Dose-Dependent Effects of Opioid Analgesics for Low Back Pain. *JAMA*  
614 *Intern. Med.* 176, 958.

615 Alkhatib, B., Rosenzweig, D.H., Krock, E., Roughley, P.J., Beckman, L., Steffen, T., Weber,  
616 M.H., Ouellet, J.A., Haglund, L., Scoliosis, M., 2014. Acute mechanical injury of the  
617 human intervertebral discs - Link to degeneration and pain. *Eur. Cell. Mater.* 28, 98–111.

618 Ankrum, J.A., Ong, J.F., Karp, J.M., 2014. Mesenchymal stem cells: immune evasive, not  
619 immune privileged. *Nat Biotechnol* 32, 252–260.

620 Baliga, S., Treon, K., Craig, N.J.A., 2015. Low back pain: Current surgical approaches. *Asian*  
621 *Spine J.* 9, 645–657.

622 Balmert, S.C., Little, S.R., 2012. Biomimetic Delivery with Micro- and Nanoparticles. *Adv.*

623 Mater. 24, 3757–3778.

624 Bartel, D.P., 2004. MicroRNAs: Genomics, Biogenesis, Mechanism, and Function. *Cell* 116,  
625 281–297.

626 Bayer, E.A., Jordan, J., Gottardi, R., Fedorchak, M., Kumta, P.N., Little, S., 2017. Programmed  
627 Platelet Derived Growth Factor- $\beta$  and Bone Morphogenetic Protein-2 Delivery from a  
628 Hybrid Calcium Phosphate/Alginate Scaffold. *Tissue Eng. Part A* Ahead of print.

629 Bedore, J., Leask, A., Séguin, C.A., 2014. Targeting the extracellular matrix: Matricellular  
630 proteins regulate cell-extracellular matrix communication within distinct niches of the  
631 intervertebral disc. *Matrix Biol.* 37, 124–130.

632 Beg, S., Rizwan, M., Sheikh, A.M., Hasnain, M.S., Anwer, K., Kohli, K., 2011. Advancement in  
633 carbon nanotubes: Basics, biomedical applications and toxicity. *J. Pharm. Pharmacol.* 63,  
634 141–163.

635 Bernkop-Schnürch, A., Dünnhaupt, S., 2012. Chitosan-based drug delivery systems. *Eur. J.*  
636 *Pharm. Biopharm.* 81, 463–469.

637 Bhardwaj, U., Sura, R., Papadimitrakopoulos, F., Burgess, D.J., 2007. Controlling acute  
638 inflammation with fast releasing dexamethasone-PLGA microsphere/pva hydrogel  
639 composites for implantable devices. *J. diabetes Sci. Technol.* 1, 8–17.

640 Blanco, J.F., Graciani, I.F., Sanchez-Guijo, F.M., Muntion, S., Hernandez-Campo, P.,  
641 Santamaria, C., Carrancio, S., Barbado, M.-V., Cruz, G., Gutierrez-Cosio, S., Herrero, C.,  
642 San Miguel, J.F., Brinon, J.G., del Canizo, M.-C., 2010. Isolation and characterization of  
643 mesenchymal stromal cells from human degenerated nucleus pulposus: comparison with  
644 bone marrow mesenchymal stromal cells from the same subjects. *Spine (Phila. Pa. 1976).*  
645 35, 2259–2265.

646 Blanquer, S.B.G., Grijpma, D.W., Poot, A. a., 2014. Delivery systems for the treatment of  
647 degenerated intervertebral discs. *Adv. Drug Deliv. Rev.* 84, 172–187.

648 Bochet, A., Fattal, E., 2012. Liposomes for intravitreal drug delivery: A state of the art. *J.*  
649 *Control. Release* 161, 628–634.

650 Bosch, X., 2011. Dendrimers to treat rheumatoid arthritis. *ACS Nano* 5, 6779–6785.

651 Bourges, X., Weiss, P., Daculsi, G., Legeay, G., 2002. Synthesis and general properties of  
652 silylated-hydroxypropyl methylcellulose in prospect of biomedical use. *Adv Colloid Interface*  
653 *Sci* 99, 215–228.

654 Chauhan, A.S., Jain, N.K., Diwan, P. V., 2009. Pre-clinical and behavioural toxicity profile of  
655 PAMAM dendrimers in mice. *Proc. R. Soc. A Math. Phys. Eng. Sci.* 466, 1535–1550.

656 Chen, J., Lee, E.J., Jing, L., Christoforou, N., Leong, K.W., Setton, L.A., 2013. Differentiation of  
657 mouse induced pluripotent stem cells (iPSCs) into nucleus pulposus-like cells in vitro. *PLoS*  
658 *One* 8, e75548.

659 Cheng, Y.H., Yang, S.H., Liu, C.C., Gefen, A., Lin, F.H., 2013. Thermosensitive hydrogel made  
660 of ferulic acid-gelatin and chitosan glycerophosphate. *Carbohydr. Polym.* 92, 1512–1519.

661 Cheng, Y.H., Yang, S.H., Su, W.Y., Chen, Y.C., Yang, K.C., Cheng, W.T., Wu, S.C., Lin, F.H.,  
662 2010. Thermosensitive chitosan-gelatin-glycerol phosphate hydrogels as a cell carrier for  
663 nucleus pulposus regeneration: an in vitro study. *Tissue Eng Part A* 16, 695–703.

- 664 Chujo, T., An, H.S., Akeda, K., 2006. Effects of Growth Differentiation Factor-5 on the  
665 Intervertebral Disc  $\gamma$ - In Vitro Bovine Study and In Vivo Rabbit Disc Degeneration Model  
666 Study. *Spine (Phila Pa 1976)* 31, 2909–2917.
- 667 Colombier, P., Camus, A., Lescaudron, L., Clouet, J., Guicheux, J., 2014. Intervertebral disc  
668 regeneration: a great challenge for tissue engineers. *Trends Biotechnol.* 32, 433–5.
- 669 Colombier, P., Clouet, J., Boyer, C., Ruel, M., Bonin, G., Lesoeur, J., Moreau, A., Fellah, B.H.,  
670 Weiss, P., Lescaudron, L., Camus, A., Guicheux, J., 2016. TGF- b 1 and GDF5 Act  
671 Synergistically to Drive the Differentiation of Human Adipose Stromal Cells toward  
672 Nucleus Pulposus -like Cells. *Stem Cells* 34, 653–667.
- 673 Colombini, A., Lombardi, G., Corsi, M.M., Banfi, G., 2008. Pathophysiology of the human  
674 intervertebral disc. *Int. J. Biochem. Cell Biol.* 40, 837–842.
- 675 Daraee, H., Etemadi, A., Kouhi, M., Alimirzalu, S., Akbarzadeh, A., 2014. Application of  
676 liposomes in medicine and drug delivery. *Artif. Cells, Nanomedicine, Biotechnol.* 1401, 1–  
677 11.
- 678 des Rieux, A., Fievez, V., Garinot, M., Schneider, Y.J., Pr at, V., 2006. Nanoparticles as  
679 potential oral delivery systems of proteins and vaccines: A mechanistic approach. *J. Control.*  
680 *Release* 116, 1–27.
- 681 Diab, R., Canilho, N., Pavel, I.A., Haffner, F.B., Girardon, M., Pasc, A., 2017. Silica-based  
682 systems for oral delivery of drugs, macromolecules and cells. *Adv. Colloid Interface Sci.* In  
683 press.
- 684 Dong, W., Huang, C., Wang, Y., Sun, Y., Ma, P., Chen, M., 2013. Superior mechanical  
685 properties of double-network hydrogels reinforced by carbon nanotubes without organic  
686 modification. *Int. J. Mol. Sci.* 14, 22380–22394.
- 687 dos Santos Giuberti, C., de Oliveira Reis, E.C., Ribeiro Rocha, T.G., Leite, E.A., Lacerda, R.G.,  
688 Ramaldes, G.A., de Oliveira, M.C., 2011. Study of the pilot production process of long-  
689 circulating and pH-sensitive liposomes containing cisplatin. *J. Liposome Res.* 21, 60–9.
- 690 Elmasry, S., Asfour, S., De Rivero Vaccari, J.P., Travascio, F., 2015. Effects of tobacco smoking  
691 on the degeneration of the intervertebral disc: A finite element study. *PLoS One* 10, 1–22.
- 692 Enochson, L., Stenberg, J., Brittberg, M., Lindahl, a, 2014. GDF5 reduces MMP13 expression in  
693 human chondrocytes via DKK1 mediated canonical Wnt signaling inhibition. *Osteoarthritis*  
694 *Cartilage* 22, 566–77.
- 695 Erwin, W.M., Ashman, K., O’Donnel, P., Inman, R.D., 2006. Nucleus pulposus notochord cells  
696 secrete connective tissue growth factor and up-regulate proteoglycan expression by  
697 intervertebral disc chondrocytes. *Arthritis Rheum* 54, 3859–3867.
- 698 Erwin, W.M., Islam, D., Inman, R.D., Fehlings, M.G., Tsui, F.W., 2011. Notochordal cells  
699 protect nucleus pulposus cells from degradation and apoptosis: implications for the  
700 mechanisms of intervertebral disc degeneration. *Arthritis Res Ther* 13, R215.
- 701 Feng, C., Liu, H., Yang, Y., Huang, B., Zhou, Y., 2015. Growth and Differentiation Factor-5  
702 Contributes to the Structural and Functional Maintenance of the Intervertebral Disc. *Cell.*  
703 *Physiol. Biochem.* 35, 1–16.
- 704 Fontana, G., See, E., Pandit, A., 2015. Current trends in biologics delivery to restore  
705 intervertebral disc anabolism. *Adv. Drug Deliv. Rev.* 84, 146–158.

706 Francisco, A.T., Hwang, P.Y., Jeong, C.G., Jing, L., Chen, J., Setton, L.A., 2014.  
707 Photocrosslinkable laminin-functionalized polyethylene glycol hydrogel for intervertebral  
708 disc regeneration. *Acta Biomater.* 10, 1102–1111.

709 Fu, W., Liu, Z., Feng, B., Hu, R., He, X., Wang, H, Meng, Y., Huang, H., Zhang, H., Wang, W.,  
710 2014. Electrospun gelatin / PCL and collagen / PLCL scaffolds for vascular tissue  
711 engineering. *Int. J. Nanomedicine* 9, 2335–2344.

712 Galbusera, F., Bellini, C.M., Zweig, T., Ferguson, S., Raimondi, M.T., Lamartina, C., Brayda-  
713 Bruno, M., Fornari, M., 2008. Design concepts in lumbar total disc arthroplasty. *Eur. Spine*  
714 *J.* 17, 1635–1650.

715 Gan, Y., Li, P., Wang, L., Mo, X., Song, L., Xu, Y., Zhao, C., Ouyang, B., Tu, B., Luo, L., Zhu,  
716 L., Dong, S., Li, F., Zhou, Q., 2017. An interpenetrating network-strengthened and  
717 toughened hydrogel that supports cell-based nucleus pulposus regeneration. *Biomaterials*  
718 136, 12–28.

719 Gan, Y., Li, S., Li, P., Xu, Y., Wang, L., Zhao, C., Ouyang, B., Tu, B., Zhang, C., Luo, L., Luo,  
720 X., Mo, X., Zhou, Q., 2016. A Controlled Release Codelivery System of MSCs  
721 Encapsulated in Dextran/Gelatin Hydrogel with TGF- $\beta$  3-Loaded Nanoparticles for Nucleus  
722 Pulposus Regeneration. *Stem Cells Int.* 2016.

723 Goldspiel, B.R., Green, L., Calis, K.A., 1993. Human gene therapy. *Clin. Pharm.* 12, 488–505.

724 Gorth, D.J., Mauck, R.L., Chiaro, J.A., Mohanraj, B., Hebel, N.M., Dodge, G.R., Elliott, D.M.,  
725 Smith, L.J., 2012. IL-1ra delivered from poly(lactic-co-glycolic acid) microspheres  
726 attenuates IL-1 $\beta$ -mediated degradation of nucleus pulposus in vitro. *Arthritis Res. Ther.* 14,  
727 R179.

728 Grad, S., Bow, C., Karppinen, J., Luk, K.D., Cheung, K.M., Alini, M., Samartzis, D., 2016.  
729 Systemic blood plasma CCL5 and CXCL6: Potential biomarkers for human lumbar disc  
730 degeneration. *Eur. Cell. Mater.* 30, 1–10.

731 Gruber, H., Marrero, E., Ingram, J., Hoelscher, G., Hanley, E., 2016. The chemokine, CXCL16,  
732 and its receptor, CXCR6, are constitutively expressed in human annulus fibrosus and  
733 expression of CXCL16 is up-regulated by exposure to IL-1 $\beta$  in vitro. *Biotech. Histochem.*  
734 295, 1–8.

735 Gruber, H.E., Hoelscher, G.L., Ingram, J.A., Bethea, S., Hanley, E.N., 2014a. Growth and  
736 differentiation factor-5 (GDF-5) in the human intervertebral annulus cells and its modulation  
737 by IL-1b and TNF-b in vitro. *Exp. Mol. Pathol.* 96, 225–229.

738 Gruber, H.E., Hoelscher, G.L., Ingram, J.A., Bethea, S., Norton, H.J., Hanley, E.N., 2014b.  
739 Production and expression of RANTES (CCL5) by human disc cells and modulation by IL-  
740 1- $\beta$  and TNF- $\alpha$  in 3D culture. *Exp. Mol. Pathol.* 96, 133–138.

741 Hadjipavlou, a G., Tzermiadianos, M.N., Bogduk, N., Zindrick, M.R., 2008. The  
742 pathophysiology of disc degeneration: a critical review. *J. Bone Joint Surg. Br.* 90, 1261–  
743 1270.

744 Harrison, B.S., Atala, A., 2007. Carbon nanotube applications for tissue engineering.  
745 *Biomaterials* 28, 344–353.

746 Henriksson, H.B., Svanvik, T., Jonsson, M., Sci, L.M., Hagman, M., Horn, M., Lindahl, A.,  
747 Brisby, H., 2009. Transplantation of Human Mesenchymal Stems Cells Into Intervertebral

748 Discs in a Xenogeneic Porcine Model. *Spine (Phila. Pa. 1976)*. 34, 141–148.

749 Henry, N., Clouet, J., Le Visage, C., Weiss, P., Gautron, E., Humber, B., Terrisse, H., Guicheux,  
750 J., Le Bideau, J., 2017. Silica nanofibers as a new drug delivery system: a study of protein-  
751 silica interactions. *J Mater Chem B* 5, 2908–2920.

752 Hirshi, K.K., Li, S., Roy, K., 2014. Induced pluripotent stem cells for regenerative medicine.  
753 *Annu Rev Biomed Eng* 16, 277–294.

754 Hiyama, A., Mochida, J., Iwashina, T., Omi, H., Watanabe, T., Serigano, K., Tamura, F., Sakai,  
755 D., 2008. Transplantation of mesenchymal stem cells in a canine disc degeneration model. *J.*  
756 *Orthop. Res.* 26, 589–600.

757 Hoffmann, F., Cornelius, M., Morell, J., Froba, M., 2006. Silica based mesoporous organic-  
758 inorganic hybrid materials. *Angew Chem Int Ed* 45, 3216–51.

759 Huynh, N.T., Passirani, C., Saulnier, P., Benoit, J.P., 2009. Lipid nanocapsules: A new platform  
760 for nanomedicine. *Int. J. Pharm.* 379, 201–209.

761 Illien-Junger, S., Grosjean, F., Laudier, D.M., Vlassara, H., Striker, G.E., Iatridis, J.C., 2013.  
762 Combined Anti-Inflammatory and Anti-AGE Drug Treatments Have a Protective Effect on  
763 Intervertebral Discs in Mice with Diabetes. *PLoS One* 8.

764 Illien-Jünger, S., Pattappa, G., Peroglio, M., Benneker, L.M., Stoddart, M.J., Sakai, D., Mochida,  
765 J., Grad, S., Alini, M., 2012. Homing of Mesenchymal Stem Cells in Induced Degenerative  
766 Intervertebral Discs in a Whole Organ Culture System. *Spine (Phila. Pa. 1976)*. 37, 1865–  
767 1873.

768 Jahan, K., Tabrizian, M., 2016. Composite biopolymers for bone regeneration enhancement in  
769 bony defects. *Biomater. Sci.* 4, 25–39.

770 Jeong, J.H., Jin, E.S., Min, J.K., Jeon, S.R., Park, C.S., Kim, H.S., Choi, K.H., 2009. Human  
771 mesenchymal stem cells implantation into the degenerated coccygeal disc of the rat.  
772 *Cytotechnology* 59, 55–64.

773 Jung, S.-M., Yoon, G.H., Lee, H.C., Shin, H.S., 2015. Chitosan nanoparticle/PCL nanofiber  
774 composite for wound dressing and drug delivery. *J. Biomater. Sci. Polym. Ed.* 26, 252–263.

775 Kaku, M., Kitami, M., Rosales Rocabado, J.M., Ida, T., Akiba, Y., Uoshima, K., 2017.  
776 Recruitment of bone marrow-derived cells to the periodontal ligament via the stromal cell-  
777 derived factor-1/C-X-C chemokine receptor type 4 axis. *J. Periodontal Res.* Ahead of print.

778 Kawaguchi, S., Yamashita, T., Katahira, G., Yokozawa, H., Torigoe, T., Sato, N., 2002.  
779 Chemokine profile of herniated intervertebral discs infiltrated with monocytes and  
780 macrophages. *Spine (Phila. Pa. 1976)*. 27, 1511–6.

781 Kepler, C.K., Markova, D.Z., Dibra, F., Yadla, S., Vaccaro, A.R., Risbud, M. V, Albert, T.J.,  
782 Anderson, D.G., 2013a. Expression and relationship of proinflammatory chemokine  
783 RANTES/CCL5 and cytokine IL-1 $\beta$  in painful human intervertebral discs. *Spine (Phila. Pa.*  
784 *1976)*. 38, 873–80.

785 Kepler, C.K., Ponnappan, R.K., Tannoury, C. a, Risbud, M. V, Anderson, D.G., 2013b. The  
786 molecular basis of intervertebral disc degeneration. *Spine J.* 13, 318–30.

787 Kohane, D.S., Tse, J.Y., Yeo, Y., Padera, R., Shubina, M., Langer, R., 2006. Biodegradable  
788 polymeric microspheres and nanospheres for drug delivery in the peritoneum. *J. Biomed.*

- 789 Mater. Res. - Part A 77, 351–361.
- 790 Kondiah, P.J., Choonara, Y.E., Kondiah, P.P.D., Marimuthu, T., Kumar, P., Du Toit, L.C., Pillay,  
791 V., 2016. A review of injectable polymeric hydrogel systems for application in bone tissue  
792 engineering. *Molecules* 21.
- 793 Kristin, C., Robert, S., Michelle, P., 2017. Effects of the intradiscal implantation of stromal  
794 vascular fraction plus platelet rich plasma in patients with degenerative disc disease. *J.*  
795 *Transl. Med.* 15, 12.
- 796 Latimer, J., Maher, C., Refshauge, K., 2004. The attitudes and beliefs of physiotherapy students  
797 to chronic back pain. *Clin. J. Pain* 20, 45–50.
- 798 Le Maitre, C.L., Hoyland, J.A., Freemont, A.J., 2007. Interleukin-1 receptor antagonist delivered  
799 directly and by gene therapy inhibits matrix degradation in the intact degenerate human  
800 intervertebral disc: an in situ zymographic and gene therapy study. *Arthritis Res. Ther.* 9,  
801 R83.
- 802 Li, C., Wang, T., Hu, L., Wei, Y., Liu, J., Mu, X., Nie, J., Yang, D., 2014. Photocrosslinkable  
803 bioadhesive based on dextran and PEG derivatives. *Mater. Sci. Eng. C* 35, 300–306.
- 804 Li, C., Wu, X., Tong, J., Yang, X., Zhao, J., Zheng, Q., Zhao, G., Ma, Z., 2015. Comparative  
805 analysis of human mesenchymal stem cells from bone marrow and adipose tissue under  
806 xeno-free conditions for cell therapy. *Stem Cell Res. Ther.* 6, 55.
- 807 Li, X., Leo, B.M., Beck, G., Balian, G., Anderson, G.D., 2004. Collagen and proteoglycan  
808 abnormalities in the GDF-5-deficient mice and molecular changes when treating disk cells  
809 with recombinant growth factor. *Spine (Phila. Pa. 1976)*. 29, 2229–34.
- 810 Li, Z., Tan, B.H., 2015. Towards the development of polycaprolactone based amphiphilic block  
811 copolymers: Molecular design, self-assembly and biomedical applications. *Mater. Sci. Eng.*  
812 *C* 45, 620–634.
- 813 Liang, C.Z., Li, H., Tao, Y.Q., Peng, L.H., Gao, J.Q., Wu, J.J., Li, F.C., Hua, J.M., Chen, Q.X.,  
814 2013. Dual release of dexamethasone and TGF- $\beta$ 3 from polymeric microspheres for stem  
815 cell matrix accumulation in a rat disc degeneration model. *Acta Biomater.* 9, 9423–9433.
- 816 Liu, S., Liang, H., Lee, S., Li, Z., Zhang, J., Fei, Q., 2016. Isolation and identification of stem  
817 cells from degenerated human intervertebral discs and their migration characteristics. *Acta*  
818 *Biochim. Biophys. Sin. (Shanghai)*. 49, 101–109.
- 819 Liu, Y., Ren, L., Wang, Y., 2013. Crosslinked collagen-gelatin-hyaluronic acid biomimetic film  
820 for cornea tissue engineering applications. *Mater. Sci. Eng. C* 33, 196–201.
- 821 Liu, Z., Ma, C., Shen, J., Wang, D., Hao, J., Hu, Z., 2016. SDF-1/CXCR4 axis induces apoptosis  
822 of human degenerative nucleus pulposus cells via the NF- $\kappa$ B pathway. *Mol. Med. Rep.* 14,  
823 783–789.
- 824 Lotz, J.C., Fields, A.J., Liebenberg, E.C., 2013. The role of the vertebral end plate in low back  
825 pain. *Glob. spine J.* 3, 153–64.
- 826 Luo, X.-W., Liu, K., Chen, Z., Zhao, M., Han, X.-W., Bai, Y.-G., Feng, G., 2016. Adenovirus-  
827 mediated GDF-5 promotes the extracellular matrix expression in degenerative nucleus  
828 pulposus cells. *J. Zhejiang Univ. Sci. B* 17, 30–42.

- 829 Luo, Z., Jin, L., Xu, L., Zhang, Z.L., Yu, J., Shi, S., Li, X., Chen, H., 2016. Thermosensitive  
830 PEG–PCL–PEG (PECE) hydrogel as an *in situ* gelling system for ocular drug delivery of  
831 diclofenac sodium. *Drug Deliv.* 23, 63–68.
- 832 Maher, C., Underwood, M., Buchbinder, R., 2017. Non-specific low back pain. *Lancet* 389, 736–  
833 47.
- 834 Majzoub, R.N., Ewert, K.K., Safinya, C.R., 2016. Cationic liposome-nucleic acid nanoparticle  
835 assemblies with applications in gene delivery and gene silencing. *Philos. Trans. A. Math.*  
836 *Phys. Eng. Sci.* 374.
- 837 Marfia, G., Campanella, R., Navone, S.E., Zucca, I., Scotti, A., Figini, M., Di Vito, C.,  
838 Alessandri, G., Riboni, L., Parati, E., 2014. Potential use of human adipose mesenchymal  
839 stromal cells for intervertebral disc regeneration: a preliminary study on biglycan-deficient  
840 murine model of chronic disc degeneration. *Arthritis Res. Ther.* 16, 457.
- 841 Maria, A.T.J., Toupet, K., Maumus, M., Fonteneau, G., Le Quellec, A., Jorgensen, C., Guilpain,  
842 P., Noël, D., 2016. Human adipose mesenchymal stem cells as potent anti-fibrosis therapy  
843 for systemic sclerosis. *J. Autoimmun.* 70, 1–9.
- 844 Maroudas, A., Stockwell, R.A., Nachemson, A., Urban, J., 1975. Factors involved in the nutrition  
845 of the human lumbar intervertebral disc: cellularity and diffusion of glucose *in vitro*. *J. Anat.*  
846 120, 113–30.
- 847 Mathieu, E., Lamirault, G., Toquet, C., Lhommet, P., Rederstorff, E., Sourice, S., Biteau, K.,  
848 Hulin, P., Forest, V., Weiss, P., Guicheux, J., Lemarchand, P., 2012. Intramyocardial  
849 Delivery of Mesenchymal Stem Cell-Seeded Hydrogel Preserves Cardiac Function and  
850 Attenuates Ventricular Remodeling after Myocardial Infarction. *PLoS One* 7, e51991.
- 851 Meisel, H.J., Siodla, V., Ganey, T., Minkus, Y., Hutton, W.C., Alasevic, O.J., 2007. Clinical  
852 experience in cell-based therapeutics: Disc chondrocyte transplantation. A treatment for  
853 degenerated or damaged intervertebral disc. *Biomol. Eng.* 24, 5–21.
- 854 Miller, D.G., Adam, M.A., Miller, A.D., 1990. Gene transfer by retrovirus vectors occurs only in  
855 cells that are actively replicating at the time of infection. *Mol. Cell. Biol.* 10, 4239–42.
- 856 Mintzer, M.A., Simanek, E.E., 2009. Nonviral vectors for gene delivery. *Chem. Rev.* 109, 259–  
857 302.
- 858 Mitragotri, S., Burke, P. a., Langer, R., 2014. Overcoming the challenges in administering  
859 biopharmaceuticals: formulation and delivery strategies. *Nat. Rev. Drug Discov.* 13, 655–  
860 672.
- 861 Miyamoto, T., Muneta, T., Tabuchi, T., Matsumoto, K., Saito, H., Tsuji, K., Sekiya, I., 2010.  
862 Intradiscal transplantation of synovial mesenchymal stem cells prevents intervertebral disc  
863 degeneration through suppression of matrix metalloproteinase-related genes in nucleus  
864 pulposus cells in rabbits. *Arthritis Res. Ther.* 12, R206.
- 865 Molinos, M., Almeida, C.R., Caldeira, J., Cunha, C., Goncalves, R.M., Barbosa, M.A., 2015.  
866 Inflammation in intervertebral disc degeneration and regeneration. *J R Soc Interface* 12,  
867 20141191.
- 868 Moritz, M., Geszke-Moritz, M., 2015. Mesoporous materials as multifunctional tools in  
869 biosciences: Principles and applications. *Mater. Sci. Eng. C* 49, 114–151.
- 870 Naves, L., Dhand, C., Almeida, L., Rajamani, L., Ramakrishna, S., Soares, G., 2017. Poly(lactic-

- 871 co-glycolic) acid drug delivery systems through transdermal pathway: an overview. *Prog.*  
872 *Biomater.* 6, 1–11.
- 873 Nettles, D.L., Chilkoti, A., Setton, L.A., 2010. Applications of Elastin-like Polypeptides in Tissue  
874 Engineering. *Adv. Drug Deliv. Rev.* 62, 1479–1485.
- 875 Nishida, K., Kang, J.D., Gilbertson, L.G., Moon, S., Suh, J., Vogt, M.T., Robbins, P.D., Evans,  
876 C.H., 1999. Modulation of the biologic activity of the rabbit intervertebral disc by gene  
877 therapy: An in vivo study of adenovirus-mediated transfer of the human transforming  
878 growth factor beta 1 encoding gene. *Spine (Phila. Pa. 1976)*. 24, 2419–2425.
- 879 Orita, S., Eguchi, Y., Kamoda, H., Arai, G., Ishikawa, T., Miyagi, M., Inoue, G., Suzuki, M.,  
880 Toyone, T., Aoki, Y., Takahashi, K., Ohtori, S., 2011. Brain-Derived Neurotrophic Factor  
881 Inhibition at the Punctured Intervertebral Disc Downregulates the Production of Calcitonin  
882 Gene-Related Peptide in Dorsal Root Ganglia in Rats. *Spine (Phila. Pa. 1976)*. 36, 1737–  
883 1743.
- 884 Orita, S., Miyagi, M., Kobori, S., Gemba, T., Ishikawa, T., Inoue, G., Toyone, T., Aoki, Y.,  
885 Eguchi, Y., Takahashi, K., Ohtori, S., 2013. IκB kinase b inhibitor downregulates pain-  
886 related neuropeptide production in the sensory neurons innervating injured lumbar  
887 intervertebral discs in the dorsal root ganglia of rats. *Spine J.* 13, 284–288.
- 888 Orozco, L., Soler, R., Morera, C., Alberca, M., Sanchez, A., Garcia-Sancho, J., 2011.  
889 Intervertebral disc repair by autologous mesenchymal bone marrow cells: a pilot study.  
890 *Transplantation* 92, 822–828.
- 891 Pattappa, G., Peroglio, M., Sakai, D., Mochida, J., Benneker, L.M., Alini, M., Grad, S., Science,  
892 S., 2014. Ccl5 / Rantes Is a Key Chemoattractant Released By Degenerative Intervertebral  
893 Discs in Organ Culture. *Eur Cell Mater* 1, 124–136.
- 894 Pereira, C.L., Gonçalves, R.M., Peroglio, M., Pattappa, G., D’Este, M., Eglin, D., Barbosa, M. a,  
895 Alini, M., Grad, S., 2014. The effect of hyaluronan-based delivery of stromal cell-derived  
896 factor-1 on the recruitment of MSCs in degenerating intervertebral discs. *Biomaterials* 35,  
897 8144–53.
- 898 Phromsopha, T., Baimark, Y., 2014. Preparation of starch/gelatin blend microparticles by a  
899 water-in-oil emulsion method for controlled release drug delivery. *Int. J. Biomater.* 2014, 1–  
900 6.
- 901 Purcell, B.P., Elser, J.A., Mu, A., Margulies, K.B., Burdick, J.A., 2012. Synergistic effects of  
902 SDF-1α chemokine and hyaluronic acid release from degradable hydrogels on directing bone  
903 marrow derived cell homing to the myocardium. *Biomaterials* 33, 7849–7857.
- 904 Qiu, Y., Park, K., 2012. Environment-sensitive hydrogels for drug delivery. *Adv. Drug Deliv.*  
905 *Rev.* 64, 49–60.
- 906 Raj, P.P., 2008. Intervertebral disc: Anatomy-physiology-pathophysiology-treatment. *Pain Pract.*  
907 8, 18–44.
- 908 Rezaee, M., Oskuee, R.K., Nassirli, H., Malaekheh-Nikouei, B., 2016. Progress in the  
909 development of lipopolyplexes as efficient non-viral gene delivery systems. *J. Control.*  
910 *Release* 236, 1–14.
- 911 Richardson, S.M., Kalamegam, G., Pushparaj, P.N., Matta, C., Memic, A., Khademhosseini, A.,  
912 Mobasher, R., Poletti, F.L., Hoyland, J.A., Mobasher, A., 2016. Mesenchymal stem cells in



913 regenerative medicine: Focus on articular cartilage and intervertebral disc regeneration.  
914 *Methods* 99, 69–80.

915 Risbud, M. V, Guttapalli, A., Tsai, T.-T., Lee, J.Y., Danielson, K.G., Vaccaro, A.R., Albert, T.J.,  
916 Gazit, Z., Gazit, D., Shapiro, I.M., 2007. Evidence for skeletal progenitor cells in the  
917 degenerate human intervertebral disc. *Spine (Phila. Pa. 1976)*. 32, 2537–2544.  
918 doi:10.1097/BRS.0b013e318158dea6

919 Roughley, P., Hoemann, C., DesRosiers, E., Mwale, F., Antoniou, J., Alini, M., 2006. The  
920 potential of chitosan-based gels containing intervertebral disc cells for nucleus pulposus  
921 supplementation. *Biomaterials* 27, 388–396.

922 Roughley, P.J., 2004. Biology of intervertebral disc aging and degeneration: involvement of the  
923 extracellular matrix. *Spine (Phila. Pa. 1976)*. 29, 2691–2699.

924 Russo, F., Hartman, R.A., Bell, K.M., Vo, N., Sowa, G.A., Kang, J.D., Vadala, G., Denaro, V.,  
925 2016. Biomechanical Evaluation of Transpedicular Nucleotomy with Intact Annulus  
926 Fibrosus. *Spine (Phila Pa 1976)* 42, E193–E201.

927 Sainoh, T., Orita, S., Miyagi, M., Inoue, G., Yamauchi, K., Suzuki, M., Sakuma, Y., Kubota, G.,  
928 Oikawa, Y., Inage, K., Sato, J., Nakata, Y., Aoki, Y., Takahashi, K., Ohtori, S., 2016. Single  
929 intradiscal injection of the interleukin-6 receptor antibody tocilizumab provides short-term  
930 relief of discogenic low back pain; prospective comparative cohort study. *J. Orthop. Sci.* 21,  
931 2–6.

932 Sakai, D., Andersson, G.B.J., 2015. Stem cell therapy for intervertebral disc regeneration:  
933 obstacles and solutions. *Nat. Rev. Rheumatol.* 11, 243–256. doi:10.1038/nrrheum.2015.13

934 Sakai, D., Grad, S., 2015. Advancing the cellular and molecular therapy for intervertebral disc  
935 disease. *Adv. Drug Deliv. Rev.* 84, 159–171.

936 Sawamura, K., Ikeda, T., Nagae, M., Okamoto, S., Mikami, Y., Hase, H., Ikoma, K., Yamada, T.,  
937 Sakamoto, H., Matsuda, K., Tabata, Y., Kawata, M., Kubo, T., 2009. Characterization of in  
938 vivo effects of platelet-rich plasma and biodegradable gelatin hydrogel microspheres on  
939 degenerated intervertebral discs. *Tissue Eng. Part A* 15, 3719–3727.

940 Seelbach, R.J., Fransen, P., Pulido, D., D’Este, M., Duttonhoefer, F., Sauerbier, S., Freiman,  
941 T.M., Niemeyer, P., Albericio, F., Alini, M., Royo, M., Mata, A., Eglin, D., 2015. Injectable  
942 Hyaluronan Hydrogels with Peptide-Binding Dendrimers Modulate the Controlled Release  
943 of BMP-2 and TGF- $\beta$ 1. *Macromol. Biosci.* 15, 1035–1044.

944 Shabbir, A., Zisa, D., Suzuki, G., Lee, T., 2009. Heart failure therapy mediated by the trophic  
945 activities of bone marrow mesenchymal stem cells: a noninvasive therapeutic regimen. *AJP*  
946 *Hear. Circ. Physiol.* 296, H1888–H1897.

947 Sionkowska, A., 2011. Current research on the blends of natural and synthetic polymers as new  
948 biomaterials: Review. *Prog. Polym. Sci.* 36, 1254–1276.

949 Sionkowska, A., Kaczmarek, B., Lewandowska, K., Grabska, S., Pokrywczynska, M.,  
950 Kloskowski, T., Drewna, T., 2016. 3D composites based on the blends of chitosan and  
951 collagen with the addition of hyaluronic acid. *Int. J. Biol. Macromol.* 89, 442–448.

952 Sun, B., Taing, A., Liu, H., Nie, G., Wang, J., Fang, Y., Liu, L., Xue, Y., Shi, J., Liao, Y.-P., Ku,  
953 J., Xia, T., Liu, Y., 2016. Nerve Growth Factor-Conjugated Mesoporous Silica  
954 Nanoparticles Promote Neuron-Like PC12 Cell Proliferation and Neurite Growth. *J.*

- 955 Nanosci. Nanotechnol. 16, 2390–3.
- 956 Sun, T., Zhang, Y.S., Pang, B., Hyun, D.C., Yang, M., Xia, Y., 2014. Engineered nanoparticles  
957 for drug delivery in cancer therapy. *Angew. Chemie - Int. Ed.* 53, 12320–12364.
- 958 Teixeira, G.Q., Leite Pereira, C., Castro, F., Ferreira, J.R., Gomez-Lazaro, M., Aguiar, P.,  
959 Barbosa, M.A., Neidlinger-Wilke, C., Goncalves, R.M., 2016. Anti-inflammatory  
960 Chitosan/Poly-gamma-glutamic acid nanoparticles control inflammation while remodeling  
961 extracellular matrix in degenerated intervertebral disc. *Acta Biomater.* 42, 168–179.
- 962 Tran, C.M., Markova, D., Smith, H.E., Susarla, B., Ponnappan, R.K., Anderson, D.G., Symes, A.,  
963 Shapiro, I.M., Risbud, M. V., 2010. Regulation of CCN2/connective tissue growth factor  
964 expression in the nucleus pulposus of the intervertebral disc: Role of smad and activator  
965 protein 1 signaling. *Arthritis Rheum.* 62, 1983–1992.
- 966 Tran, C.M., Schoepflin, Z.R., Markova, D.Z., Kepler, C.K., Anderson, D.G., Shapiro, I.M.,  
967 Risbud, M. V., 2014. CCN2 Suppresses Catabolic Effects of Interleukin-1 $\beta$  through  $\alpha$ 5 $\beta$ 1  
968 and  $\alpha$ V $\beta$ 3 Integrins in Nucleus Pulposus Cells. *J. Biol. Chem.* 289, 7374–7387.
- 969 Tran, V.T., Karam, J.P., Garric, X., Coudane, J., Benoit, J.P., Montero-Menei, C.N., Venier-  
970 Julienne, M.C., 2012. Protein-loaded PLGA-PEG-PLGA microspheres: a tool for cell  
971 therapy. *Eur J Pharm Sci* 45, 128–137.
- 972 Tuakli-Wosornu, Y.A., Terry, A., Boachie-Adjei, K., Harrison, J.R., Gribbin, C.K., LaSalle, E.E.,  
973 Nguyen, J.T., Solomon, J.L., Lutz, G.E., 2016. Lumbar Intradiskal Platelet-Rich Plasma  
974 (PRP) Injections: A Prospective, Double-Blind, Randomized Controlled Study. *PM R* 8, 1–  
975 10.
- 976 Vallet-Regi, M., Balas, F., 2008. Silica materials for medical applications. *Open Biomed Eng J* 2,  
977 1–9.
- 978 van Rijt, S., Habibovic, P., 2017. Enhancing regenerative approaches with nanoparticles. *J. R.*  
979 *Soc. Interface* 14.
- 980 Vasiliadis, E., Pneumaticos, S., 2014. Biologic treatment of mild and moderate intervertebral disc  
981 degeneration. *Mol. Med.* 20, 1.
- 982 Vinatier, C., Gauthier, O., Fatimi, A., Merceron, C., Masson, M., Moreau, A., Moreau, F., Fellah,  
983 B., Weiss, P., Guicheux, J., 2009. An injectable cellulose-based hydrogel for the transfer of  
984 autologous nasal chondrocytes in articular cartilage defects. *Biotechnol. Bioeng.* 102, 1259–  
985 1267.
- 986 Vos, T., Allen, C., Arora, M., Barber, R.M., Bhutta, Z.A., Brown, A., Carter, A., Casey, D.C.,  
987 Charlson, F.J., Chen, A.Z., Coggeshall, M., Cornaby, L., Dandona, L., Dicker, D.J., Dilegge,  
988 T., Erskine, H.E., Ferrari, A.J., Fitzmaurice, C., Fleming, T., Forouzanfar, M.H., Fullman,  
989 N., Gething, P.W., Goldberg, E.M., Graetz, N., Haagsma, J.A., Hay, S.I., Johnson, C.O.,  
990 Kassebaum, N.J., Kawashima, T., Kemmer, L., Khalil, I.A., Kinfu, Y., Kyu, H.H., Leung, J.,  
991 Liang, X., Lim, S.S., Lopez, A.D., Lozano, R., Marczak, L., Mensah, G.A., Mokdad, A.H.,  
992 Naghavi, M., Nguyen, G., Nsoesie, E., Olsen, H., Pigott, D.M., Pinho, C., Rankin, Z.,  
993 Reinig, N., Salomon, J.A., Sandar, L., Smith, A., Stanaway, J., Steiner, C., Teeples, S.,  
994 Thomas, B.A., Troeger, C., Wagner, J.A., Wang, H., Wanga, V., Whiteford, H.A., Zocckler,  
995 L., Abajobir, A.A., Abate, K.H., Abbafati, C., Abbas, K.M., Abd-Allah, F., Abraham, B.,  
996 Abubakar, I., Abu-Raddad, L.J., Abu-Rmeileh, N.M.E., Ackerman, I.N., Adebisi, A.O.,  
997 Ademi, Z., Adou, A.K., Afanvi, K.A., Agardh, E.E., Agarwal, A., Kiadaliri, A.A.,

998 Ahmadieh, H., Ajala, O.N., Akinyemi, R.O., Akseer, N., Al-Aly, Z., Alam, K., Alam,  
 999 N.K.M., Aldahri, S.F., Alegretti, M.A., Alemu, Z.A., Alexander, L.T., Alhabib, S., Ali, R.,  
 1000 Alkerwi, A., Alla, F., Allebeck, P., Al-Raddadi, R., Alsharif, U., Altirkawi, K.A., Alvis-  
 1001 Guzman, N., Amare, A.T., Amberbir, A., Amini, H., Ammar, W., Amrock, S.M., Andersen,  
 1002 H.H., Anderson, G.M., Anderson, B.O., Antonio, C.A.T., Aregay, A.F., Ärnlov, J.,  
 1003 Artaman, A., Asayesh, H., Assadi, R., Atique, S., Avokpaho, E.F.G.A., Awasthi, A.,  
 1004 Quintanilla, B.P.A., Azzopardi, P., Bacha, U., Badawi, A., Balakrishnan, K., Banerjee, A.,  
 1005 Barac, A., Barker-Collo, S.L., Bärnighausen, T., Barregard, L., Barrero, L.H., Basu, A.,  
 1006 Bazargan-Hejazi, S., Beghi, E., Bell, B., Bell, M.L., Bennett, D.A., Bensenor, I.M., Benzian,  
 1007 H., Berhane, A., Bernabé, E., Betsu, B.D., Beyene, A.S., Bhala, N., Bhatt, S., Biadgilign, S.,  
 1008 Bienhoff, K., Bikbov, B., Biryukov, S., Bisanzio, D., Bjertness, E., Blore, J., Borschmann,  
 1009 R., Boufous, S., Brainin, M., Brazinova, A., Breitborde, N.J.K., Brown, J., Buchbinder, R.,  
 1010 Buckle, G.C., Butt, Z.A., Calabria, B., Campos-Nonato, I.R., Campuzano, J.C., Carabin, H.,  
 1011 Cárdenas, R., Carpenter, D.O., Carrero, J.J., Castañeda-Orjuela, C.A., Rivas, J.C., Catalá-  
 1012 López, F., Chang, J.-C., Chiang, P.P.-C., Chibueze, C.E., Chisumpa, V.H., Choi, J.-Y.J.,  
 1013 Chowdhury, R., Christensen, H., Christopher, D.J., Ciobanu, L.G., Cirillo, M., Coates,  
 1014 M.M., Colquhoun, S.M., Cooper, C., Cortinovis, M., Crump, J.A., Damtew, S.A., Dandona,  
 1015 R., Daoud, F., Dargan, P.I., das Neves, J., Davey, G., Davis, A.C., Leo, D. De, Degenhardt,  
 1016 L., Gobbo, L.C. Del, Dellavalle, R.P., Deribe, K., Deribew, A., Derrett, S., Jarlais, D.C. Des,  
 1017 Dharmaratne, S.D., Dhillon, P.K., Diaz-Torné, C., Ding, E.L., Driscoll, T.R., Duan, L.,  
 1018 Dubey, M., Duncan, B.B., Ebrahimi, H., Ellenbogen, R.G., Elyazar, I., Endres, M., Endries,  
 1019 A.Y., Ermakov, S.P., Eshrati, B., Estep, K., Farid, T.A., Farinha, C.S. e S., Faro, A., Farvid,  
 1020 M.S., Farzadfar, F., Feigin, V.L., Felson, D.T., Fereshtehnejad, S.-M., Fernandes, J.G.,  
 1021 Fernandes, J.C., Fischer, F., Fitchett, J.R.A., Foreman, K., Fowkes, F.G.R., Fox, J., Franklin,  
 1022 R.C., Friedman, J., Frostad, J., Fürst, T., Futran, N.D., Gabbe, B., Ganguly, P., Gankpé,  
 1023 F.G., Gebre, T., Gebrehiwot, T.T., Gebremedhin, A.T., Geleijnse, J.M., Gessner, B.D.,  
 1024 Gibney, K.B., Ginawi, I.A.M., Giref, A.Z., Giroud, M., Gishu, M.D., Giussani, G., Glaser,  
 1025 E., Godwin, W.W., Gomez-Dantes, H., Gona, P., Goodridge, A., Gopalani, S.V., Gotay,  
 1026 C.C., Goto, A., Gouda, H.N., Grainger, R., Greaves, F., Guillemin, F., Guo, Y., Gupta, R.,  
 1027 Gupta, R., Gupta, V., Gutiérrez, R.A., Haile, D., Hailu, A.D., Hailu, G.B., Halasa, Y.A.,  
 1028 Hamadeh, R.R., Hamidi, S., Hammami, M., Hancock, J., Handal, A.J., Hankey, G.J., Hao,  
 1029 Y., Harb, H.L., Harikrishnan, S., Haro, J.M., Havmoeller, R., Hay, R.J., Heredia-Pi, I.B.,  
 1030 Heydarpour, P., Hoek, H.W., Horino, M., Horita, N., Hosgood, H.D., Hoy, D.G., Htet, A.S.,  
 1031 Huang, H., Huang, J.J., Huynh, C., Iannarone, M., Iburg, K.M., Innos, K., Inoue, M., Iyer,  
 1032 V.J., Jacobsen, K.H., Jahanmehr, N., Jakovljevic, M.B., Javanbakht, M., Jayaraman, S.P.,  
 1033 Jayatilleke, A.U., Jee, S.H., Jeemon, P., Jensen, P.N., Jiang, Y., Jibat, T., Jimenez-Corona,  
 1034 A., Jin, Y., Jonas, J.B., Kabir, Z., Kalkonde, Y., Kamal, R., Kan, H., Karch, A., Karema,  
 1035 C.K., Karimkhani, C., Kasaeian, A., Kaul, A., Kawakami, N., Keiyoro, P.N., Kemp, A.H.,  
 1036 Keren, A., Kesavachandran, C.N., Khader, Y.S., Khan, A.R., Khan, E.A., Khang, Y.-H.,  
 1037 Khera, S., Khoja, T.A.M., Khubchandani, J., Kieling, C., Kim, P., Kim, C., Kim, D., Kim,  
 1038 Y.J., Kisson, N., Knibbs, L.D., Knudsen, A.K., Kokubo, Y., Kolte, D., Kopec, J.A., Kosen,  
 1039 S., Kotsakis, G.A., Koul, P.A., Koyanagi, A., Kravchenko, M., Defo, B.K., Bicer, B.K.,  
 1040 Kudom, A.A., Kuipers, E.J., Kumar, G.A., Kutz, M., Kwan, G.F., Lal, A., Laloo, R.,  
 1041 Lallukka, T., Lam, H., Lam, J.O., Langan, S.M., Larsson, A., Lavados, P.M., Leasher, J.L.,  
 1042 Leigh, J., Leung, R., Levi, M., Li, Y., Li, Y., Liang, J., Liu, S., Liu, Y., Lloyd, B.K., Lo,  
 1043 W.D., Logroscino, G., Looker, K.J., Lotufo, P.A., Lunevicius, R., Lyons, R.A., Mackay,  
 1044 M.T., Magdy, M., Razek, A. El, Mahdavi, M., Majdan, M., Majeed, A., Malekzadeh, R.,

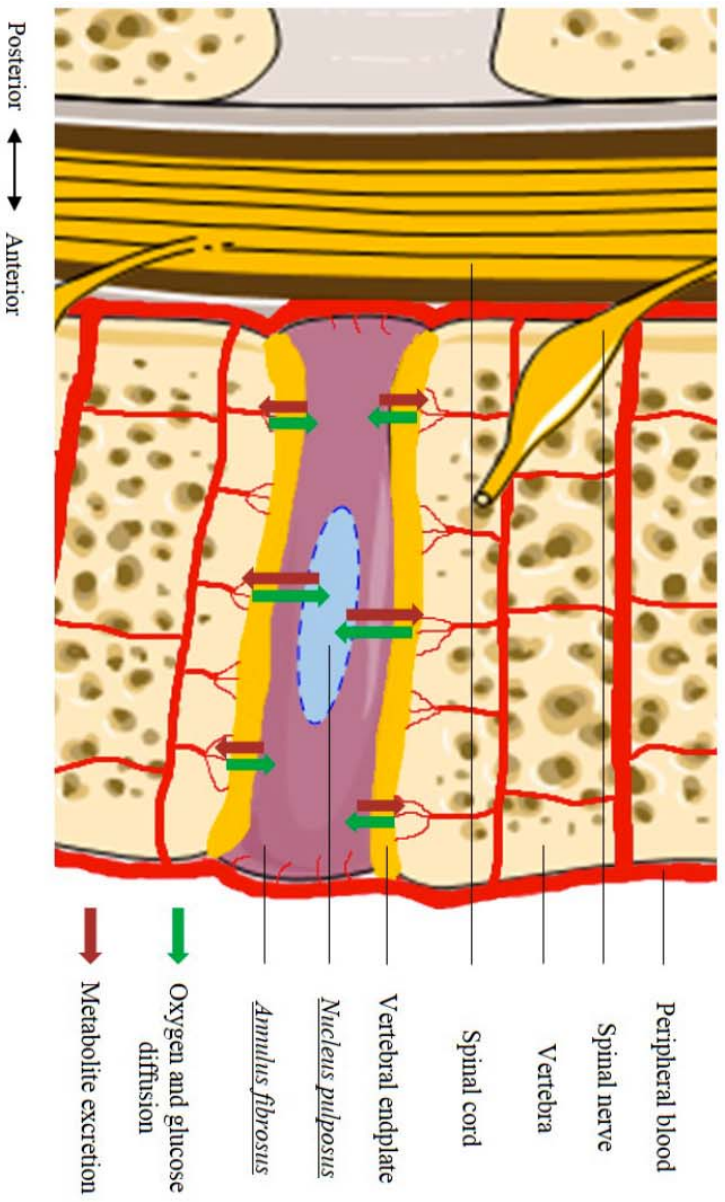
1045 Marcenes, W., Margolis, D.J., Martinez-Raga, J., Masiye, F., Massano, J., McGarvey, S.T.,  
 1046 McGrath, J.J., McKee, M., McMahon, B.J., Meaney, P.A., Mehari, A., Mejia-Rodriguez, F.,  
 1047 Mekonnen, A.B., Melaku, Y.A., Memiah, P., Memish, Z.A., Mendoza, W., Meretoja, A.,  
 1048 Meretoja, T.J., Mhimbira, F.A., Millea, A., Miller, T.R., Mills, E.J., Mirarefin, M.,  
 1049 Mitchell, P.B., Mock, C.N., Mohammadi, A., Mohammed, S., Monasta, L., Hernandez,  
 1050 J.C.M., Montico, M., Mooney, M.D., Moradi-Lakeh, M., Morawska, L., Mueller, U.O.,  
 1051 Mullany, E., Mumford, J.E., Murdoch, M.E., Nachega, J.B., Nagel, G., Naheed, A., Naldi,  
 1052 L., Nangia, V., Newton, J.N., Ng, M., Ngalesoni, F.N., Nguyen, Q. Le, Nisar, M.I., Pete,  
 1053 P.M.N., Nolla, J.M., Norheim, O.F., Norman, R.E., Norrving, B., Nunes, B.P., Ogbo, F.A.,  
 1054 Oh, I.-H., Ohkubo, T., Olivares, P.R., Olusanya, B.O., Olusanya, J.O., Ortiz, A., Osman, M.,  
 1055 Ota, E., PA, M., Park, E.-K., Parsaeian, M., de Azeredo Passos, V.M., Caicedo, A.J.P.,  
 1056 Patten, S.B., Patton, G.C., Pereira, D.M., Perez-Padilla, R., Perico, N., Pesudovs, K.,  
 1057 Petzold, M., Phillips, M.R., Piel, F.B., Pillay, J.D., Pishgar, F., Plass, D., Platts-Mills, J.A.,  
 1058 Polinder, S., Pond, C.D., Popova, S., Poulton, R.G., Pourmalek, F., Prabhakaran, D., Prasad,  
 1059 N.M., Qorbani, M., Rabiee, R.H.S., Radfar, A., Rafay, A., Rahimi, K., Rahimi-Movaghar,  
 1060 V., Rahman, M., Rahman, M.H.U., Rahman, S.U., Rai, R.K., Rajsic, S., Ram, U., Rao, P.,  
 1061 Refaat, A.H., Reitsma, M.B., Remuzzi, G., Resnikoff, S., Reynolds, A., Ribeiro, A.L.,  
 1062 Blancas, M.J.R., Roba, H.S., Rojas-Rueda, D., Ronfani, L., Roshandel, G., Roth, G.A.,  
 1063 Rothenbacher, D., Roy, A., Sagar, R., Sahathevan, R., Sanabria, J.R., Sanchez-Niño, M.D.,  
 1064 Santos, I.S., Santos, J.V., Sarmiento-Suarez, R., Sartorius, B., Satpathy, M., Savic, M.,  
 1065 Sawhney, M., Schaub, M.P., Schmidt, M.I., Schneider, I.J.C., Schöttker, B., Schwebel, D.C.,  
 1066 Scott, J.G., Seedat, S., Sepanlou, S.G., Servan-Mori, E.E., Shackelford, K.A., Shaheen, A.,  
 1067 Shaikh, M.A., Sharma, R., Sharma, U., Shen, J., Shepard, D.S., Sheth, K.N., Shibuya, K.,  
 1068 Shin, M.-J., Shiri, R., Shiue, I., Shrimme, M.G., Sigfusdottir, I.D., Silva, D.A.S., Silveira,  
 1069 D.G.A., Singh, A., Singh, J.A., Singh, O.P., Singh, P.K., Sivonda, A., Skirbekk, V., Skogen,  
 1070 J.C., Sliagar, A., Sliwa, K., Soljak, M., Søreide, K., Sorensen, R.J.D., Soriano, J.B., Sposato,  
 1071 L.A., Sreeramareddy, C.T., Stathopoulou, V., Steel, N., Stein, D.J., Steiner, T.J., Steinke, S.,  
 1072 Stovner, L., Stroumpoulis, K., Sunguya, B.F., Sur, P., Swaminathan, S., Sykes, B.L.,  
 1073 Szoeki, C.E.I., Tabarés-Seisdedos, R., Takala, J.S., Tandon, N., Tanne, D., Tavakkoli, M.,  
 1074 Taye, B., Taylor, H.R., Ao, B.J. Te, Tedla, B.A., Terkawi, A.S., Thomson, A.J., Thorne-  
 1075 Lyman, A.L., Thrift, A.G., Thurston, G.D., Tobe-Gai, R., Tonelli, M., Topor-Madry, R.,  
 1076 Topouzis, F., Tran, B.X., Truelsen, T., Dimbuene, Z.T., Tsilimbaris, M., Tura, A.K., Tuzcu,  
 1077 E.M., Tyrovolas, S., Ukwaja, K.N., Undurraga, E.A., Uneke, C.J., Uthman, O.A., van Gool,  
 1078 C.H., Varakin, Y.Y., Vasankari, T., Venketasubramanian, N., Verma, R.K., Violante, F.S.,  
 1079 Vladimirov, S.K., Vlassov, V.V., Vollset, S.E., Wagner, G.R., Waller, S.G., Wang, L.,  
 1080 Watkins, D.A., Weichenthal, S., Weiderpass, E., Weintraub, R.G., Werdecker, A.,  
 1081 Westerman, R., White, R.A., Williams, H.C., Wiysonge, C.S., Wolfe, C.D.A., Won, S.,  
 1082 Woodbrook, R., Wubshet, M., Xavier, D., Xu, G., Yadav, A.K., Yan, L.L., Yano, Y.,  
 1083 Yaseri, M., Ye, P., Yebyo, H.G., Yip, P., Yonemoto, N., Yoon, S.-J., Younis, M.Z., Yu, C.,  
 1084 Zaidi, Z., Zaki, M.E.S., Zeeb, H., Zhou, M., Zodpey, S., Zuhlke, L.J., Murray, C.J.L., 2016.  
 1085 Global, regional, and national incidence, prevalence, and years lived with disability for 310  
 1086 diseases and injuries, 1990–2015: a systematic analysis for the Global Burden of Disease  
 1087 Study 2015. *Lancet* 388, 1545–1602.

1088 Wallach, C.J., Sobajima, S., Watanabe, Y., Kim, J.S., Georgescu, H.I., Robbins, P., Gilbertson,  
 1089 L.G., Kang, J.D., 2003. Gene transfer of the catabolic inhibitor TIMP-1 increases measured  
 1090 proteoglycans in cells from degenerated human intervertebral discs. *Spine* (Phila. Pa. 1976).

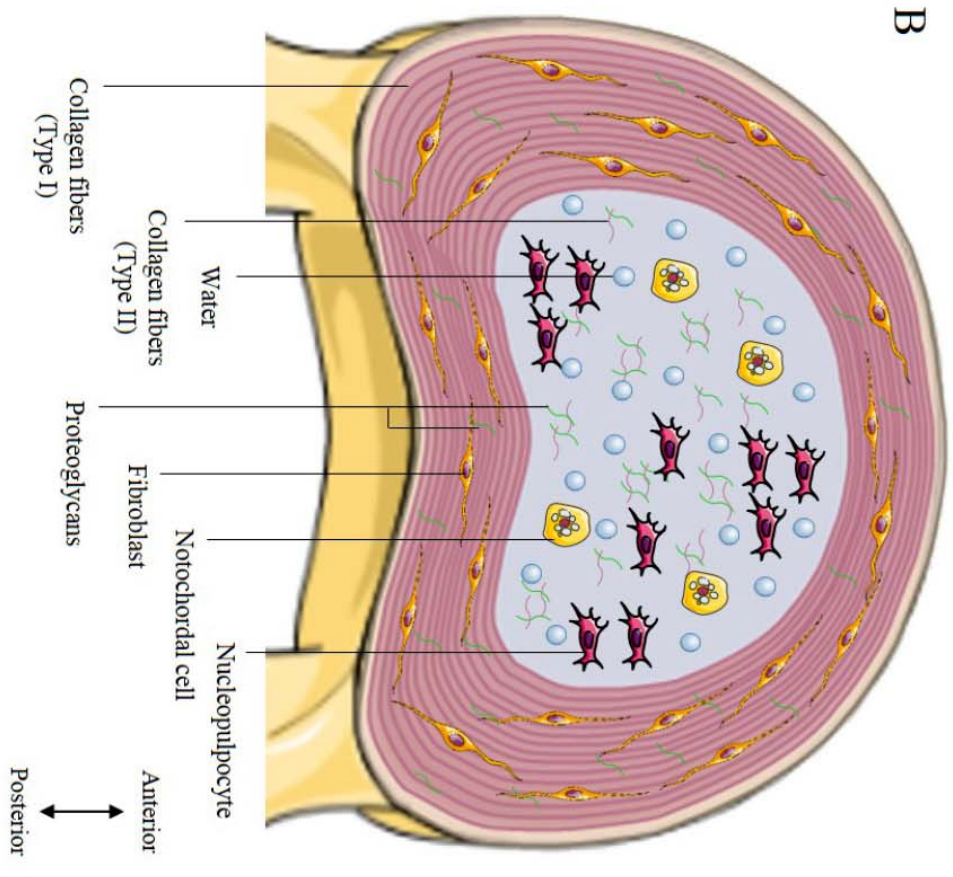
- 1091 28, 2331–2337.
- 1092 Walter, B. a, Purmessur, D., Likhitpanichkul, M., Weinberg, A., Cho, S.K., Qureshi, S. a., Hecht,  
1093 A.C., Iatridis, J.C., 2015. Inflammatory Kinetics and Efficacy of Anti-inflammatory  
1094 Treatments on Human Nucleus Pulposus Cells. *Spine (Phila. Pa. 1976)*. 40, 1.
- 1095 Wan, S., Borland, S., Richardson, S.M., Merry, C.L.R., Saiani, A., Gough, J.E., 2016. Self-  
1096 assembling peptide hydrogel for intervertebral disc tissue engineering. *Acta Biomater.* 46,  
1097 29–40.
- 1098 Wang, S., Chang, Q., Lu, J., Wang, C., 2015. Growth factors and platelet-rich plasma: promising  
1099 biological strategies for early intervertebral disc degeneration. *Int. Orthop.* 39, 927–934.
- 1100 Wang, W.-J., Yu, X.-H., Wang, C., Yang, W., He, W.-S., Zhang, S.-J., Yan, Y.-G., Zhang, J.,  
1101 2015. MMPs and ADAMTSs in intervertebral disc degeneration. *Clin. Chim. Acta.* 448.
- 1102 Wei, X.W., Gong, C.Y., Gou, M.L., Fu, S.Z., Guo, Q.F., Shi, S., Luo, F., Guo, G., Qiu, L.Y.,  
1103 Qian, Z.Y., 2009. Biodegradable poly( $\epsilon$ -caprolactone)-poly(ethylene glycol) copolymers as  
1104 drug delivery system. *Int. J. Pharm.* 381, 1–18.
- 1105 Wenk, E., Meinel, A.J., Wildy, S., Merkle, H.P., Meinel, L., 2009. Microporous silk fibroin  
1106 scaffolds embedding PLGA microparticles for controlled growth factor delivery in tissue  
1107 engineering. *Biomaterials* 30, 2571–2581.
- 1108 Whatley, B.R., Wen, X., 2012. Intervertebral disc (IVD) : Structure, degeneration, repair and  
1109 regeneration. *Mater. Sci. Eng. C* 32, 61–77.
- 1110 Wilczewska, A.Z., Niemirowicz, K., Markiewicz, K.H., Car, H., 2012. Nanoparticles as drug  
1111 delivery systems. *Pharmacol Rep* 64, 1020–1037.
- 1112 Willems, N., Yang, H.-Y., Langelaan, M.L.P., Tellegen, A.R., Grinwis, G.C.M., Kranenburg, H.-  
1113 J.C., Riemers, F.M., Plomp, S.G.M., Craenmehr, E.G.M., Dhert, W.J.A., Papen-Botterhuis,  
1114 N.E., Meij, B.P., Creemers, L.B., Tryfonidou, M.A., 2015. Biocompatibility and intradiscal  
1115 application of a thermoreversible celecoxib-loaded poly-N-isopropylacrylamide MgFe-  
1116 layered double hydroxide hydrogel in a canine model. *Arthritis Res. Ther.* 17, 214.
- 1117 Wismer, N., Grad, S., Fortunato, G., Ferguson, S.J., Alini, M., Eglin, D., 2014. Biodegradable  
1118 electrospun scaffolds for annulus fibrosus tissue engineering: effect of scaffold structure and  
1119 composition on annulus fibrosus cells in vitro. *Tissue Eng. Part A* 20, 672–82.
- 1120 Wu, J., Guan, T., Zheng, S., Grosjean, F., Liu, W., Xiong, H., Gordon, R., Vlassara, H., Striker,  
1121 G.E., Zheng, F., 2011. Inhibition of inflammation by pentosan polysulfate impedes the  
1122 development and progression of severe diabetic nephropathy in aging C57B6 mice. *Lab.*  
1123 *Investig.* 91, 1459–1471.
- 1124 Yan, J., Yang, S., Sun, H., Guo, D., Wu, B., Ji, F., Zhou, D., 2013. Effects of releasing  
1125 recombinant human growth and differentiation factor-5 from poly(lactic-co-glycolic acid)  
1126 microspheres for repair of the rat degenerated intervertebral disc. *J. Biomater. Appl.* 29, 72–  
1127 80.
- 1128 Yang, H., Gao, F., Li, X., Wang, J., Liu, H., Zheng, Z., 2015. TGF- $\beta$ 1 antagonizes TNF- $\alpha$   
1129 induced up-regulation of matrix metalloproteinase 3 in nucleus pulposus cells: role of the  
1130 ERK1/2 pathway. *Connect. Tissue Res.* 8207, 1–8.
- 1131 Yang, Y., Yu, C., 2016. Advances in silica based nanoparticles for targeted cancer therapy.  
1132 *Nanomedicine* 12, 317–32.

- 1133 Zeng, Y., Kurokawa, Y., Win-Shwe, T.-T., Zeng, Q., Hirano, S., Zhang, Z., Sone, H., 2016.  
1134 Effects of PAMAM dendrimers with various surface functional groups and multiple  
1135 generations on cytotoxicity and neuronal differentiation using human neural progenitor  
1136 cells. *J. Toxicol. Sci.* 41, 351–70.
- 1137 Zhang, H., Zhang, L., Chen, L., Li, W., Li, F., Chen, Q., 2014. Stromal cell-derived factor-1 and  
1138 its receptor CXCR4 are upregulated expression in degenerated intervertebral discs. *Int. J.*  
1139 *Med. Sci.* 11, 240–245.
- 1140 Zhao, Y., Fan, X., Liu, D., Wang, Z., 2011. PEGylated thermo-sensitive poly(amidoamine)  
1141 dendritic drug delivery systems. *Int. J. Pharm.* 409, 229–236.
- 1142 Zhongyi, S., Sai, Z., Chao, L., Jiwei, T., 2015. Effects of nuclear factor kappa B signaling  
1143 pathway in human intervertebral disc degeneration. *Spine (Phila. Pa. 1976)*. 40, 224–32.
- 1144 Zhou, X., Chen, L., Grad, S., Alini, M., Pan, H., Yang, D., Zhen, W., Li, Z., Huang, S., Peng, S.,  
1145 2017. The roles and perspectives of microRNAs as biomarkers for intervertebral disc  
1146 degeneration. *J. Tissue Eng. Regen. Med.* Ahead of print.
- 1147 Zhou, Z., Gao, M., Wei, F., Liang, J., Deng, W., Dai, X., Zhou, G., Zou, X., 2014. Shock  
1148 absorbing function study on denucleated intervertebral disc with or without hydrogel  
1149 injection through static and dynamic biomechanical tests in vitro. *Biomed Res. Int.* 2014,  
1150 461724.
- 1152

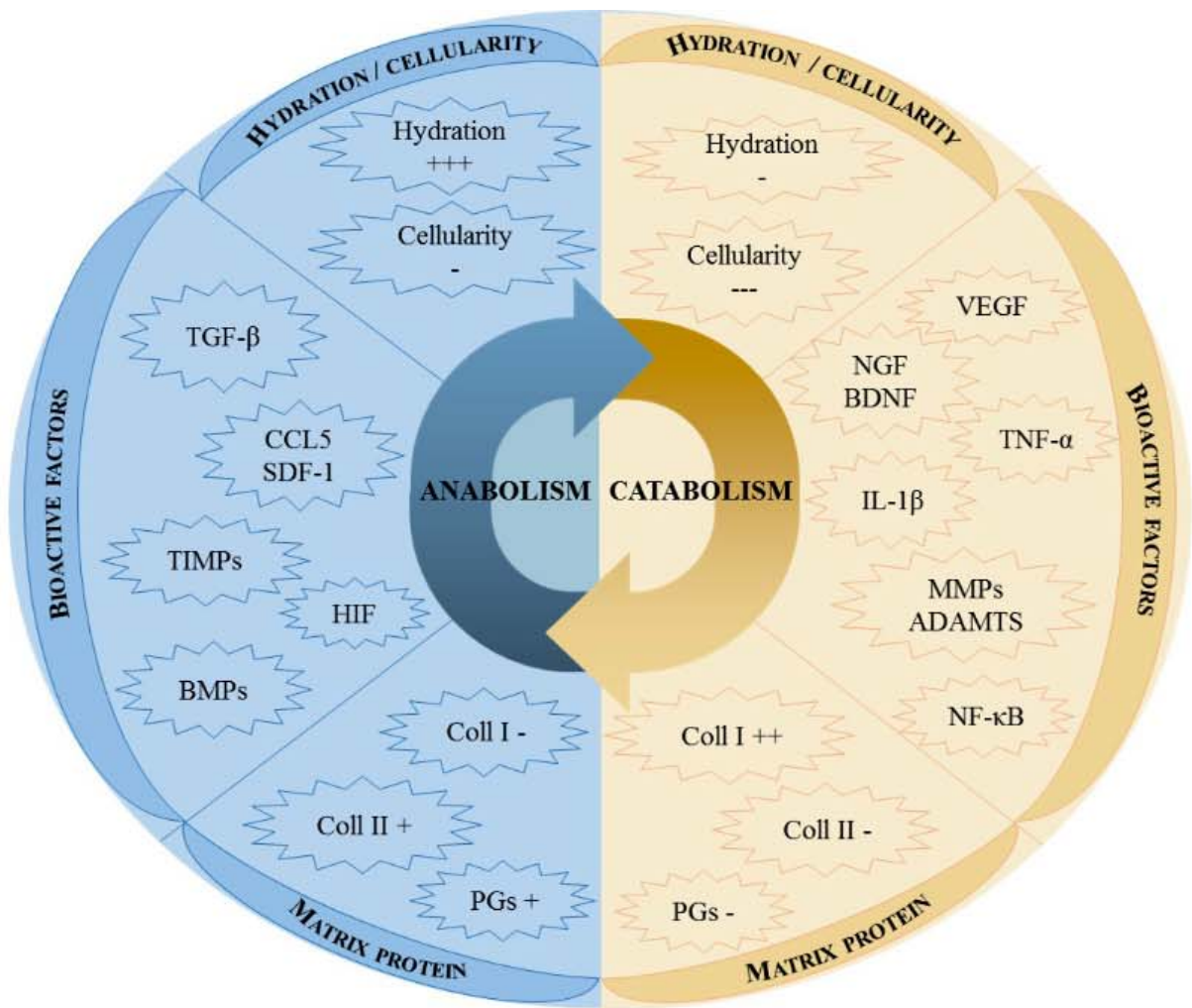
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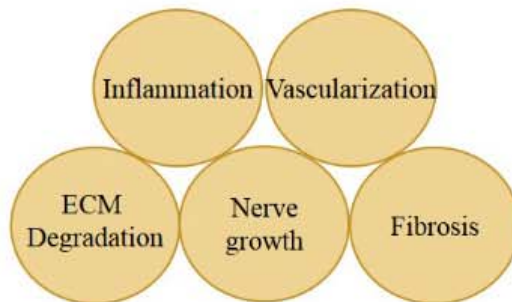
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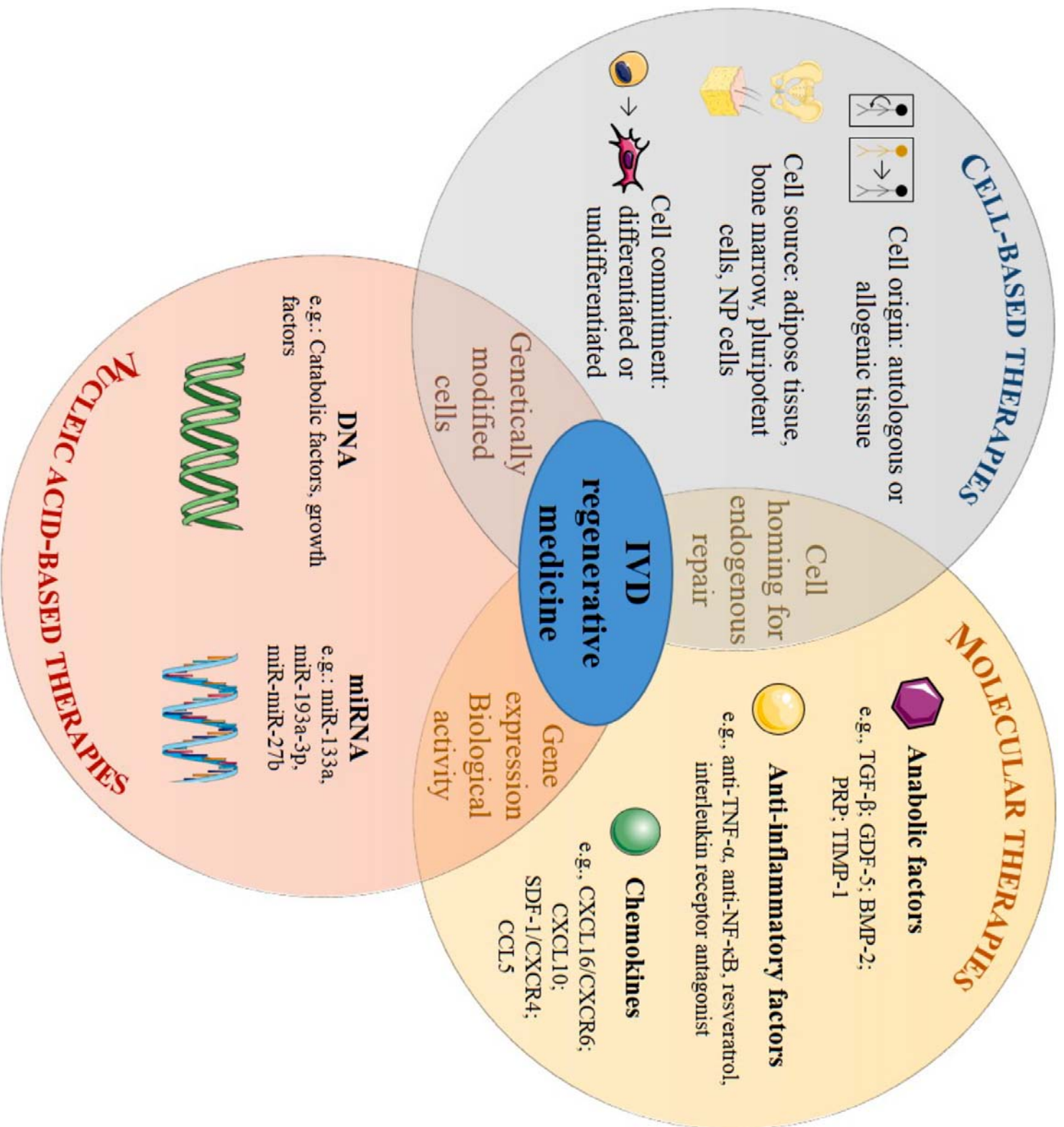




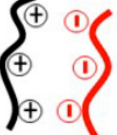
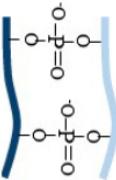





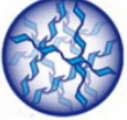

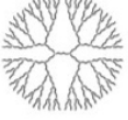


↓ Anabolism + Catabolism ↑



**DISC DEGENERATIVE DISEASE**





| <i>In situ</i> forming systems   | Ready-to-use systems   |  |
|--|--|--|
| <div style="display: flex; justify-content: space-between; align-items: center;"> <div style="width: 30%; text-align: center;"> <p><b>Macro-scaled scaffold</b></p> </div> <div style="width: 40%; text-align: center;"> <p><b>Micro-scaled particles</b></p> </div> <div style="width: 30%; text-align: center;"> <p><b>Nano-scaled particles</b></p> </div> </div>   |  |  |
| <p>Physical crosslinking</p> <div style="display: flex; justify-content: space-around;"> <div style="text-align: center;"> <br/>pH         </div> <div style="text-align: center;"> <br/>Temperature         </div> <div style="text-align: center;"> <br/>Ionic interactions         </div> </div> <p>Chemical crosslinking</p> <div style="display: flex; justify-content: space-around;"> <div style="text-align: center;"> <br/>Crosslinker         </div> <div style="text-align: center;"> <br/>Silanol groups         </div> <div style="text-align: center;"> <br/>UV light         </div> </div> | <div style="display: flex; flex-direction: column; align-items: center;"> <div style="text-align: center; margin-bottom: 20px;"> <br/>Microsphere         </div> <div style="text-align: center; margin-bottom: 20px;"> <br/>Microcapsule         </div> <div style="text-align: center;"> <br/>Porous microparticle         </div> </div> | <div style="display: flex; flex-direction: column; align-items: center;"> <div style="text-align: center; margin-bottom: 20px;"> <br/>Matrix         </div> <div style="text-align: center; margin-bottom: 20px;"> <br/>Liposome         </div> <div style="text-align: center; margin-bottom: 20px;"> <br/>Dendrimer         </div> <div style="text-align: center; margin-bottom: 20px;"> <br/>Carbon nanotubes         </div> <div style="text-align: center;"> <br/>Silica nanofibers         </div> </div> |
| <p>Injectable and <i>in situ</i> crosslinkable</p> <p>Low invasive approach</p> <p>Cell and molecule protection</p>  | <p>Physicochemical properties easily modified for well-adapted sustained delivery</p>  | <p>Suitable carriers for lipophilic drugs and gene delivery; mechanical reinforcement of hydrogel scaffold</p>   |

**MULTISCALE SYSTEMS**

