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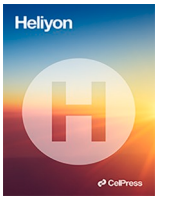

Review article

Multifunctional injectable hydrogels with controlled delivery of bioactive factors for efficient repair of intervertebral disc degeneration

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ABSTRACT

Millions of people worldwide suffer from intervertebral disc degeneration (IVDD), which imposes a significant socioeconomic burden on society. There is an urgent clinical demand for more effective treatments for IVDD because conventional treatments can only alleviate the symptoms rather than preventing the progression of IVDD. Hydrogels, a class of elastic biomaterials with good biocompatibility, are promising candidates for intervertebral disc repair and regeneration. In recent years, various hydrogels have been investigated in vitro and *in vivo* for the repair of intervertebral discs, some of which are ready for clinical testing. This review summarizes the latest findings and developments in using bioactive factors-released bioactive injectable hydrogels for the repair and regeneration of intervertebral discs. It focuses on the analysis and summary of the use of multifunctional injectable hydrogels to delivery bioactive factors (cells, exosomes, growth factors, genes, drugs) for disc regeneration, providing guidance for future study. Finally, we discussed and analyzed the optimal timing for the application of controlled-release hydrogels in the treatment of IVDD to meet the high standards required for intervertebral disc regeneration and precision medicine.

1. Introduction

As the human lifespan continues to lengthen, the prevalence and impact of age-related diseases are escalating. Recent comprehensive global surveys encompassing 50 chronic pathological conditions have unequivocally identified low back pain (LBP) as the foremost cause of disability, persisting over several years [1]. This affliction exerts a significant clinical and socioeconomic burden upon society [2]. While a number of potential etiologies, including spinal stenosis, radiculopathy, or sciatica, have been recognized [3,4], intervertebral disc degeneration (IVDD) remains most closely associated with the condition, accounting for an estimated 40 % of all LBP cases [5].

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The precise mechanisms underlying the development of IVDD remain elusive, although it is postulated to involve a complex interplay of apoptosis, inflammatory cytokines, and extracellular matrix catabolism [6]. Existing surgical and conservative interventions often fail to meet the expectations of a majority of patients, as their primary focus is on symptomatic relief rather than halting disease progression or restoring the structure and function of degenerated intervertebral discs [7]. Urgent attention is required to devise innovative treatment strategies promptly, aiming to improve this predicament by inhibiting the progression and regenerative degradation of IVDD for the definitive cure of chronic low back pain. However, a prerequisite for the discovery of novel interventions is a comprehensive understanding of the pathogenesis underlying IVDD.

The intervertebral disc (IVD) encompasses the nucleus pulposus (NP), annulus fibrosus (AF), and cartilaginous endplates (CEPs), collectively contributing to its structural integrity. Among these components, the NP assumes a pivotal role in maintaining homeostasis through the secretion of a complex extracellular matrix (ECM) (type II collagen and proteoglycans). This intricate ECM composition is indispensable for the physiological viscoelastic properties of the IVD [8]. Notably, IVDD originates from alterations within the NP and is primarily characterized by modifications in ECM constituents [9].

According to extensive research conducted over the past decade, a prominent pathological characteristic of IVDD is the disruption of the immune microenvironment within the IVD. This aberration primarily manifests as the infiltration of inflammatory cells and upregulation of inflammatory factors [10,11]. The dysregulation of the IVD immune microenvironment contributes to the degeneration of nucleus pulposus cells (NPCs) and concurrently precipitates ECM breakdown and anabolic imbalances, resulting in progressive ECM degradation and reduced proteoglycan synthesis [12]. These changes further amplify the inflammatory response, perpetuating a vicious cycle that perpetuates the advancement of the IVDD process [13](Fig. 1). Given the absence of blood vessels [14], nerves [15], or lymphoid tissue within the IVD tissue, the self-repair capability of degenerated NPCs is inherently limited. Consequently, early intervention during the initial stages of IVDD becomes crucial to mitigate the degenerative process or even reverse it, thus achieving the concept of "prevention before disease." In recent years, the limitations of conservative and surgical treatments, coupled with an enhanced understanding of the pathophysiology of intervertebral discs, have fostered the emergence of biological regenerative treatment modalities. These approaches, which integrate biomaterials with cell therapy, gene therapy, and growth factor, have instilled renewed hope for addressing IVDD [16–18]. Among a wide variety of biological materials, biomedical hydrogels currently are a hot spot in disc replacement, repair, and regeneration treatments due to their similar physiological functions, potential hydrophilicity, and cell compatibility with the nucleus pulposus tissues [10]. Among the numerous types of hydrogel, injectable hydrogel is an exciting hydrogel type, which is more attractive to clinicians and patients because of its advantages of less side effects, lower cost, diminished pain, minimal tissue damage and enhanced ease of use [19]. The injectability of hydrogels facilitates their minimally invasive delivery into early-stage degenerated intervertebral disc tissue, enabling the adjustment of the microenvironment associated with IVDD and facilitating the repair and regeneration of the affected tissue. Injectable hydrogels create an aqueous environment in situ, promoting rehydration of the compromised microenvironment and partially restoring the mechanical properties of the intervertebral disc tissue. This feature holds particular significance for the regeneration of the highly hydrated nucleus pulposus tissue. Moreover, injectable hydrogels can be laden with diverse bioactive substances, allowing for potent reparative effects through cell delivery, drug delivery, gene delivery, and other modalities [20].

This review presents a comprehensive review of the latest discoveries and advancements in the field of injectable bioactive hydrogels for intervertebral disc repair and regeneration. It provides a detailed analysis and summary, while also focusing on the application of controlled-release hydrogels in intervertebral disc regeneration. Additionally, a discussion and analysis are conducted to determine the optimal timing for the application of controlled-release hydrogels in treating degenerative disc conditions, aiming to meet the high standards required for intervertebral disc regeneration and precision medicine.

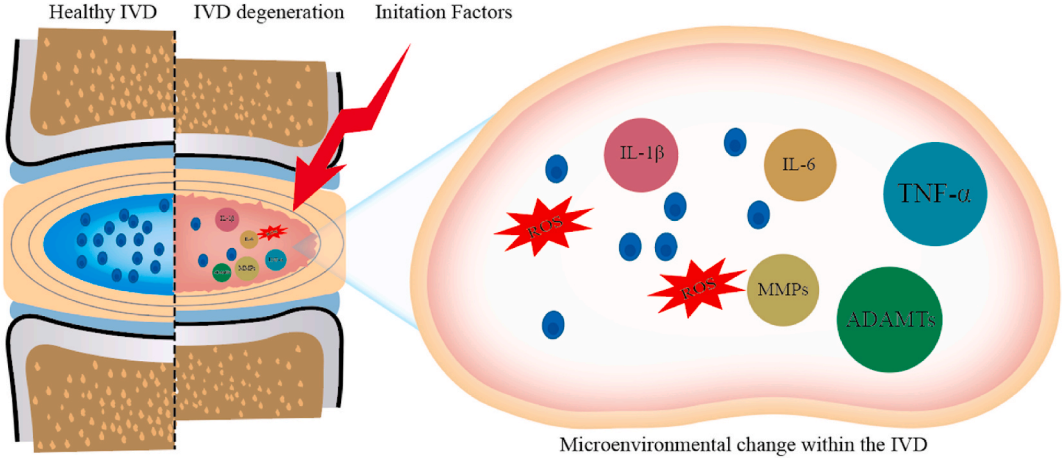

Fig. 1. Schematic illustration of the microenvironmental change within the IVD.

2. Principle of injectable hydrogels for IVD regeneration

The intervertebral disc is the significant tissue without blood supply in the human body. Its oxygen supply and nutrients mainly come from the capillary diffusion in the lateral annulus fibrosus and the endplates [21], resulting in the limited repair capacity after injuries. Fortunately, biotherapies utilizing biomaterials provide promising prospects for intervertebral disc regeneration [22]. Among the various biomaterials, hydrogels constructed from biocompatible polymers like collagen and hyaluronic acid exhibit exceptional biocompatibility and biodegradability, making them an appealing choice. These hydrogels offer distinct advantages in intervertebral disc repair and regeneration by creating a conducive three-dimensional microenvironment that supports cell survival, proliferation, and tissue regeneration. Furthermore, their adjustable degradation rates allow for gradual replacement by newly formed tissue, thereby facilitating intervertebral disc restoration and regeneration. These unique characteristics establish hydrogels as promising materials in the field of intervertebral disc repair and regeneration [23,24]. Currently, injectable hydrogels employed for intervertebral disc degeneration (IVDD) repair and regeneration operate on several principles. Firstly, as hydrophilic polymer networks, injectable hydrogels possess high water absorption capacity. They can fill the damaged intervertebral disc region, provide mechanical support, and create a porous scaffold that supports cell survival and proliferation, thereby promoting cell regeneration and repair [25]. Secondly, injectable hydrogels typically exhibit excellent biocompatibility, resulting in minimal immune or toxic reactions. This characteristic enables them to interact harmoniously with surrounding tissues and cells, without adverse effects on the body, while providing a suitable environment for cells and facilitating the regeneration and repair process. Additionally, injectable hydrogels possess the advantage of injectability, allowing them to be minimally invasively injected into the intervertebral disc. Pre- and post-injection, these hydrogels undergo sol-gel transition, which alleviates patient discomfort, reduces trauma, and effectively prevents hydrogel leakage [26]. Lastly, the encapsulation and controlled release of therapeutic drugs further enhance the therapeutic potential. Currently, most injectable hydrogels used for intervertebral disc repair act as carriers, encapsulating cells, growth factors, small molecule drugs, etc., and injecting them into degenerated intervertebral discs. Through appropriate release mechanisms, these substances are gradually released, providing sustained therapeutic effects and promoting cell proliferation, differentiation, and matrix synthesis, thereby facilitating intervertebral disc regeneration and repair [27]. However, this treatment approach presents some limitations. Firstly, hydrogels solely serve as carriers for cells or drugs and lack direct therapeutic effects, limiting the treatment's maximum efficiency. Secondly, current multifunctional hydrogels face challenges such as complex compositions, cumbersome synthesis steps, and difficulties in clinical translation [28]. Therefore, the development of biologically active multifunctional hydrogels with simple compositions and synthesis methods for tissue regeneration has become a research hotspot. We have summarized some standards and recommendations to optimize the use of hydrogels for intervertebral disc tissue regeneration and clinical applications.

i. Biocompatibility. The biocompatibility of hydrogels intended for intervertebral disc regeneration is of paramount importance, particularly when targeting the nucleus pulposus region. It is crucial to meticulously select materials devoid of any constituents that may trigger undesirable inflammatory responses or immune reactions. To ensure a favorable host response and mitigate potential complications, it is imperative to steer clear of components that may provoke adverse biological reactions.
ii. Cell affinity. Given the pivotal role of cells in the regenerative process of intervertebral discs, the material employed should exhibit a high degree of cell affinity. Facilitating the facile attachment and integration of cells within the hydrogel matrix is essential for promoting optimal cellular behavior and tissue regeneration. Thus, the material composition should be carefully tailored to enable efficient cell adhesion and subsequent cell encapsulation within the hydrogel network.
iii. Mechanical properties. Superior mechanical properties and remarkable plasticity are crucial for reinstating intervertebral space height, enduring mechanical loads, and preserving the spine's range of motion. By possessing these desired characteristics, the hydrogel material can effectively contribute to the restoration of structural integrity and biomechanical functionality within the intervertebral disc.
iv. Biodegradability. The hydrogel material employed should exhibit a remarkable ability to biodegrade and metabolize alongside the surrounding tissue, while concurrently aligning with the pace of tissue regeneration. It is imperative that both the hydrogel and its degradation products do not compromise the delicate microenvironment within the degenerative disc, thereby fostering a favorable milieu for tissue repair and regeneration.
v. In situ curing to avoid leakage. In situ curing is highly advantageous to avert any undesired leakage of the hydrogel material. The ability to undergo in situ curing offers a strategic advantage, allowing for precise placement and conformal filling within the intervertebral disc defect or cavity. By circumventing the risk of leakage, in situ curing ensures the efficient encapsulation of the hydrogel material, enhancing its therapeutic efficacy and minimizing potential complications.
vi. Multifunctional bioactivities. The hydrogel material should possess exceptional capabilities in promoting cell adhesion, proliferation, and differentiation, while also exhibiting outstanding anti-inflammatory, anti-oxidative, and antibacterial properties. These multifaceted bioactive attributes are instrumental in creating a favorable microenvironment for tissue regeneration. By facilitating robust cellular responses, including enhanced cell attachment, proliferation, and differentiation, the hydrogel material can actively contribute to the regenerative process. Additionally, its remarkable anti-inflammatory, anti-oxidative, and antibacterial abilities play a pivotal role in mitigating detrimental factors and safeguarding the regenerating tissue from adverse environmental influences, thereby fostering an optimal regenerative milieu.

Table 1
Application of cell-load injectable hydrogels in regeneration of IVDD.

Materials	Hydrogel composition	Hydrogel-encapsulated agent	Biofunctions	Disease model	Ref
Odex/Teleostean/CEC	combined triple interpenetrating network hydrogel (comprised of dextran, chitosan and teleostean)	MSCs	promote cell adhesion, migration and proliferation , Anti-inflammation	goat model of disc degeneration	[43]
CP-CS	chitosan–poly(hydroxybutyrate-*co*-valerate) with chondroitin sulfate nanoparticles	adipose derived rat mesenchymal stem cells (ADMSCs)	promote stem cell proliferation and differentiation	in vitro studies with ADMSCs cells	[44]
HAMA	1 % HAMA	bone marrow mesenchymal stem cells (BMSCs)	anti-apoptosis , promote the differentiation of stem cell into NP cells	rat model of disc degeneration	[45]
GDH	genipin cross-linked decellularized nucleus pulposus hydrogel (GDH)	adipose-derived mesenchymal stem cells (ADSCs)	good biocompatibility, inducibility of expressing NP-related genes	rat model of disc degeneration	[46]
GC/poly (EO-*co*-Gly)	poly (EO-*co*-Gly)-CHO and glycol chitosan (GC) hydrogel	human-induced pluripotent stem cells (hiPSCs)	promote cell proliferation and differentiation	rat model of disc degeneration	[47]
ECM-Gels	rat tail collagen hydrogels (Gels) modified by costal cartilage extracellular matrix (ECM-Gels)	lentivirus-engineered cartilage endplate stem cells (CESCs)	continuous release of engineered exosomes	rat model of disc degeneration	[48]
PEG-LM	peptide-functionalized poly(ethylene glycol) (PEG)-based hydrogel	primary NP cells	promote ECM secretion, Promotes cell proliferation	rat model of disc degeneration	[49]
HA-pNIPAM	covalently cross-linked HA (HA-BDDE) and HA-poly(N-isopropylacrylamide) (HA-pNIPAM) hydroge	NP cells	Promote NP cell proliferation and differentiation, promote ECM secretion	in ex vivo studies with IVD organ culture model	[50]
CCS	type II collagen/chondroitin sulfate (CS) composite hydrogel	adipose-derived stem cell (ADSC)	promote the expressions of NP-specific genes, biocompatible	rat model of disc degeneration	[51]
gelatin colloidal gels	nanostructured gelatin colloidal hydrogels	MSCs	cytocompatible, biodegradable, Promote cell differentiation	rabbit IVDD model	[52]
dextran/gelatin hydrogel	oxidative dextran (Oxi-Dex) and the amino gelatin (amino-Gel)	MSCs	Promote cell differentiation and proliferation	in vitro studies with MSCs cells	[53]
CS/HA	chitosan (CS) and hyaluronic acid (HA) crosslinked with glycerol phosphate (GP) hydrogel	ADSCs	Promote cell proliferation and differentiation	in vitro studies with ADSCs cells	[54]

3. Classification and applications of bioactive injectable hydrogels for intervertebral disc regeneration

Based on the various crosslinking methods employed, hydrogels can be categorized into two distinct types: physical crosslinking and chemical crosslinking. Physical crosslinking involves altering the structure and properties of hydrogels through the application of physical forces or conditions, such as temperature, pH value, ion concentration, among others. Conversely, chemical crosslinking involves the creation of a crosslinked structure through chemical reactions, thereby imparting enhanced stability and mechanical strength to the hydrogel. Furthermore, hydrogels can be further classified into three categories based on the origin of their constituent components: natural, synthetic, and composite hydrogels (Table 1). Natural hydrogel refers to the material extracted or separated from nature, which has natural characteristics and ingredients. Common natural hydrogels include collagen [29]、Alginate [30]、Gelatin [31]、Hyaluronic acid [32]、Chitosan [33]、gellan gum [34]. These materials find extensive applications in the fields of tissue engineering and regenerative medicine owing to their favorable biocompatibility and low cytotoxicity. Notably, natural substances such as collagen and hyaluronic acid are integral components of the ECM within the intervertebral disc, facilitating the repair of the nucleus pulposus [35]. However, natural hydrogels also have some disadvantages, such as relatively low mechanical strength and single function. In order to overcome these limitations, scientists have developed synthetic hydrogels and composite hydrogels. Synthetic materials include Poly(lactic-*co*-glycolic) acid [36]、Polyvinyl alcohol [37]、Polyurethane [38]、Polyethylene glycol [39] etc. Compared with natural materials, synthetic materials have the advantages of solid controllability, easy design, and overcoming the insufficient mechanical properties of natural hydrogels, and are also widely used in the regeneration and repair of fibrous rings [40]. However, its disadvantage is that some synthetic hydrogels need to add toxic ingredients in the preparation process, resulting in low biocompatibility. These shortcomings limit the application of synthetic hydrogels in the repair of intervertebral discs. In order to overcome these problems, composite hydrogels have been introduced as a potential solution. Composite hydrogels are based on the combination of natural materials and synthetic materials, such as fibrin-polyurethane [41], hyaluronic acid-polyethylene glycol [42], etc. Composite hydrogels can combine the advantages of natural/synthetic hydrogels, have good biocompatibility and mechanical properties, and are an essential direction for future research on intervertebral disc regeneration, repair, and replacement.

Currently, the prevailing approach in the practical application of nucleus pulposus tissue repair and regeneration involves utilizing hydrogels as carriers to encapsulate cellular or non-cellular components (such as growth factor, drugs, and nucleic acids). By adjusting the cross-linking degree and pore structure of the hydrogel, the release rate of these components can be controlled, thereby achieving a targeted therapeutic effect in the repair and regeneration of nucleus pulposus tissue. Fig. 2 illustrates the specific application of hydrogels in the regeneration and repair of degenerative intervertebral discs.

3.1. Cell-loaded hydrogels

IVDD arises due to a series of cellular, structural, and biomechanical changes that are triggered by a decrease in NPCs [55]. The loss of resident NPCs is considered a crucial initial factor in the regression of the intervertebral disc. This leads to a disruption in the balance between anabolic and catabolic processes in ECM synthesis, ultimately resulting in degenerative alterations associated with biomechanical stress [56]. Consequently, the restoration of NPC quantity and functionality emerges as a critical aspect in addressing IVDD. Repairing and replenishing NPCs becomes a top priority in the treatment of IVDD [57,58]. Indeed, investigations in the realm of Regenerative Medicine have demonstrated the feasibility of generating ECM constituents resembling the distinguishing attributes of

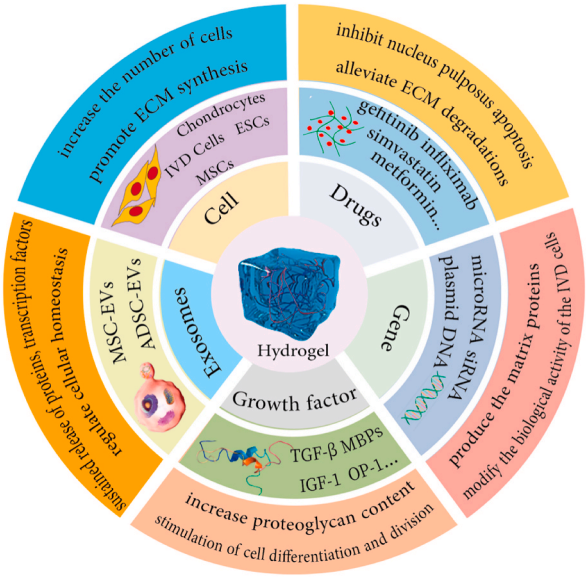

Fig. 2. Schematic diagram of the application of injectable hydrogel in IVDD.

nucleus pulposus through the implantation of designated active cells within intervertebral discs. Cell-based cell therapy for intervertebral discs has received extensive attention in recent years. Its purpose is to: i. replenish the number of cells and restore the ECM of the intervertebral disc; ii. use cells to supplement nutrition; iii. restore the biomechanical function of the intervertebral disc; iv. exert an anti-inflammatory effect [59]. The currently available cell types mainly include MSCs, nucleus pulposus cells, notochordal cells, chondrocytes, etc. Among them, MSCs are the most widely used due to their relative ease of isolation, high safety, and ability to adopt a phenotype similar to natural NP cells [60].

The utilization of hydrogel as a carrier for encapsulated cells has been substantiated as the pivotal factor in the successful treatment of degenerative intervertebral disc cells. Notably, studies have unequivocally confirmed the capability of nucleus pulposus cells to retain their phenotype within a three-dimensional environment. Hydrogels, functioning as macromolecular biomaterials capable of uniformly dispersing and transporting cells, assume a critical role in establishing a conducive environment for cell attachment and proliferation, while also serving as an extracellular matrix during the regenerative process of intervertebral discs. Consequently, these advancements hold substantial promise and present significant application prospects in the field [61].(Table .1). Initially, a range of hydrogel scaffolds incorporating cell seeds were sequentially developed, including polymer-based bio-composites utilizing PLGA, PLLA, Polycaprolactone, and nano cellulose. Nevertheless, studies have revealed challenges in achieving uniform distribution of seed cells within these preformed biomaterials. Furthermore, the implantation of such preformed hydrogels also entails invasive procedures [62]. In recent times, there has been a growing emphasis on injectable hydrogel materials within the domain of intervertebral disc tissue engineering. A substantial body of literature has predominantly focused on the selection of chitosan [43], collagen [63], and hyaluronic acid [64] as the primary materials in intervertebral disc tissue engineering. These materials possess favorable attributes of biocompatibility and biodegradability. They are capable of forming a stable gel structure and can be directly injected into the intervertebral disc through a non-invasive approach. This approach provides an advantageous means to establish a supportive mechanical framework and a conducive cellular attachment environment within the intervertebral disc. Importantly, it circumvents the risks of trauma and complications typically associated with traditional surgical procedures.

In cell therapy utilizing hydrogel as a carrier, the mechanical strength of the hydrogel is a crucial consideration. The hydrogel must possess sufficient mechanical strength to provide robust mechanical support and maintain a stable gel structure. This capability is essential for facilitating cell attachment, proliferation, and differentiation, as well as withstanding certain mechanical loads. Simultaneously, the hydrogel should possess adequate compressive and shear strength to resist the mechanical stresses exerted within the intervertebral disc environment. This is imperative for preserving the structural integrity of the hydrogel and ensuring the survival of the encapsulated cells. Achieving a balance between biocompatibility and mechanical properties in hydrogels is of paramount importance, considering the prevalent challenge of low strength in commonly used hydrogel materials. Recent studies have focused on

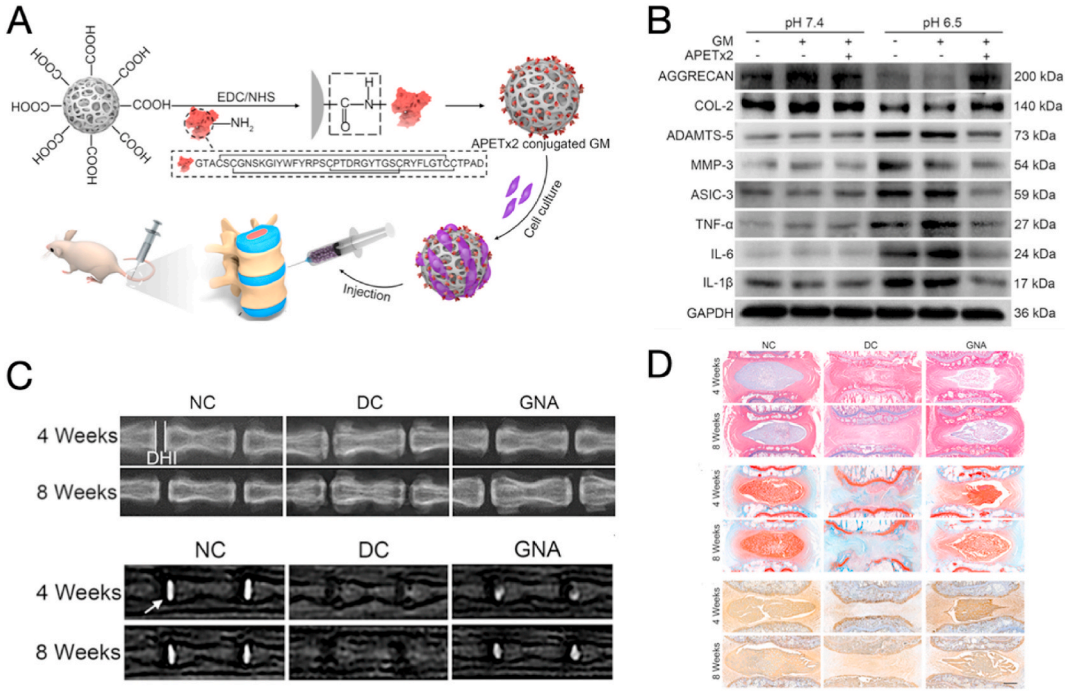

Fig. 3. Schematic illustration of injectable GelMA hydrogel microspheres combined with nucleus pulposus cells for the treatment of IVDD in Rats. A) The preparation and injectable process of GelMA hydrogel microspheres with nucleus pulposus cells in the IVD degeneration rat model. B) The protein expression of inflammatory factor, ECM degrading enzymes, and ECM proteins. C) Representative X-ray images and MRI images of intervertebral discs at 4 and 8 weeks. D) Histological evaluation of animal experiment (H&E, Safranin O-Fast Green staining and Immunohistochemistry staining of Col II) [69]. Copyright 2021, American Chemical Society.

enhancing the mechanical strength of hydrogels through the incorporation of various strategies, including the introduction of nanostructures, topological structures, and double-crosslinked network structures. Zhang et al. [65] inserted MSCs into a triple interpenetrating network hydrogel and injected them into a goat model of IVDD. Discs treated with MSC and hydrogel showed significant improvement in disc height index by 10 % after two weeks, as well as improvements in histology. According to this study, hydrogels impregnated with MSCs may provide a clinically feasible and minimally invasive treatment for IVDD. Nair et al. [44] developed a composite hydrogel comprising chitosan/polyhydroxybutyrate-*co*-valerate (PHBV) for NP tissue engineering, eliminating the need for crosslinkers. This hydrogel demonstrated water absorption capacity and viscoelasticity similar to that of native tissue. Moreover, it exhibited the ability to withstand varying stress levels associated with everyday activities such as sitting (0.5 MPa), lying down (0.01 MPa), and standing (1.0 MPa) under dynamic conditions. Furthermore, the hydrogel facilitated the survival and adhesion of rat adipose-derived stem cells.

In addition to highlighting mechanical properties, significant attention has been directed towards the essential aspects of biocompatibility and non-immunogenicity. Over the past years, peptide-based hydrogels have gained escalating popularity due to their inherent biocompatibility and non-immunogenic characteristics [66]. Barcellona et al. [67] suggest that the encapsulation of adult degenerative human NP cells into a stiff hydrogel functionalized with laminin-mimetic peptides IKVAV and AG73 could promote cell viability and increase biosynthetic activity for this population in 3D culture in vitro. As an injectable hydrogel scaffold, it has excellent potential to deliver nucleus pulposus cells *in vivo*. Wang et al. [68]used a polysaccharide hydrogel-loaded NP-derived mesenchymal stem cell (NPMSC) modified with 3D RGD peptide to treat rats' IVDD. The results confirmed that RGD peptides formed cell adhesion structures in hydrogels, greatly enhanced long-term cell viability in 3D cultures, and induced the differentiation of NPMSCs into NP cells. Recently, Chen et al. [69] reported an injectable "peptide-cell-hydrogel" microspheres through covalently coupling APETx2 to methacrylylated gelatin (GelMA) and further loading nucleus pulposus cells (Fig. 3A). The injectable hydrogel microspheres not only showed excellent anti-inflammatory ability, but also regulated the metabolic balance of ECM, giving an effective way for tissue repair under excessive active inflammatory response (Fig. 3B). The radiological data and histological evaluation of animal experiments consistently verified that the hydrogel microspheres had a certain ability to repair the intervertebral disc (Fig. 3C and D). In conclusion, the research structure of applying cell-loaded hydrogels for the treatment of IVDD is exciting. However, there is still a long way to go

Table 2
Application of drug-load injectable hydrogels in regeneration of IVDD.

Materials	Hydrogel composition	Hydrogel-encapsulated agent	Biofuctions	Disease model	Ref
HAMA	1 % HAMA	salvianolic acid B (SalB)	anti-oxidation , regulate ECM metabolism	rat model of disc degeneration	[45]
PNIPAAm	N-isopropylacrylamide-based thermosensitive hydrogels	SHP099	expression of key proteins (collagen II and aggrecan), reverse the degeneration of NP cells	rat model of disc degeneration	[79]
GelMA	ASP-liposomes @ gelatin-methacryloyl (GelMA) hydrogels	aspirin	relieve local inflammation	rabbit model of disc degeneration	[80]
pNIPAAM MgFe-LDH	poly-N-isopropylacrylamide MgFe-layered double hydroxide (pNIPAAM MgFe-LDH) hydrogel	celecoxib (CXB)	relieve local inflammation	canine model of spontaneous mild IVD degeneration	[81]
PCLA–PEG–PCLA	poly(ε-caprolactone-*co*-lactide)-*b*-poly (ethylene glycol)-bpoly (ε-caprolactone-co-lactide) PCLA–PEG–PCLA hydrogel	celecoxib (CXB)	reduct back pain	client-owned dogs with chronic low back pain related to IVD degeneration	[82]
TSPBA/PVA	N,N,N′,N′-tetramethyl-1,3-propanediamine/4-(bromomethyl) phenylboronic acid/PVA	rapamycin	promote M2-like macrophage phenotype polarization, scavenge ROS	rat model of disc degeneration	[83]
Col-JK1	collagen/JK1 solution	hydrogen sulfide (H2S)	inhibition of nucleus pulposus apoptosis, inhibition of matrix degradation , anti-inflammatory	rat model of disc degeneration	[84]
AHA-g-PNIPAAm	aminated hyaluronic acid-g-poly(N-isopropylacrylamide)	gefitinib	inhibit cartilage base degrade, promote Type II collagen synthesis	rat model of disc degeneration	[85]
FibGen/CHS	genipin crosslinked fibrin/collagen type I	infliximab	anti-inflammatory	in vitro studies with human AF cells	[86]
Gel-PEG-tyramine	gelatin/poly-(ethylene glycol)/tyramine	simvastatin	promote the production of ECM	rat model of disc degeneration	[87]
C/G/GP	chitosan/gelatin/glycerol phosphate	ferulic acid	reduce oxidative stress	in vitro studies with NPCs	[88]
RTNPs/F-127	chitosan hydrochloride (CS)/ carboxymethyl chitosan (CMCS)/Pluronic F127	thalidomide/ ruxolitinib	anti-inflammatory, promote the production of ECM	rat model of disc degeneration	[89]
HA/PEG	hyaluronic acid (HA)/poly(ethylene glycol) diacrylate (PEG)/phenylboronic acid	metformin	anti-inflammation and promotion of ECM synthesis	rat model of disc degeneration	[90]

before the widespread clinical application of cell therapy. In conclusion, hydrogel-based cell therapy provides a new approach for treating degenerative disc disease, with potential therapeutic value for IVD regeneration.

3.2. Drug-loaded hydrogels

In recent years, many small molecule drugs have been proved to have the effect of relieving IVDD. Different drugs can intervene in the biological cascade in the process of IVDD, help restore the homeostasis of the intervertebral disc, and provide a new idea for the treatment of IVDD (Table .2). However, a simple injection of drugs has the following problems: i. Drugs are easy to leak and have low bioavailability. ii. The sustained release of the drug cannot be achieved, and the drug effect lasts for a short time. iii. Repeated injections will aggravate disc degeneration. Fortunately, the therapeutic strategy of hydrogel-carrying drugs can solve the above problems well, which has become a research hotspot in recent years. The etiology of IVDD is unclear. However, it is generally believed that intradiscal inflammation and oxidative stress are common pathological mechanisms in the occurrence and development of IVDD [70]. Therefore, targeting the inflammatory process is a therapeutic strategy for treating disc degeneration [71]. Studies have recently discovered a small molecule compound MR409 with a strong ability to inhibit IL-1β synthesis and immune cell infiltration, effectively inhibiting disease-related inflammation [72]. Zheng et al. [73] constructed an injectable thermosensitive PLGA-PEG-PLGA injectable hydrogel as a sustainable release system for MR409. After treatment with IL-1β-induced nucleus pulposus cells, the expression of ACAN and SOX9 was up-regulated, and the expression of MMP-13 and ADATMS5 was down-regulated. Similarly, Bai et al. [74] developed a reactive oxygen species-responsive hydrogel scaffold containing rapamycin. In an environment rich in reactive oxygen species, the hydrogel scaffold continuously released rapamycin while consuming reactive oxygen species, promoting M2 cell polarization, reducing inflammation, and ultimately preventing IVDD. This represents a new method that regulate the local inflammatory microenvironment to improve IVD regeneration. Pan et al. [75] found that a sustained-release gefitinib controlled-release injection heat-sensitive hydrogel can promote the production of the ECM of nucleus pulposus cells and promote the synthesis of type II collagen in the degenerated intervertebral disc of mice. It shows that gefitinib has excellent application prospects in treating IVDD. Diabetes-induced IVDD has received increasing attention in recent years [76]. Localized glucose overload and disc fluctuations

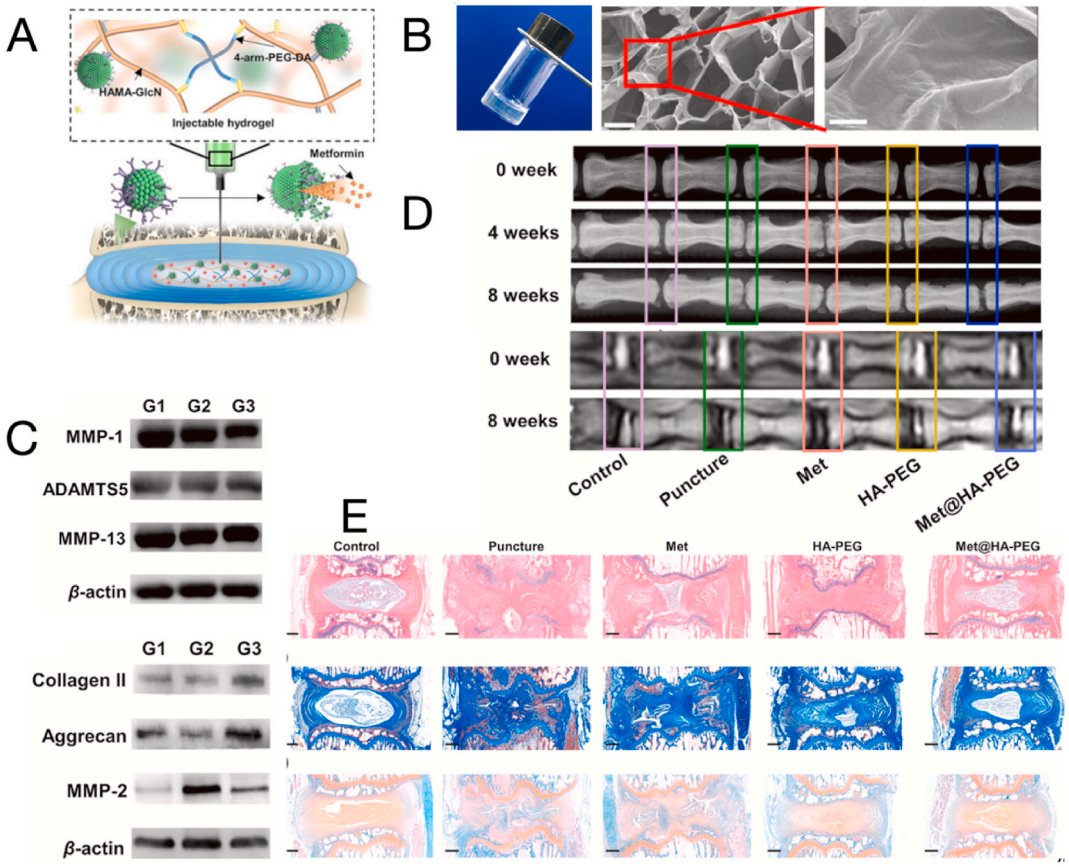

Fig. 4. Injectable "Metformin-hydrogel" regulate inflammation to treat IVDD. A) The injectable process of Metformin-hydrogel in the IVD degeneration rat model. B) Crosslinked HA-PEG hydrogel and SEM image of HA-PEG hydrogel. C) The protein expression of in vitro NPCs cultured in high glucose and Met@HA- PEG. D) The radiology evaluation of *in vivo* IVDD model in diabetic rats. E) Histological evaluation of animal experiment (H&E, Masson staining and Safranin O-Fast Green staining) [78]. Copyright 2022, Elsevier.

Table 3
Application of growth factor-load injectable hydrogels in regeneration of IVDD.

Materials	Hydrogel composition	Hydrogel-encapsulated agent	Biofuctions	Disease model	Ref
dextran/gelatin	oxidative dextran (Oxi-Dex) and the amino gelatin (amino-Gel)	TGF-β3	Promote cell differentiation and proliferation	in vitro studies with MSCs cells	[53]
GC/poly (EO-*co*-Gly)	poly (EO-*co*-Gly)-CHO and glycol chitosan (GC) hydrogel	growth differentiation factor-5 (GDF5)	Promote cell proliferation and differentiation	rat model of disc degeneration	[47]
CS/HA	chitosan (CS) and hyaluronic acid (HA) crosslinked with glycerol phosphate (GP) hydrogel	KGN	Promote cell proliferation and differentiation	in vitro studies with adipose-derived stem cell (ADSCs) cells	[54]
chitosan	chitosan hydrogel	basic fibroblast growth factor (bFGF) , TGF-β1	promote the proliferation of NPCs	rat model of disc degeneration	[108]
chitosan	chitosan hydrogel	TGF-β3 , BMP4 , TIMP-2	regenerative repair of acute intervertebral disc injury	rabbit model of disc degeneration	[96]
GO-FEFKFEFK	graphene oxide/self-assembling peptide FEFKFEFK (F: phenylalanine; K: lysine; E: glutamic acid)	TGF-β3	promote the activity of NPCs	in vitro studies with NPCs	[109]
FBG-HA	Fibrin/hyaluronan	BMP-2/7 heterodimer	promote the expression of type II collagen and the synthesis of glycosaminoglycans	Bovine Caudal IVDs model	[110]
alginate	PRP alginate coupled hydrogel	PRP	promote cell proliferation and synthesis of extracellular matrix	in vitro studies with hNPCs	[102]
Si-HPMC	silanized hydroxypropyl methylcellulose	TGF-β1 , GDF-5	promote the proliferation of NPCs	in vitro	[111]

aggravate the chronic inflammatory microenvironment of IVDD, making it more difficult for it to regenerate [77]. Zheng et al. [78] developed a Metformin-hydrogel with glucose responsiveness, which protects mitochondria from ROS, thus inhibiting the activation of the NLRP3 inflammatory pathway and increasing the amount of ECM produced in the nucleus pulposus. (Fig. 4). The schematic diagram of Metformin-hydrogel construction is shown in Fig. 4A. Fig. 4B shows the optical photos and SEM images of Metformin-hydrogel respectively. As shown in Fig. 4C, metformin hydrogel inhibits the expression of matrix metalloproteinases and promotes the production of extracellular matrix. The radiological data and histological evaluation of animal experiments had consistently verified that the metformin-hydrogel had a certain ability to repair the intervertebral disc (Fig. 4D and E). Therefore, safe and controllable drug-loaded hydrogels are very important in the biomedical field, such as treating early IVDD. Although satisfactory results have been obtained in a variety of animal experiments, there is still a distance from clinical translation.

3.3. Growth factor-loaded hydrogels

Recent studies have found that IVDD is associated with proteins or factors in the ECM of the intervertebral disc, which maintain the homeostasis of the ECM by maintaining the balance of anabolism and catabolism in the intervertebral disc cells. Bioactive factors that regulate the anabolic state of the intervertebral disc include transforming growth factor-beta (TGF-β) [91], bone morphogenetic proteins (MBPs) [92],insulin-like growth factor (IGF-1) [93], osteogenic proteins −1 (OP-1) [94],etc. There have been many studies by injecting growth factors directly into the intervertebral disc, aiming to stimulate the anabolism of the intervertebral disc and provide an environment conducive to bioremediation [95]. Injectable hydrogels can be mixed or cross-linked with growth factors to provide longer-lasting anabolic effects than growth factors alone (Table .3). Gandhi et al. [96] studied the effect of chitosan hydrogels loaded with TGF-β 3 and BMP-4 on a rabbit model of acute intervertebral disc injury. The results show that both can stimulate the survival and proliferation of nucleus pulposus cells, and promote the regeneration and repair of intervertebral discs. Gan et al. [53]constructed a dextran/gelatin hydrogel using poly(D, L-lactide-*co*-glycolide) (PLGA) nanoparticles as a controlled release carrier of TGF-β3. The drug-loading system can induce MSCs to differentiate into nucleus pulposus-like cells in situ by stably releasing active TGF-β3 for a long time, and promoting the synthesis of the ECM. Recent studies have found that platelet-rich plasma containing high levels of various growth factors has a positive effect on the regeneration and repair of intervertebral discs [97,98], and may represent a new strategy for IVDD biotherapy. Studies have shown that in vitro, Platelet-Rich Plasma (PRP) stimulates the proliferation and matrix synthesis of porcine intervertebral disc cells [99] and stimulates isolated human intervertebral disc cells to form nucleus pulposus-like tissue [100]. Sawamura et al. [101] investigated the therapeutic effect of platelet-rich plasma-loaded gelatin hydrogel microspheres (PRP-GHMs) on rabbit IVD. After 8 weeks of treatment, the disc water content and height were well preserved on MRI images.

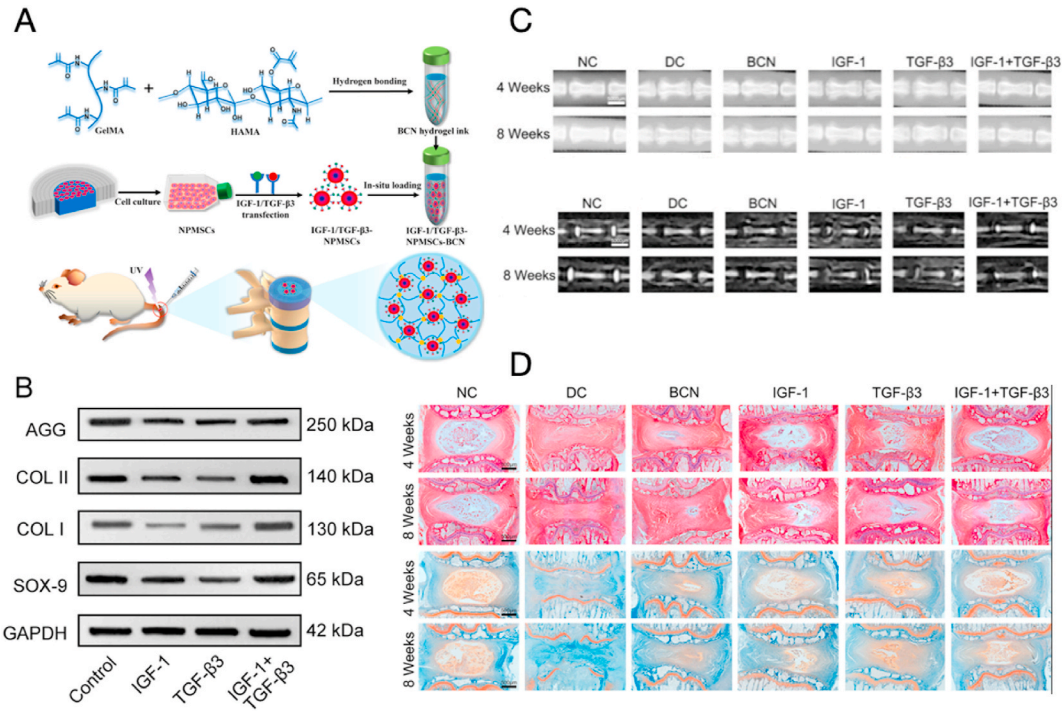

Fig. 5. Injectable "BCN hydrogel" upregulate ECM expression to treat IVDD. A) Schematic diagram of the synthesis of BCN hydrogel loaded with IGF-1/TGF-β 3-NPMSCs and its injection into rat IVDD model. B) Protein expression levels for the transfected cells in the BCN hydrogel for the three groups. C) Representative X-ray images and MRI images of intervertebral discs in 6 groups at 4 and 8 weeks. D) Histological evaluation of animal experiment (H&E, Safranin O-Fast Green staining) [107]. Copyright 2023, Elsevier.

Consistent with this, mRNA expression of proteoglycan core protein and collagen type II was significantly increased after PRP-GHMs treatment, and the number of apoptotic cells in the nucleus pulposus was significantly decreased. Similarly, GROWNEY et al. [102] developed a platelet-rich plasma-functionalized alginate hydrogel, which has not only good mechanical strength but also has superior performance in stimulating intervertebral disc cell proliferation and synthesizing ECM. With the maturity of injectable hydrogel technology, formulating hydrogels with high stability and biocompatibility as well as low immunogenicity is a new development direction in the field of hydrogels [103,104]. In this context, De novo synthesized self-assembling peptides have been the focus of significant interest [105]. Ligorio et al. [106]recently developed an injectable graphene oxide- (GO-) self-assembly peptide FeFKFeFK (F: phenylalanine; K: lysine; E: glutamate) hydrogel. This study demonstrates the ability of GO flakes in this hydrogel as a carrier to sequester and control the delivery of transforming growth factor β3 to promote intervertebral disc repair and regeneration with good biocompatibility. In order to use controlled release to maximize their efficacy and to rationalize the use of growth factors in IVD tissue engineering, Ligorio et al. [107] constructed genetically engineered NPMSCs by transfecting IGF-1 and TGF-β3 into NPMSCs using lentiviral vectors. Then, the genetically engineered NPMSCs were encapsulated in a two-component polymer network (BCN) hydrogel based on gelatin methacrylate (GelMA) and hyaluronic acid methacrylate (HAMA) to deliver the genetically engineered NPMSCs to target tissues (Fig. 5A). This study shows that the hydrogel can effectively upregulate the expression of ECM by continuously expressing IGF-1 and TGF-β3, thus providing theoretical and experimental basis for the etiological treatment of IVDD (Fig. 5B). The radiological data and histological evaluation of animal experiments consistently verified that the hydrogel had a certain ability to repair the intervertebral disc (Fig. 5C and D). Substantial evidence supports the role and efficacy of growth factor-loaded hydrogels in the treatment of IVDD. However, this approach works in the early stages of disc degeneration, when enough cells respond positively to growth factor stimulation. In the middle and late stages of degeneration, the cells are further damaged, and the ability of matrix secretion is severely reduced. Treatment with bioactive factors alone cannot effectively inhibit IVDD. At this time, it is often necessary to combine cell transplantation to increase the cell number and secretory activity to achieve the best therapeutic effect.

3.4. Gene delivery hydrogel

Compared with the direct injection of drugs and bioactive factors into the intervertebral disc, gene therapy can prolong the action time, promote the continuous synthesis of matrix proteins and ECM, and has the advantage of a more stable curative effect, Gene therapy is also an effective way to fundamentally modulate the metabolic balance of the ECM of the intervertebral disc [112]. Wehling et al. [113] first reported the application of gene therapy in IVDD in 1997. In this study, a viral vector expressing anti-inflammatory genes was transfected into isolated and cultured intervertebral disc cells, which proved for the first time that gene therapy could be applied to the treatment of IVDD. With the continuous development of gene sequencing technology, genes related to IVDD repair have been discovered through differential gene expression analysis of degenerative nucleus pulposus tissue, thus promoting the development of gene therapy. To solve the defects of low gene transfection efficiency and easy degradation by nucleases, hydrogel systems with efficient, continuous, and controllable gene delivery have been successively developed [114], which has also promoted the development of gene therapy for IVDD (Table .4). Ge et al. [115] uses polyethylene glycol and poly-N-isopropyl acrylamide to prepare thermally reactive multiphase coronal polyionic micellar hydrogels, and deliver of the heme oxygenase gene through this system can alleviate the inflammatory response caused by interleukins, promote the expression of proteoglycan, and promote the regeneration of the intervertebral disc. The hydrogel carrier can be used as a safe and efficient non-viral carrier for gene therapy of IVDD. In a study, Wang et al. [116] used gelatin hydrogel as a carrier to transfect miRNA-21 inhibitor (Antagomir-21) into SD rat nucleus pulposus cells and found that the transfected nucleus pulposus cells significantly up-regulated the expression of proteoglycans expression, and significantly down-regulated the expression of MMP. This experiment demonstrates that the hydrogel gene carrier can postpone the course of IVDD and support IVDD regeneration by sustaining the release of Antagomir-21, inhibiting ECM degradation, and restoring the ECM synthesis/catabolism balance in NPCs (Fig. 6). The schematic diagram of the synthesis process of Gel-BA-CD and mPEG-TK-PLGA molecules is shown in Fig. 6A. Fig. 6B shows the dynamic properties and microstructure of the hydrogel. As shown in Fig. 6C, the hydrogel had a strong inhibitory effect on ROS generation. Fig. 6D and E respectively showed that the hydrogel had significant anti-inflammatory ability and could effectively promote the polarization of macrophages to the M2 phenotype. Finally, the X-ray and MRI images verified the role of this hydrogel in repairing the intervertebral disc (Fig. 6F). At the same time, this “gene hydrogel” could also be used to treatiing other diseases, expanding the application scope of gene therapy. In recent years, smart responsive hydrogels have been developed to address the adverse effects caused by the instantaneous and rapid release of genes. It can respond positively, rapidly, and accurately to changes in the body’s microenvironment, improve the effect of gene therapy on IVDD, and reduce unnecessary side effects. Ge et al. [117] introduced the MMP-responsive GPLGVRG peptide into the miRNA-29a transfection system to form the MMP-responsive miRNA transfection vector PEG-GPLGVRG-PAsp(DET)-Chole (PGPC). Experiments showed that the block polymer PGPC complexed miRNA-29a to form a stable structure. Under the action of MMPs, the PEG shell was removed, realizing the on-demand release of miRNA-29a and significantly improving the endocytosis and transfection efficiency of miRNA, further improving the durability and targeting of the treatment. In conclusion, as an efficient and stable gene delivery system, hydrogel improves the therapeutic efficiency and safety of functional genes, prolongs the effective functional time of genes, and shows great application potential in the treatment of IVDD.

3.5. Loaded with exosomes

In recent years, with the in-depth research in the fields of regenerative medicine such as cell therapy, gene-targeted therapy, and tissue engineering, the therapeutic value of exosomes for IVDD has attracted more and more attention [122]. The term “exosome” was

Table 4
Application of gene-load injectable hydrogels in regeneration of IVDD.

Materials	Hydrogel composition	Hydrogel-encapsulated agent	Biofuctions	Disease model	Ref
PEG	polyethylene glycol (PEG) hydrogel	miRNA874	regulate the synthesis/catabolism balance of ECM	rat model of disc degeneration	[118]
MPMs	PEG-b-PAsp(DET) and poly(N-isopropylacrylamide)-*block*-PAsp (DET) [PNIPAM-*b*-PAsp(DET)]	loading HO-1 plasmid DNA	increase the NP phenotype-associated genes such as aggrecan, type II collagen, and SOX-9	rat model of disc degeneration	[115]
HA-CHO/ PAMAM	aldehyde hyaluronic acid/poly(amidoamine)	siSTING	ease the IVD inflammation and degeneration	rat model of disc degeneration	[119]
Gel-BA-CD-TA	Gelatin/phenylboronic acid/β-cyclodextrin/Tannic acid	miRNA-21 inhibitor (Antagomir-21)	anti-inflammation and promotion of ECM	rat model of disc degeneration	[116]
Alginate	Alginate hydrogels	GDF-5 mRNA	improves the tissue microenvironment regeneration	in vitro studies with hNPCs	[120]
PGPC	PEG-GPLGVRG-PAsp(DET)-Chole (PGPC) MMP-degradable hydrogels	miRNA-29a	possesses potent fibrosis suppression apability, reverse IVDD	rat model of disc degeneration	[117]
ZOGA	zinc-oxidized sodium alginate-gelatin	Antagomir-204–3p	inhibit the apoptosis of NPCs, maintain the metabolic balance of the ECM	rat model of disc degeneration	[121]

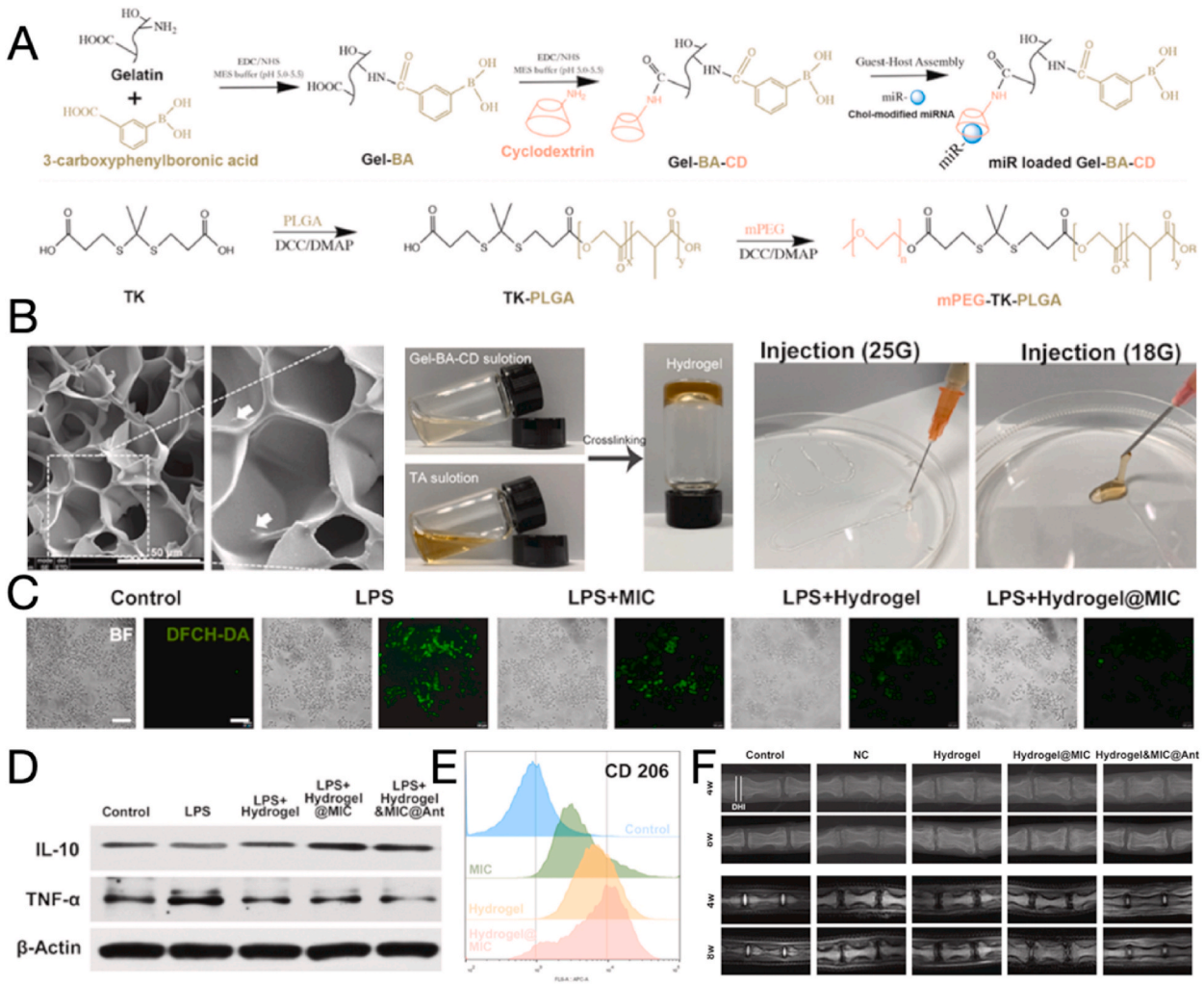

Fig. 6. The anti-inflammation and ECM regeneration behavior of gene delivery hydrogel positively promoted IVDD repair. A) Schematic diagram of the synthesis procedure of Gel-BA-CD and mPEG-TK-PLGA molecules. B) Dynamic characteristics and microstructures of hydrogels. C) Confocal images of ROS generation in LPS-infected macrophages after DCFH-DA staining. D) IL-10 and TNF-α expression levels were evaluated by Western blot. E) Morphological and phenotypic changes in macrophages after hydrogel treatment. F) X-ray and MRI images of rat coccygeal vertebral discs in different groups at 4 and 8 week [116]. Copyright 2022, Elsevier.

first coined by Trams et al., in 1981 [123]. Because of its powerful biological activity and cellular function, exosomes are now used as a cell-free therapy solution with unique advantages such as lower immunogenicity than cells, stable contents, and easy preservation. Exosomes are a class of tiny vesicles with a diameter of 40–100 nm that can deliver biomolecules such as lipids, carbohydrates, proteins, mRNA, miRNA, and DNA from one cell to another. And then play the role of anti-inflammatory, and antioxidant, inhibit cell apoptosis, promote cell proliferation and maintain ECM homeostasis, and finally achieve the effect of treating IVDD [124,125]. However, in the face of the microenvironment of hypertonicity, hypoxia, low pH, and low nutrition in the intervertebral disc tissue, the simple use of exosomes in the treatment process will face problems such as high clearance rate and short half-life, which limit the efficacy of exosomes [126]. Hydrogel-loaded exosomes can not only avoid the rapid removal of exosomes but also maintain the biological activity of exosomes and the controlled release of effective concentrations, which has potential application value in the treatment of IVDD [127,128] (Table .5). Xing et al. [129] constructed an injectable thermosensitive ECM hydrogel without toxic cross-linking agents, and the novel hydrogel exhibited good biocompatibility and similar properties to the nucleus pulposus tissue. In this study, The authors combined exosomes extracted from adipose-derived mesenchymal stem cells (ADSCs) with ECM hydrogels and confirmed through *in vivo* and in vitro experiments that dECM@exo can promote ECM regeneration through anti-inflammatory and anti-apoptosis. This thermosensitive dECM@exo hydrogel system can provide not only in situ gelations to replenish ECM leakage in NPCs but also an environment for the growth of NPCs(Fig. 7A). Fig. 7B shows the SEM images of ECM hydrogel. As shown in Fig. 7C, ECM hydrogel has good cytocompatibility. Fig. 7D shows that ECM hydrogel has anti-inflammatory and anti-apoptotic abilities. In addition, the radiological data and histological evaluation of animal experiments also verified that the hydrogel had a certain ability to

Table 5
Application of exosomes-load injectable hydrogels in regeneration of IVDD.

Materials	Hydrogel composition	Hydrogel-encapsulated agent	Biofuctions	Disease model	Ref
ECM-Gels	rat tail collagen hydrogels (Gels) modified by costal cartilage extracellular matrix	lentivirus-engineered cartilage endplate stem cells (CESCs) exosomes	continuous release of engineered exosomes	rat model of disc degeneration	[48]
dECM@exo	injectable thermosensitive ECM hydrogel	adipose-derived mesenchymal stem cell (ADSC) exosomes	mitigate the inflammatory response in discs, promote the proliferation and inhibit the apoptosis of IVD cells	rat model of disc degeneration	[129]
FEC	Pluronic F127/decellularized extracellular matrix	MSC-derived small extracellular vesicles	maintain the metabolic balance of ECM	rat model of disc degeneration	[131]
M2c-Exos@HA	hyaluronic acid	Exosomes from M2c macrophages	maintain the metabolic balance of ECM	rat model of disc degeneration	[132]

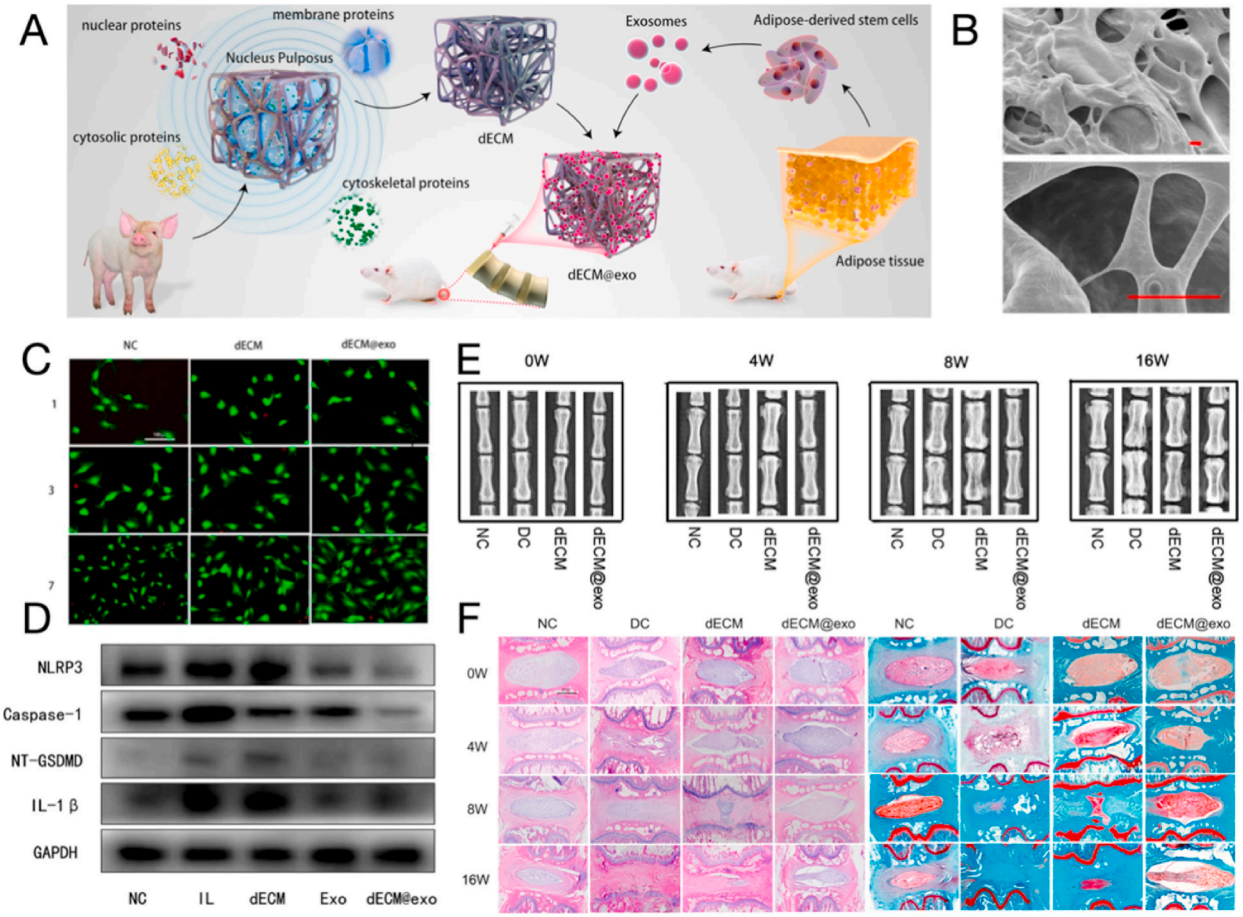

Fig. 7. Injectable exosome-functionalized ECM hydrogel for metabolism balance and pyroptosis regulation in IVDD. A) Schematic diagram of the synthesis of exosome-functionalized ECM hydrogel and its injection into rat IVDD model. B) SEM analysis of dECM@exo. C) Fluorescence images of living (green) and dead (red) cells in each group. D) NLRP3, cleaved Caspase-1, NT-GSDMD, and IL-1β expression levels were evaluated by Western blot. E) X-ray and MRI images of rat coccygeal vertebral discs in different groups at 4,8 and 16 week. F) Histological evaluation of animal experiment (H&E, Safranin O-Fast Green staining) [129].

repair the intervertebral disc (Fig. 7E and F). Similarly, Luo et al. [130] developed temperature controlled rat tail collagen hydrogels and encapsulated functionalized exosomes in hydrogels. The exosomes express Sphingosine kinase 2 (Sphk2), and when injected into the degenerative disc, the hydrogel can continuously and stably release exosomes, which can transport Sphk2 into NPCs by activating PI3K/AKT signaling pathway to regulate the autophagy in NPCs, slow nucleus pulposus cell senescence and prevent IVDD. The above results provide further support for hydrogel-loaded exosomes as a potential therapeutic tool for preventing IVDD and provide a new strategy for the development of IVDD biotherapeutics.

4. Optimal timing of hydrogel injections in IVDD

Hydrogels, which represent biotherapeutics, have heightened interest in disc regeneration due to their bio-therapeutic properties [133]. Recent trials have shown that intervertebral discs have great regenerative potential. However, there is no consensus regarding the best time to treat disc degeneration. Clearly, regenerative therapy is no longer effective when the disc space is wholly lost, or the facet joints are irreversibly damaged. Once facet joint disease and spinal instability occur, no matter how much the nucleus pulposus regenerates, the damage to the physiological function of the spine will not be reversed. Patients often do not have any symptoms in the early stage of the disease, and traditional imaging techniques, such as CT, X-ray, and MRI, cannot detect degenerative changes in their early stages. It is not easy to measure the optimal biological treatment period. Hence, a diagnostic system that is accurate, simple, and reproducible is required to determine when IVDD biological intervention therapy should be conducted. Recently, the rapid development of molecular imaging technology can help us in the early diagnosis of IVDD. Liu et al. [134] reported that they used a rhesus monkey model of IVDD and T1rho [135,136] MR imaging to find out the optimal timing of injections of biocompatible hydrogel for the treatment of IVDD. They found a rapid degenerative stage in the early IVDD process of rhesus monkeys, which had been demonstrated to coincide with the immediate degeneration process of human lumbar intervertebral discs between Pfirrmann grades II and III [137].

The moderate degenerative stage of IVDD (T1r values from 95 to 80 ms) was the good time for the injection of hydrogel at the regenerative intervention. This is undoubtedly excellent news. The development of molecular imaging technology allows us to diagnose early IVDD and take timely measures for intervention and early treatment, making it possible to reverse IVDD.

5. Challenges and outlook

In the field of intervertebral disc regeneration, it is widely recognized that the natural healing process is hindered by the absence of blood vessels, nerves, and lymphatic tissues. Hydrogels can act as scaffolds, elastomers, drug carriers to help the regeneration of intervertebral disc, and play an important role in the regeneration, repair and replacement of the intervertebral disc nucleus pulposus. But there are also many problems and challenges: i. Injection injury. Compared with traditional surgical treatment, although fine-needle injection of hydrogel reduces iatrogenic trauma, the puncture injection itself will also cause damage to the annulus fibrosus of the intervertebral disc. Injection therapy has the potential to cause disc degeneration [138]. At present, the relationship between injection wound and IVDD has not been demonstrated in the literature. ii. Hydrogel spillage. Due to the high pressure in the intervertebral disc [139] , the hydrogel injected into the intervertebral disc is prone to leak, namely hydrogel spillage [140] , and the hydrogel spillage leads to a decrease in the therapeutic effect, or even a complete loss of the therapeutic effect [141,142] , more seriously, if the gel leaks into the spinal canal, osteophytes may be formed in the spinal canal, resulting in iatrogenic spinal stenosis, nerve entrapment, etc., leading to serious consequences [143]. iii. Insufficient strength. The pressure on the lumbar intervertebral disc when a normal person stands is about 1.0 MPa [44] , ordinary hydrogel materials are often unable to withstand such high pressures. How to make the mechanical strength of the hydrogel meet the requirements of the normal motion of the lumbar intervertebral disc without affecting the performance of the gel is also a huge challenge for the hydrogel material. Furthermore, although hydrogels have made significant strides in the treatment of IVDD, their application remains predominantly confined to cell and animal experiments. Clinical implementation is still a considerable distance away. Consequently, for hydrogels to serve as biomaterials for the regeneration and repair of the nucleus pulposus, the ideal hydrogel should not only possess favorable mechanical properties but also actively promote the proliferation and differentiation of intervertebral disc cells, while seamlessly integrating with surrounding tissues without exerting toxic effects. Thus, achieving good biocompatibility and mechanical properties that closely resemble those of the normal nucleus pulposus tissue remains a crucial direction for future advancements in hydrogel materials. Moreover, considering the complex microenvironment encountered within degenerative intervertebral disc tissue, characterized by factors such as hypoxia, acidity, and poor nutrition, stimuli-responsive hydrogels designed to address these degenerative microenvironments exhibit promising prospects for future development. Lastly, it is worth noting that many of the hydrogels reported thus far feature complex compositions and intricate synthesis procedures, which hinder their clinical translation. Therefore, an important future consideration lies in the development of bioactive multifunctional hydrogels composed of simpler constituents, facilitating their application in biomedical settings.

Data availability statement

Data will be made available on request.

CRediT authorship contribution statement

Hao Han: Data curation, Investigation, Methodology, Writing – original draft. **Xiaoming Zhao:** Formal analysis, Methodology. **Hongyun Ma:** Methodology. **Yingang Zhang:** Funding acquisition, Writing – review & editing. **Bo Lei:** Conceptualization, Supervision, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2023.e21867.

References

[1] S.L. James, D. Abate, K.H. Abate, S.M. Abay, C. Abbafati, N. Abbasi, H. Abbastabar, F. Abd-Allah, J. Abdela, A. Abdelalim, Global, regional, and national incidence, prevalence, and years lived with disability for 354 diseases and injuries for 195 countries and territories, 1990–2017: a systematic analysis for the Global Burden of Disease Study 2017, Lancet 392 (2018) 1789–1858.

[2] T. Vos, A.A. Abajobir, K.H. Abate, C. Abbafati, K.M. Abbas, F. Abd-Allah, R.S. Abdulkader, A.M. Abdulle, T.A. Abebo, S.F. Abera, Global, regional, and national incidence, prevalence, and years lived with disability for 328 diseases and injuries for 195 countries, 1990–2016: a systematic analysis for the Global Burden of Disease Study 2016, Lancet 390 (2017) 1211–1259.

[3] M.A. Adams, P.J. Roughley, What is intervertebral disc degeneration, and what causes it? Spine 31 (2006) 2151–2161.

[4] Y. Zhang, H.S. An, C. Tannoury, J.-M.T. Eugene, M.K. Freedman, D.G. Anderson, Biological treatment for degenerative disc disease: implications for the field of physical medicine and rehabilitation, Am. J. Phys. Med. Rehabil. 87 (2008) 694–702.

[5] T. Vos, R.M. Barber, B. Bell, A. Bertozzi-Villa, S. Biryukov, I. Bolliger, F. Charlson, A. Davis, L. Degenhardt, D. Dicker, Global, regional, and national incidence, prevalence, and years lived with disability for 301 acute and chronic diseases and injuries in 188 countries, 1990–2013: a systematic analysis for the Global Burden of Disease Study 2013, Lancet 386 (2015) 743–800.

[6] C. Feng, H. Liu, M. Yang, Y. Zhang, B. Huang, Y. Zhou, Disc cell senescence in intervertebral disc degeneration: causes and molecular pathways, Cell Cycle 15 (2016) 1674–1684.

[7] F. Wang, F. Cai, R. Shi, X.-H. Wang, X.-T. Wu, Aging and age related stresses: a senescence mechanism of intervertebral disc degeneration, Osteoarthritis Cartilage 24 (2016) 398–408.

[8] J. Wang, Y. Tian, K.L. Phillips, N. Chiverton, G. Haddock, R.A. Bunning, A.K. Cross, I.M. Shapiro, C.L. Le Maitre, M.V. Risbud, Tumor necrosis factor α–and interleukin-1β–dependent induction of CCL3 expression by nucleus pulposus cells promotes macrophage migration through CCR1, Arthritis Rheum. 65 (2013) 832–842.

[9] M.-l. Ji, H. Jiang, X.-j. Zhang, P.-l. Shi, C. Li, H. Wu, X.-t. Wu, Y.-t. Wang, C. Wang, J. Lu, Preclinical development of a microRNA-based therapy for intervertebral disc degeneration, Nat. Commun. 9 (2018) 5051.

[10] S. Blanquer, D.W. Grijpma, A.A. Poot, Delivery systems for the treatment of degenerated intervertebral discs, Adv. Drug Deliv. Rev. 84 (2015) 172–187.

[11] H. Che, J. Li, Y. Li, C. Ma, H. Liu, J. Qin, J. Dong, Z. Zhang, C.J. Xian, D. Miao, p16 deficiency attenuates intervertebral disc degeneration by adjusting oxidative stress and nucleus pulposus cell cycle, Elife 9 (2020), e52570.

[12] A. Freemont, A. Watkins, C. Le Maitre, M. Jeziorska, J. Hoyland, Current understanding of cellular and molecular events in intervertebral disc degeneration: implications for therapy, J. Pathol.: A Journal of the Pathological Society of Great Britain and Ireland 196 (2002) 374–379.

[13] G. Fontana, E. See, A. Pandit, Current trends in biologics delivery to restore intervertebral disc anabolism, Adv. Drug Deliv. Rev. 84 (2015) 146–158.

[14] O.A. Boubriak, N. Watson, S.S. Sivan, N. Stubbens, J.P. Urban, Factors regulating viable cell density in the intervertebral disc: blood supply in relation to disc height, J. Anat. 222 (2013) 341–348.

[15] S.K. Mirza, A.A. White III, Anatomy of intervertebral disc and pathophysiology of herniated disc disease, J. Clin. Laser Med. Surg. 13 (1995) 131–142.

[16] P. Priyadarshani, Y. Li, L. Yao, Advances in biological therapy for nucleus pulposus regeneration, Osteoarthritis Cartilage 24 (2016) 206–212.

[17] D. Sakai, G.B. Andersson, Stem cell therapy for intervertebral disc regeneration: obstacles and solutions, Nat. Rev. Rheumatol. 11 (2015) 243–256.

[18] E.A. Levicoff, L.G. Gilbertson, J.D. Kang, Gene therapy for disc repair, Spine J. 5 (2005) S287–S296.

[19] M. Liu, X. Zeng, C. Ma, H. Yi, Z. Ali, X. Mou, S. Li, Y. Deng, N. He, Injectable hydrogels for cartilage and bone tissue engineering, Bone Research 5 (2017) 1–20.

[20] Y.J. Chuah, Y. Peck, J.E.J. Lau, H.T. Hee, D.-A. Wang, Hydrogel based cartilaginous tissue regeneration: recent insights and technologies, Biomater. Sci. 5 (2017) 613–631.

[21] A. Malandrino, D. Lacroix, C. Hellmich, K. Ito, S.J. Ferguson, J. Noailly, The role of endplate poromechanical properties on the nutrient availability in the intervertebral disc, Osteoarthritis Cartilage 22 (2014) 1053–1060.

[22] P. Colombier, A. Camus, L. Lescaudron, J. Clouet, J. Guicheux, Intervertebral disc regeneration: a great challenge for tissue engineers, Trends Biotechnol. 32 (2014) 433–435.

[23] R. Dimatteo, N.J. Darling, T. Segura, In situ forming injectable hydrogels for drug delivery and wound repair, Adv. Drug Deliv. Rev. 127 (2018) 167–184.

[24] U.Y. Choi, H.P. Joshi, S. Payne, K.T. Kim, J.W. Kyung, H. Choi, M.J. Cooke, S.Y. Kwon, E.J. Roh, S. Sohn, An Injectable Hyaluronan–Methylcellulose (HAMC) Hydrogel combined with Wharton's jelly-derived mesenchymal Stromal cells (WJ-MSCs) promotes degenerative disc repair, Int. J. Mol. Sci. 21 (2020) 7391.

[25] Y. Li, H.Y. Yang, D.S. Lee, Advances in biodegradable and injectable hydrogels for biomedical applications, J. Contr. Release 330 (2021) 151–160.

[26] S. Basu, S. Pacelli, A. Paul, Self-healing DNA-based injectable hydrogels with reversible covalent linkages for controlled drug delivery, Acta Biomater. 105 (2020) 159–169.

[27] Q. Feng, Q. Li, H. Wen, J. Chen, M. Liang, H. Huang, D. Lan, H. Dong, X. Cao, Injection and self-assembly of bioinspired stem cell-laden gelatin/hyaluronic acid hybrid microgels promote cartilage repair in vivo, Adv. Funct. Mater. 29 (2019), 1906690.

[28] T. Gorai, U. Maitra, Luminescence resonance energy transfer in a multiple-component, self-assembled supramolecular hydrogel, Angew. Chem. Int. Ed. 56 (2017) 10730–10734.

[29] N. Isobe, T. Komamiya, S. Kimura, U.-J. Kim, M. Wada, Cellulose hydrogel with tunable shape and mechanical properties: from rigid cylinder to soft scaffold, Int. J. Biol. Macromol. 117 (2018) 625–631.

[30] T. Tsujimoto, H. Sudo, M. Todoh, K. Yamada, K. Iwasaki, T. Ohnishi, N. Hirohama, T. Nonoyama, D. Ukeba, K. Ura, An acellular bioresorbable ultra-purified alginate gel promotes intervertebral disc repair: a preclinical proof-of-concept study, EBioMedicine 37 (2018) 521–534.

[31] H. Xu, M. Sun, C. Wang, K. Xia, S. Xiao, Y. Wang, L. Ying, C. Yu, Q. Yang, Y. He, Growth differentiation factor-5–gelatin methacryloyl injectable microspheres laden with adipose-derived stem cells for repair of disc degeneration, Biofabrication 13 (2020), 015010.

[32] R.D. Altman, A. Bedi, J. Karlsson, P. Sancheti, E. Schemitsch, Product differences in intra-articular hyaluronic acids for osteoarthritis of the knee, Am. J. Sports Med. 44 (2016) 2158–2165.

[33] Y. Alinejad, A. Adoungotchodo, M.P. Grant, L.M. Epure, J. Antoniou, F. Mwale, S. Lerouge, Injectable chitosan hydrogels with enhanced mechanical properties for nucleus pulposus regeneration, Tissue Eng. 25 (2019) 303–313.

[34] J. Silva-Correia, J.M. Oliveira, S. Caridade, J. Oliveira, R. Sousa, J. Mano, R. Reis, Gellan gum-based hydrogels for intervertebral disc tissue-engineering applications, Journal of Tissue Engineering and Regenerative Medicine 5 (2011) e97–e107.

[35] E.C. Collin, S. Grad, D.I. Zeugolis, C.S. Vinatier, J.R. Clouet, J.J. Guicheux, P. Weiss, M. Alini, A.S. Pandit, An injectable vehicle for nucleus pulposus cell-based therapy, Biomaterials 32 (2011) 2862–2870.

[36] H. Zhang, L. Wang, J.B. Park, P. Park, V.C. Yang, S.J. Hollister, F. La Marca, C.-Y. Lin, Intradiscal injection of simvastatin retards progression of intervertebral disc degeneration induced by stab injury, Arthritis Res. Ther. 11 (2009) 1–10.

[37] A.S. Oliveira, O. Seidi, N. Ribeiro, R. Colaço, A.P. Serro, Tribomechanical comparison between PVA hydrogels obtained using different processing conditions and human cartilage, Materials 12 (2019) 3413.

[38] Y.-C. Huang, J.P. Urban, K.D. Luk, Intervertebral disc regeneration: do nutrients lead the way? Nat. Rev. Rheumatol. 10 (2014) 561–566.

[39] B.V. Sridhar, J.L. Brock, J.S. Silver, J.L. Leight, M.A. Randolph, K.S. Anseth, Development of a cellularly degradable PEG hydrogel to promote articular cartilage extracellular matrix deposition, Adv. Healthcare Mater. 4 (2015) 702–713.

[40] N. Wismer, S. Grad, G. Fortunato, S.J. Ferguson, M. Alini, D. Eglin, Biodegradable electrospun scaffolds for annulus fibrosus tissue engineering: effect of scaffold structure and composition on annulus fibrosus cells in vitro, Tissue Eng. 20 (2014) 672–682.

[41] C. Mauth, E. Bono, S. Haas, G. Paesold, H. Wiese, G. Maier, N. Boos, U. Graf-Hausner, Cell-seeded polyurethane-fibrin structures: a possible system for intervertebral disc regeneration, Eur. Cell. Mater. 18 (2009) 27–39.

[42] C.G. Jeong, A.T. Francisco, Z. Niu, R.L. Mancino, S.L. Craig, L.A. Setton, Screening of hyaluronic acid–poly (ethylene glycol) composite hydrogels to support intervertebral disc cell biosynthesis using artificial neural network analysis, Acta Biomater. 10 (2014) 3421–3430.

[43] C. Zhang, S.E. Gullbrand, T.P. Schaer, S. Boorman, D.M. Elliott, W. Chen, G.R. Dodge, R.L. Mauck, N.R. Malhotra, L.J. Smith, Combined hydrogel and mesenchymal stem cell therapy for moderate-severity disc degeneration in goats, Tissue Eng. 27 (2021) 117–128.
[44] M.B. Nair, G. Baranwal, P. Vijayan, K.S. Keyan, R. Jayakumar, Composite hydrogel of chitosan–poly (hydroxybutyrate-co-valerate) with chondroitin sulfate nanoparticles for nucleus pulposus tissue engineering, Colloids Surf. B Biointerfaces 136 (2015) 84–92.
[45] J. Hu, C. Li, S. Jin, Y. Ye, Y. Fang, P. Xu, C. Zhang, Salvianolic acid B combined with bone marrow mesenchymal stem cells piggybacked on HAMA hydrogel re-transplantation improves intervertebral disc degeneration, Front. Bioeng. Biotechnol. 10 (2022), 950625.
[46] L. Yu, Y. Liu, J. Wu, S. Wang, J. Yu, W. Wang, X. Ye, Genipin cross-linked decellularized nucleus pulposus hydrogel-like cell delivery system induces differentiation of ADSCs and retards intervertebral disc degeneration, Front. Bioeng. Biotechnol. 9 (2021).
[47] A. Hu, R. Xing, L. Jiang, Z. Li, P. Liu, H. Wang, X. Li, J. Dong, Thermosensitive hydrogels loaded with human-induced pluripotent stem cells overexpressing growth differentiation factor-5 ameliorate intervertebral disc degeneration in rats, J. Biomed. Mater. Res. B Appl. Biomater. 108 (2020) 2005–2016.
[48] L. Luo, J. Gong, Z. Wang, Y. Liu, J. Cao, J. Qin, R. Zuo, H. Zhang, S. Wang, P. Zhao, Injectable cartilage matrix hydrogel loaded with cartilage endplate stem cells engineered to release exosomes for non-invasive treatment of intervertebral disc degeneration, Bioact. Mater. 15 (2022) 29–43.
[49] M.N. Barcellona, J.E. Speer, L. Jing, D.S. Patil, M.C. Gupta, J.M. Buchowski, L.A. Setton, Bioactive in situ crosslinkable polymer-peptide hydrogel for cell delivery to the intervertebral disc in a rat model, Acta Biomater. 131 (2021) 117–127.
[50] W. Guo, L. Douma, M.H. Hu, D. Eglin, M. Alini, A. Šećerović, S. Grad, X. Peng, X. Zou, M. D'Este, Hyaluronic acid-based interpenetrating network hydrogel as a cell carrier for nucleus pulposus repair, Carbohydrate Polymers 277 (2022), 118828.
[51] X. Zhou, J. Wang, W. Fang, Y. Tao, T. Zhao, K. Xia, C. Liang, J. Hua, F. Li, Q. Chen, Genipin cross-linked type II collagen/chondroitin sulfate composite hydrogel-like cell delivery system induces differentiation of adipose-derived stem cells and regenerates degenerated nucleus pulposus, Acta Biomater. 71 (2018) 496–509.
[52] Y. Wang, Y. Zhang, K. Chen, F. Shao, Y. Wu, C. Guo, H. Wu, D. Zhang, W. Li, Q. Kong, Injectable nanostructured colloidal gels resembling native nucleus pulposus as carriers of mesenchymal stem cells for the repair of degenerated intervertebral discs, Mater. Sci. Eng. C 128 (2021), 112343.
[53] Y. Gan, S. Li, P. Li, Y. Xu, L. Wang, C. Zhao, B. Ouyang, B. Tu, C. Zhang, L. Luo, A controlled release codelivery system of MSCs encapsulated in dextran/gelatin hydrogel with TGF-β3-loaded nanoparticles for nucleus pulposus regeneration, Stem Cell. Int. 2016 (2016).
[54] Y. Zhu, J. Tan, H. Zhu, G. Lin, F. Yin, L. Wang, K. Song, Y. Wang, G. Zhou, W. Yi, Development of kartogenin-conjugated chitosan–hyaluronic acid hydrogel for nucleus pulposus regeneration, Biomater. Sci. 5 (2017) 784–791.
[55] S. Richardson, A. Mobasheri, A. Freemont, J. Hoyland, Intervertebral Disc Biology, Degeneration and Novel Tissue Engineering and Regenerative Medicine Therapies, Histology and Histopathology, 2007.
[56] W. Rea, S. Kapur, H. Mutagi, Intervertebral disc as a source of pain, continuing education in anaesthesia, Critical Care & Pain 12 (2012) 279–282.
[57] J. Clouet, M. Fusellier, A. Camus, C. Le Visage, J. Guicheux, Intervertebral disc regeneration: from cell therapy to the development of novel bioinspired endogenous repair strategies, Adv. Drug Deliv. Rev. 146 (2019) 306–324.
[58] Z. Bian, J. Sun, Development of a KLD-12 polypeptide/TGF-β1-tissue scaffold promoting the differentiation of mesenchymal stem cell into nucleus pulposus-like cells for treatment of intervertebral disc degeneration, Int. J. Clin. Exp. Pathol. 8 (2015) 1093.
[59] P. Hematti, A. Keating, Mesenchymal Stromal Cells in Regenerative Medicine: a Perspective, Mesenchymal Stromal Cells, Springer, 2013, pp. 3–16.
[60] J. Stoyanov, B. Gantenbein-Ritter, A. Bertolo, N. Aebli, M. Baur, M. Alini, S. Grad, Role of hypoxia and growth and differentiation factor-5 on differentiation of human mesenchymal stem cells towards intervertebral nucleus pulposus-like cells, Eur. Cell. Mater. 21 (2011) e47.
[61] A. Kremer, I. Ribitsch, J. Reboredo, J. Dürr, M. Egerbacher, F. Jenner, H. Walles, Three-dimensional coculture of meniscal cells and mesenchymal stem cells in collagen type I hydrogel on a small intestinal matrix—a pilot study toward equine meniscus tissue engineering, Tissue Eng. 23 (2017) 390–402.
[62] S. Wan, S. Borland, S.M. Richardson, C.L. Merry, A. Saiani, J.E. Gough, Self-assembling peptide hydrogel for intervertebral disc tissue engineering, Acta Biomater. 46 (2016) 29–40.
[63] A. Friedmann, A. Baertel, C. Schmitt, C. Ludtka, J. Milosevic, H.-J. Meisel, F. Goehre, S. Schwan, Intervertebral disc regeneration injection of a cell-loaded collagen hydrogel in a sheep model, Int. J. Mol. Sci. 22 (2021) 4248.
[64] L.-Y. Chan, C.-C. Chang, P.-L. Lai, T. Maeda, H.-C. Hsu, C.-Y. Lin, S.-J. Kuo, Cre/LoxP genetic recombination sustains cartilage anabolic factor expression in hyaluronan encapsulated MSCs alleviates intervertebral disc degeneration, Biomedicines 10 (2022) 555.
[65] C. Zhang, S.E. Gullbrand, T.P. Schaer, S. Boorman, D.M. Elliott, W. Chen, G.R. Dodge, R.L. Mauck, N.R. Malhotra, L.J. Smith, Combined hydrogel and mesenchymal stem cell therapy for moderate-severity disc degeneration in goats, Tissue Eng Part A 27 (2021) 117–128.
[66] J.E. Gough, A. Saiani, A.F. Miller, Peptide hydrogels: mimicking the extracellular matrix, Bioinspired, Biomimetic Nanobiomaterials 1 (2012) 4–12.
[67] M.N. Barcellona, J.E. Speer, L. Jing, D.S. Patil, M.C. Gupta, J.M. Buchowski, L.A. Setton, Bioactive in situ crosslinkable polymer-peptide hydrogel for cell delivery to the intervertebral disc in a rat model, Acta Biomater. 131 (2021) 117–127.
[68] F. Wang, L.P. Nan, S.F. Zhou, Y. Liu, Z.Y. Wang, J.C. Wang, X.M. Feng, L. Zhang, Injectable hydrogel combined with nucleus pulposus-derived mesenchymal stem cells for the treatment of degenerative intervertebral disc in rats, Stem Cells Int 2019 (2019), 8496025.
[69] J. Bian, F. Cai, H. Chen, Z. Tang, K. Xi, J. Tang, L. Wu, Y. Xu, L. Deng, Y. Gu, Modulation of local overactive inflammation via injectable hydrogel microspheres, Nano Lett. 21 (2021) 2690–2698.
[70] A. Srivastava, I.L.M. Isa, P. Rooney, A. Pandit, Bioengineered three-dimensional diseased intervertebral disc model revealed inflammatory crosstalk, Biomaterials 123 (2017) 127–141.
[71] D. Sakai, S. Grad, Advancing the cellular and molecular therapy for intervertebral disc disease, Adv. Drug Deliv. Rev. 84 (2015) 159–171.
[72] X. Zhang, T. Cui, J. He, H. Wang, R. Cai, P. Popovics, I. Vidaurre, W. Sha, J. Schmid, B. Ludwig, Beneficial effects of growth hormone-releasing hormone agonists on rat INS-1 cells and on streptozotocin-induced NOD/SCID mice, Proc. Natl. Acad. Sci. USA 112 (2015) 13651–13656.
[73] Q. Zheng, H. Shen, Z. Tong, L. Cheng, Y. Xu, Z. Feng, S. Liao, X. Hu, Z. Pan, Z. Mao, Y. Wang, A thermosensitive, reactive oxygen species-responsive, MR409-encapsulated hydrogel ameliorates disc degeneration in rats by inhibiting the secretory autophagy pathway, Theranostics 11 (2021) 147–163.
[74] J. Bai, Y. Zhang, Q. Fan, J. Xu, H. Shan, X. Gao, Q. Ma, L. Sheng, X. Zheng, W. Cheng, D. Li, M. Zhang, Y. Hao, L. Feng, Q. Chen, X. Zhou, C. Wang, Reactive oxygen species-scavenging scaffold with rapamycin for treatment of intervertebral disk degeneration, Adv Healthc Mater 9 (2020), e1901186.
[75] Z. Pan, H. Sun, B. Xie, D. Xia, X. Zhang, D. Yu, J. Li, Y. Xu, Z. Wang, Y. Wu, X. Zhang, Y. Wang, Q. Fu, W. Hu, Y. Yang, V. Bunpetch, W. Shen, B.C. Heng, S. Zhang, H. Ouyang, Therapeutic effects of gefitinib-encapsulated thermosensitive injectable hydrogel in intervertebral disc degeneration, Biomaterials 160 (2018) 56–68.
[76] C.-H. Park, K.-B. Min, J.-Y. Min, D.H. Kim, K.M. Seo, D.-K. Kim, Strong association of type 2 diabetes with degenerative lumbar spine disorders, Sci. Rep. 11 (2021) 1–11.
[77] M. Yao, J. Zhang, Z. Li, S. Guo, X. Zhou, W. Zhang, Marein protects human nucleus pulposus cells against high glucose-induced injury and extracellular matrix degradation at least partly by inhibition of ROS/NF-κB pathway, Int. Immunopharm. 80 (2020), 106126.
[78] D. Zheng, W. Chen, H. Ruan, Z. Cai, X. Chen, T. Chen, Y. Zhang, W. Cui, H. Chen, H. Shen, Metformin-hydrogel with glucose responsiveness for chronic inflammatory suppression, Chem. Eng. J. 428 (2022).
[79] J. Wang, L. Huang, Y. Huang, Y. Jiang, L. Zhang, G. Feng, L. Liu, Therapeutic effect of the injectable thermosensitive hydrogel loaded with SHP099 on intervertebral disc degeneration, Life Sci. 266 (2021), 118891.
[80] Y. Liu, J. Du, P. Peng, R. Cheng, J. Lin, C. Xu, H. Yang, W. Cui, H. Mao, Y. Li, Regulation of the inflammatory cycle by a controllable release hydrogel for eliminating postoperative inflammation after discectomy, Bioact. Mater. 6 (2021) 146–157.
[81] N. Willems, H.-y. Yang, M.L. Langelaan, A.R. Tellegen, G. Grinwis, H.-J.C. Kranenburg, F.M. Riemers, S.G. Plomp, E.G. Craenmehr, W.J. Dhert, Biocompatibility and intradiscal application of a thermoreversible celecoxib-loaded poly-N-isopropylacrylamide MgFe-layered double hydroxide hydrogel in a canine model, Arthritis Res. Ther. 17 (2015) 1–16.

[82] A.R. Tellegen, N. Willems, M. Beukers, G.C. Grinwis, S.G. Plomp, C. Bos, M. van Dijk, M. de Leeuw, L.B. Creemers, M.A. Tryfonidou, Intradiscal application of a PCLA–PEG–PCLA hydrogel loaded with celecoxib for the treatment of back pain in canines: what's in it for humans? Journal of Tissue Engineering and Regenerative Medicine 12 (2018) 642–652.

[83] J. Bai, Y. Zhang, Q. Fan, J. Xu, H. Shan, X. Gao, Q. Ma, L. Sheng, X. Zheng, W. Cheng, Reactive oxygen species-scavenging scaffold with rapamycin for treatment of intervertebral disk degeneration, Adv. Healthcare Mater. 9 (2020), 1901186.

[84] Z. Zheng, A. Chen, H. He, Y. Chen, J. Chen, A.A. Albashari, J. Li, J. Yin, Z. He, Q. Wang, pH and enzyme dual-responsive release of hydrogen sulfide for disc degeneration therapy, J. Mater. Chem. B 7 (2019) 611–618.

[85] Z. Pan, H. Sun, B. Xie, D. Xia, X. Zhang, D. Yu, J. Li, Y. Xu, Z. Wang, Y. Wu, Therapeutic effects of gefitinib-encapsulated thermosensitive injectable hydrogel in intervertebral disc degeneration, Biomaterials 160 (2018) 56–68.

[86] M. Likhitpanichkul, Y. Kim, O.M. Torre, E. See, Z. Kazezian, A. Pandit, A.C. Hecht, J.C. Iatridis, Fibrin-genipin annulus fibrosus sealant as a delivery system for anti-TNFα drug, Spine J. 15 (2015) 2045–2054.

[87] K.D. Than, S.U. Rahman, L. Wang, A. Khan, K.A. Kyere, T.T. Than, Y. Miyata, Y.-S. Park, F. La Marca, H.M. Kim, Intradiscal injection of simvastatin results in radiologic, histologic, and genetic evidence of disc regeneration in a rat model of degenerative disc disease, Spine J. 14 (2014) 1017–1028.

[88] Y.-H. Cheng, S.-H. Yang, F.-H. Lin, Thermosensitive chitosan-gelatin-glycerol phosphate hydrogel as a controlled release system of ferulic acid for nucleus pulposus regeneration, Biomaterials 32 (2011) 6953–6961.

[89] F. Hu, Z. Pan, C. Liu, X. Dong, Z. Zhang, Q. Ji, W. Hu, S. Zhang, Y. Zhang, Z. Sun, Identification of inflammatory regulation roles of thalidomide/ruxolitinib in nucleus pulposus and construction of polyelectrolyte nanocomplexes-impregnated injectable hydrogels for synergistic intervertebral disk degeneration treatment, Nano Today 44 (2022), 101462.

[90] D. Zheng, W. Chen, H. Ruan, Z. Cai, X. Chen, T. Chen, Y. Zhang, W. Cui, H. Chen, H. Shen, Metformin-hydrogel with glucose responsiveness for chronic inflammatory suppression, Chem. Eng. J. 428 (2022), 131064.

[91] S. Chen, S. Liu, K. Ma, L. Zhao, H. Lin, Z. Shao, TGF-β signaling in intervertebral disc health and disease, Osteoarthritis Cartilage 27 (2019) 1109–1117.

[92] M. Okada, J.H. Kim, S.T. Yoon, W.C. Hutton, Pulsed electromagnetic field (PEMF) plus BMP-2 upregulates intervertebral disc-cell matrix synthesis more than either BMP-2 alone or PEMF alone, Clinical Spine Surgery 26 (2013) E221–E226.

[93] W.-C. Lo, C.-S. Chiou, F.-C. Tsai, C.-H. Chan, S. Mao, Y.-H. Deng, C.-Y. Wu, B.-Y. Peng, W.-P. Deng, Platelet-derived biomaterials inhibit nicotine-induced intervertebral disc degeneration through regulating IGF-1/AKT/IRS-1 signaling Axis, Cell Transplant. 30 (2021), 09636897211045319.

[94] K. Miyamoto, K. Masuda, J.G. Kim, N. Inoue, K. Akeda, G.B. Andersson, H.S. An, Intradiscal injections of osteogenic protein-1 restore the viscoelastic properties of degenerated intervertebral discs, Spine J. 6 (2006) 692–703.

[95] J.C. Kennon, M.E. Awad, N. Chutkan, J. DeVine, S. Fulzele, Current insights on use of growth factors as therapy for intervertebral disc degeneration, Biomol. Concepts 9 (2018) 43–52.

[96] S.D. Gandhi, T. Maerz, S. Mitchell, C. Bachison, D.K. Park, J.S. Fischgrund, K.C. Baker, Intradiscal delivery of anabolic growth factors and a metalloproteinase inhibitor in a rabbit acute lumbar disc injury model, International Journal of Spine Surgery 14 (2020) 585–593.

[97] Y. Chang, M. Yang, S. Ke, Y. Zhang, G. Xu, Z. Li, Effect of Platelet-Rich Plasma on Intervertebral Disc Degeneration in Vivo and in Vitro: a Critical Review, Oxidative Medicine and Cellular Longevity 2020, 2020.

[98] S. Mohammed, J. Yu, Platelet-rich plasma injections: an emerging therapy for chronic discogenic low back pain, Journal of Spine Surgery 4 (2018) 115.

[99] K. Akeda, H.S. An, R. Pichika, M. Attawia, J.-M.T. Eugene, M.E. Lenz, A. Uchida, K. Masuda, Platelet-rich plasma (PRP) stimulates the extracellular matrix metabolism of porcine nucleus pulposus and anulus fibrosus cells cultured in alginate beads, Spine 31 (2006) 959–966.

[100] W.H. Chen, W.C. Lo, J.J. Lee, C.H. Su, C.T. Lin, H.Y. Liu, T.W. Lin, W.C. Lin, T.Y. Huang, W.P. Deng, Tissue-engineered intervertebral disc and chondrogenesis using human nucleus pulposus regulated through TGF-β1 in platelet-rich plasma, J. Cell. Physiol. 209 (2006) 744–754.

[101] K. Sawamura, T. Ikeda, M. Nagae, S.-i. Okamoto, Y. Mikami, H. Hase, K. Ikoma, T. Yamada, H. Sakamoto, K.-i. Matsuda, Characterization of in vivo effects of platelet-rich plasma and biodegradable gelatin hydrogel microspheres on degenerated intervertebral discs, Tissue Eng. 15 (2009) 3719–3727.

[102] E.A. Growney, H.R. Linder, K. Garg, J.G. Bledsoe, S.A. Sell, Bio-conjugation of platelet-rich plasma and alginate through carbodiimide chemistry for injectable hydrogel therapies, J. Biomed. Mater. Res. B Appl. Biomater. 108 (2020) 1972–1984.

[103] K. Burgess, C. Frati, K. Meade, J. Gao, L.C. Diaz, D. Madeddu, G. Graiani, S. Cavalli, A. Miller, D. Oceandy, Functionalised peptide hydrogel for the delivery of cardiac progenitor cells, Mater. Sci. Eng. C 119 (2021), 111539.

[104] A. Imere, C. Ligorio, M. O'Brien, J.K. Wong, M. Domingos, S.H. Cartmell, Engineering a cell-hydrogel-fibre composite to mimic the structure and function of the tendon synovial sheath, Acta Biomater. 119 (2021) 140–154.

[105] R.V. Ulijn, A.M. Smith, Designing peptide based nanomaterials, Chem. Soc. Rev. 37 (2008) 664–675.

[106] C. Ligorio, M. O'Brien, N.W. Hodson, A. Mironov, M. Iliut, A.F. Miller, A. Vijayaraghavan, J.A. Hoyland, A. Saiani, TGF-beta3-loaded graphene oxide - self-assembling peptide hybrid hydrogels as functional 3D scaffolds for the regeneration of the nucleus pulposus, Acta Biomater. 127 (2021) 116–130.

[107] Y. Ye, P. Xu, C. Li, S. Jin, J. Hu, Y. Fang, K. Zhu, G. Xu, Z. Han, Z. Zhang, Bioactive hydrogel encapsulated dual-gene engineered nucleus pulposus stem cells towards intervertebral disc tissue repair, Chem. Eng. J. (2022), 139717.

[108] C. Jiang, D. Li, Z. Zhang, H. Shu, L. Hu, Z. Li, Y. Huang, Effect of basic fibroblast growth factor and transforming growth factor-β1 combined with bone marrow mesenchymal stem cells on the repair of degenerated intervertebral discs in rat models, Zhongguo yi xue ke xue Yuan xue bao, Acta Acad. Med. Sin. 37 (2015) 456–465.

[109] C. Ligorio, M. O'Brien, N.W. Hodson, A. Mironov, M. Iliut, A.F. Miller, A. Vijayaraghavan, J.A. Hoyland, A. Saiani, TGF-β3-loaded graphene oxide-self-assembling peptide hybrid hydrogels as functional 3D scaffolds for the regeneration of the nucleus pulposus, Acta Biomater. 127 (2021) 116–130.

[110] Z. Li, G. Lang, L.S. Karfeld-Sulzer, K.T. Mader, R.G. Richards, F.E. Weber, C. Sammon, H. Sacks, A. Yayon, M. Alini, Heterodimeric BMP-2/7 for nucleus pulposus regeneration—in vitro and ex vivo studies, J. Orthop. Res. 35 (2017) 51–60.

[111] N. Henry, J. Clouet, A. Fragale, L. Griveau, C. Chédeville, J. Véziers, P. Weiss, J. Le Bideau, J. Guicheux, C. Le Visage, Pullulan microbeads/Si-HPMC hydrogel injectable system for the sustained delivery of GDF-5 and TGF-β1: new insight into intervertebral disc regenerative medicine, Drug Deliv. 24 (2017) 999–1010.

[112] J. Chen, H. Zhu, J. Xia, Y. Zhu, C. Xia, Z. Hu, Y. Jin, J. Wang, Y. He, J. Dai, High-performance multi-dynamic bond cross-linked hydrogel with spatiotemporal siRNA delivery for gene–cell combination therapy of intervertebral disc degeneration, Adv. Sci. (2023), 2206306.

[113] P. Wehling, K.-P. Schulitz, P.D. Robbins, C.H. Evans, J.A. Reinecke, Transfer of genes to chondrocytic cells of the lumbar spine: proposal for a treatment strategy of spinal disorders by local gene therapy, Spine 22 (1997) 1092–1097.

[114] H. Madry, L. Gao, A. Rey-Rico, J.K. Venkatesan, K. Müller-Brandt, X. Cai, L. Goebel, G. Schmitt, S. Speicher-Mentges, D. Zurakowski, Thermosensitive hydrogel based on PEO–PPO–PEO poloxamers for a controlled in situ release of recombinant adeno-associated viral vectors for effective gene therapy of cartilage defects, Adv. Mater. 32 (2020), 1906508.

[115] G. Feng, H. Chen, J. Li, Q. Huang, M.J. Gupte, H. Liu, Y. Song, Z. Ge, Gene therapy for nucleus pulposus regeneration by heme oxygenase-1 plasmid DNA carried by mixed polyplex micelles with thermo-responsive heterogeneous coronas, Biomaterials 52 (2015) 1–13.

[116] Y. Wang, M. Deng, Y. Wu, C. Hu, B. Zhang, C. Guo, H. Song, Q. Kong, Y. Wang, Sustained gene delivery from inflammation-responsive anti-inflammatory hydrogels promotes extracellular matrix metabolism balance in degenerative nucleus pulposus, Compos. B Eng. 236 (2022), 109806.

[117] G. Feng, Z. Zha, Y. Huang, J. Li, Y. Wang, W. Ke, H. Chen, L. Liu, Y. Song, Z. Ge, Sustained and bioresponsive two-stage delivery of therapeutic miRNA via polyplex micelle-loaded injectable hydrogels for inhibition of intervertebral disc fibrosis, Adv. Healthcare Mater. 7 (2018), 1800623.

[118] W. Chen, H. Chen, D. Zheng, H. Zhang, L. Deng, W. Cui, Y. Zhang, H.A. Santos, H. Shen, Gene-hydrogel microenvironment regulates extracellular matrix metabolism balance in nucleus pulposus, Adv. Sci. 7 (2020), 1902099.

[119] J. Chen, H. Zhu, Y. Zhu, C. Zhao, S. Wang, Y. Zheng, Z. Xie, Y. Jin, H. Song, L. Yang, Injectable self-healing hydrogel with siRNA delivery property for sustained STING silencing and enhanced therapy of intervertebral disc degeneration, Bioact. Mater. 9 (2022) 29–43.

[120] C. Bucher, A. Gazdhar, L.M. Benneker, T. Geiser, B. Gantenbein-Ritter, Nonviral gene delivery of growth and differentiation factor 5 to human mesenchymal stem cells injected into a 3D bovine intervertebral disc organ culture system, Stem Cells International 2013 (2013).

[121] T. Chen, Q. Qian, P. Makvandi, E.N. Zare, Q. Chen, L. Chen, Z. Zhang, H. Zhou, W. Zhou, H. Wang, Engineered high-strength biohydrogel as a multifunctional platform to deliver nucleic acid for ameliorating intervertebral disc degeneration, Bioact. Mater. 25 (2023) 107–121.
[122] W. Li, S. Zhang, D. Wang, H. Zhang, Q. Shi, Y. Zhang, M. Wang, Z. Ding, B. Gao, M. Yan, Exosomes immunity strategy: a novel approach for ameliorating intervertebral disc degeneration, Front. Cell Dev. Biol. (2021) 3855.
[123] E.G. Trams, C.J. Lauter, J.N. Salem, U. Heine, Exfoliation of membrane ecto-enzymes in the form of micro-vesicles, Biochimica et Biophysica Acta (BBA)-Biomembranes 645 (1981) 63–70.
[124] Y.G. Kim, J. Choi, K. Kim, Mesenchymal stem cell-derived exosomes for effective cartilage tissue repair and treatment of osteoarthritis, Biotechnol. J. 15 (2020), 2000082.
[125] E. Marbán, The secret life of exosomes: what bees can teach us about next-generation therapeutics, J. Am. Coll. Cardiol. 71 (2018) 193–200.
[126] H. Xiang, W. Su, X. Wu, W. Chen, W. Cong, S. Yang, C. Liu, C. Qiu, S.-Y. Yang, Y. Wang, Exosomes derived from human urine-derived stem cells inhibit intervertebral disc degeneration by ameliorating endoplasmic reticulum stress, Oxid. Med. Cell. Longev. 2020 (2020), 6697577.
[127] D.G. Phinney, M.F. Pittenger, Concise review: MSC-derived exosomes for cell-free therapy, Stem Cell. 35 (2017) 851–858.
[128] K. Lu, H.-y. Li, K. Yang, J.-l. Wu, X.-w. Cai, Y. Zhou, C.-q. Li, Exosomes as potential alternatives to stem cell therapy for intervertebral disc degeneration: in-vitro study on exosomes in interaction of nucleus pulposus cells and bone marrow mesenchymal stem cells, Stem Cell Res. Ther. 8 (2017) 1–11.
[129] H. Xing, Z. Zhang, Q. Mao, C. Wang, Y. Zhou, X. Zhou, L. Ying, H. Xu, S. Hu, N. Zhang, Injectable exosome-functionalized extracellular matrix hydrogel for metabolism balance and pyroptosis regulation in intervertebral disc degeneration, J. Nanobiotechnol. 19 (2021) 1–15.
[130] Luo L., Gong J., Wang Z., Liu Y., Cao J., Qin J., Zuo R., Zhang H., Wang S., Zhao P., Injectable cartilage matrix hydrogel loaded with cartilage endplate stem cells engineered to release exosomes for non-invasive treatment of intervertebral disc degeneration, Bioact. Mater. 15(2021)2943.
[131] Z. Liao, W. Ke, H. Liu, B. Tong, K. Wang, X. Feng, W. Hua, B. Wang, Y. Song, R. Luo, Vasorin-containing small extracellular vesicles retard intervertebral disc degeneration utilizing an injectable thermoresponsive delivery system, J. Nanobiotechnol. 20 (2022) 1–20.
[132] Y. Liu, M. Xue, Y. Han, Y. Li, B. Xiao, W. Wang, J. Yu, X. Ye, Exosomes from M2c Macrophages Alleviate Intervertebral Disc Degeneration by Promoting Synthesis of the Extracellular Matrix via MiR-124/CILP/TGF-β, Bioengineering & Translational Medicine, 2023, e10500.
[133] F. Wei, R. Zhong, L. Wang, Z. Zhou, X. Pan, S. Cui, H. Sun, X. Zou, M. Gao, B. Jiang, Pingyangmycin-induced in vivo lumbar disc degeneration model of rhesus monkeys, Spine 40 (2015) E199–E210.
[134] Z. Liu, J. Li, M. Hu, X. Wang, N. Chen, S. Cui, S. Li, H. Liu, G. Chen, X. Pan, The optimal timing of hydrogel injection for treatment of intervertebral disc degeneration: quantitative analysis based on T1ρ MR imaging, Spine 45 (2020) E1451–E1459.
[135] W. Johannessen, J.D. Auerbach, A.J. Wheaton, A. Kurji, A. Borthakur, R. Reddy, D.M. Elliott, Assessment of human disc degeneration and proteoglycan content using T1ρ-weighted magnetic resonance imaging, Spine 31 (2006) 1253.
[136] G. Blumenkrantz, J. Zuo, X. Li, J. Kornak, T.M. Link, S. Majumdar, In vivo 3.0-tesla magnetic resonance T1ρ and T2 relaxation mapping in subjects with intervertebral disc degeneration and clinical symptoms, Magn. Reson. Med. 63 (2010) 1193–1200.
[137] Z. Zhou, B. Jiang, Z. Zhou, X. Pan, H. Sun, B. Huang, T. Liang, S. Ringgaard, X. Zou, Intervertebral disk degeneration: T1ρ MR imaging of human and animal models, Radiology 268 (2013) 492–500.
[138] A. Thorpe, V. Boyes, C. Sammon, C. Le Maitre, Thermally triggered injectable hydrogel, which induces mesenchymal stem cell differentiation to nucleus pulposus cells: potential for regeneration of the intervertebral disc, Acta Biomater. 36 (2016) 99–111.
[139] Y. Zeng, C. Chen, W. Liu, Q. Fu, Z. Han, Y. Li, S. Feng, X. Li, C. Qi, J. Wu, Injectable microcryogels reinforced alginate encapsulation of mesenchymal stromal cells for leak-proof delivery and alleviation of canine disc degeneration, Biomaterials 59 (2015) 53–65.
[140] W.-Y. Su, Y.-C. Chen, F.-H. Lin, Injectable oxidized hyaluronic acid/adipic acid dihydrazide hydrogel for nucleus pulposus regeneration, Acta Biomater. 6 (2010) 3044–3055.
[141] Y.-C. Huang, V.Y. Leung, W.W. Lu, K.D. Luk, The effects of microenvironment in mesenchymal stem cell–based regeneration of intervertebral disc, Spine J. 13 (2013) 352–362.
[142] R.L.-H. Yim, J.T.-Y. Lee, C.H. Bow, B. Meij, V. Leung, K.M. Cheung, P. Vavken, D. Samartzis, A systematic review of the safety and efficacy of mesenchymal stem cells for disc degeneration: insights and future directions for regenerative therapeutics, Stem Cell. Dev. 23 (2014) 2553–2567.
[143] G. Vadalà, G. Sowa, M. Hubert, L.G. Gilbertson, V. Denaro, J.D. Kang, Mesenchymal stem cells injection in degenerated intervertebral disc: cell leakage may induce osteophyte formation, Journal of Tissue Engineering and Regenerative Medicine 6 (2012) 348–355.