

REVIEW ARTICLE

Hydrogel microsphere-mediated treatment of intervertebral disc degeneration and organoid construction

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Abstract

Intervertebral disc degeneration is a complex pathological process driven by multiple factors, involving cellular pathological changes, an imbalance in the inflammatory microenvironment, and oxidative stress mechanisms, and significantly impacts patients' quality of life. Current treatments lack therapeutic strategies targeting tissue regeneration and repair. With excellent biocompatibility, injectability, tunable mechanical properties, and smart responsive capabilities, hydrogel microspheres serve as ideal carriers for drug, gene, and cell therapies in intervertebral disc degeneration. This paper systematically reviews the preparation techniques and properties of hydrogel microspheres, focusing on their key mechanisms and advantages in gene delivery, drug delivery, and cell-scaffold applications. Subsequently, it summarizes cutting-edge advances in the construction of disc organoids, highlighting the advantages of hydrogel microspheres in mimicking extracellular matrices, constructing 3D biomimetic microenvironments, supporting cell proliferation and differentiation, and recapitulating degenerative microenvironments, thus providing a new platform for disease modeling and personalized therapy. Finally, future directions for hydrogel microsphere-based therapies for disc degeneration are envisioned, including multimodal smart-response design, personalized manufacturing, integrated organoid applications, and non-invasive, precise delivery technologies. This review aims to advance the management of disc degeneration from symptom relief toward functional restoration, thereby promoting clinical translation and the development of precision regenerative medicine.

Keywords: Organoids; Hydrogel microspheres; Intervertebral disc degeneration; Gene therapy; Drug therapy; Cell therapy

1. Introduction

Intervertebral disc degeneration (IVDD) is a highly prevalent chronic degenerative spinal disorder affecting individuals across all age groups, serving as a primary structural cause of low back and neck pain. According to the Global Burden of Disease Study 2021, low back pain caused by IVDD has become a leading cause of disability among musculoskeletal disorders globally, affecting approximately 628.8 million

people and standing as a primary cause of years lived with disability.¹ The Autoregressive Integrated Moving Average (ARIMA) model forecasts a sustained increase in the global prevalence of low back pain from 2022 to 2050, projecting that approximately 890 million individuals will be affected, with 380 million incident cases anticipated by 2050.¹ This condition not only significantly reduces patients' quality of life but also incurs substantial healthcare expenditures

and productivity losses, placing heavy burdens on global healthcare systems and socioeconomic development.

Intervertebral disc degeneration is a chronic degenerative disease caused by multiple factors, with its precise mechanisms not yet fully elucidated.² Its pathological features manifest as the destruction of disc tissue structural integrity and progressive loss of physiological function.^{3,4} Current clinical treatments for IVDD primarily include conservative management and surgical intervention. The former focuses on alleviating symptoms such as pain, while the latter emphasizes restoring or maintaining spinal structural stability. However, existing therapies have failed to address the fundamental biological issue of disc degeneration and restore the loss of structure and function in the degenerated disc itself.⁵ Conservative treatment offers only temporary symptom relief without reversing or halting the degenerative process. While surgical intervention is applicable for severe cases, it compromises spinal segmental mobility and may lead to adjacent segmental injury alongside intraoperative and postoperative complications. Neither approach achieves disc tissue regeneration or functional restoration.^{5,6} Consequently, developing novel therapeutic strategies that promote disc repair and regeneration has become a critical focus in current disc degeneration research.

Advancements in biomaterials provide critical technological support for regenerative therapies targeting disc degeneration. Among various biomaterials, hydrogels exhibit significant potential for treating disc degeneration due to their physicochemical properties, which are similar to those of nucleus pulposus tissue, and their excellent biocompatibility. By mimicking the composition and structure of the disc matrix, they provide a biomimetic microenvironment for cell growth. Additionally, they serve as delivery vehicles for drugs, genes, and cells, enabling targeted therapy.^{7,8} As a key derivative of hydrogel materials, hydrogel microspheres further overcome the limitations of traditional bulk hydrogels in delivery efficiency and adaptability.⁹ Hydrogel microspheres offer irreplaceable core advantages in treating IVDD. They exhibit excellent biocompatibility and the ability to construct biomimetic microenvironments that precisely mimic the physicochemical characteristics of the disc extracellular matrix. Furthermore, their controllable particle size enables delivery *via* minimally invasive injection, adapting to the disc's unique anatomical structure. In addition, they exhibit superior loading capacity, stably encapsulating bioactive components, such as drugs, genes, stem cells, and organoids, while effectively resisting degradation from the harsh pathological microenvironment of the intervertebral disc.¹⁰⁻¹²

In recent years, with the development and integration of regenerative medicine, materials science, and

bioengineering, hydrogel microspheres have not only achieved significant progress in traditional regenerative therapies, such as gene therapy, drug delivery, and cell therapy, but also demonstrated tremendous potential for the construction of intervertebral disc organoids. Organoids are 3D multicellular models that mimic the structure and function of natural tissues. Hydrogel microspheres, with their unique physicochemical properties and ability to construct biomimetic microenvironments, have become core scaffold materials for organoid culture. The combination of these two elements provides a novel platform for studying the mechanisms of IVDD, drug screening, and personalized treatment.¹³

This review introduces the complex pathological mechanisms of IVDD and existing treatment bottlenecks, summarizes the application of hydrogel microspheres in treating disc degeneration, and explores their mechanisms, progress, and advantages and disadvantages in therapeutic scenarios such as gene delivery, sustained drug release, and cell carriers. Furthermore, integrating cutting-edge research on disc organoid construction, this review analyzes the advantages and application prospects of hydrogel microspheres in simulating extracellular matrices, supporting organoid formation and functional expression, and modeling degenerative microenvironments. It explores the integration of hydrogel microspheres into organoid therapy, aiming to provide insights for advancing disc degeneration treatment from symptom relief to functional restoration and for implementing precision medicine and regenerative medicine.

2. Normal physiological structure and function of the intervertebral disc

As shown in [Figure 1](#), the intervertebral disc is a complex fibrocartilaginous structure composed of three components: the nucleus pulposus, annulus fibrosus, and cartilaginous endplates, which are structurally and functionally interdependent.¹⁴⁻¹⁶

The nucleus pulposus, located at the disc's center, is a water-rich gelatinous tissue primarily composed of hydrophilic proteoglycans and type II collagen.¹⁷ It also contains small amounts of type I collagen, elastin, and glycosaminoglycans. These proteoglycans possess strong hydrophilic properties, enabling them to bind large amounts of water and maintain the nucleus pulposus in a highly hydrated state. The water content of a healthy nucleus pulposus can reach 80–85%.¹⁸ The nucleus's high-water content effectively absorbs and disperses axial loads on the spine, acting as a shock absorber.^{19,20} Through its integration with the cartilaginous endplates, the nucleus facilitates the exchange of nutrients, including water, ions, and small molecules.

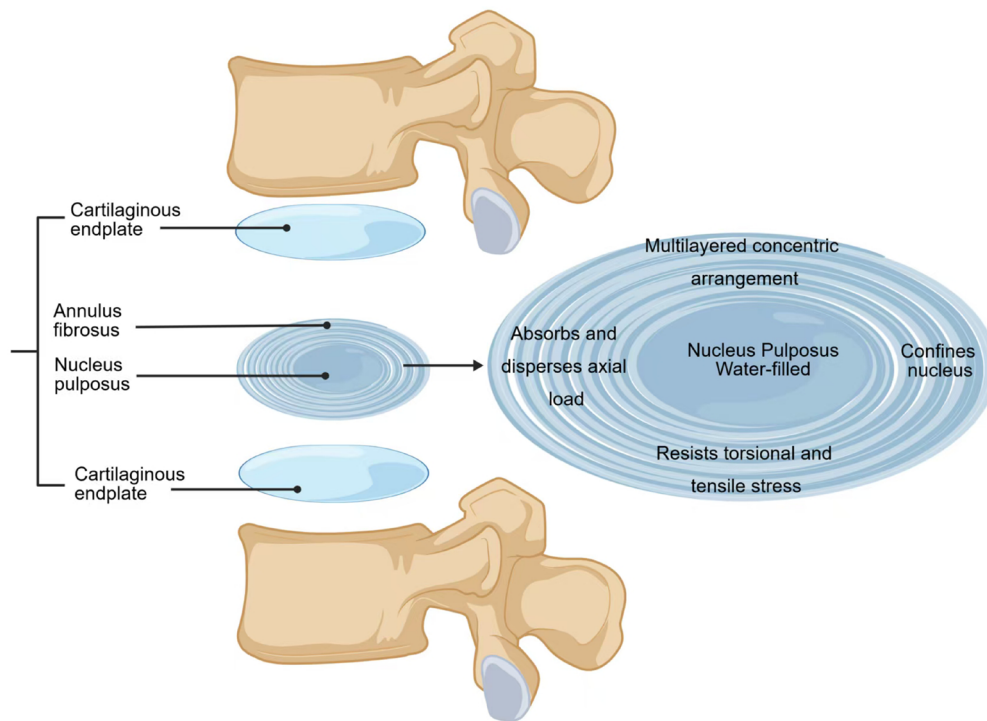


Figure 1. Schematic of the intervertebral disc structure. An intervertebral disc is a fibrocartilaginous joint between adjacent vertebrae, consisting of the central nucleus pulposus, the surrounding annulus fibrosus, and the superior and inferior cartilaginous endplates. This structure provides mechanical support, load distribution, and spinal flexibility.¹⁴ Created with BioGDP.com

The annulus fibrosus is the outer fibrous tissue surrounding the nucleus pulposus, composed of multiple concentric layers of fibrous cartilage rings.²¹ This structure not only confines the nucleus pulposus centrally but also resists torsional and tensile stresses, maintaining internal pressure to prevent disc protrusion.²² It also works synergistically with the nucleus pulposus to convert axial compressive loads into tensile stresses within the annulus fibrosus, thereby effectively dispersing mechanical pressure.²³

The cartilaginous endplate is a thin, dense layer of hyaline cartilage covering the upper and lower surfaces of the vertebral body.²⁴ It serves both as the structural interface between the intervertebral disc and the vertebral body and as a semipermeable membrane, allowing substances to diffuse into and out of the intervertebral disc. Reduced vascularization of the endplates compromises disc nutrition, accelerating disc degeneration.¹⁸ The cartilaginous endplates are crucial for maintaining disc cell survival and function.^{25,26} They also serve a connecting function, anchoring the annulus fibrosus and the nucleus pulposus to the vertebral body, forming a complete structural unit.²⁷ The nucleus pulposus, annulus fibrosus,

and cartilaginous endplates of the intervertebral disc do not operate independently but function synergistically as a complex biomechanical and biological unit.

Dysfunction among these three components is often interdependent and causally linked, and the impairment in any one may trigger a cascade of events, accelerating overall IVDD.²⁸ This interconnected nature enables healthy discs to withstand complex mechanical stresses and exhibit a degree of resilience.²⁹ Given the close interconnection of the internal structures of the intervertebral disc and its functional characteristics, using organoids as a research model shows great promise for understanding the mechanisms of IVDD and developing regenerative therapies.³⁰

3. Pathological mechanisms of intervertebral disc degeneration

Intervertebral disc degeneration is a complex pathological process driven by multiple factors, and its precise mechanisms remain incompletely elucidated.³¹ Current research generally recognizes that several interrelated theories collectively explain the onset and progression of disc degeneration, with the following core theories being

most representative:

(i) The nucleus pulposus initiation theory posits that disc degeneration originates from the functional decline of the nucleus pulposus.³² With aging or abnormal mechanical loading, the number of nucleus pulposus cells decreases, and their function deteriorates, leading to apoptosis, senescence, and phenotypic dedifferentiation. This impairs the synthesis of proteoglycans and type II collagen while simultaneously causing the overexpression of degradative enzymes, such as matrix metalloproteinases (MMPs) and a disintegrin and metalloproteinase with thrombospondin motifs (ADAMTS), leading to an imbalance in the extracellular matrix.^{33,34}

(ii) The annular rupture theory posits that chronic overuse, or acute trauma, can induce microfractures, delamination, or rupture in the annulus fibrosus. This compromise in structural integrity weakens its containment of the nucleus pulposus, facilitating disc displacement or herniation. It may also expose internal matrix components, triggering localized inflammatory responses.^{35,36} Following annular injury, inflammatory mediators accumulate at the site, impairing mitochondrial energy metabolism in annular cells. This impairs annular regeneration, creating a vicious cycle that accelerates disc degeneration.³⁷

(iii) The cartilage endplate degeneration theory posits that the cartilage endplates covering the upper and lower surfaces of the vertebral bodies serve as the primary conduit for intervertebral disc nutrient exchange. Once calcification, thinning, or microfractures occur, their permeability significantly decreases, leading to hypoxia and glucose deprivation within the disc. This accelerates nucleus pulposus cell apoptosis and extracellular matrix degradation.³⁸⁻⁴⁰

(iv) The inflammatory theory posits that the inflammatory microenvironment is a key driver of disc degeneration. Under trauma, mechanical stress, or biochemical stimuli, nucleus pulposus cells upregulate pro-inflammatory factors, such as interleukin (IL)-1 β , tumor necrosis factor- α (TNF- α), and IL-6, and activate signaling pathways, including nuclear factor-kappa B (NF- κ B) and mitogen-activated protein kinase (MAPK). These processes create a vicious cycle of “inflammation–extracellular matrix degradation.”⁴¹ Concurrently, inflammatory mediators recruit immune cells, such as M1 macrophages and neutrophils, promoting angiogenesis and nerve invasion, thereby exacerbating pain and degeneration.⁴²

(v) The oxidative stress theory posits that oxidative stress imbalance is a key pathological mechanism mediating disc degeneration, closely linked to and synergistically interacting with the other theories. Under conditions of hypoxia, abnormal mechanical loading, or inflammatory stimulation, increased reactive oxygen species (ROS)

production in disc cells exceeds the clearance capacity of the cell's antioxidant system, triggering oxidative stress responses.⁴³ Excessive ROS damages cellular DNA, lipids, and proteins, inducing apoptosis and senescence in nucleus pulposus and annulus fibrosus cells. This inhibits extracellular matrix synthesis while promoting degradative enzyme expression, further exacerbating extracellular matrix imbalance.^{44,45} Oxidative stress can also activate signaling pathways such as NF- κ B and p38 MAPK, promoting the release of pro-inflammatory factors and amplifying inflammatory responses. This creates a vicious cycle of “oxidative stress–inflammation–cell damage,” continuously driving the progression of disc degeneration.^{46,47}

(vi) The abnormal stress theory holds that abnormal mechanical loading is the core external trigger for the initiation and amplification of the aforementioned pathological processes, as well as a crucial initiating factor in the onset of IVDD. Abnormal mechanical loads caused by long-term poor spinal posture, excessive weight bearing, repeated flexion, extension, and torsion, or trauma—including excessive static compression, high-amplitude dynamic mechanical stimulation, non-physiological torsional stretching, and uneven distribution of mechanical loads—disrupt the physiological mechanical balance of intervertebral disc tissue.⁴⁸ This exceeds the physiological tolerance threshold of disc cells, serving as a trigger for a cascade of pathological changes. Abnormal mechanical stress can directly mediate mitochondrial and death receptor apoptotic pathways in nucleus pulposus and annulus fibrosus cells. This is achieved by activating mechanosensitive receptors, such as Piezo1, leading to excessive apoptosis of functional cells and directly initiating the pathological process of functional decline in the nucleus pulposus.⁴⁹ Abnormal mechanical stress can also directly upregulate the expression of degradative enzymes and inhibit extracellular matrix synthesis through the NF- κ B and p38 MAPK pathways, resulting in an imbalance in matrix metabolism.^{5,50} Furthermore, abnormal mechanical loading induces mitochondrial dysfunction in disc cells and promotes the massive production of ROS, acting as a core mechanical stimulator of oxidative stress. Meanwhile, it activates inflammatory signaling pathways downstream of mechanoreceptors, promoting the release of pro-inflammatory factors and laying the foundation for the formation of an inflammatory microenvironment. In turn, inflammation and oxidative stress exacerbate mechanical stress-induced cell damage and matrix degradation, forming a vicious cycle of “abnormal mechanical stress–inflammation–oxidative stress.”

Beyond these theories, additional hypotheses exist, including the infection theory, cellular senescence theory, and circadian rhythm disruption theory.⁵¹⁻⁵⁵ However,

current clinical and basic research widely recognizes the nucleus pulposus initiation theory as the core mechanism of disc degeneration. Its early functional impairment can cascade into annulus fibrosus rupture, endplate degeneration, and chronic inflammation, ultimately leading to complete structural and functional impairment of the entire disc (Figure 2).⁵⁶

4. Clinical management of disc degeneration

The current clinical treatment options for IVDD are primarily divided into early conservative management and late surgical intervention. Early conservative treatment aims to alleviate symptoms and improve quality of life, primarily suitable for patients with mild degeneration and localized symptoms. It focuses on symptomatic relief, mainly including pain management, anti-inflammatory therapy, physical therapy, and lifestyle adjustments, with medication being the primary treatment modality.⁵⁷ For example, nonsteroidal anti-inflammatory drugs inhibit cyclooxygenase activity to reduce prostaglandin synthesis, thereby achieving anti-inflammatory and analgesic

effects. Muscle relaxants relieve spasm in the muscles surrounding the spine, reducing discomfort caused by nerve compression.⁵⁸ Physical therapy modalities, such as lumbar traction and therapeutic massage, improve local blood circulation and enhance spinal stability, serving as common conservative treatment options.⁵⁹ However, conservative management only delays symptom progression and cannot reverse key pathological processes, such as nucleus pulposus cell apoptosis, extracellular matrix degradation, and annulus fibrosus rupture. While most patients experience short-term symptom relief after conservative treatment, the disease typically continues to progress over time.

Surgical intervention is considered only for severe cases unresponsive to conservative treatment. Common surgical approaches include discectomy and spinal fusion.⁶⁰ Discectomy involves removing protruding nucleus pulposus tissue *via* minimally invasive or open techniques, directly relieving nerve compression and rapidly alleviating pain symptoms. Spinal fusion, involving the implantation of fusion devices or bone grafts, stabilizes the affected vertebrae with adjacent ones to restore spinal stability. It is

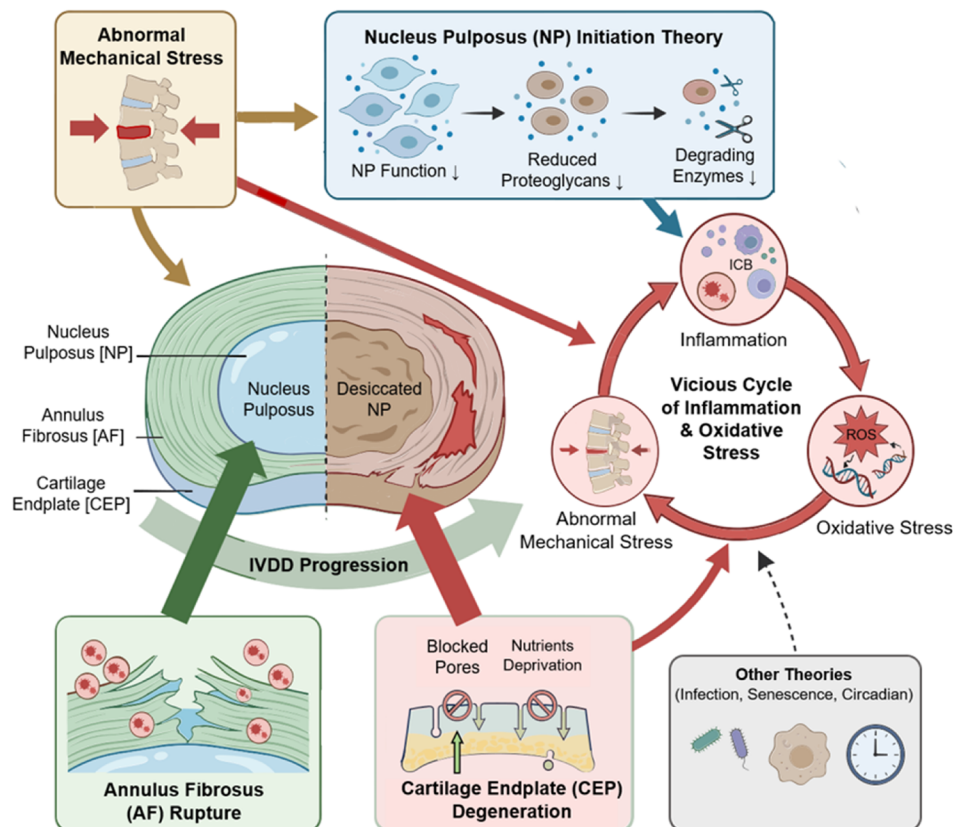


Figure 2. Intervertebral disc degeneration (IVDD) is driven by multiple interconnected mechanisms, among which the nucleus pulposus initiation theory is central. These mechanisms are not independent but interact synergistically, ultimately leading to structural and functional degeneration of the intervertebral disc. Image created by the authors with Microsoft PowerPoint.

Abbreviation: ICB: Inflammatory cell burden.

suitable for patients with degenerative changes accompanied by spinal instability. However, surgery inevitably causes iatrogenic damage to soft tissues such as ligaments and muscles, compromising the spine's original structural and functional integrity.⁶¹ Surgical interventions carry high risks of postoperative complications, particularly spinal fusion procedures, which frequently involve adjacent segment degeneration, infection, and implant-related issues.⁶² The long-term efficacy of surgical treatments remains insufficiently validated, with some patients continuing to experience chronic pain and disability post-surgery.⁶³

Currently, both conservative and surgical treatments have limitations, failing to fundamentally reverse the degenerative process of intervertebral discs or effectively restore their normal physiological structure and biomechanical function.⁶⁴ Meanwhile, artificial intelligence (AI) and machine learning have been widely applied in preoperative evaluation, surgical planning, and prognostic prediction of spinal degenerative diseases, providing objective and quantitative tools to improve the accuracy and personalization of clinical decision-making for IVDD.^{65,66} Therefore, exploring novel therapeutic approaches that can precisely target the lesion site, promote nucleus pulposus tissue regeneration, and achieve structural and functional repair has become a key direction in treating disc degeneration.

Against this backdrop, hydrogel microspheres leverage their unique physicochemical properties and biological functions to load bioactive substances, such as cells and growth factors. They offer advantages such as minimally invasive delivery, sustained-release drug delivery, and excellent tissue compatibility. This provides a novel approach to address the challenge of nucleus pulposus repair—unattainable with traditional therapies—and research on their application in treating disc degeneration is progressively unfolding.

5. Application of hydrogel microspheres in intervertebral disc degeneration

5.1. Preparation techniques for hydrogel microspheres

Hydrogel microspheres can be prepared through diverse methods. Current mainstream techniques include microfluidics, emulsion polymerization, electrospray technology, and photolithography, each with distinct characteristics and applicable scenarios.

Microfluidic technology enables precise control over the flow of multiphase fluids within microchannels, achieving controllable preparation of hydrogel microspheres. It is the most widely adopted method in current research.⁶⁷ Its core advantage lies in the high monodispersity of microspheres, with particle size precisely controlled by adjusting the flow

rate ratio between the oil and water phases and the channel dimensions.⁶⁸ Microfluidic technology can also enhance production through parallel channel design. In addition, microspheres prepared *via* microfluidics exhibit excellent injectability, enabling cell encapsulation, drug encapsulation, and functionalization.⁶⁹ Microfluidics further simulate *in vivo* microenvironments, enabling time-controlled release of bioactive factors by regulating microsphere degradation rates. It also promotes nutrient exchange and structural uniformity in organoid production.⁷⁰ However, the preparation of hydrogel microspheres via microfluidic technology currently has several limitations. In terms of devices, traditional microfluidic chips require sophisticated fabrication techniques, such as photolithography and soft lithography, which involve significant equipment investment and long processing cycles. The design and fabrication of some advanced structures (e.g., multi-compartment microspheres and parallelized channels) pose even higher barriers, making them difficult to implement in ordinary laboratories. In terms of production, it is challenging to balance high throughput with the uniformity of microspheres: increasing throughput leads to poorer uniformity, while ensuring uniformity restricts yield. Although a parallelized design can improve throughput, the single-device yield in microfluidics remains low compared with traditional industrial production methods, making it difficult to meet large-scale industrial demands.⁷¹

Emulsion polymerization is a traditional and straightforward preparation method. It involves mechanically mixing a hydrogel precursor solution with an oil phase to form droplets, followed by photopolymerization or temperature-induced crosslinking and curing.⁷² This method requires minimal equipment, offers rapid production, and is operationally straightforward. However, the resulting microspheres exhibit a broad particle size distribution and poor morphological uniformity, making precise size control challenging.⁷³ Batch emulsion polymerization requires surfactants to stabilize emulsions, and residual oil phase and surfactants can compromise cell viability, making this method unsuitable for fabricating microspheres loaded with highly active cells.⁷⁴

Electrospray technology overcomes solution surface tension by applying a high-voltage electric field, forming a Taylor cone at the needle tip to eject droplets that crosslink into microspheres.⁷⁵ The advantages of this method include mild preparation conditions that preserve the functionality of active substances, such as cells and proteins, and the ability to adjust particle size within a certain range by controlling process parameters.⁷⁶ However, significant drawbacks, including high polydispersity, the need for specialized equipment, and a challenging droplet-collection process, limit their application in scenarios demanding high precision.⁷⁷

Lithography technology fabricates hydrogel microspheres *via* photopolymerization on micro-scale templates. Its advantages include the ability to achieve customized morphologies, such as non-spherical shapes and high microsphere uniformity, thereby meeting specific morphological requirements.⁷⁸ Disadvantages include low production efficiency, difficult demolding operations, and material limitations primarily to acrylate-functionalized polymers, hindering widespread application.⁷⁹

In recent years, manufacturing technologies for hydrogel microspheres have been continuously innovated, and emerging processes such as 3D bioprinting and supercritical fluid technology have gradually emerged. The preparation of hydrogel microspheres via 3D printing enables precise control of size, morphology, and internal structure and allows for batch and uniform preparation. It can also stably encapsulate bioactive substances, such as cells and growth factors, within microspheres, reducing damage to these substances during the printing process. Meanwhile, it can be combined with microfluidic technology to improve preparation efficiency. The prepared microspheres can better simulate the *in vivo* microenvironment and meet the personalized demands of biomedical applications, including tissue engineering and drug delivery. Moreover, the printing process is easy to automate and standardize, reducing the risk of contamination.^{80,81} Supercritical fluid technology uses CO₂ as the core medium. With no organic solvent residues and mild preparation conditions, it can maximally retain the biological activity of loaded cells or proteins. It can regulate particle size, construct porous structures, and efficiently load hydrogel microspheres, while maintaining their high porosity and structural integrity through supercritical drying.⁸²

However, these advanced technologies still face non-negligible preparation challenges. 3D printing equipment and supporting materials are expensive, the parameter-optimization process is complicated, and high-level

operating skills are required. Some printing methods may affect the activity of internal bioactive substances due to shear forces, photo-crosslinking, and other factors. High-viscosity hydrogel materials are prone to nozzle clogging during printing. Large-scale and rapid preparation of microspheres remains limited by low efficiency. It is difficult to control the printing accuracy and stability of some composite-structure microspheres. Precise regulation of key indicators, such as mechanical properties and degradation rate of microspheres after printing, also presents certain difficulties.^{80,81} Although supercritical CO₂ drying can produce high-porosity chitosan microsphere aerogels, it suffers from cumbersome steps, time-consuming solvent exchange, high energy consumption and cost, and limited adaptability for processing polar natural polymers.⁸² In addition, the lack of standardized preparation processes and quality control standards makes it difficult to ensure batch-to-batch consistency of hydrogel microspheres, hindering their clinical translation.

As shown in Table 1, a variety of methods are currently available for preparing hydrogel microspheres. Different preparation methods significantly influence the morphology, particle size distribution, and functional properties of microspheres by regulating material crosslinking, droplet formation, and curing processes. Therefore, the selection of hydrogel microsphere preparation methods must comprehensively consider both dimensional precision and functional requirements, laying the foundation for their applications in gene therapy, drug delivery, cell therapy, and tissue engineering.

5.2. Key properties of hydrogel microspheres

As core carriers for cell delivery and drug loading, hydrogel microspheres critically depend on properties such as specific surface area, porosity, injectability, biocompatibility, leak resistance, and mechanical performance. These properties synergistically ensure optimal conditions for cell growth, material exchange, and delivery (Figure 3).

Table 1. Comparison of common preparation methods for hydrogel microspheres

Preparation method	Advantages	Key disadvantages	Application scenarios
Microfluidic technology	i. Precise control of particle size with high monodispersity ii. Excellent biocompatibility	i. High equipment investment ii. Difficult for large-scale production	i. Precision drug delivery ii. Organoid construction
Emulsion polymerization	i. Simple operation with low cost ii. High production efficiency	i. Wide particle size distribution ii. Poor biocompatibility	i. Mass production of conventional carriers ii. Low-cost basic materials
Electrospray technology	i. Mild preparation conditions ii. Preservation of the function of bioactive substances	i. Poor particle size consistency ii. Challenging collection process	i. Cell loading ii. Active protein delivery
Lithography technology	i. Customized special morphologies ii. High precision	i. Extremely low production efficiency ii. Complicated process	i. Preparation of microspheres with special morphologies ii. High-precision fundamental research

Specific surface area and porosity are key properties enabling hydrogel microspheres to support cell growth and material exchange. A well-developed porous structure provides an optimal microenvironment for cell adhesion and proliferation while ensuring nutrient diffusion and metabolic waste removal. For example, the epigallocatechin-3-gallate-loaded gelatin hydrogel (GPE), designed by Liu *et al.*⁸³, exhibited interconnected porous structures suitable for cell growth and mass transfer under scanning electron microscopy observation. Cheng *et al.*⁸⁴ prepared RGD-SA/HA-His-Sr (rSA-HHS) hydrogel microspheres that retained a porous structure after freeze-drying, with uniformly distributed pore sizes facilitating drug loading and nutrient diffusion. Mechanically reinforced bioactive sodium alginate composite hydrogel microspheres exhibit high specific surface area and permeability, facilitating cellular nutrient metabolism exchange.⁸⁵

Injectability is a crucial property for achieving minimally invasive delivery with hydrogel microspheres. This property fundamentally requires excellent flowability for delivery *via* minimally invasive needles while maintaining structural integrity post-injection. Xiao *et al.*⁸⁶ employed air-jet microfluidic fabrication to produce mineralized hydrogel

microspheres with uniform and controllable particle sizes. These microspheres demonstrated compatibility with standard clinical needles for injection delivery, showcasing excellent injectability and clinical adaptability.

Biocompatibility is primarily reflected in hydrogel microspheres' compatibility with cells and their ability to interact with them. It is a prerequisite for ensuring the safe *in vivo* application of carriers and promoting cellular function. GPE hydrogels showed no toxic effects on nucleus pulposus cell proliferation, with cell survival rates approaching those of the control group, demonstrating excellent biocompatibility.⁸³ rSA-HHS microspheres effectively enhance bone marrow cell proliferation by specifically binding to integrin subtypes such as $\alpha\text{v}\beta 3$.⁸⁴ Tests indicate higher survival rates and significantly improved migration capacity in the rSA-HHS group. These results highlight the superior biocompatibility of rSA-HHS microspheres. Despite the excellent overall biocompatibility of hydrogel microspheres, rigorous biosafety concerns remain a critical translational hurdle that necessitates specific optimization strategies. One primary concern is the potential cytotoxicity induced by residual chemical crosslinkers (such as glutaraldehyde)

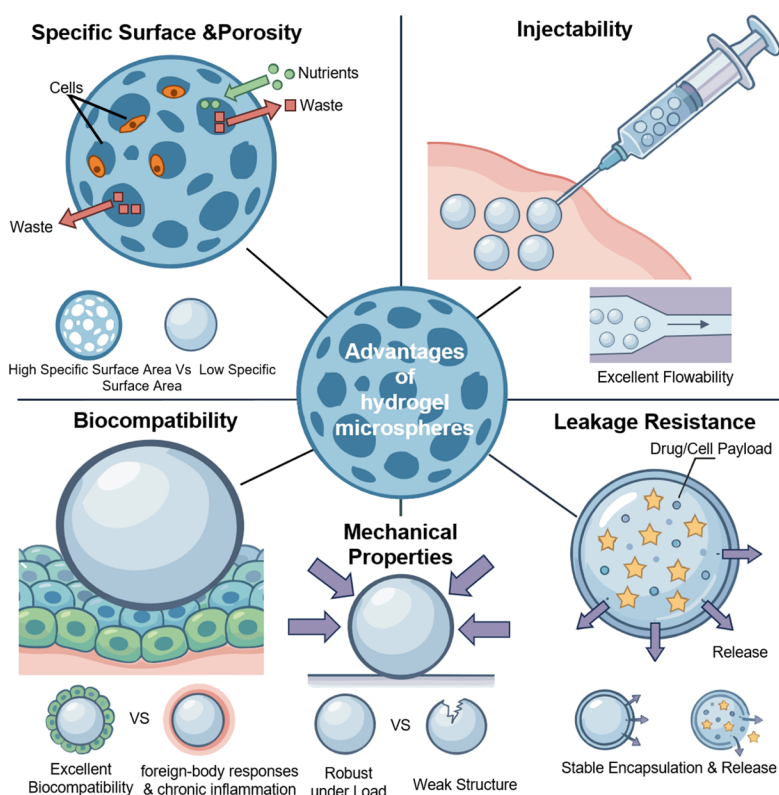


Figure 3. Hydrogel microspheres exhibit multiple favorable properties, including injectability, high specific surface area and porosity, tunable mechanical properties, leakage resistance, and excellent biocompatibility, making them promising carriers for the treatment of intervertebral disc degeneration. Image created by the authors with Microsoft PowerPoint.

and unreacted components during fabrication processes.⁸⁷ Prolonged accumulation of non-degradable components in the avascular intervertebral disc microenvironment can trigger foreign-body responses and chronic inflammation.⁸⁸ To address these issues, researchers are increasingly adopting safer crosslinking mechanisms, such as visible-light-induced photopolymerization, enzymatic crosslinking, and bio-orthogonal click chemistry, which can effectively reduce the generation of toxic byproducts.⁸⁷ Furthermore, utilizing highly purified, naturally derived polymers (e.g., gelatin methacryloyl [GelMA], hyaluronic acid, and alginate) and optimizing degradation kinetics to match the tissue regeneration rate are effective strategies to prevent local accumulation. In complex delivery systems, surface modifications—such as poly(ethylene glycol)ylation and biomimetic cell-membrane coating—are widely employed to mask immunogenic nanoparticles, thereby evading macrophage clearance and improving *in vivo* biosafety.⁸⁹ Nevertheless, long-term biosafety evaluation and standardized assessment frameworks remain required to fully support clinical translation.

The leakproof nature of hydrogel microspheres ensures stable release of drugs or cells during delivery and action. rSA-HHS hydrogel microspheres exhibit low swelling rates and slow degradation rates.⁸⁴ Specifically, strontium ion release under low pH conditions demonstrates sustained, pH-dependent release, mimicking the acidic microenvironment of degenerative intervertebral discs. This enables targeted drug release while reducing leakage risks. Mineralized hydrogel microspheres retained approximately 25% of their initial mass during a two-week *in vitro* simulated enzymatic degradation test, indicating suitable biodegradation rates and stable leak-proof performance.⁸⁶

Mechanical properties are core parameters for hydrogel microspheres. Sufficient mechanical strength enables them to withstand *in vivo* mechanical stress and maintain structural integrity. The compressive modulus of phenylboronic acid-modified GelMA (GP) and GPE hydrogels exceeds the 3–6 kPa range of native nucleus pulposus tissue, indicating adequate mechanical strength for nucleus pulposus replacement scaffolds.⁸³ The GelMA/alginate methacrylate/hydroxyapatite composite hydrogel, developed by Wang *et al.*⁹⁰, exhibited significantly enhanced mechanical properties compared to the single-component GelMA hydrogel, making it suitable for tissue repair scenarios with higher mechanical demands.

5.3. Gene therapy applications of hydrogel microspheres in intervertebral disc degeneration

Hydrogel microspheres, with their outstanding biocompatibility, injectable minimally invasive properties, and localized controlled-release capabilities, have become highly promising therapeutic gene delivery carriers in

orthopedic clinical settings. As an important delivery platform for IVDD repair, microsphere systems have been proven to efficiently achieve targeted local delivery of genes, drugs, and cells, providing a novel strategy for the regenerative treatment of degenerative intervertebral discs.⁹¹ Current research on delivering various gene types *via* hydrogel microspheres has made significant progress, primarily involving microRNAs (miRNAs/miR), small-interfering RNAs (siRNAs), and gene-editing tools. However, differences exist in the design concepts and methods of different delivery systems, such as targeting modification approaches, responsive mechanisms, and synergistic therapeutic characteristics.

MicroRNAs play pivotal roles in nucleus pulposus cell apoptosis, inflammation regulation, and extracellular matrix metabolism. However, standalone miRNA delivery is prone to degradation by nucleases and suffers from poor targeting, making it difficult to achieve synergistic delivery with other therapeutic agents. This represents a core challenge in miRNA delivery system design. Against this backdrop, Wang *et al.*⁹² developed an injectable tannic acid-loaded hydrogel system encapsulating multifunctional mitochondrial-protective gene nanocarriers, establishing a tripartite miRNA delivery system integrating targeting, responsiveness, and synergy. The system's design innovation lies in its hyaluronic acid oxidation coating, which targets CD44-high degenerative nucleus pulposus cells for precise lesion localization and resolves miRNA targeting deficiencies. The study employed MMP-2-sensitive peptide crosslinkers to construct methacrylated hyaluronic acid-based hydrogels, enabling pathologically responsive drug release that prevents premature leakage and efficacy loss. Concurrently, ss-31 peptide modification enhanced mitochondrial targeting, combining miR-21 inhibitor-mediated apoptosis suppression with mitochondrial protection to synergistically regulate extracellular matrix metabolic balance. This innovative multifunctional, multi-responsive hydrogel gene platform holds promise for overcoming longstanding challenges in IVDD therapy, including weak targeting, adverse microenvironments, and limited therapeutic efficacy. Qingxin *et al.*⁹³ developed a DNA hydrogel that further overcomes the limitations of traditional carriers. With sequence programmability and ultra-high biocompatibility, it enabled long-term stable delivery of miR-5590, remodeled extracellular matrix metabolism by regulating the autophagy–apoptosis balance, and simultaneously solved the core problems of easy degradation and post-injection leakage of naked miRNA, significantly improving the retention and repair efficacy of gene therapy in degenerative intervertebral discs.

Small-interfering RNA can precisely regulate the function of nucleus pulposus cells by specifically silencing target genes. However, achieving sustained siRNA

release, reducing off-target effects, and simultaneously improving the inflammatory microenvironment remain key challenges in current siRNA delivery research. Guo *et al.*⁹⁴ designed a smart microgel gene delivery system (dopamine-grafted hyaluronic acid [HADA]-encapsulated siGrem1 nanoparticle [HSGN]-loaded microspheres [MHSGN]) that leverages the synergistic effects of target gene silencing and ROS scavenging. By encapsulating siGrem1 within polyethyleneimine and modifying it with HADA, the system achieved CD44-targeted delivery while dopamine groups scavenged ROS. By downregulating apoptosis-related genes and upregulating extracellular matrix synthesis-related genes, it improved the pathological microenvironment of degenerated nucleus pulposus cells to exert therapeutic effects. In contrast, the GelMA hydrogel microsphere delivery system constructed by Zheng *et al.*⁹⁵ placed greater emphasis on integrating microenvironmental responsiveness with anti-inflammatory functions. By loading IL-33 siRNA onto phenylboric acid (PBA)-modified polyethyleneimine (PEI-PBA) and leveraging the MMP-responsive properties of GelMA hydrogel microspheres to modulate the degenerative disc microenvironment, they simultaneously suppressed inflammatory responses through IL-33 gene silencing, thereby indirectly protecting extracellular matrix components. Both studies employed a combined approach of targeted modification and responsive drug delivery, reflecting current research trends toward targeted, responsive, and multifunctional siRNA delivery systems. Chen *et al.*⁹⁶ further constructed a multifunctional responsive hydrogel cross-linked by multiple dynamic bonds. The siRNA targeting P65 was complexed with PBA-modified G5 poly(amidoamine) nanocarriers and then encapsulated in the hydrogel system, enabling pH-responsive release in the acidic microenvironment of degenerative intervertebral discs and sustained local release of siRNA for more than 28 days. This system significantly inhibited the inflammatory storm by silencing the P65/NOD-, LRR- and pyrin domain-containing protein 3 (NLRP3) inflammatory pathway. When combined with nucleus pulposus cell transplantation, it achieved long-term recovery of disc height and matrix in a rat degeneration model, providing a high-performance delivery platform for siRNA-mediated gene-cell synergistic therapy for IVDD.

In recent years, research on gene-editing tool delivery systems for IVDD has primarily focused on carrier functions adapted to pathological microenvironments and precise molecular targeting mechanisms. The BLNP@GF system developed by Gong *et al.*⁹⁷ innovatively integrates biomimetic material design with pathological intervention requirements. Leveraging the porous structure of GelMA/fucoidan (FU) dual-component hydrogel microspheres, it achieved both efficient encapsulation and sustained release of brachyury (BRY) messenger RNA (mRNA)-

loaded lipid nanoparticles (LNPs). The anti-inflammatory properties of the FU component created a stable repair environment for nucleus pulposus cells. The LNP carrier fundamentally improved the extracellular matrix metabolic imbalance by upregulating type II collagen and aggrecan synthesis while inhibiting MMP-3 activity. This synergistic design of anti-inflammatory pretreatment and metabolic regulation adapts the carrier's function to the pathological environment, enhancing therapeutic efficacy. Zhang *et al.*⁴⁰ innovatively identified *LOXL2* as a key regulatory gene in disc degeneration by cross-validating three machine learning algorithms on aging-related genes, providing a precise target for gene therapy. Building upon this, they developed an ROS-responsive poly(vinyl alcohol)-transient/self-healing PBA hydrogel delivery system. This system ingeniously utilizes elevated ROS levels in the degenerative microenvironment to trigger drug release, enabling lysyl oxidase-like protein 2 (LOXL2) to target nucleus pulposus cells. By directly binding to the *NOTCH1* promoter and inhibiting the NOTCH pathway, it achieved multiple effects: reducing cellular senescence, improving mitochondrial function, and promoting extracellular matrix synthesis. This approach resolves issues such as imprecise targeting and disordered release inherent in traditional gene delivery methods.

As an innovative approach for treating disc degeneration, hydrogel microsphere-mediated gene therapy demonstrates advantages in precise microenvironment responsiveness and targeted delivery. These microspheres respond to pathological microenvironment features of disc degeneration to trigger gene release. For example, GelMA microspheres' specific response to MMPs enables precise release of IL-33 siRNA at degenerative sites.⁹⁵ Regarding gene protection and efficient transfection, composite carriers combining hydrogel microspheres with nanoparticles effectively shield nucleic acid drugs from enzymatic degradation. For example, PEI-PBA nanoparticles enhance siRNA internalization efficiency through electrostatic encapsulation⁹⁵, while LNP, as an mRNA carrier, protects *BRY* mRNA from nuclease degradation and facilitates intracellular delivery.⁹⁷ Additionally, they can exhibit multifunctional synergistic therapeutic effects. For example, the MHSGN system integrates targeted delivery, ROS scavenging, anti-inflammatory, and gene silencing functions to multidimensionally improve the survival environment of nucleus pulposus cells⁹⁴, yielding more significant effects than those of single-gene therapy or drug treatment alone. Hydrogel microspheres also exhibit excellent injectability, enabling local puncture administration to avoid open surgical trauma. Naturally derived materials such as GelMA, hyaluronic acid methacryloyl (HAMA), and FU demonstrate superior biocompatibility and produce non-toxic degradation products. Their mechanical properties

closely resemble those of normal nucleus pulposus tissue, providing temporary mechanical support.^{95,97}

However, this therapeutic approach still faces significant limitations. The low proliferative capacity of nucleus pulposus cells and the avascular environment of the intervertebral disc constrain gene delivery depth. Directly injected nanoparticles are prone to loss due to disc mechanical loading.⁹⁵ The degradation rate of hydrogel microspheres is difficult to precisely match the gene action cycle; for example, GelMA/FU microspheres exhibit diminished anti-inflammatory effects during long-term *in vivo* treatment.⁹⁷ Although the MHSGN system achieves sustained release, the retention time of siRNA within the disc still requires extension.⁹⁴ Safety concerns remain unresolved, including potential cytotoxicity of cationic polymers and LNPs, off-target risks of siRNA, and immunogenicity of mRNA. Pathological mechanisms of disc degeneration involve intertwined factors, including inflammation, oxidative stress, and mechanical injury. Single-gene targets struggle to comprehensively cover the pathological network, while existing carriers primarily address single or dual microenvironment signals, making them ill-suited for dynamically evolving pathological processes.⁹⁸ These bottlenecks require further investigation through the development of multi-responsive carriers and validation in large animal models. Furthermore, recent reviews in nanomedicine have highlighted that microsphere-based delivery systems are the core strategy for overcoming the avascular nature of intervertebral discs and achieving precise gene delivery. Meanwhile, key issues such as carrier safety, transfection efficiency, and clinical translation also need to be systematically addressed.⁹⁹

5.4. Therapeutic applications of hydrogel microspheres in intervertebral disc degeneration

Hydrogel microspheres, with their excellent biocompatibility and controllable drug-release capabilities, serve as ideal carriers for drug delivery systems in IVDD. Current therapeutic approaches based on hydrogel microspheres primarily involve loading bioactive substances with diverse mechanisms, such as antioxidant, anti-inflammatory, and pro-differentiation effects, to target improvements in the disc's inflammatory microenvironment, suppress oxidative stress, and promote nucleus pulposus matrix synthesis.¹⁰⁰

Hydrogel microspheres loaded with antioxidant components effectively address the limitations of traditional antioxidant drugs. Current antioxidant therapy research primarily follows two approaches: direct scavenging of ROS and activation of endogenous antioxidant pathways. The SeNPs@GelMA hydrogel microspheres, designed by Lv *et al.*¹⁰¹, utilize GelMA as a carrier to load selenium nanoparticles (SeNPs) and achieve

controlled release of selenium, offering a key advantage by activating the endogenous antioxidant enzyme glutathione peroxidase 1 (GPX1). This approach fundamentally maintained mitochondrial function in nucleus pulposus cells, providing insights for endogenous antioxidant therapy research. Meanwhile, Jin *et al.*¹⁰² designed GelMA@CINPs hydrogel microspheres by innovatively selecting naturally sourced cuttlefish ink nanoparticles (CINPs) as antioxidant components, establishing a dual antioxidant mechanism of direct scavenging and endogenous activation, thereby effectively addressing the limitations of single-antioxidant approaches. Shen *et al.*⁴⁹ developed MS@MCL nanozyme-functionalized hydrogel microspheres by covalently binding manganese dioxide (MnO₂)-lactate oxidase (LOX) composite nanozymes to HAMA microspheres, achieving synergistic regulation of lactate consumption, anti-inflammation, and antioxidant effects. Further overcoming the limitations of conventional antioxidant therapies, this approach integrates antioxidant effects with the improvement of the lactic acid-enriched microenvironment in degenerative intervertebral discs, establishing a multi-step synergistic regulatory system that combines pathological microenvironment optimization with antioxidant treatment. The GM@CS-BP system by Li *et al.*¹⁰³ and the Fu@GelMA-MS microspheres by Li *et al.*¹⁰⁴ advance the mechanistic understanding of antioxidant therapy. Based on the pathological characteristics of disc degeneration—where oxidative stress and inflammation mutually reinforce and exacerbate matrix degradation—these approaches integrate antioxidant, anti-inflammatory, and matrix metabolic regulation, demonstrating superior efficacy compared to monotherapy. Wang *et al.*¹⁰⁵ constructed a fibrin composite platelet vesicle hydrogel, FG@PEV, that further expanded the regulatory dimension. This system significantly inhibited NLRP3/gasdermin D pathway-mediated pyroptosis and reduced the release of inflammatory factors, such as IL1 β , by addressing fatty acid metabolism disorders in nucleus pulposus cells. *In vitro*, it scavenged ROS and reduced lactate accumulation; *in vivo*, it effectively maintained the disc height index and nucleus pulposus T2 signal, and significantly retarded the degenerative process within eight weeks, providing a new paradigm for hydrogel therapy targeting pyroptosis and metabolic disorders.

The imbalance in extracellular matrix metabolism triggered by excessive release of inflammatory factors is also a key factor in IVDD. Therefore, targeting inflammatory pathways with anti-inflammatory factors or inhibitors has become another core direction for hydrogel microsphere drug delivery. Hong *et al.*¹⁰⁶ developed chondroitin sulfate-functionalized GelMA microspheres that synergistically achieve anti-inflammatory effects and matrix protection by loading IL-1ra, overcoming the limitations of single-

agent anti-inflammatory therapies. The dual-drug microsphere system designed by Cheng *et al.*¹⁰⁷ enabled sequential synergistic anti-inflammatory effects of IL-4 and kartogenin (KGN). Its innovation lies in precisely capturing the inflammatory characteristics of different stages of disc degeneration, achieving phased inflammatory regulation through sequential delivery, and demonstrating superior therapeutic specificity compared to single-drug delivery. Ma *et al.*¹⁰⁸ developed pH-responsive HAMA microspheres enabling targeted, on-demand release of the IL-1 receptor antagonist. This advances inflammation regulation toward intelligent responsiveness by leveraging the acidic microenvironment of degenerated discs for directed drug delivery. This approach enhances therapeutic precision while minimizing drug accumulation in healthy tissues. Wang *et al.*¹⁰⁹ constructed a GP hydrogel and established a quercetin-loaded GP system by dynamically conjugating quercetin via boronate ester bonds. This system enables pH-responsive drug release in the acidic microenvironment of degenerative intervertebral discs. It not only efficiently eliminated senescent nucleus pulposus cells and downregulated senescence markers, including p16 and p21, but also inhibited the expression of senescence-associated secretory phenotype-related inflammatory factors, such as IL1 β and IL6, while restoring the synthesis of type II collagen and proteoglycans. A single injection in rat models significantly maintained disc height, improved the water content of the nucleus pulposus, and alleviated degeneration-related mechanical low back pain, providing an important paradigm for intelligent responsive delivery systems with both anti-senescence and anti-inflammatory functions.

The essence of disc degeneration lies in the functional decline of nucleus pulposus cells and loss of matrix. Therefore, loading bioactive molecules that promote stem cell differentiation and induce mesenchymal stem cells to differentiate into nucleus pulposus-like cells is key to achieving long-term repair and represents a core focus of current research. Chen *et al.*¹¹⁰ grafted Foxy5 and antioxidative peptide onto GelMA, loaded with bone marrow-derived mesenchymal stem cells, achieving triple synergistic therapy that effectively addressed the challenges of stem cell apoptosis and low differentiation efficiency in harsh pathological microenvironments. Wang *et al.*¹¹¹ selected adipose-derived stem cells as seed cells to establish a KGN–platelet-rich plasma (PRP) synergistic regenerative system. The widely available and easily accessible adipose-derived stem cells lower clinical application barriers, while PRP supplementation enhances growth factor signaling, further refining the regenerative mechanism. The dual-protein synergistic delivery strategy proposed by Bello *et al.*¹¹² enabled precise regulation of stem cell differentiation

direction, addressing the critical challenge of abnormal terminal differentiation. Through the synergistic action of transforming growth factor (TGF)- β 3 and matrilin-3, it promoted cartilage differentiation while preventing calcification, aligning with intervertebral disc tissue characteristics.

The circadian clock is a ubiquitous endogenous timing system regulating approximately 24-h physiological and behavioral rhythms. Recent studies indicate that circadian rhythm disruption is closely associated with the onset and progression of various chronic diseases, including IVDD and disc cell dysfunction. Nucleus pulposus cells also possess an intrinsic circadian clock, and dysfunction of this clock may contribute to disc degeneration.¹¹³ Delivering circadian rhythm-modulating factors *via* hydrogel microspheres can activate disrupted biological clocks in degenerated nucleus pulposus cells, thereby improving their physiological function and promoting disc regeneration.^{53,54} Alternatively, hydrogel microspheres can release therapeutic agents at specific time points to maximize therapeutic efficacy while minimizing side effects.¹¹⁴

Hydrogel microspheres have established a well-defined system for drug therapy in IVDD, evolving from single-drug delivery to multi-component, multi-mechanism synergistic delivery. Carrier materials and preparation techniques are also undergoing continuous optimization. As the core carrier for drug delivery in IVDD, hydrogel microspheres demonstrate significant advantages through designs tailored to pathological characteristics and clinical needs. Their precise targeting capability enables minimally invasive injection directly into degenerated disc sites, avoiding systemic side effects. The controlled degradation properties of these carriers enable on-demand, sustained drug release, overcoming the challenge posed by the short half-lives of bioactive molecules. Examples include SeNPs@GelMA microspheres sustaining 28-day drug release and IL-1 α -loaded microspheres maintaining effective concentrations for 20 days.¹⁰⁶ However, existing sustained-release systems predominantly exhibit single-rate release and cannot dynamically adjust to varying degeneration severity, which represents a key focus for future optimization. Hydrogel microspheres can also leverage multifunctional synergistic therapy advantages. Through carrier functionalization or dual-drug loading, a single system can integrate multiple functions, such as antioxidant, anti-inflammatory, and pro-differentiation effects. For example, microspheres consisting of Wnt5a-mimetic peptide Foxy5- and the antioxidative peptide-grafted gelatin methacryloyl matrix (GFA) simultaneously possess ROS scavenging, inflammation suppression, and stem cell differentiation induction capabilities¹¹⁰, while GelMA-chondroitin sulfate

(CS)-IL-1ra microspheres combine the anti-inflammatory effects of IL-1ra with the pro-matrix synthesis properties of CS¹⁰⁶, significantly enhancing therapeutic efficacy. Drug-loaded carrier materials, predominantly composed of highly biocompatible components such as GelMA and CS, reduce the risk of immune rejection.^{106,107} Microfluidic technology enables precise control over microsphere size and pore structure, matching their mechanical properties to natural nucleus pulposus tissue. For example, SeNPs@GelMA microspheres exhibit a loss factor similar to that of rat nucleus pulposus tissue, providing cells with an appropriate mechanical microenvironment.¹⁰¹

Despite significant advantages, hydrogel microspheres face multiple limitations. Insufficient drug loading and release efficiency are most prominent, with physical adsorption or electrostatic binding often causing drug burst release. For example, IL-4-poly(lactic-co-glycolic acid) (PLGA) microspheres exhibit 82% drug release within 10 days.¹⁰⁷ Additionally, the intervertebral disc microenvironment affects carrier degradation, leading to a mismatch between drug release and pathological progression. The key to overcoming this challenge lies in optimizing binding mechanisms and developing smart, responsive, sustained-release systems. *In vivo* retention and insufficient targeting further limit therapeutic efficacy. The avascular nature of the intervertebral disc makes microspheres prone to displacement under pressure or movement¹⁰⁶, and existing systems lack lesion-specific recognition, leading to drug wastage. Future approaches may enhance targeting through microsphere surface modification and reduce displacement by optimizing adhesion properties.

The gap between large animal validation and clinical translation remains a significant barrier. Most existing studies rely on rat caudal vertebra models^{101,102}, which differ markedly from human lumbar vertebrae. Furthermore, the lack of long-term safety and efficacy data in large animals, along with unresolved issues, such as the metabolism of microsphere degradation products, hinders clinical translation. There is a need to establish large-animal models more closely aligned with clinical conditions and to refine preparation and quality control standards. An incomplete understanding of therapeutic mechanisms also impacts efficacy. The pathological process of disc degeneration is complex, and most microsphere systems can only intervene in 1–2 stages. For example, GFA microspheres do not intervene in cellular senescence¹¹⁰, and KGN-PLGA microspheres do not regulate the cellular senescence process.¹¹¹ Moreover, most studies are confined to animal or *in vitro* models, with clinical applicability yet to be validated. This limitation stems from insufficient

exploration of underlying molecular mechanisms.¹¹⁵

5.5. Cell therapy applications of hydrogel microspheres in intervertebral disc degeneration

Cell therapy represents a core direction for IVDD repair. A key therapeutic bottleneck lies in the degenerative disc microenvironment, which readily induces seed cell apoptosis, inadequate adhesion, and functional loss. Hydrogel microspheres can mimic the extracellular matrix, provide bioactive support, and modulate the local microenvironment. Leveraging this unique advantage, hydrogel microspheres have become the preferred choice for carrier construction in cell therapy. Current therapeutic designs focus on adapting carriers to seed cell characteristics, improving the degenerative microenvironment, and maintaining cellular function. This enables enhanced survival, adhesion, and functional preservation of seed cells such as nucleus pulposus cells and mesenchymal stem cells.

The core requirements for nucleus pulposus cell carrier development include mimicking extracellular matrix properties, resisting oxidative stress, and promoting cell adhesion and matrix secretion. Existing research advances around these core objectives, though design emphases and practical applicability vary significantly. Yang *et al.*¹¹⁶ utilized microfluidic technology to prepare TBA@Gel&Chs hydrogel microspheres. Their gelatin component provides arginine–glycine–aspartic acid (RGD) sequences to promote cell adhesion, while chitosan sulfate mimics the extracellular matrix and confers antioxidant properties. Structural stability is enhanced through Schiff base cross-linking, significantly improving nucleus pulposus cell adhesion, proliferation, and extracellular matrix secretion. Additionally, it protects cell viability by scavenging extracellular ROS and upregulating endogenous antioxidant proteins sirtuin 3 (SIRT3) and superoxide dismutase 2 (SOD2). In 2023, Tang *et al.*¹¹⁷ developed GelMA/HA-His-Mg²⁺ (GHHM) dual-network hydrogel microspheres incorporating Mg²⁺ to achieve multifaceted protection for nucleus pulposus cells, overcoming the limitations of standalone antioxidant approaches. Dai *et al.*¹¹⁸ designed GelMA/HAMA-KGN composite hydrogel microspheres (GHKM) that released KGN *via* esterase-responsive mechanisms. This activated the nuclear factor erythroid 2–related factor 2 (NRF2) antioxidant pathway, promoted extracellular matrix synthesis (aggrecan and type II collagen alpha 1 chain) in nucleus pulposus cells, and suppressed degradative enzyme (MMP-13) expression. In 2024, Wang *et al.*¹¹⁹ developed SCGP hydrogels combining sodium alginate with gelatin microcapsules. Dual crosslinking enhanced mechanical properties, while the sustained-release peptide P2 synergistically protected nucleus pulposus cells from oxidative stress, mitigating

mitochondrial damage and apoptosis.

Mesenchymal stem cells serve as ideal seed cells for intervertebral disc cell therapy. The core of carrier construction lies in enhancing cell retention rates, resisting adverse microenvironments, and inducing directed differentiation. Current research has evolved from simple protection to precise regulation. In 2024, Zhao *et al.*¹²⁰ demonstrated that the porous structure of hydrogels can precisely localize mesenchymal stem cells and shield them from mechanical injury. Composite hydrogels, such as alginate containing polyethylene glycol diacrylate microgel, prolonged the retention and survival time of mesenchymal stem cells under high-pressure intervertebral disc conditions while promoting extracellular matrix synthesis. In the same year, Peng *et al.*¹²¹ addressed previous limitations in weight-bearing protection and nutrient deficiency by microfluidically fabricating LMGDNP microspheres, which were generated by immersing LOX-MnO₂ nanozyme into glucose-enriched decellularized nucleus pulposus matrix (DNPM) hydrogel microspheres. They provided nutrients to mesenchymal stem cells while consuming lactate, improving the cellular survival environment and enhancing core cell phenotype. In 2025, Chen *et al.*¹²² utilized photolithography to fabricate 3% agarose microcavity structures, promoting the self-assembly of bone marrow mesenchymal stem cells into spherical organoids. This 3D microenvironment enhanced cell survival, extracellular matrix secretion, and cartilage marker expression, outperforming traditional 2D culture. Zhou *et al.*¹²³ started from the physiological hypoxic microenvironment of the intervertebral disc and fabricated interpenetrating network hypoxia-inducible hydrogel microspheres using microfluidic technology. Through laccase-mediated oxygen consumption reaction, the local oxygen tension was reduced by one-third, and the hypoxic state was stably maintained for up to five days, effectively activating the phosphoinositide 3-kinase (PI3K)/AKT/hypoxia-inducible factor (HIF)-1 α pathway in endogenous stem cells. Meanwhile, neural stem cell exosomes were loaded to recruit endogenous progenitor cells, synergistically promoting the directed differentiation of stem cells into nucleus pulposus-like cells and significantly upregulating the expression of type II collagen and proteoglycans. In the rat degeneration model, the structural and functional maintenance of the intervertebral disc was achieved for up to eight weeks, providing a novel strategy for IVDD repair via hypoxic microenvironment remodeling combined with endogenous stem cells. In 2026, Tang *et al.*¹²⁴ highlighted that natural polymer hydrogels, such as alginate and hyaluronic acid, possess extracellular matrix-like properties. These were further optimized through modification with bioactive molecules to enhance the survival, proliferation, and differentiation of

mesenchymal stem cells.

Through optimized composition, structure, and functional modifications, hydrogel microspheres provide suitable 3D scaffolds for various seed cell types. Combined with strategies such as antioxidation, anti-inflammation, nutrient supply, and differentiation induction, they effectively address critical issues of cell survival and functional maintenance within the degenerative disc microenvironment.¹²⁵ Cell therapy applications in disc degeneration offer multifaceted advantages but also pose certain challenges. Regarding cell survival rates, hydrogel microspheres as carriers significantly enhance cellular viability. For example, SeNPs@GelMA microspheres exhibited excellent biocompatibility, supporting high survival rates of nucleus pulposus cells and significantly promoting their proliferation at high doses.¹⁰¹ Decellularized tissue matrices hydrogel derived from spinal cord (DSCM gel), characterized by high porosity and coarse nanofiber diameter, facilitated nucleus pulposus cell survival.¹²⁶ Regarding cellular function maintenance, hydrogel microspheres effectively support normal physiological functions. SeNPs@GelMA microspheres significantly enhanced the extracellular matrix synthesis capacity of nucleus pulposus cells, promoting the expression of key genes and proteins, such as aggrecan, type II collagen, and SRY-box transcription factor 9 (SOX9), while simultaneously suppressing the expression of matrix-degrading enzymes MMP-13 and ADAMTS5.¹⁰¹ Regarding immune response regulation and inflammation suppression, the carrier system demonstrates notable advantages. In IL-1 β -induced inflammatory environments, SeNPs@GelMA microspheres protected nucleus pulposus cells from inflammation-induced matrix degradation and mitochondrial dysfunction, suppressed inflammation-related enzyme expression, and maintained selenoprotein levels (e.g., GPX1), exhibiting regulatory benefits for cellular immune responses.¹⁰¹ GelMA hydrogel microsphere carriers exhibit excellent biocompatibility and sensitivity to extracellular enzymatic reactions, overcoming the limitations of traditional microsphere carriers in drug delivery stability and immune responses. However, challenges remain in cell therapy. Research on SeNPs@GelMA microspheres indicates that precisely regulating cellular abnormalities and immune responses remains a critical challenge.¹⁰¹ Furthermore, most current carriers focus on single-function regulation, lacking comprehensive synergistic design encompassing adhesion, protection, nutrition, and differentiation—an area warranting continued exploration.

As shown in Table 2, each of the three therapeutic approaches has unique characteristics, advantages, and disadvantages. With excellent biocompatibility, injectability, tunable mechanical properties, and smart,

Table 2. Comparison of microsphere-based systems for gene, drug, and cell therapy

Comparison dimension	Microspheres for gene therapy	Microspheres for drug therapy	Microspheres for cell therapy
Core payload	miRNA, siRNA, mRNA, gene-editing tools	Antioxidants, anti-inflammatory factors, pro-differentiation small molecules, growth factors	Nucleus pulposus cells, bone marrow/adipose mesenchymal stem cells
Key function	Targeted inhibition of pathogenic genes, regulation of cell senescence/inflammation/metabolism	ROS scavenging, anti-inflammation, promotion of stem cell differentiation, rhythm regulation	Protection of cell survival, promotion of adhesion/proliferation, enhancement of extracellular matrix synthesis
Biocompatibility	Good; degradation products are non-toxic	Excellent; no obvious immunogenicity	Excellent; supports cell colonization and functional expression
Delivery efficiency	Good nucleic acid protection, improved transfection efficiency	High drug loading, minimal burst release, long release cycle	High cell retention rate, resistance to mechanical extrusion/loss
Microenvironment adaptation	Alleviates inflammation, inhibits matrix degradation, anti-apoptosis	Antioxidation, anti-inflammation, lactic acid/nutrient metabolism regulation	Antioxidation, anti-inflammation, provides nutritional support
Therapeutic limitation	Limited efficacy for a single target, <i>in vivo</i> retention needs improvement	Prone to drug leakage, insufficient targeting ability	Prone to cell apoptosis, heterogeneous differentiation
Representative material system	MeHA, GelMA-PEI, PVA-tsPBA	GelMA, HAMA, sodium alginate, PLGA	GelMA/chitosan, sodium alginate, agarose

Abbreviations: GelMA: Gelatin methacryloyl; HAMA: Hyaluronic acid methacryloyl; MeHA: Methacrylated hyaluronic acid; miRNA: MicroRNA; mRNA: Messenger RNA; PEI: Polyethylenimine; PLGA: Poly(lactic-co-glycolic acid); PVA: Poly(vinyl alcohol); ROS: Reactive oxygen species; siRNA: Small-interfering RNA; tsPBA: Transient/self-healing phenylboric acid.

responsive capabilities, hydrogel microspheres serve as ideal carriers for gene therapy, drug therapy, and cell therapy in IVDD. Hydrogel microspheres can be prepared using microfluidics and other diverse techniques, and possess key properties including a high specific surface area, porosity, and anti-leakage capacity. They enable minimally invasive and targeted delivery of nucleic acid drugs, antioxidants, anti-inflammatories, and pro-differentiation bioactive substances, as well as seed cells, such as nucleus pulposus cells and mesenchymal stem cells, thereby enabling spatiotemporally controlled release of genes, sustained drug release, and maintenance of cell survival and function. Hydrogel microspheres can multidimensionally improve the inflammatory and oxidative stress microenvironments in degenerative intervertebral discs and promote extracellular matrix synthesis. Nevertheless, current applications still face challenges such as limited delivery depth, difficulty in matching the degradation rate to the therapeutic cycle, insufficient standardization of preparation processes, a lack of preclinical data in large-animal models, and the failure of single carriers to cover the complex pathological network.

In summary, hydrogel microspheres serve as multifunctional delivery platforms that enable sustained and targeted release of therapeutic agents for pharmacotherapy, gene therapy, and cell therapy in the treatment of IVDD (Figure 4). Their unique functionalities continue to expand their applications in IVDD, facilitating the construction of organoids that mimic the structure and function of the intervertebral disc.

6. Application prospects of hydrogel microspheres in intervertebral disc organoid construction

6.1. Definition and function of organoids

An organoid is a 3D multicellular structure formed by stem cells or tissue-specific precursor cells in a 3D culture system through cell-cell adhesion, self-assembly, and differentiation. It possesses self-renewal and self-organization capabilities, capable of mimicking the complex structure, cellular composition, spatial architecture, and partial physiological functions of the corresponding organ or tissue.^{127,128} Organoid construction relies on bio-inspired microenvironments, including mechanical support provided by bioactive materials and soluble factors regulating cell development, proliferation, and fate determination.^{129,130} As a new generation of *in vitro* biomimetic models, organoids are established based on decades of technological advances in epithelial stem cell research and are combined with basement membrane-derived hydrogel 3D culture systems. They have become a key bridge connecting traditional 2D cell culture and animal models, achieving significant improvements in physiological simulation fidelity.¹³¹

As 3D miniature organ models derived from stem cells, organoids can highly recapitulate the structural and functional complexity of human organs, providing unparalleled advantages over traditional 2D culture for

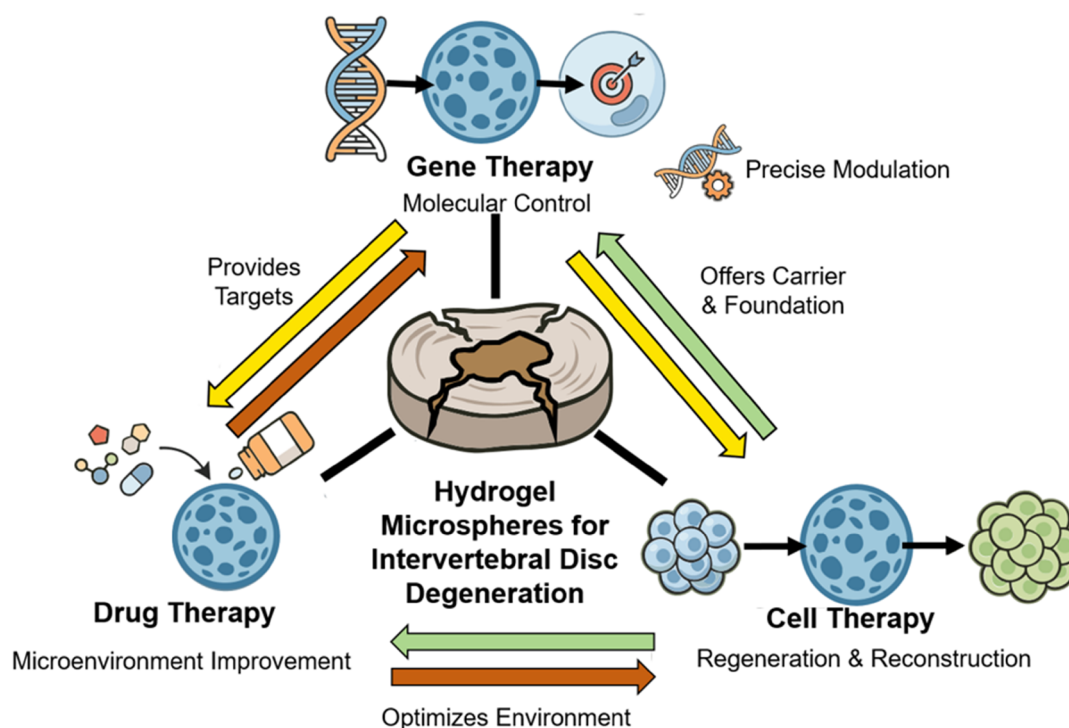


Figure 4. Hydrogel microspheres serve as multifunctional delivery platforms, enabling sustained and targeted release of therapeutic agents for drug therapy, gene therapy, and cell therapy in the treatment of intervertebral disc degeneration. Image created by the authors with Microsoft PowerPoint.

studies of tissue development, disease progression, and therapeutic intervention.¹³² Leveraging their structural and functional properties, organoids offer unique advantages in studying disease mechanisms. They can mimic interactions among multiple cell types within tissues and their microenvironments, facilitating research into developmental mechanisms, disease progression, and drug responses. For example, in the study of IVDD mechanisms, researchers used intervertebral disc organoids to simulate the degeneration process of nucleus pulposus cells *in vivo*.¹³³ It was observed that nucleus pulposus cells within organoids exhibited morphological changes, reduced matrix synthesis, and high expression of inflammatory factors consistent with the degenerative state *in vivo*, clearly revealing the molecular pathways by which inflammatory factors, such as TNF- α and IL-1 β , regulate nucleus pulposus cell apoptosis, thereby providing a precise *in vitro* model for investigating the pathological mechanisms of IVDD. Osteochondral organoids mimic biological interactions between bone and cartilage¹³⁴; bone organoids reproduce bone mineralization and mechanical responses in bone tissue, while rheumatoid arthritis synovial organoids simulate cellular communication among fibroblasts, endothelial cells, and macrophages in inflammatory environments. They provide physiologically relevant *in vitro* platforms for disease mechanism research, filling gaps

in traditional *in vitro* models.

Organoids also demonstrate significant value in drug screening. Patient-derived organoids can be used directly for personalized drug-sensitivity testing, providing innovative tools for precision medicine and tailored drug development.¹³⁵ Organoids derived from gastric cancer patients fully retain the histological features and gene expression profiles of the primary tumor. Their sensitivity to multiple chemotherapeutic agents has been validated through animal models and clinical patients.¹³⁶ Compared with models such as cell line-derived xenografts and patient-derived xenografts, organoids better retain patient tumor heterogeneity and microenvironmental features, significantly improve the accuracy of drug screening and toxicity assessment, and reduce the risk of failure in late-stage clinical development.¹³¹ Compared to traditional models, they more accurately reproduce human tissue characteristics, enhancing the reliability and efficiency of drug screening while reducing clinical drug risks.

In *in vitro* model development, organoids bridge the gap between traditional 2D cell cultures and animal models. They overcome limitations such as the lack of complex cell-cell interactions in 2D cultures and species differences in animal models, providing a more physiologically relevant 3D microenvironment. For example, musculoskeletal

organoids more realistically simulate human physiological functions and 3D structures¹³⁷, while intestinal organoids can form crypt-villus-like structures, making them ideal models for disease modeling, developmental biology research, and regenerative medicine.¹³⁸ Combined with technologies such as 3D bioprinting, microfluidic chips, and gene editing, organoids have seen significant improvements in spatial precision, structural complexity, and functional simulation capabilities, further advancing their applications in precision medicine and regenerative medicine.¹³⁹

6.2. Research progress in intervertebral disc organoid construction

The construction of intervertebral disc organoids holds significant importance for deepening our understanding of the biological characteristics and disease mechanisms of intervertebral discs, as well as for developing novel therapeutic approaches. These organoids can mimic the complex structure and function of *in vivo* discs, which is crucial for investigating the pathological mechanisms underlying disc degeneration. Currently, diverse methods exist for constructing disc organoids, primarily encompassing directed stem cell induction, biomaterial integration, and tissue engineering techniques. These approaches aim to mimic natural disc tissue at both structural and functional levels. Research primarily centers on two core objectives: structural biomimicry and functional recreation. Based on construction logic and methodology, these approaches can be broadly categorized into two mainstream types. These approaches differ in design philosophy, with distinct strengths and limitations^{140,141}:

The first category involves engineered organoid precursor construction. This approach centers on the synergistic regulation of materials, cells, and functional factors, utilizing deliverable and implantable organoid precursors as the core component. It represents the current mainstream direction in clinically oriented research. Chen *et al.*¹⁴¹ developed a minimally invasive injectable nucleus pulposus organoid precursor. This design employs extracellular matrix particles derived from decellularized natural nucleus pulposus tissue as a scaffold, integrating TGF- β 3 and titanium carbide nanoenzymes to achieve dual anti-inflammatory and nucleus pulposus differentiation functions. By co-inducing these components to form 3D cell spheroids, they successfully constructed a nucleus pulposus organoid precursor with microenvironment regulation capabilities. This strategy is highly consistent with the concept of intervertebral disc tissue engineering repair. Synergizing biomimetic scaffolds and bioactive factors to simulate the physiological microenvironment of

the intervertebral disc enables the functional development of organoid precursors. Wang *et al.*¹⁴² addressed the insufficient biocompatibility of traditional scaffolds. The constructed organoid precursor exhibits self-organizing properties and microenvironment regulation capabilities, alleviating oxidative stress and inflammatory responses *in vivo*, thereby providing a feasible approach for minimally invasive clinical treatment. The core advantage of this construction method lies in its alignment with minimally invasive clinical treatment demands, enabling organoid *in vivo* implantation and intervertebral disc tissue regeneration. However, it faces common bottlenecks, such as poor batch-to-batch consistency in scaffold preparation, insufficient *in vivo* sustained-release efficacy of functional factors, and inadequate validation of the metabolic safety of the nanomaterials used. These issues collectively hinder its translation into clinical applications.

The second category involves multipotent stem cell-directed differentiation approaches. Signaling pathways, including those involving Wnt, TGF- β , and bone morphogenetic protein (BMP), are the core pathways regulating the directed differentiation of stem cells into intervertebral disc-related cells. Temporal regulation of these pathways enables precise guidance of stem cell fate determination.¹⁴³ Using multipotent stem cell-like cells as starting material, these approaches guide the cells through sequential modulation of signaling pathways to differentiate into disc-specific cells, which subsequently self-assemble into organoids. This method addresses the shortage of seed cells in organoid construction. For example, Su *et al.*¹⁴⁴ systematically reviewed mainstream approaches in this category. Starting with pluripotent or embryonic stem cells, the sequential addition of small-molecule signaling regulators, such as CHIR99021 and retinoic acid, supported by biological scaffolds, can effectively induce 3D organoids mimicking notochord-derived nucleus pulposus tissue and its extracellular microenvironment. Wei *et al.*¹⁴⁵ employed a dual-fluorescent reporter gene system in human expanded pluripotent stem cells. They first induced primordial chondrocytes through 2D culture, then transferred them to a 3D environment. By applying relevant factors in stages, they ultimately generated an organoid model exhibiting hypertrophic cartilage characteristics. The core logic of these approaches relies on the sequential addition of small-molecule signaling factors, supported by biological scaffolds, to induce stem cell differentiation. While incorporating fluorescent reporter gene systems has indeed enhanced construction precision, key limitations remain: the synergistic mechanisms between signaling factors remain poorly understood, and there is no standardized induction protocol. Consequently, results vary significantly across laboratories, leading to high cellular heterogeneity within organoids.

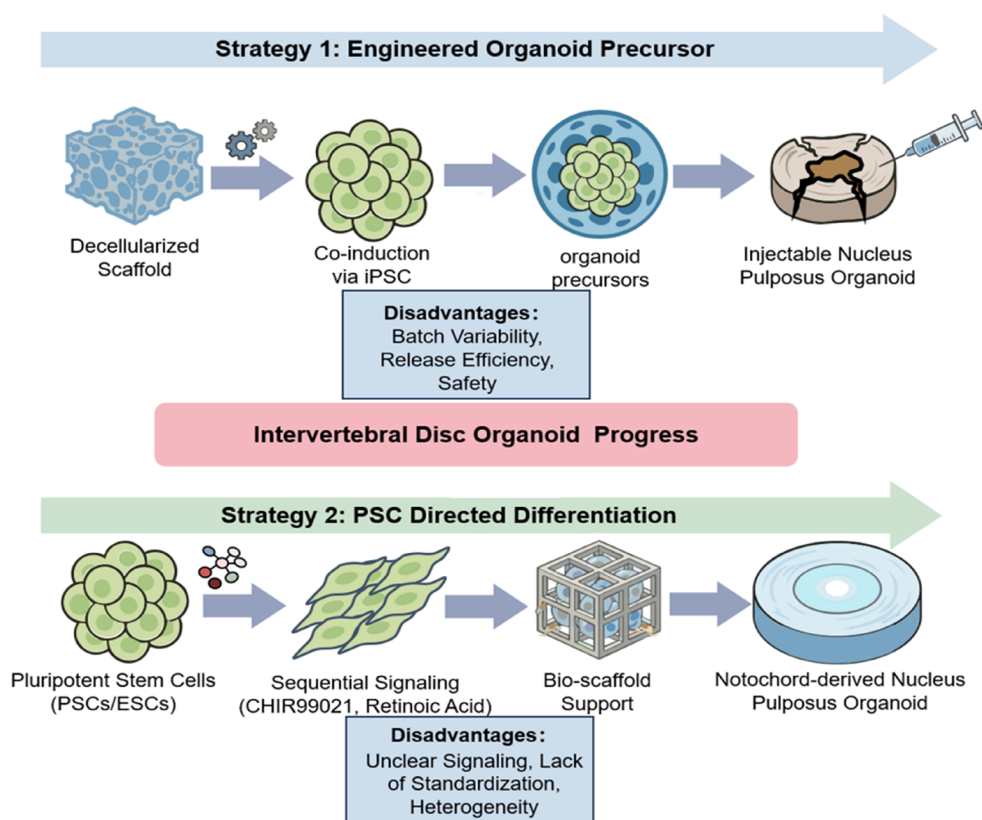


Figure 5. Schematic of two strategies for intervertebral disc nucleus pulposus organoid construction. Image created by the authors with Microsoft PowerPoint.

Abbreviations: ESC: Embryonic stem cell; iPSC: Induced pluripotent stem cell; PSC: Pluripotent stem cell.

As illustrated in Figure 5, these two approaches are not mutually exclusive but rather complementary and synergistic. The engineered organoid precursor approach emphasizes clinical delivery feasibility, with a focus on *in vivo* transplantation and regenerative efficacy. On the other hand, the pluripotent stem cell-directed differentiation method prioritizes seed cell optimization to address the scarcity of seed cells in organoid construction. The combination of directed differentiation of pluripotent stem cells with 3D bioprinting allows the generation of disc-specific cells, followed by the construction of structurally and functionally biomimetic intervertebral disc organoids using biomimetic bioinks and printing processes, representing an important integrated direction in current research.¹⁴⁶ The combination of engineered scaffolds with directed stem cell differentiation further advances the construction of intervertebral disc models, which orderly integrate nucleus pulposus, annulus fibrosus, and cartilage endplate organoids to achieve biomimetic reconstruction of the entire intervertebral disc structure and function.¹⁴² Both approaches share common bottlenecks: low standardization, insufficient functional maturity, and

significant challenges in clinical translation. These remain core areas requiring breakthroughs in current intervertebral disc organoid development.

A comprehensive analysis of research outcomes from both approaches reveals that while intervertebral disc organoid construction has achieved phased breakthroughs, significant gaps remain before fully mimicking the structure and function of natural intervertebral discs and achieving clinical translation. As a carrier material combining biocompatibility, structural tunability, and functional potential, intervertebral disc hydrogel microspheres offer unique advantages in cell protection, microenvironment regulation, and precise delivery. These properties specifically address multiple shortcomings in current disc organoid construction, providing insights for optimizing organoids and enabling clinical translation.

6.3. Role and advantages of hydrogel microspheres in intervertebral disc organoids

Hydrogel microspheres demonstrate potential and advantages in constructing 3D microenvironments,

supporting cell growth, and simulating degenerative tissues within intervertebral disc organoids.

As core scaffolds for mimicking the extracellular matrix, hydrogel microspheres can modify the microenvironment by adjusting physical, chemical, and mechanical properties, providing critical support for cell growth and differentiation within organoids.¹⁴⁷ Unlike the uniform construction approach of traditional scaffolds, hydrogel microspheres demonstrate adaptability through high controllability and biomimetic precision. Hydrogel microspheres with different modifications offer targeted improvements that address distinct organoid construction challenges. Methacrylated DNPM hydrogels retain functional extracellular matrix components, such as type II collagen and glycosaminoglycans, from the natural nucleus pulposus matrix.¹⁴⁸ Combined with photopolymerization for rapid prototyping, they achieved dual simulation of composition and mechanics in a 3D microenvironment. This effectively addresses issues in traditional “top-down” methods, such as uneven cell distribution within scaffolds and inadequate microenvironment simulation. DNPM and chitosan composite hydrogel can highly mimic the composition and microstructure of the natural nucleus pulposus extracellular matrix. It is rich in collagen and polysaccharide components, with suitable elasticity and rheological properties, providing an ideal biomimetic microenvironment for the adhesion, proliferation, and directed differentiation of nucleus pulposus stem cells.¹⁴⁹ Additionally, LMGDNP feature nanofibrillar structures that mimic the natural extracellular matrix, providing a biomimetic structural foundation for cell adhesion, proliferation, and directed differentiation.¹⁵⁰ Furthermore, hydrogel microspheres exhibit a high surface-to-volume ratio, facilitating oxygen and nutrient transport while preventing cellular necrosis within bulk scaffolds.¹⁵¹ This further optimizes material exchange efficiency within the 3D microenvironment. Collectively, these multifaceted structural advantages and functional modifications endow hydrogel microspheres with superior capabilities for constructing 3D microenvironments.

In supporting cell growth, hydrogel microspheres play a pivotal role by integrating cellular support with regulation, rather than merely serving as growth carriers. This distinguishes them from traditional scaffold materials. They comprehensively promote cell survival, proliferation, and functional expression by precisely providing suitable adhesion sites, sustained nutrient support, and flexible signaling regulation. Functionalized hydrogel microspheres represent personalized optimizations tailored to diverse cellular growth requirements. For example, SeNPs@GelMA microspheres specifically enhanced the matrix synthesis capacity of nucleus pulposus cells, significantly increasing proteoglycan and type II collagen secretion—

core functions for maintaining nucleus pulposus-like tissue functionality.¹⁰¹ Meanwhile, the dual-network hydrogel microsphere system continuously released magnesium ions to optimize nucleus pulposus cell adhesion and proliferation, addressing issues of cell detachment and slow proliferation in organoid construction.¹¹⁷ Chitosan-based hydrogel microspheres are rich in amino and hydroxyl groups on the surface, which can be easily modified with cell adhesion sites to promote the adhesion and spreading of intervertebral disc cells.¹⁵² Moreover, their cationic nature can interact with anionic groups on the cell surface, improving the cell colonization of the microsphere surface. A particularly notable advance is the fibronectin-modified GelMA microsphere system developed by Chen *et al.*¹⁵³ Without requiring exogenous biochemical inducers, these microspheres regulated mechanical signals to direct the differentiation of intervertebral disc stem cells into nucleus pulposus-like cells by modulating their own mechanical properties and cell adhesion sites. This inducer-independent differentiation regulation approach reduces the cost and complexity of organoid construction while minimizing interference from exogenous factors on cellular function, potentially becoming a key direction for standardized organoid development in the future.

In simulating degenerative tissue microenvironments, hydrogel microspheres effectively mimic and ameliorate pathological states of degenerative intervertebral discs through dynamic responsiveness, anti-inflammatory and antioxidant effects, and metabolic regulation. This achieves integrated simulation and repair—a feat challenging for traditional scaffold materials. Li *et al.*¹⁴⁸'s methacrylated DNPM hydrogel, rich in nucleus pulposus matrix components, specifically alleviated inflammation in degenerative microenvironments by suppressing inflammatory gene expression and blocking the p38 MAPK pathway, thereby promoting extracellular matrix metabolic homeostasis and fundamentally enhancing pathological simulation efficacy in organoids. Guo *et al.*⁹⁴'s MHSGN dynamically modulated the degenerative microenvironment by pH- and ROS-stimulated release of siGrem1 particles, which scavenged ROS and reduced nucleus pulposus cell apoptosis. Additionally, Gong *et al.*⁹⁷'s BLNP@GF microspheres loaded with *BRY* mRNA continuously released FU in inflammatory environments to reduce nucleus pulposus cell inflammatory activity while upregulating extracellular matrix synthesis, thereby simulating and repairing degenerative tissue function. Moreover, by regulating the stiffness and stress-relaxation properties of hydrogel microspheres, it is possible to mimic the mechanical phenotype of matrix stiffening in aged intervertebral discs, activate cell mechanosensing pathways, induce cellular senescence phenotypes, and improve the authenticity of pathological models.¹³³

3D bioprinting enables the regional printing of such functionalized hydrogel microspheres to construct heterogeneous intervertebral disc organoid models containing both healthy and degenerated regions. This approach more accurately recapitulates the pathological process of IVDD, providing a more realistic model for disease mechanism research and drug screening.¹⁵⁴ Collectively, these properties confer hydrogel microspheres with powerful capabilities to simulate physiological and pathological microenvironments in intervertebral disc organoid construction, providing an ideal platform for tissue regeneration and disease model research.

In summary, the three core functions of hydrogel microspheres in intervertebral disc organoid construction—creating a 3D microenvironment, supporting cell growth, and simulating degenerative tissue—are not mutually exclusive. They form a complete synergistic system encompassing environmental support, cellular regulation, and pathological simulation. The precise construction of the 3D microenvironment provides the foundation for cell growth and pathological simulation. Support for cell growth enables organoids to maintain a stable structure and function, making them a reliable platform for simulating pathology. The precise simulation and dynamic regulation of degenerative microenvironments bring organoids closer to the actual *in vivo* state of IVDD, significantly enhancing their applicability in disease mechanism research and drug screening. The advantages of hydrogel microspheres precisely address the current challenges in intervertebral disc organoid construction—insufficient microenvironment biomimicry, unstable cell function, and imprecise pathological state simulation—offering solutions for advancing organoids from basic research to clinical translation (Figure 5). Hydrogel microspheres offer distinct advantages and can precisely address current challenges in intervertebral disc organoid construction: an insufficient biomimetic microenvironment, unstable cellular function, and inaccurate pathological simulation. Nevertheless, they still have limitations, including difficulty recapitulating the complex multilayer structure of the intervertebral disc, low functional maturity of organoids, poor batch-to-batch consistency in scaffold preparation, immature large-scale culture techniques, and a lack of safety and efficacy data in large animal models, which limit their clinical translation.

7. Conclusion

The pathological mechanisms of IVDD are complex. Current therapeutic approaches primarily focus on alleviating early symptoms and reconstructing structures through surgery in later stages, with limited interventions targeting tissue regeneration. Hydrogel microspheres, leveraging their excellent biocompatibility, tunable physical and mechanical properties, and smart responsiveness,

achieve precise coupling between the carrier function and action mechanisms in gene, drug, and cell therapies. This offers novel strategies for targeted regulation and synergistic intervention within degenerative microenvironments. Diverse fabrication techniques for hydrogel microspheres exist, with advanced methods such as microfluidics significantly enhancing morphological uniformity and size control, laying the foundation for mass production and functional customization. Through chemical modification and physical structural optimization, diverse natural and synthetic polymer materials and their composite systems enable precise regulation of hydrogel microsphere mechanical strength, biodegradability, pore structure, and microenvironment responsiveness, meeting the performance demands of intervertebral disc therapies. The microspheres' high specific surface area, porous structure, and excellent injectability effectively ensure efficient cell growth, drug delivery, and material exchange.

In gene therapy applications, hydrogel microspheres provide multilayer protection and intelligent response systems to achieve stable encapsulation, targeted delivery, and spatiotemporal-controlled release of nucleic acid-based gene drugs. They synergistically regulate apoptosis, senescence, and metabolic pathways in nucleus pulposus cells, significantly enhancing delivery efficiency and safety in gene therapy. In drug therapy, hydrogel microspheres deliver diverse medications to address disc degeneration through multidimensional approaches, including antioxidant, anti-inflammatory, and differentiation-promoting effects. Their sustained-release properties, minimally invasive nature, and synergistic advantages offer novel clinical treatment strategies. For cell therapy, these microspheres provide biomimetic 3D scaffolds for nucleus pulposus cells and various mesenchymal stem cells, enhancing cell adhesion, proliferation, and differentiation.

In organoid engineering, hydrogel microspheres serve as core scaffold materials for constructing 3D disc microenvironments. They mimic the composition and mechanical properties of natural extracellular matrices, providing stem cells with optimal growth conditions and biochemical signals to promote autonomous tissue organization and functional expression. Their dynamic responsiveness and porous structure further facilitate the simulation of microenvironmental differences between degenerative and healthy tissues, offering an effective *in vitro* platform for disease modeling, drug screening, and regenerative therapy with significant clinical application potential. Despite their numerous advantages in treating disc degeneration, hydrogel microspheres require further research and optimization in several areas: matching degradation rates with tissue regeneration rhythms, ensuring stability and precision in drug-controlled release, addressing complexities in large-scale manufacturing

processes, and enhancing translational efficacy from animal models to clinical applications.

Future advancements in hydrogel microspheres will integrate deeply with materials science, bioengineering, and clinical medicine, evolving toward intelligent, personalized, and multifunctional therapies. To further overcome translational bottlenecks in material design, AI and machine learning are emerging as powerful tools. AI-driven approaches enable efficient screening and optimization of hydrogel systems, including composition, degradation behavior, and mechanical properties, thereby improving both design precision and reproducibility. Research combining hydrogel microspheres with organoids will focus on three core directions: First, creating microsphere-organoid composite systems that utilize microsphere carriers to deliver growth factors, drugs, or stem cells, accelerating organoid maturation while enhancing post-transplantation survival rates and *in vivo* integration efficiency. Second, integrating 3D bioprinting technology to precisely assemble organoid-loaded microspheres into biomimetic intervertebral disc structures, achieving simultaneous structural repair and functional regeneration to meet personalized clinical treatment demands. Third, developing responsive smart control systems. Utilizing microspheres sensitive to pH, temperature, or mechanical signals enables on-demand release of growth factors. Combined with physiological feedback from the organoids, this dynamically regulates the degradation rate and delivery rhythm of the microspheres, establishing a bidirectional closed-loop interaction mechanism.

Although significant challenges remain in scaling organoid cultivation and ensuring long-term biosafety, overcoming the translational bottleneck of organoid standardization is of paramount importance. It is imperative to establish rigorous quality control protocols to ensure high batch-to-batch consistency in microsphere-mediated organoids, thereby guaranteeing reproducible biological functions and structural integrity for clinical applications. In addition, standardizing fabrication protocols, culture conditions, and evaluation criteria will be essential to improve comparability across studies and facilitate future clinical translation. Nevertheless, the synergistic advancement of materials science and regenerative medicine holds promise. This innovative hydrogel microsphere-organoid therapy may transform disc degeneration treatment paradigms, shifting from traditional symptomatic relief toward regenerative repair of diseased tissues, ultimately delivering more precise and sustained therapeutic outcomes.

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Conflict of interest

The authors declare they have no competing interests.

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Ethics approval and consent to participate

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Consent for publication

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Availability of data

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