REVIEW

Stimuli-Responsive Delivery Systems for Intervertebral Disc Degeneration

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Abstract: As a major cause of low back pain, intervertebral disc degeneration is an increasingly prevalent chronic disease worldwide that leads to huge annual financial losses. The intervertebral disc consists of the inner nucleus pulposus, outer annulus fibrosus, and sandwiched cartilage endplates. All these factors collectively participate in maintaining the structure and physiological functions of the disc. During the unavoidable degeneration stage, the degenerated discs are surrounded by a harsh microenvironment characterized by acidic, oxidative, inflammatory, and chaotic cytokine expression. Loss of stem cell markers, imbalance of the extracellular matrix, increase in inflammation, sensory hyperinnervation, and vascularization have been considered as the reasons for the progression of intervertebral disc degeneration. The current treatment approaches include conservative therapy and surgery, both of which have drawbacks. Novel stimuli-responsive delivery systems are more promising future therapeutic options than traditional treatments. By combining bioactive agents with specially designed hydrogels, scaffolds, microspheres, and nanoparticles, novel stimuli-responsive delivery systems can realize the targeted and sustained release of drugs, which can both reduce systematic adverse effects and maximize therapeutic efficacy. Trigger factors are categorized into internal (pH, reactive oxygen species, enzymes, etc.) and external stimuli (photo, ultrasound, magnetic, etc.) based on their intrinsic properties. This review systematically summarizes novel stimuli-responsive delivery systems for intervertebral disc degeneration, shedding new light on intervertebral disc therapy.

Keywords: stimulus response, delivery system, intervertebral disc degeneration, biomaterial

Introduction

Intervertebral disc (IVD) degeneration (IVDD) is a major cause (40%) of low back pain (LBP), with a prevalence of over 11.9% in the population worldwide (over 568 million), which leads to huge economic losses estimated at 70 billion euros annually.[1–4] Epidemiological research in 195 countries found that LBP is the primary cause of years lived with disability in 126 countries.[5] As an essential structure in the human body, the intervertebral disc allows correct spatial alignment of vertebrae and facet joints, provides spinal mechanical stability, and enables segment movement.[6] The intervertebral disc comprises the nucleus pulposus (NP), annulus fibrosus (AF), and cartilage endplates (CEP). All these factors collectively participate in maintaining the structure and physiological functions of the disc.

The pathology of IVDD mainly includes dysfunction of intervertebral disc cells and an imbalance between anabolism and catabolism of the extracellular matrix (ECM).[7–10] Current IVDD treatments can be classified into conservative therapy (physical exercise and therapy, oral medication, and pain-relieving injections) and surgery.[11] Surgical treatments include intervertebral disc fusion (regarded as the standard method) and total disc replacement.[11] However, these IVDD treatment categories have unavoidable disadvantages. It is often difficult to achieve effective therapeutic outcomes with conservative physical treatments.[12] Moreover, owing to the avascular nature of the IVDs, it is difficult to achieve therapeutic concentrations of bioactive agents by systemic administration. The high doses of drugs required may cause multiple systemic side effects. Although local injections and interventional treatment can avoid this dilemma, repeated

injections can cause adverse effects.[13] Simultaneously, the degenerated intervertebral disc features a harsh microenvironment, including acidic, oxidative, inflammatory, and chaotic cytokines, which is not conducive to medication.[14] However, surgical treatment is expensive, risky, and relapsable. IVD fusion eliminates the movement of the adjacent vertebral body, while total disc replacement induces a series of complications, including facet dislocation, pedicle fracture, device dislocation, and vertebral split fracture.[15–17]

Biomaterial-based treatments have been widely researched to effectively alleviate the symptoms of IVDD and restore the disc structure. Various scaffolds, hydrogels, nanoparticles, and microspheres have been designed to relieve pain and restrain disc degeneration.[18,19] Biomaterial-assisted interventions offer a significant advancement over traditional methods for treating IVDD, as they eliminate the need for multiple injections and circumvent the risk of widespread adverse effects, thereby providing a more efficient and safer therapeutic strategy. Moreover, biomaterials, such as hydrogels, have excellent biocompatibility and hypotoxicity.[20] Typically, biomaterials are effective for tissue regeneration and repair, which conventional treatments lack.[21] However, few biomaterials have shown significant effects in clinical trials, failing to address discogenic pain and maintain sustained therapeutic effects.[21] Therefore, novel smart therapeutic methods are needed to treat IVDD.

Conventional drug delivery systems include systemic administration (oral and intravenous) and local injections. Recently, researchers have focused on novel stimuli-responsive drug delivery systems that exhibit targeted and sustained drug release, with fewer invasive features.[22] By specially modifying functional polymers and wrapping them into various stimuli-responsive "shells", these synthetics can effectively relieve pain and restore the metabolism of degenerated discs. Stimuli-responsive drug delivery systems are categorized based on internal stimuli, including pH, enzymes, and oxidative agents, and external stimuli, including temperature, photo, ultrasound, and magnetic field. In conclusion, special stimuli-responsive system presents a superior alternative to conventional IVDD treatment options, offering a more effective, safer, and personalized approach to managing IVDD. This review systematically summarizes novel stimuli-responsive drug delivery systems based on different stimulating factors, shedding new light on the intervertebral disc therapy field. This review encompasses a systematic search of Web of Science, Scopus, and PubMed from utilizing keywords including stimuli responsive, stimulus response, stimulation, disc, intervertebral disc degeneration, pH, enzyme, oxidative agents, photo, light, temperature, ultrasound, and magnetic. The search strategy was designed to be as inclusive as possible, ensuring a broad spectrum of available research is represented.

Anatomical and Molecular Structure of the Intervertebral Discs

The IVD is a major component of the spine. IVDs account for approximately 15–20% of the length of the spinal column, and their percentage depends on age, time of day, occupation, and disease state.[23] From the cervical region to the sacrum, at least 23 IVDs exist between the adjacent surfaces of the vertebrae to unite them, which is important for movement and load distribution.[24] Each IVD is structurally characterized by three integrated tissues: NP, AF, and CEP.[25] The AF surrounds the NP, and both are sandwiched between the CEP of the adjacent vertebrae.[23]

AF

AF consists of highly organized collagen fibers arranged in 10–20 lamellae.[26] The thickness of each lamella ranges from 100 to 500 μm, in which the inner lamellae are thinner than the outer lamellae.[27,28] Each lamella is inclined at approximately 30° from one vertebra to the other.[27] The fibers of the adjacent lamellae cross diagonally at angles >60°. Such arrangement enables limited rotation and bending between adjacent vertebrae and allows the IVD to withstand circumferential loads.[27,29] Water represents 60–70% of AF[30] and the dry weight contains approximately 20% proteoglycan, 50–70% collagen, and 2% elastin.[23] AF can be further divided into an inner fibrocartilaginous region and an outer or peripheral fibrous zone.[31] The inner zone contains mainly type II collagen fibers generated by round chondrocyte-like cells, whereas the outer zone primarily contains type I collagen fibers produced by elongated, fusiform, fibroblast-like cells of mesenchymal origin.[29,32,33] Other differences between the inner and outer regions include the distribution of decorin and biglycan, which are mainly found in the outer annulus.[23]

NP

As the core of the IVD, the NP is derived from the notochord and cartilaginous cells, with a few irregular collagen fibers disseminated in a semiliquid medium.[34] The fluidic nature of NP enables their deformation under pressure without volume changes. Regarding chemical composition, 70–90% of NP is water, and the dry weight consists of approximately 35–65% proteoglycan, 5–20% type II collagen, and a small amount of non-collagenous proteins and elastin.[24] Proteoglycan can store water and reduce the stress of IVD by distributing the hydraulic pressure to the ECM.[35,36] NP cells are a hybrid group of cells in different phases of maturation that sustain tissue homeostasis.[37] In newborn and premature tissues, NP cells are large and highly vacuolated (often referred to as "notochordal cells"), occurring in clusters with tight intercellular junctions and a compact cytoskeletal network.[38–40] In adults, the cell density of NP is low, and large notochordal cells morphologically transform into small fibrochondrocyte-like cells.[41] Tang et al confirmed a new set of NP markers (brain abundant membrane attached signal protein 1 (BASP1), neurochondrin (NCDN), neuropilin 1 (NRP1), cluster of differentiation (CD)24, CD155, CD221, and Brachyury T) and one non-NP marker (CD90), which have the potential to reveal shifts in NP cell phenotype and function during degeneration.[42] With respect to the ECM, the NP[23] features abundant collagen types I and II and scarce types IX and XI. Collagen type X is present during degeneration.[23] Based on the physiological characteristics of the blood supply, the disc exhibits low oxygen tension. In this case, the NP cells rely almost completely on the glycolytic pathway to generate energy.[43]

End Plates

End plates form the boundaries of adjacent intervertebral discs and are composed of bony endplates (BEP) and CEP. The actual width of the CEP is approximately 0.6–1 mm. Moreover, CEP have a water content of about 60%, and the main dry-weight components are type II collagen and proteoglycans.[24] The predominant cell type within the CEP is chondrocytes.[23] CEP form a functional structure that connects the IVD and adjacent vertebrae via the adjacent BEP.[25] Moreover, CEP serve as a mechanical barrier between the NP and vertebrae, which prevents direct pressure on the bone.[23] CEP also serve as a gateway for nutrient transport into the disc.[44] In addition, hyaline cartilage is present in neonates and children, whereas adult CEP are fibrocartilage.[34] Collagen fibers in the CEP and vertebrae are originally separated and can be dislocated by excessive horizontal stresses.[45,46]

Blood Supply and Innervation

In general, vessels and nerves are concomitant within the IVD. IVD is an avascular tissue with a limited blood supply to the peripheral fibers of the AF.[47] The CEP have branched blood vessels from the spinal artery in the fetus that disappear with aging.[48] Nutrients and metabolic waste products are delivered by diffusion from outer annulus blood vessels.[49] Regarding innervation in the IVDs, the NP has no nerve supply,[50] while the AF has limited nerves that are mainly restricted to the outer lamellae, with some terminating in the proprioceptor.[51] The nerves are branches of the sinus vertebral nerve, which consists of a combination of somatic nerve roots from the ventral ramus and autonomic nerve roots from the gray ramus.[47] Some nerves in the IVD contain glial support cells or Schwann cells. Clinically, many pathological changes, such as osteosclerosis, altered blood flow, and CEP calcification, can affect vascular transportation, which, in turn, may lead to cell death. The NP has the poorest blood supply in the IVD and is consequently susceptible to injury.[49]

Pathological Alterations and Microenvironments in IVDD
AF Degeneration

The major pathological alteration of the AF in IVDD is a change in the distribution of collagen fibers. The mechanism theory is the "wear and tear", which means that the ECM remolding can be accelerated by high mechanical stress.[52] In IVDD, the level of outer tension-resisting type I collagen increases, while that of inner compression force-combating type II collagen decreases.[53,54] Consequently, with the changes in the types of collagen, the ability of AF to resist shear stress decreases, which leads to morphological changes in IVDs and the progression of IVDD.[55] Moreover, the drastic microenvironment in IVDD restrains AF regeneration. Early AF repair may be an ideal therapeutic strategy for IVDD.[56]

Mechanistically, overexpression of cytokines, including cyclooxygenase-2, interleukin (IL)-6, and IL-8, leads to the formation of an inflammatory microenvironment and changes in collagen types.[57,58]

NP Degeneration

IVD can lead to long-term spinal dysfunction and instability. Generally, compared to a healthy NP, a degenerated NP features unorganized fibrous tissue, which makes it difficult to bind water under compression.[59] Consequently, both the pressure in the NP and the disc height decrease.[60,61] Degenerative factors include biological and biomechanical factors that play crucial roles in the pain signaling pathway and cause clinical symptoms.[62] IVDD can develop as early as the first decade of life. The notochordal cells in the NP gradually disappear as the body grows, and this reduction is considered the initiation process of disc degeneration.[6,63] Apoptosis and autophagy cause these cellular alterations. The cells in the NP change from notochordal cells to nuclear chondrocytes.[3] Mature nuclear chondrocytes mainly produce collagen type I and reduce the amount of water-attracting proteoglycans and collagen type II, resulting in the transition to fibrillated NP tissue.[64] Such cell type changes cause a decrease in proteoglycan synthesis; thus, tissue hydration is lost, which further affects the ability of the NP to maintain its structure and composition.[65,66]

Furthermore, in IVDD, the balance between ECM synthesis and catabolism is disrupted due to a decrease in cell number.[67] Inflammation also mediates ECM degradation. As demonstrated, the proinflammatory factors, such as IL-1β and tumor necrosis factor (TNF)-α,[68,69] could accelerate the matrix remodeling in IVDD. IL-1β and TNF-α can stimulate the matrix-degrading enzymes, including matrix metalloproteinase (MMP)-2, MMP-1, MMP-13, MMP-3, MMP-14, a disintegrin and metalloproteinases with thrombospondin motifs (ADAMTS)-4, and ADAMTS-5.[70,71] In comparison, the levels of ECM enzyme inhibitors of MMPs tissue inhibitor of metalloproteinase (TIMP)-1, and TIMP-2 are reduced.[72] Long-lasting proteoglycan loss and collagen type alterations contribute to the degeneration of the NP.

Endplate Degeneration

Moore et al pointed out that blood vessels in the CEP become narrowed, constricted, or even disappear as they mature, a change that is likely to affect the nutrient supply to the cartilage and discs.[73] With aging, the endplate gradually undergoes calcification, which also affects the nutrient supply to the IVD. Moreover, the endplate becomes thinner with age, increasing the risk of endplate fracture.[74] Furthermore, the displacement of the damaged CEP leads to a decreased NP pressure (estimated at 30–50%) and an uneven load distribution.[75] The magnetic resonance imaging (MRI) signal of the vertebral endplate and subchondral bone changes in patients with IVDD, providing complementary diagnostic evidence.[76] Such MRI changes are named "Modic" changes. Type 1 Modic changes refer to hypointensity on T1-weighted imaging (T1WI) and hyperintensity on T2-weighted imaging (T2WI); Type 2 changes refer to hyperintensity on T1WI and isointensity or slight hyperintensity on T2WI; Type 3 changes refer to hypointensity on both T1WI and T2WI.[77]

Overall, compared to a non-degenerated IVD, a degenerated IVD features a loss of disc height, a fibrous dehydrated nucleus, inward and outward buckling of annulus fibers, extensive endplate damage, and sclerosis of the subchondral bone (Figure 1).

Harsh Microenvironment in IVDD

As is widely acknowledged, degenerated IVDs exhibit harsh microenvironments, such as inflammatory, oxidative, and acidic environments. The production of other bioactive agents, such as enzymes and cytokines is also abnormal. Meanwhile, both NP and AF cells secrete high levels of proinflammatory cytokines, such as TNF-α, interferon-γ (IFN-γ), IL-1β, IL-10, IL-4, IL-6, IL-17, IL-2, IL-8, and C-C chemokine ligand 20 (CCL20).[79,80] Such harsh microenvironments are detrimental to disc cell metabolism and tissue regeneration. The inflammatory microenvironment exacerbates the dysfunction and abnormal apoptosis of disc cells, leading to further ECM degradation and disruption. Based on these features, a novel and promising stimuli-responsive drug delivery system can be specially constructed, which produces precise and sustained IVDD treatment while reducing adverse impact factors.
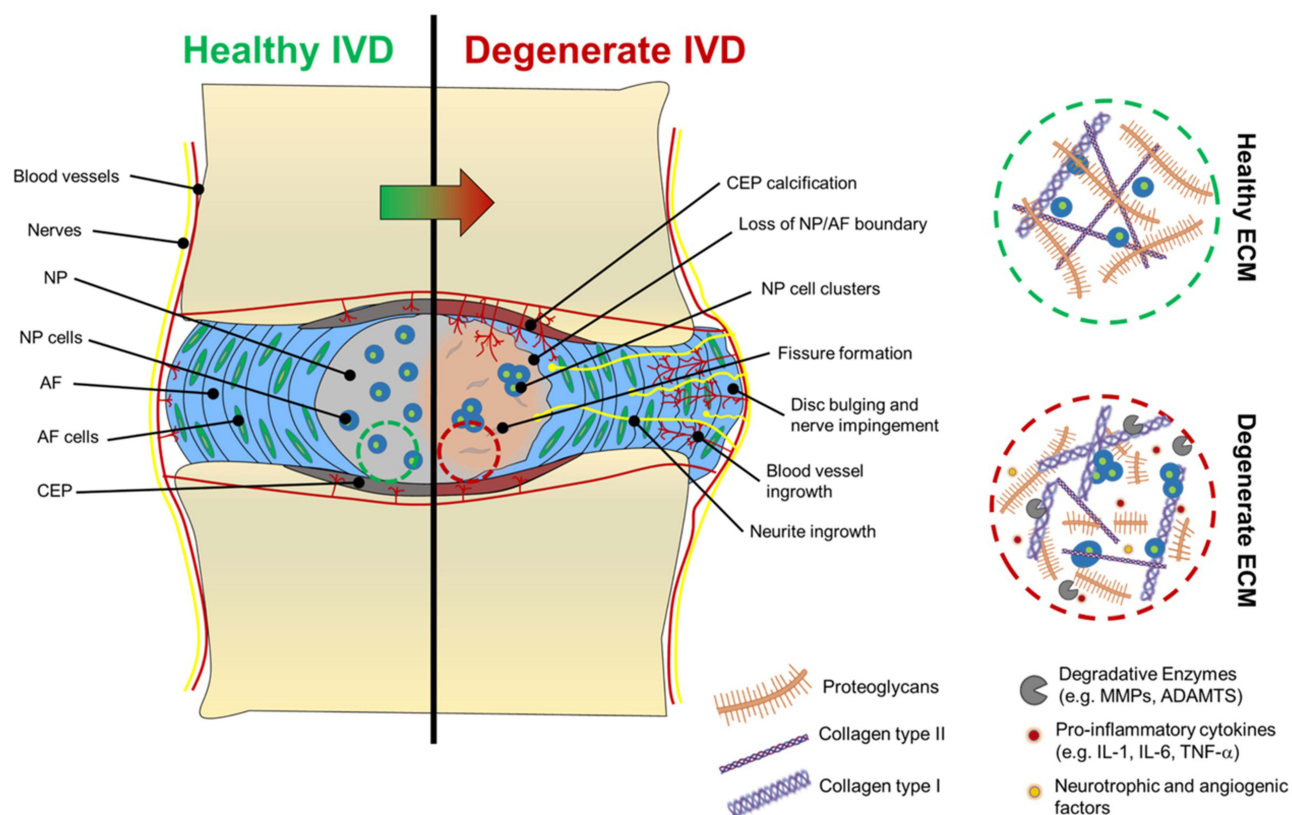
Figure 1 Macroscopic and microscopic changes of the IVDD during degeneration. Characteristic changes of the NP's ECM after degeneration are illustrated in the dashed circles. ECM of degenerated discs (dashed red circle) shows shorter aggrecan macromolecules and more collagen type I fibres (thicker fibre bundles) than collagen type II (thinner collagen bundles), which are largely abundant in the ECM of healthy discs (dashed green circle). Reprinted from Costăchescu B, Niculescu AG, Teleanu RI, et al. Recent advances in managing spinal intervertebral discs degeneration. *Int J Mol Sci.* 2022;23(12):6460. Creative Commons.[78]

Internal Stimuli-Responsive Delivery Systems for IVDD
Redox-Responsive System

The IVDD microenvironment features an increased expression of reactive oxygen species (ROS), which induces the progression and deterioration of IVDD. ROS includes oxygen-derived molecules and high-reactivity free radicals, including hydrogen peroxide (H_2O_2), hydroxyl radicals (•OH), and superoxide anion radicals ($O_2^{-•}$).[81,82] The excessive level of ROS leads to the overexpression of catabolic factors, such as TNF-α, MMPs, and aggrecanases, causing dramatic histological changes. Moreover, decreased levels of antioxidant substances aggravate the oxidative microenvironment in IVDD.[83] Thus, therapeutic strategies for reducing ROS and ROS-induced inflammation are considered promising options for attenuating disc catabolism and restoring disc anabolism. High levels of ROS can be a suitable stimulus for drug delivery systems.

Zhang et al constructed ROS-responsive magnesium-containing microspheres (Mg@PLPE MSs) as a new ROS-responsive drug delivery system that could realize the controlled release of hydrogen gas (H_2).[84] Constructed in a core-shell format, the core component is magnesium microparticles, and the exposed shell is developed using the ROS-responsive polymers poly(polybutylene terephthalate [PBT]-co-ethylene glycol dimethacrylate (EGDM) and poly(lactic-co-glycolic acid) (PLGA). The specific design and combination of PBT-co-EGDM and PLGA can achieve ROS-targeted migration and a hydrophobic-to-hydrophilic transition through a reaction with the overproduced H_2O_2 in the degenerated disc. Then, the Mg-water reaction starts, and H_2 is produced continuously, which further reduces the ROS level of the degenerated discs by reacting with oxidative substances. The efficiency of this ROS-responsive drug delivery system was verified in vivo using

a puncture-induced rat IVDD model, and the results showed biocompatibility, negligible toxicity, and excellent treatment outcomes.[84]

Moreover, Wang et al constructed a special ROS-responsive vesicle hydrogel to alleviate disc degeneration in a rat model, which featured a peptide core component named mineralocorticoid receptor (MR)409.[85] The underlying mechanism is that MR409 modulates the secretory autophagy of NP cells by regulating disease-associated inflammation, immune infiltration, and IL-1β synthesis.[85–88] MR409 was loaded onto a special ROS-responsive poly(propylene sulfide) (PPS)-poly(ethylene glycol) (PEG) (PPS-PEG) amphiphilic polymer. Eventually, the MR409-loading vesicles were embedded in a thermosensitive PLGA-PEG-PLGA hydrogel (Figure 2). This special hydrogel is thermosensitive and protects the internal materials from harsh microenvironments. The MR409-vesicle-hydrogel was tested in a rat model and demonstrated to be valid for disc degeneration treatment.[85]

Isoginkgetin (IGK), a natural flavonoid extracted from Ginkgo biloba leaves, can enhance autophagy and scavenge ROS. Yu et al constructed a ROS-responsive IGK-loaded nanodelivery system (IGK@SeNP) and achieved its smart release under the stimulation of excessive ROS.[89] The IGK@SeNP system contained Se-Se bonds and exhibited excellent biocompatibility. Se-Se bonds and IGK play a joint role in clearing ROS. Simultaneously, the targeted and controlled release of IGK increases autophagy in NPCs, which maintains the balance between the anabolism and catabolism of the ECM. Further testing in a rat needle puncture IVDD model showed that this intelligent system has superior efficacy for treating IVDD in vivo.[89]

Bai et al designed an in situ-formed rapamycin-loaded ROS-scavenging scaffold and offered a new strategy for promoting IVDD tissue regeneration.[90] In an oxidative environment, the scaffold consumed ROS and gradually released rapamycin. Rapamycin reduces the inflammatory response and promotes the regeneration of disc cells by promoting macrophage polarization toward an anti-inflammatory M2-like phenotype. In a rat IVDD model, this ROS-scavenging scaffold showed significant effects in in vivo experiments.[90]

Enzyme-Responsive System

Enzyme-responsive features have excellent biocompatibility and high catalytic efficacy, which enable the targeted migration of therapeutic substances to injury sites.[88] In the pathological state of IVDD, enzymes with elevated levels include MMP, hyaluronidase (HA), and ADAMTS.[91–93] Possessing the ability to catalyze chemical reactions, enzymes overexpressed in IVDD are ideal stimuli for enzyme-responsive delivery systems.

The micro-ribonucleic acid (RNA) (miRNA)-29 (miR-29) family (miR-29a, miR-29b, and miR-29c) is involved in tissue fibrosis and is crucial for inhibiting abnormal fibrosis and collagen transition.[94–98] Ge et al designed a valuable complex named MMP-responsive miR-29a polyplex micelles to achieve precise and efficient delivery while maintaining the integrity of miRNAs.[99] This MMP-responsive shell is composed of MMP-responsive PEG/ CGPLGVRGC peptide hydrogels and PEG-GPLGVRG-poly{N′-[N-(2-aminoethyl)-2-aminoehtyl]aspartamide}-cholesteryl (PEG-GPLGVRG-PAsp(DET)-Chole) (PGPC). The peptide linkage (GPLGVRG) of the shell can be decomposed by the overproduction of MMPs. This special delivery system includes two MMP-responsive processes and enables a more accurate and efficient miRNA delivery for IVDD treatment[99] (Figure 3).

In addition, stem cell therapy using mesenchymal stem cells (MSCs) and pluripotent stem cells is a crucial IVDD treatment approach that features fewer autoimmune reactions and fewer side effects.[100,101] NP progenitor cells (NPPCs) are ideal tissue-specific stem cells for IVDD regeneration. However, because of the harsh microenvironment (hypoxia, acidity, and inflammation) of the degenerated disc, stem cell therapy often results in poor outcomes.[101] To overcome this obstacle, Tang et al designed an esterase-responsive ibuprofen copolymer nanomicelle (PEG- polyisobutene) that modified embryo-derived NPPCs for IVDD treatment. As an effective inflammatory inhibitor, ibuprofen is suitable for protecting NPPCs. To resolve the fast degradation speed of direct ibuprofen injection, researchers constructed esterase-responsive nanomicelle-premodified NPPCs, which achieved a sustained release of ibuprofen and protected NPPCs during IVDD regeneration.[102] The production process of this special delivery system is illustrated in Figure 4.

Hydrogen sulfide (H_2S) has been used to treat many diseases, including cardiac diseases, skin injury, and neuronal degeneration.[103–105] H_2S participates in signal transduction and protects the cells from apoptosis. Some studies have shown that it can upregulate antioxidant expression and reduce inflammation through macrophage polarization.[103] However, although H_2
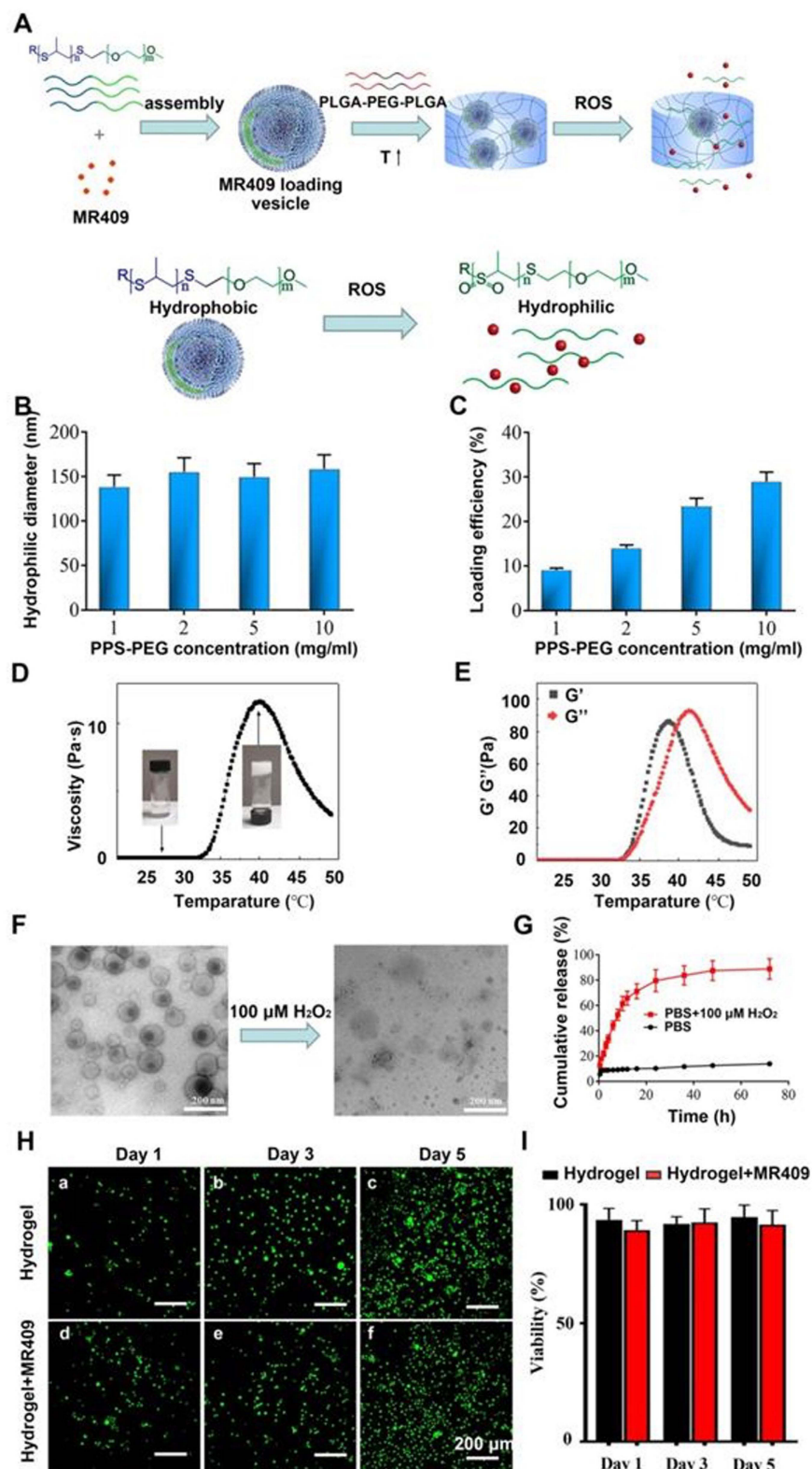
Figure 2 A new injectable thermosensitive hydrogel with ROS-sensitive MR409-loaded vesicles demonstrates sustained controlled release and biocompatibility. (**A**) Schematic illustration of the thermosensitive hydrogel loaded with ROS-responsive PPS-PEG vesicles for controlled release of MR409. (**B**) Hydrodynamic diameters and (**C**) MR409-loading efficiencies of PPS-PEG vesicles. (**D**) Viscosity of the PLGA-PEG-PLGA solution containing MR409-loaded vesicles as a function of temperature. (**E**) G' and G'' of the PLGA-PEG-PLGA solution containing MR409-loaded vesicles as a function of temperature. (**F**) Change in the morphology of MR409-loaded vesicles in the presence of external H_2O_2 (100 μM). (**G**) Cumulative release of MR409 from PPS-PEG vesicles or hydrogel containing vesicles in the presence of 100 μM H_2O_2. (**H** and **I**) Images of live/dead cell staining (**H**) and quantitation (**I**) of rat NP cells cultured with hydrogel confirming good biocompatibility. Reprinted from Zheng Q, Shen H, Tong Z, et al. A thermosensitive, reactive oxygen species-responsive, MR409-encapsulated hydrogel ameliorates disc degeneration in rats by inhibiting the secretory autophagy pathway. *Theranostics*. 2021;11(1):147–163. Creative Commons.[85]
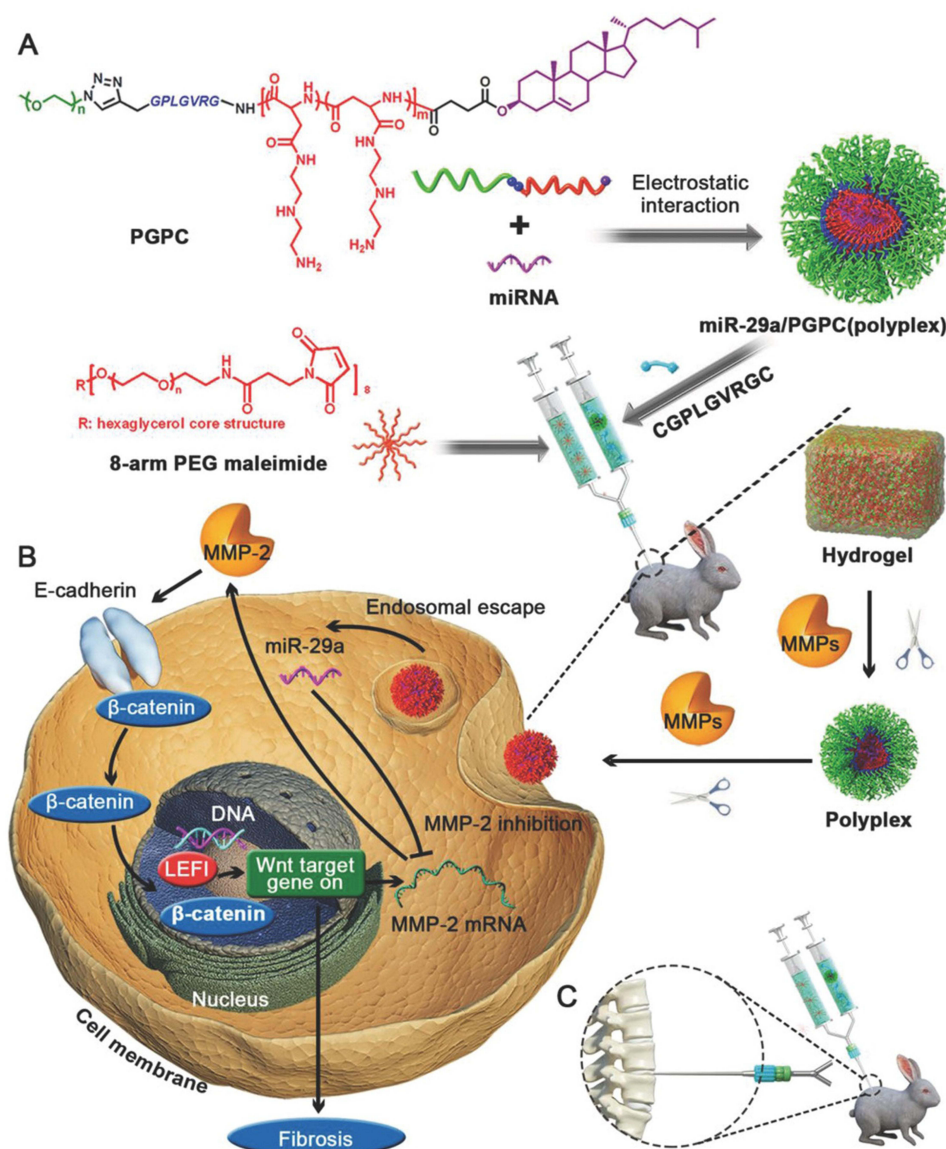
Figure 3 (**A**) Schematic illustration for formation of miRNA/PGPC polyplex micelles. (**B**) Encapsulation of miRNA/PGPC polyplexes in PEG hydrogels in an injectable manner and molecular mechanism of MMP-2 silence in nucleus pulposus cells for fibrosis inhibition. (**C**) Injection sites in the IVDs of rabbits. Reprinted from Feng G, Zha Z, Huang Y, et al. Sustained and Bioresponsive Two-Stage Delivery of Therapeutic miRNA via Polyplex Micelle-Loaded Injectable Hydrogels for Inhibition of Intervertebral Disc Fibrosis. *Adv Healthc Mater.* 2018 Nov;7(21):e1800623. VCH Verlag GmbH & Co. KGaA, Weinheim.[99]

S has been demonstrated to be effective in suppressing the degeneration process of NP cells, there is a huge obstacle to the production and accumulation of H_2S, and the quick volatilization of H_2S gas limits its clinical usage. Considering these difficulties, Xiao et al developed a pH and enzyme dual-responsive H_2S release system, named collagen-JK1 hydrogel (Col-JK1), for IVDD treatment.[106] This specially designed H_2S release system included the core component of a pH-responsive H_2S donor, named JK1, and a carrier of the MMP-responsive collagen hydrogel. This unique combination resulted in sustained H_2S release and retention in situ. The in vivo results showed that the Col-JK1 significantly reduced the apoptosis of NP cells. H_2S participated in and regulated the nuclear factor (NF)-κB signaling pathway.[106]
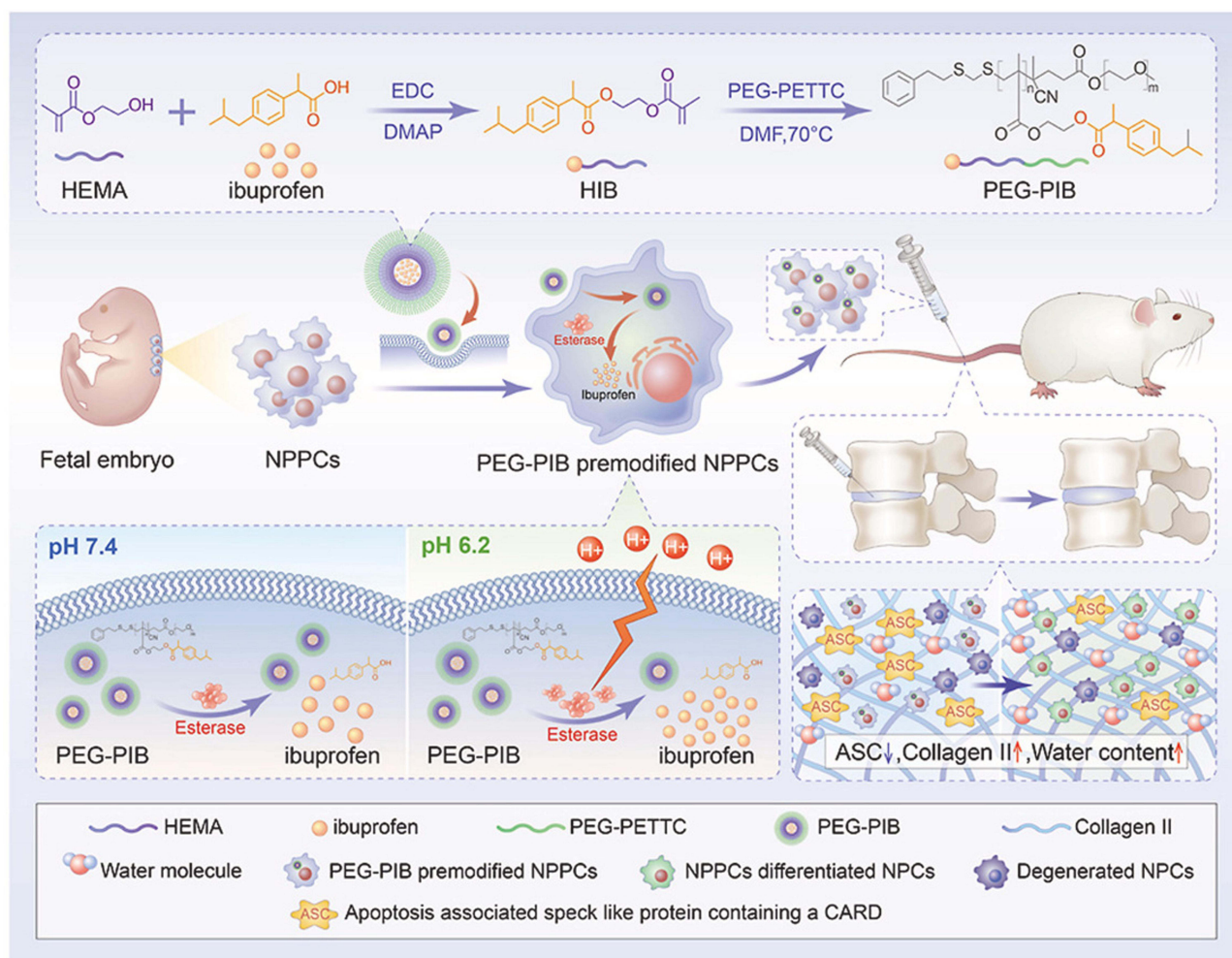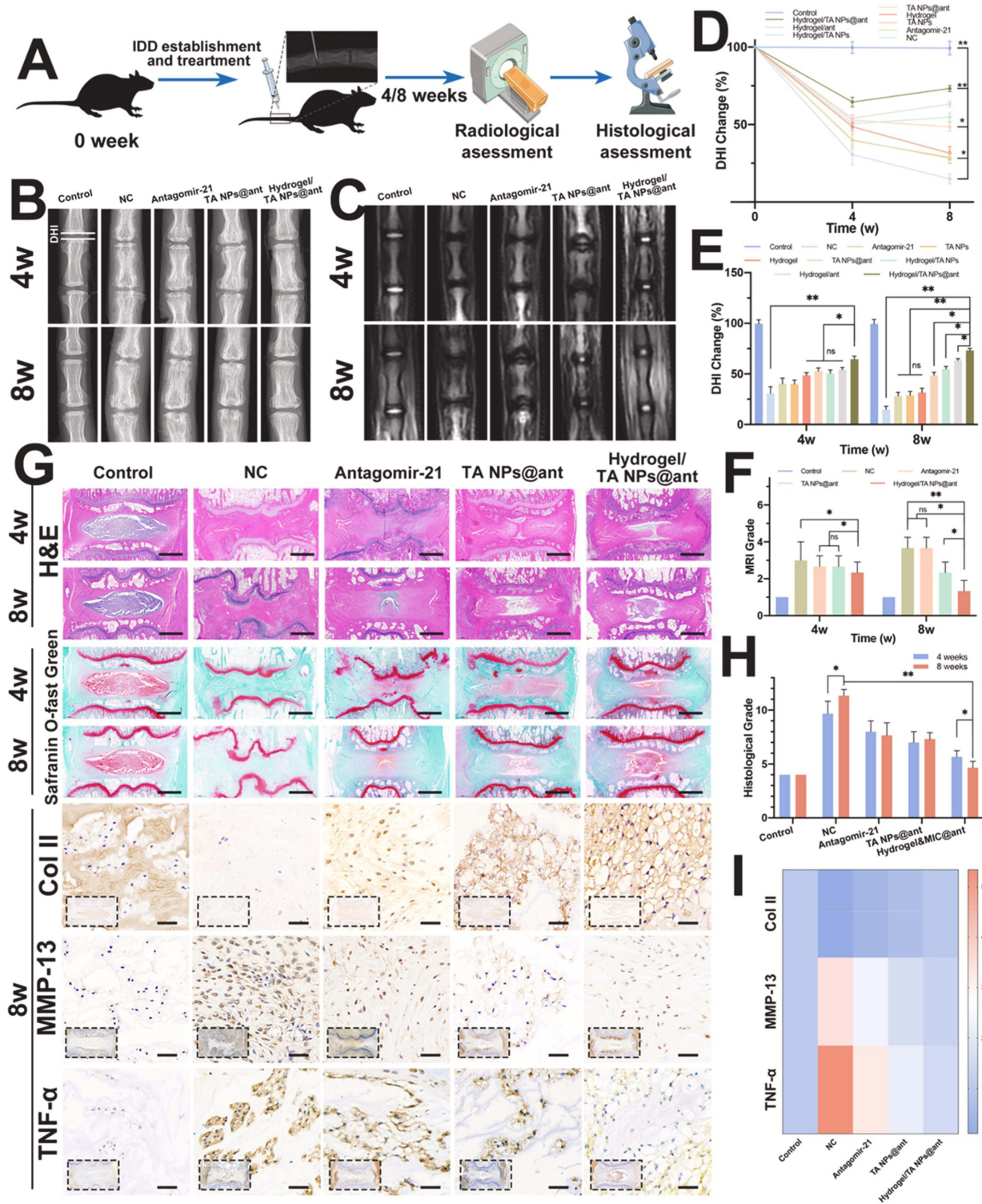
Figure 4 General schematic of synthesis of esterase-responsive ibuprofen nano-micelles (PEG-PIB) to pre-modify embryo-derived long-term expandable nucleus pulposus progenitor cells (NPPCs) for synergistic transplantation in intervertebral disc degeneration. Reprinted from Xia KS, Li DD, Wang CG, et al. An esterase-responsive ibuprofen nano-micelle pre-modified embryo derived nucleus pulposus progenitor cells promote the regeneration of intervertebral disc degeneration. *Bioact Mater*. 2023;21:69–85. Creative Commons.[102]

pH-Responsive System

The normal human tissues possess an extracellular pH of 7.4.[107] The pH of the normal IVD is estimated to be 7.1; however, the pH in mildly, moderately, and severely degenerated IVDs is reported as 6.8, 6.5, and 6.2, respectively.[108,109] The acidic microenvironment of NP tissues is also a detrimental factor in IVDD. Accordingly, acidic microenvironments can lead to abnormal cell damage and apoptosis.[110] Other studies have demonstrated that lower pH results in increased expression of acid-sensing ion channel-3 (ASIC-3), which is considered novel therapeutic targets.[109]

Zeolite imidazolate framework-8 is a metal–organic framework (MOF) with a large surface area and good degradability in acidic microenvironments. Protocatechuic acid (PCA) is a phenolic compound extracted from traditional Chinese herbs that promotes chondrocyte regeneration. Recently, Ding et al designed an intelligent drug delivery system named MOF@PH that could release PCA under the stimulation of an acidic environment at the degenerated disc. MOF@PH satisfactorily inhibited IVDD progression in a rat model.[111]

Wang et al associated H_2 therapy with hollow polydopamine (HPDA) to achieve sustained H_2 release from a degenerated disc. They used HPDA as a pH-responsive carrier and developed ammonia borane (AB) as a source of H_2. This specially designed complex, called AB@HPDA, is characterized by a large surface area, high biocompatibility, and permeability.[112] The complex is incorporated into the nanoparticles and can be injected at degenerated positions. In vivo experiments showed that this injectable nanoparticle significantly reduced tissue oxidative damage by the pH-responsive delivery of H_2 and restored normal physiological functions.[112]

Figure 5 In vivo therapeutic effects in a rat IVDD model. (**A**) Schematic illustration showing the overall processes of animal experiments. (**B**) X-ray and (**C**) MRI images of rat coccygeal vertebral discs at different time points indicated that the height and water content of the degenerative intervertebral disc were significantly improved after treatment with the Hydrogel/TA NPs@ant. (**D** and **E**) The changes in DHI and (**F**) MRI grade at 4 and 8 w quantitatively confirmed the repair effect of IVDD implanted with Hydrogel/TA NPs@ant. (**G**) Representative images of H&E, safranin-O/fast green, Col II, MMP-13 and TNF-α immunohistochemical staining of different treatments at 4 and 8 w (scale bars, 1 mm (for H&E, safranin-O/fast green), 50 μm (for immunohistochemical staining)). The histological results further confirmed the role of the Hydrogel/TA NPs@ant in improving the metabolic balance of the ECM in degenerative nucleus pulposus and inhibiting the infiltration of inflammatory factors. (H) The histological grades in different groups further proved the synergistic effect of antagomir-21, TA NPs and the hydrogel on nucleus pulposus regeneration. (I) Heatmap represents the total intensity of Col II, MMP-13 and TNF-α immunohistochemical staining in nucleus pulposus of different treatments at 8 w. Reprinted from *Biomaterials*, volume: 298, Wang Y, Wu Y, Zhang B, et al. Repair of degenerative nucleus pulposus by polyphenol nanosphere-encapsulated hydrogel gene delivery system. 122132. Copyright 2023, with permission from Elsevier.[113]

Wang et al designed another novel injectable and pH-responsive miRNA delivery system, Ta nanoparticles@anta-gomir-21, which has great potential for regulating ECM metabolism in NP tissues. The surrounding hydrogel is formed by glycidyl methacrylate (GMA)-modified carboxy-methyl chitosan (CMC-GMA), which can achieve a two-stage inflammation-triggered on-demand delivery of antagomir-21.[113] In vivo experiments confirmed the efficacy of the multifunctional delivery system (Figure 5). Researchers have also explored the mechanism of antagomir-21 in degenerated NP cells, demonstrating that antagomir-21 is a mitogen-activated protein kinase (MAPK)/extracellular signal-regulated kinase (ERK) signaling pathway regulator.[113]

Temperature-Responsive System

In general, the properties of a temperature-responsive delivery system can shift to another morphology as the surrounding temperature changes, and the embedded cargo can then be released. Temperature-sensitive hydrogels are ideal matrices for engineered cartilage and spine construction.[114] Temperature-sensitive materials are critical components of the temperature-responsive delivery system, which mainly include poly(N-isopropylacrylamide),[115] poly(amidoamine),[116] poly(2-oxazoline),[117] and poly[2-(2-methoxyethoxy) ethyl methacrylate].[118] Based on previous studies, the currently promising temperature-responsive delivery systems for IVDD treatment can be classified into three categories.

Cell-Encapsulated Temperature-Responsive Delivery System

Based on modified polysaccharides with temperature-sensitive polyamides, poly(N-isopropylacrylamide) (pNIPAM), the temperature-sensitive hydrogel (TR-HG) is liquid at room temperature and hardens over 32 °C (Figure 6). Malonzo et al used a papain-induced disc degeneration model to assess a temperature-sensitive hydrogel cell therapeutic approach. The results showed no significant changes in the activity of NP cells after TR-HG+NP cells were injected into the discs, illustrating the cytocompatibility and practicability of TR-HG. By further examining gene expression in AF tissues from TR-HG and TR-HG+NP cell-treated discs, researchers found that the IVD tissues responded differently to the gel+cell mixtures than to the pure gel. This phenomenon indicates that the potential of TR-HG partly depends on the cell signaling (IL-6β and IL-1β) of the NP cells.[119]

HA is the preferred cellular delivery platform because it is a major component of the ECM in connective, epithelial, and neural tissues. However, the unmodified hyaluronic acid solutions exhibited rapid degradation and clearance after implantation.[120] Based on the above backgrounds, Guo et al introduced an HA-based hybrid interpenetrating polymer network (IPN), which could be used to deliver NP cells and repair nucleotomized IVDs. The components of HA–IPN include pNIPAM and 1.4-butanediol diglycidyl ether (BDDE). Both pNIPAM and BDDE supported the survival and differentiation of embedded NP cells. The characteristics of HA-IPN, such as its swelling properties, cytocompatibility, and support for ECM deposition, were excellent in vitro.[121]
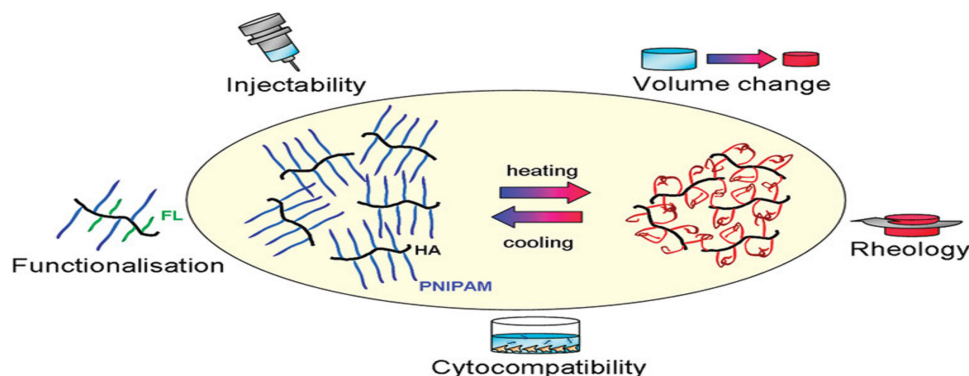

Figure 6 Various properties of the temperature-sensitive material hyaluronan-poly(N-isopropylacrylamide) HA-pNIPAM. Reprinted with permission from Mortisen D, Peroglio M, Alini M, Eglin D. Tailoring thermoreversible hyaluronan hydrogels by "click" chemistry and RAFT polymerization for cell and drug therapy. *Biomacromolecules*. 2010;11(5):1261–1272. Copyright {2010} American Chemical Society.[114]

Nucleic Acid-Encapsulated Temperature-Responsive Delivery System

miRNAs are a class of small, non-coding, endogenous RNA molecules that direct the function of corresponding messenger RNAs.[122] Previous studies have demonstrated that miRNAs are crucial for IVDD progression.[123,124] The miRNA-25-3p could precisely silence the targeted metal regulatory transcription factor 1 (MTF1) protein expression and inhibit IL-1β-induced effects.[125] However, miRNAs and negatively charged mimics cannot cross cell membranes, and the "naked" miRNAs are highly susceptible to degradation in serum.[126]

Based on this background, Huang et al constructed a novel temperature-responsive carrier formed by mixed cationic block copolymers (MCBCs), PEG-b-poly [Poly(L-Aspartic acid) (PAsp)(DET)], and PNIPAM-b-PAsp(DET).[125] As the temperature increased from room temperature (25 °C) to physiological temperature (37 °C), the PNIPAM-b-PAsp(DET) formed a hydrophobic PNIPAM core in the micellar structure, which facilitated gene transfection and stability.[127,128]
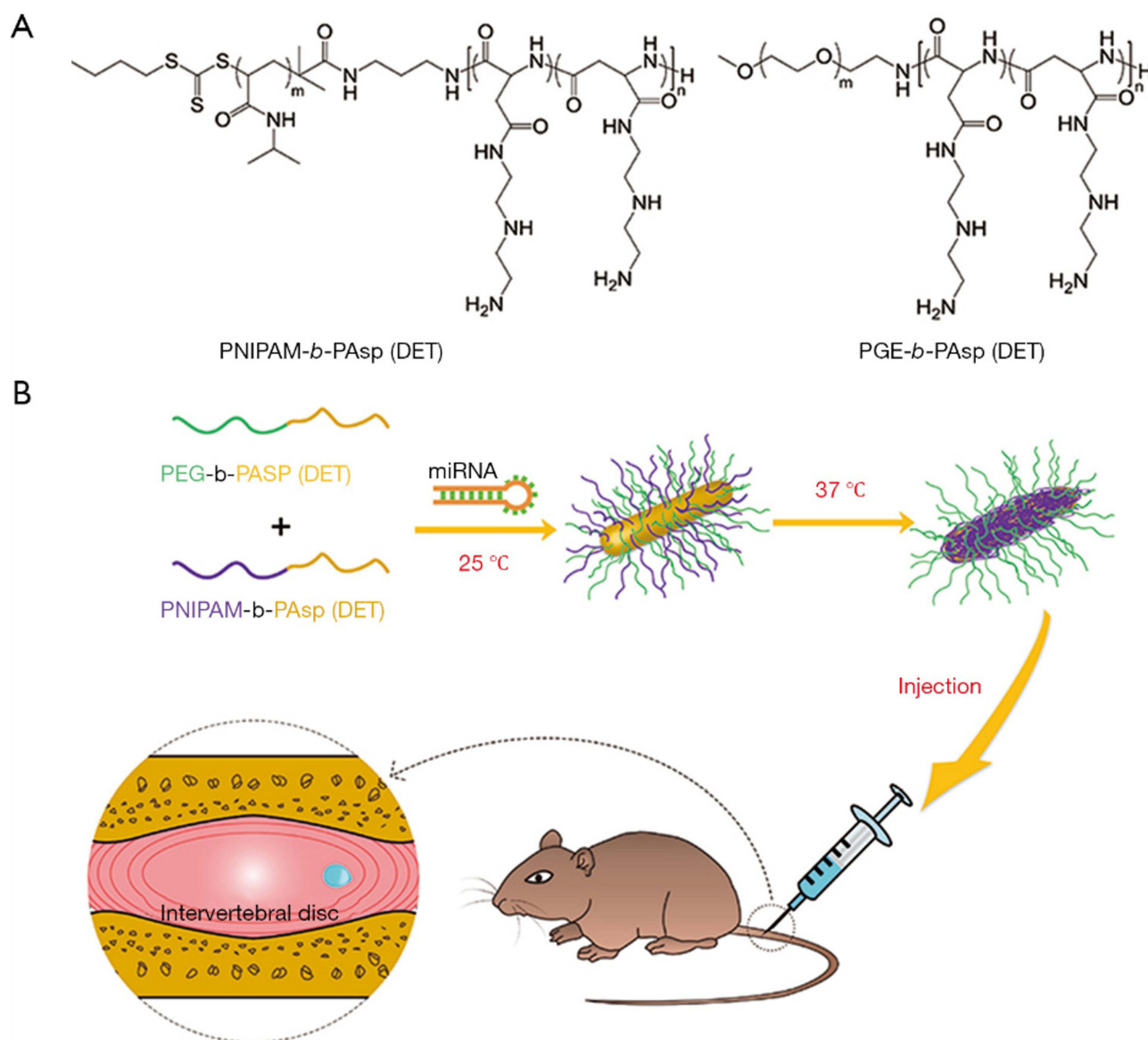

Figure 7 Schematic illustration of miRNA-based gene therapy delivered by a thermo-responsive vector for intervertebral disc degeneration (IVDD). (**A**) The chemical structure of cationic block copolymers: PNIPAM-b-PAsp (DET) and PEG-b-PAsp (DET). (**B**) The mixed cationic block copolymers (MCBC) PNIPAM-b-PAsp (DET) and PEG-b-PAsp (DET) as vectors for miRNA to form mixed polyplex micelles. With a temperature increase from room temperature (25 °C) to physiological temperature (37 °C), thermo-sensitivity of PNIPAM segments become hydrophobic and form more compact mixed polyplex micelles with hydrophobic and hydrophilic heterogeneous surfaces. The mixed cationic block copolymers delivered miRNA-25-3p for IVDD gene therapy. Reprinted from Huang Y, Huang L, Li L, et al. MicroRNA-25-3p therapy for intervertebral disc degeneration by targeting the IL-1β/ZIP8/ MTF1 signaling pathway with a novel thermo-responsive vector. *Ann Transl Med*. 2020;8(22):1500. Creative Commons.[125]

High efficiency of the temperature-responsive vector MCBC was observed, and miRNA-25-3p was effectively delivered in rat models[125] (Figure 7).

Bioactive Agent-Encapsulated Temperature-Responsive Delivery System

Previous studies have demonstrated that bioactive agents, such as simvastatin, alleviate IVDD deterioration by stimulating endogenous bone morphogenetic protein (BMP)-2 expression.[129] To further test and promote therapeutic efficacy, researchers have combined simvastatin with novel delivery systems. Zhang et al constructed a special simvastatin-loaded PEG-PLGA-PEG gel, which showed significant effects on disc regeneration.[130] By incorporating simvastatin into the temperature-sensitive liquid PEG-PLGA-PEG gel, the exposure of IVD cells to simvastatin was confined to the disc core, as the compound is converted to the gel phase after reaching body temperature.[130] Moreover, the use of the PEG-PLGA-PEG hydrogel reduced the instantaneous drug concentration and enabled sustained therapy. Such triblock polymers are widely used as drug-controlled vehicles for gene delivery.[131–133]

External Stimuli-Responsive Delivery Systems for IVDD
Photo-Responsive System

Photo, as a stimulus for biomaterials, provides non-invasive precision and physiological benignity, essential for advanced biomedical applications.[134] The cargo delivered by the photoresponsive system includes cells and bioactive substances. Photosensitive biomaterial-embedded agents are activated by photoisomerization, photolysis, photo-crosslinking, photo-oxidative reduction (which can be applied to photodynamic therapy), and photothermal triggering (which is related to photothermal therapy).[135]

Chen et al combined a photo-crosslinked gelatin (Gel)-HA methacrylate (MA) (HAMA) (GelHA) hydrogel with adipose stromal cells (ASCs),[136] which possess the potential for NP-like cell differentiation.[137,138] The addition of HAMA increases the mechanical properties of photocrosslinked GelMA hydrogels, and the whole complex promotes the expression of articular cartilage ECM while avoiding the formation of osteophytes caused by direct injection of stem cells. The efficacy of the GelHA hydrogel and ASCs was evaluated in an IVDD rat model.[136] Kumar et al embedded human bone marrow-derived MSC (hMSC) in a novel three-dimensional photo-curable, biodegradable synthetic polymer hydrogel, in which polyhydroxyethyl MA-co-N-(3-aminopropyl)methacrylamide was grafted with polyamidoamine.[139] Their results showed that a special photo-curable hydrogel enhanced the differentiation ability of hMSC toward the NP cell type. (Figure 8)
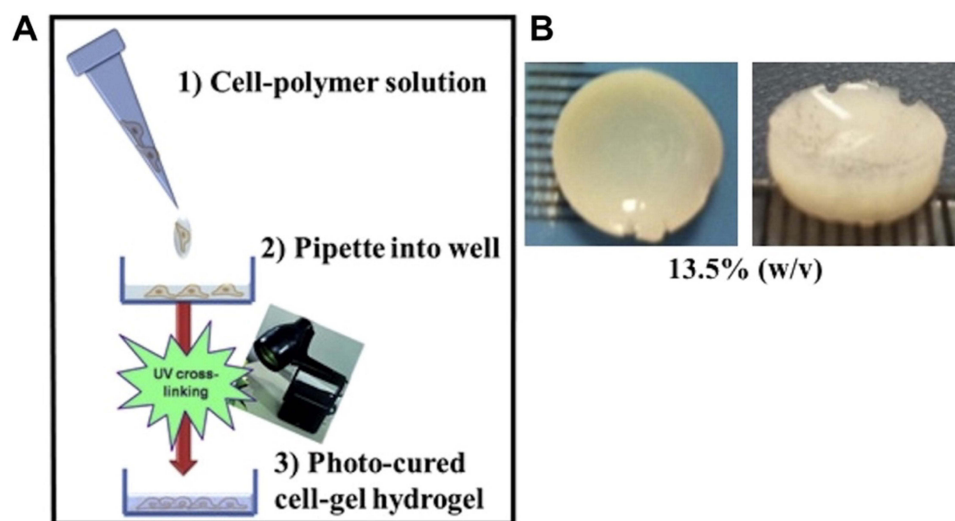

Figure 8 Schematic showing the process of photo-curing of hMSC encapsulated within p(HEMA-co-APMA) g PAA hydrogels: (**A**) procedure of Three-dimensional hMSC encapsulation in polymer to produce hydrogel; (**B**) representative image of a 13.5% (w/v) photo-cured hydrogel with dimensions 1 cm Ø and 3 mm thick). Reprinted from Kumar D, Gerges I, Tamplenizza M, Lenardi C, Forsyth NR, Liu Y. Three-dimensional hypoxic culture of human mesenchymal stem cells encapsulated in a photocurable, biodegradable polymer hydrogel: a potential injectable cellular product for nucleus pulposus regeneration. *Acta Biomater.* 2014;10(8):3463–3474. Creative Commons.[139]

Ultrasound-Responsive System

Ultrasound is considered a prospective external stimulus for delivery systems that exhibits effective energy focus, deep penetration, ease of handling, security, low cost, and portability of ultrasound instruments.[140,141] Ultrasonication improves the delivery efficiency of bioactive substances and genes through thermal and non-thermal effects.[140,142] Tissues with higher ultrasound absorption coefficients, such as bones, expand in temperature more rapidly than muscles with lower absorption coefficients.[143] Moreover, non-thermal effects include ultrasound pressure, shockwaves, acoustic streaming, liquid microjets, and ultrasound-induced oscillations or cavitation.[144–146]

Previous studies have shown that ultrasound enhances the penetration of agents into living cells.[147] Based on this characteristic, microbubbles have been widely applied as cavitation nuclei in ultrasound-mediated delivery systems.[148,149] Common microbubbles have a gas core and phospholipid, polymer, and protein shells.[150] Microbubbles commonly circulate with red blood cells and have a short circulation time in the bloodstream because of their size, which limits their transportation between blood vessels and target tissues. Recently, nanoscale bubbles, droplets, micelles, and nanoliposomes have been developed as novel nanomaterials in ultrasound-responsive delivery systems.[151–153]

Guo et al constructed special resveratrol (RES) 3,5,4′-trihydroxystilbene-loaded anti-cadherin 2 (CDH2) antibody-decorated PLGA nanobubbles, which are effectively imploded by external ultrasound.[154] The released microjets led to the propulsion of the delivered drugs, which was applied in rat models. Relevant synthetic process is illustrated in Figure 9. Additionally, Kobayashi et al demonstrated that low-intensity pulsed ultrasound (LIPUS) stimulation upregulated proteoglycan production in human NP cells, which showed potential in IVDD treatment.[155] Relevant studies have found that the mechanisms underlying the therapeutic effects of LIPUS include activation of the focal adhesion kinase (FAK)/phosphoinositide 3-kinase (PI3K)/AKT pathway and upregulation of aggrecan, collagen II, and SRY-box
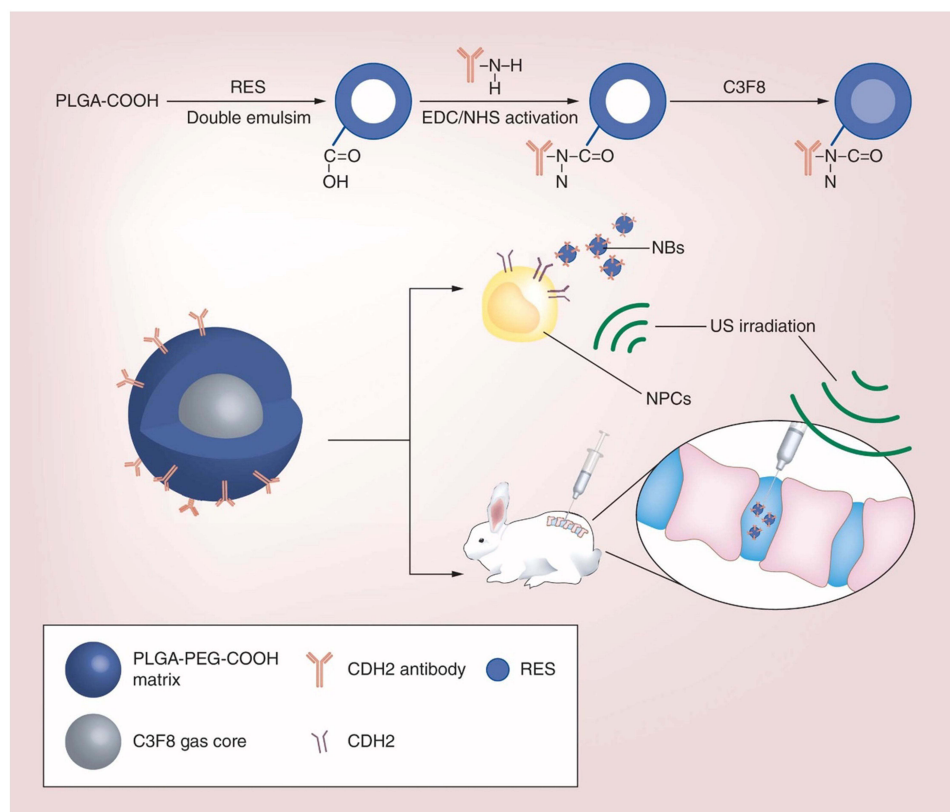

Figure 9 A schematic illustration for the experiment design and the fabrication process of RES-loaded, CDH2-decorated nanobubbles. Reprinted from Shen J, Zhuo N, Xu S et al. Resveratrol delivery by ultrasound-mediated nanobubbles targeting nucleus pulposus cells. *Nanomedicine (Lond)*. 2018 Jun;13(12):1433–1446.[154] Copyright 2018 Future Medicine Ltd; permission conveyed through Copyright Clearance Center, Inc.
Abbreviations: CDH2, N-cadherin; NB, Nanobubble; NPC, Nucleus pulposus cell; PLGA, Poly(lactic-co-glycolic acid); RES: Resveratrol; US, Ultrasound.

transcription factor 9 (SOX9) expression.[156] Furthermore, Horne et al and Chen et al found that LIPUS downregulated the expression of TNF-α gene in NP cells and upregulated the metabolic rate of AF cells.[157,158]

Magnetic-Responsive System

Magnetically responsive materials respond to changes in the magnetic fields by switching their properties. Owing to its changeable nature, magnetically responsive drug delivery can be achieved under the guidance of a magnetic field. Magnetic materials generate heat when exposed to alternating magnetic fields (AMFs), and magnetic NP cells generate heat through two methods: hysteresis loss (ferromagnetic particles) and the oscillation of their magnetic moment owing to Néel and Brownian relaxation (superparamagnetic particles).

"Small" magnets have larger magnetic moments and are more sensitive to weak stimuli (static or alternating magnetic fields) than molecular magnets.[159] Composites have a variety of merging properties, including deformability, magnetically guided action, and magnetothermal effects[159] (Figure 10). Activation approaches for magnetically responsive polymer composites (MRPCs) involve the application of static magnetic fields or AMFs. The magnetothermal effect of the MRPCs can be used for controlled drug release. The underlying mechanism is attributed to the heat generated by the oscillating AMF, which causes structural changes in the nanocarrier structure, such as an increase in shell or double-layer pores,[160,161] disintegration of the Fe_3O_4 core,[162] and deformation of the single-crystal nanoshell lattice.[163]
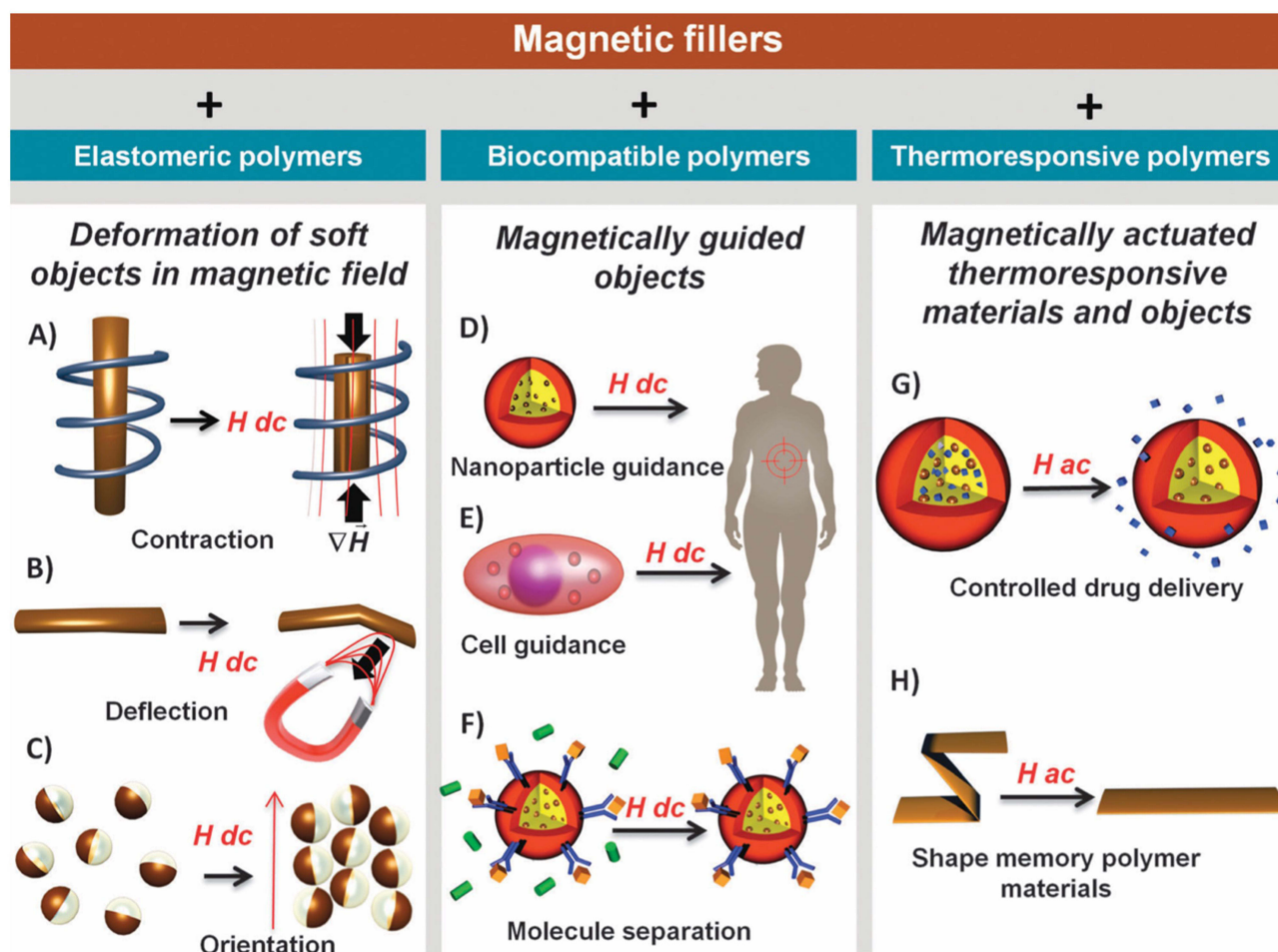

Figure 10 Schemes depicting the different types of magnetic responsive materials obtained from the doping of various polymers with magnetic particles and illustration of their response when exposed to a static magnetic field (H dc) or to an alternating magnetic field (H ac). From left to right: composites made from elastomeric polymers can be deformed in homogeneous fields or gradients in a controlled fashion (**A-C**); MRPC particles made of polymers designed for biomedical applications can be used for magnetic guidance for drug delivery or separation purpose (**D-F**); MRPC from thermo-responsive polymers can be activated by magnetic induction using alternating fields (**G** and **H**). Reprinted from Thévenot J, Oliveira H, Sandre O, Lecommandoux S. Magnetic responsive polymer composite materials. *Chem Soc Rev.* 2013 Sep 7;42(17):7099–116.[159] Copyright 2013 Royal Society of Chemistry; permission conveyed through Copyright Clearance Center, Inc.
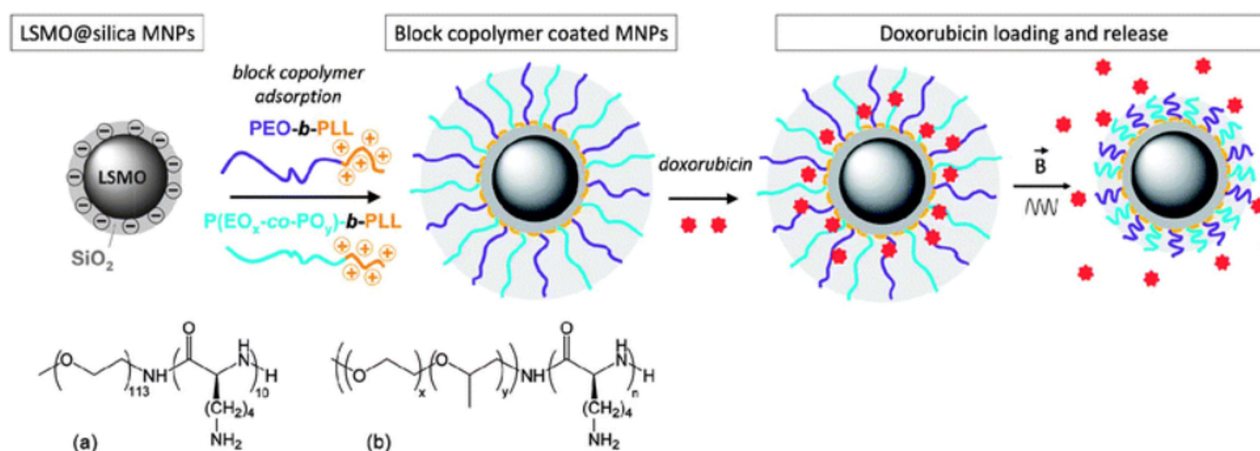
Figure 11 Preparation of magnetic responsive drug delivery systems (DDS) based on lanthanum strontium manganese oxide (LSMO) MNPs and polymer brush with a tunable LCST (top left). Chemical structures of the block copolymers used: (a) poly (ethylene glycol)-b-poly(lysine), and (b) poly (ethylene glycol-co-propylene glycol)-b-poly(lysine), with x = 6 and y = 29. Principle of drug loading and alternating magnetic field (AMF) triggered drug release (right). Reprinted from Louguet S, Rousseau B, Epherre R, et al. Thermoresponsive polymer brush-functionalized magnetic manganite nanoparticles for remotely triggered drug release. 10.1039/C2PY20089A. *Polym Chem*. 2012;3(6):1408–1417.[165] Copyright 2013 Royal Society of Chemistry; permission conveyed through Copyright Clearance Center, Inc.

Figure 11 shows the release of the drug in relation to the contraction of the polymer brushes when the temperature exceeds the lower critical solution temperature. Moreover, Okada et al found that a pulsed electromagnetic field enhances the function of BMP-2 in IVD-cell matrix regeneration,[164] which explored the usage of the magnetic field. Although relevant studies have been limited to IVDD treatment, magnetically responsive drug delivery systems are a new research direction.

Summary and Prospects

Compared to traditional systemic and local administration methods, newly developed stimuli-responsive delivery systems hold promise for treating a variety of diseases. These systems, such as hydrogels, scaffolds, nanoparticles, and micro-spheres, when combined with therapeutic molecules and specially modified, enable the precise release of therapeutic agents into degenerated intervertebral discs. This novel approach to stimuli-responsive delivery provides a more precise and sustained treatment for intervertebral disc degeneration (IVDD), aiming to reduce adverse effects. As research continues to advance and our understanding of biomaterials deepens, it is expected that the conception of stimuli-responsive will play an increasingly vital role in the future of IVDD therapy, ultimately leading to improved patient outcomes and quality of life.

However, emerging problems still need to be addressed. Low biocompatibility and high toxicity are the prime limitations of molecular delivery vectors. Thus, with their great biocompatibility and far lower toxicity, hydrogels are better applied as delivery systems than NPs and MSs. One novel approach to address the limitations of molecular delivery vectors is the integration of biomimicry principles. By mimicking the body's natural mechanisms of healing and drug transport, we can design delivery systems that not only enhance biocompatibility but also reduce toxicity. For instance, the use of biologically derived materials, such as natural polymers and proteins, can create a more hospitable environment for the encapsulated bioactive agents, thereby preserving their therapeutic efficacy and reducing the risk of adverse reactions.

The diffusion ability of bioactive agents in a combined complex is another limiting factor for stimuli-responsive delivery systems. Current delivery systems usually use shell-core structures that feature special stimuli-responsive surface shells and curable core components. Following the reaction between the shell and stimulatory factors, the diffusive ability determines the therapeutic functions of the core bioactive agents. As the synthetic process may suppress the migration capability of the core component, it is vital to avoid additional restrictions on the main bioactive agents. More importantly, the intrinsic properties of core components should remain intact under all circumstances. Chemical synthesis can easily change the natural properties of the core components, which are crucial for their therapeutic ability. The synthesis process should be carefully optimized to

ensure that the natural properties and therapeutic potential of the core components are not compromised. Employing gentle synthesis techniques and carefully selecting the appropriate materials can help maintain the integrity of the bioactive agents.

In conclusion, the future of stimuli-responsive drug delivery systems lies in the integration of biomimetic designs, smart materials, multifunctional capabilities, gentle synthesis techniques, and personalized medicine approaches. By pursuing these innovative strategies, we can overcome existing limitations and enter a new era of effective, safe, and patient-centered treatments for IVDD, ultimately improving patient outcomes and quality of life.

Abbreviations

IVD, intervertebral disc; IVDD, intervertebral disc degeneration; LBP, low back pain; NP, nucleus pulposus; AF, annulus fibrosus; CEP, cartilage endplates; ECM, extracellular matrix; BASP1, brain abundant membrane attached signal protein 1; NCDN, neurochondrin; NRP1, neuropilin 1; CD, cluster of differentiation; BEP, bony endplate; IL, interleukin; TNF, tumor necrosis factor; MMP, matrix metalloproteinase; ADAMTS, a disintegrin and metalloproteinase with thrombospondin motifs; TIMP, tissue inhibitors of metalloproteinase; MRI, magnetic resonance imaging; T1WI, T1-weighted imaging; T2WI, T2-weighted imaging; IFN-γ, interferon-γ; CCL20, C-C chemokine ligand 20; ROS, reactive oxygen species; H_2O_2, hydrogen peroxide; •OH, hydroxyl radicals; $O_2^{-\bullet}$, superoxide anion radicals; H_2, hydrogen gas; PLGA, poly(lactic-co-glycolic acid); EGDM, ethylene glycol dimethacrylate; PBT, polybutylene terephthalate; MR, mineralocorticoid receptor; PPS, poly(propylene sulfide); PEG, poly(ethylene glycol); IGK, Isoginkgetin; HA, hyaluronidase; miRNA, micro-ribonucleic acid; RNA, ribonucleic acid; PGPC, PEG-GPLGVRG-poly{N′-[N-(2-aminoethyl)-2-aminoehtyl]aspartamide}-cholesteryl; MSCs, mesenchymal stem cells; NPPCs, NP progenitor cells; H_2S, Hydrogen sulfide; Col-JK1, collagen-JK1 hydrogel; NF, nuclear factor; ASIC-3, acid-sensing ion channel-3; MOF, metal–organic framework; PCA, Protocatechuic acid; HPDA, hollow polydopamine; AB, ammonia borane; GMA, glycidyl methacrylate; CMC, carboxy-methyl chitosan; MAPK, mitogen-activated protein kinase; ERK, extracellular signal-regulated kinase; pNIPAM, poly(N-isopropylacrylamide); TR-HG, temperature-sensitive hydrogel; IPN, interpenetrating polymer network; BDDE, 1.4-butanediol diglycidyl ether; MTF1, metal regulatory transcription factor 1; MCBCs, mixed cationic block copolymers; MA, methacrylate; Gel, gelatin; ASCs, adipose stromal cells; hMSC, human bone marrow-derived MSC; RES, resveratrol; CDH2, 3,5,4′-trihydroxystilbene-loaded anti-cadherin 2; FAK, focal adhesion kinase; LIPUS, low-intensity pulsed ultrasound; PI3K, phosphoinositide 3-kinase; SOX9, SRY-box transcription factor 9; AMFs, alternating magnetic fields; MRPCs, magnetically responsive polymer composites.

Data Sharing Statement

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Acknowledgments

Author Contributions

All authors have contributed significantly to the work presented, spanning from its conception and study design to involvement in drafting, revising, and critically reviewing the article. All authors gave final approval of the version to be published, and have agreed on the journal to which the article has been submitted. All authors agree to take responsibility and be accountable for the contents of the article.

Disclosure

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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