Hindawi
Journal of Clinical Pharmacy and Therapeutics
Volume 2023, Article ID 1105664, 36 pages
https://doi.org/10.1155/2023/1105664

Review Article

Hydrogel-Loaded Exosomes: A Promising Therapeutic Strategy for Musculoskeletal Disorders

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Received 10 February 2023; Revised 16 June 2023; Accepted 18 October 2023; Published 13 November 2023

Academic Editor: Simon Ngamli Fewou

Clinical treatment strategies for musculoskeletal disorders have been a hot research topic. Accumulating evidence suggests that hydrogels loaded with MSC-derived EVs show great potential in improving musculoskeletal injuries. The ideal hydrogels should be capable of promoting the development of new tissues and simulating the characteristics of target tissues, with the properties matching the cell-matrix constituents of autologous tissues. Although there have been numerous reports of hydrogels loaded with MSC-derived EVs for the repair of musculoskeletal injuries, such as intervertebral disc injury, tendinopathy, bone fractures, and cartilage injuries, there are still many hurdles to overcome before the clinical application of modified hydrogels. In this review, we focus on the advantages of the isolation technique of EVs in combination with different types of hydrogels. In this context, the efficacy of hydrogels loaded with MSC-derived EVs in different musculoskeletal injuries is discussed in detail to provide a reference for the future application of hydrogels loaded with MSC-derived EVs in the clinical treatment of musculoskeletal injuries.

1. Introduction

According to the World Health Organization in 2021 [1], approximately 1.71 billion people worldwide suffer from musculoskeletal disorders. Due to population growth and aging, the number of people with musculoskeletal disorders is increasing rapidly, leading to early retirement, reduced quality of life, and reduced ability to participate in society. There are over 150 musculoskeletal disorders [2]. Due to the difference between the severity of the injury and the healing ability of the injured tissue, these diseases can cause short-term dysfunction in patients (such as stability fractures [3], muscle strains [4], and sprain) and limited continuing function [5], which can even lead to permanent disability. Musculoskeletal disorders account for the largest proportion of rehabilitation needs worldwide and need to be addressed urgently. Given that these tissues (cartilage, tendon, and bone) lack the necessary vasculature and mechanical stimulation for full regeneration, their capacity for healing is constrained. Surgery and physical therapy offer only limited benefits for patients who are unable to heal [6, 7]. These strategies frequently result in incomplete mechanical strength and function restoration of the repaired cartilage and tendons, as well as failure to achieve long-term healing of the musculoskeletal system. Therefore, clinical studies suggest that for the treatment of musculoskeletal disorders, attention should be paid to the maximum healing of tissue structure and the improvement of function. Currently, extracellular vesicles (EVs) are confirmed to have great application potential in the treatment of various diseases. However, the characteristics of a short half-life hinder further clinical transformation. With the rapid development of tissue engineering in recent years, controlled release systems prepared from biomaterials, such as hydrogels, have solved the dilemma of short half-life of EVs [8, 9]. Hydrogel-loaded EVs can achieve prolonged controlled release in

locally injured tissues. Cumulative studies have shown satisfactory efficacy of hydrogel systems loaded with EVs in difficult-to-heal musculoskeletal injuries [10–13]. However, the current research on hydrogel-loaded EVs therapy is not mature enough, and the best-recommended standard for clinical application has not been established. Therefore, in this paper, by searching the literature related to the application of hydrogel-controlled release EVs to promote the repair of musculoskeletal injuries in recent years, the current preparation strategy of hydrogels and the application of specific tissues were elaborated to provide ideas and theoretical support for future research and promote the clinical transformation of this therapy in the field of musculoskeletal disorders.

2. Literature Screening and Methods

2.1. Literature Sources

2.1.1. Time of Literature Search. Searching was carried out by the authors in December 2022.

2.1.2. Time Limit of References. The time limit of references is from January 2015 to December 2022.

2.1.3. Database and Keywords

(1) Databases. Scopus, Pubmed, and Web of Science.

(2) Keywords. "Exosomes, EVs, Hydrogel, Musculoskeletal injuries, IVD, osteoarthritis, Tendon injuries, Bone, Biomaterial scaffold."

2.1.4. Number of Initial Literature Obtained. An initial search of the literature yielded 2479 articles, including original research and reviews.

2.2. Literature Selection

2.2.1. Literature Inclusion Criteria. The inclusion criteria were are follows: (i) literature describing the characteristics of EVs; (ii) literature related to the treatment of musculoskeletal injuries with different cell-derived EVs; (iii) literature related to the extraction and culture of EVs, pretreatment, and imposition of interventions; (iv) literature related to the construction of hydrogel delivery systems; (v) original research on the application of biomaterial scaffolds other than hydrogels for the treatment of musculoskeletal injuries; and (vi) research on the application of hydrogel-loaded EVs in areas other than musculoskeletal injuries.

2.2.2. Literature Exclusion Criteria. The exclusion criteria are as follows: (i) literature that is not relevant to the purpose of this review; (ii) retracted literature; and (iii) literature that lacks timeliness and has an unreasonable experimental design.

2.3. Literature Quality Assessment and Data Extraction. The literature was searched to obtain 2,479 articles. 459 articles were removed according to the inclusion and exclusion criteria by reading the titles and abstracts of the articles and excluding those with poor relevance to the study content of this review and duplicate articles. Further reading of the original text resulted in the exclusion of 1663 articles and the retention of 357 articles. The literature screening process is shown in Figure 1.

3. Overview of Extracellular Vesicles

EVs are small membranous vesicles released by cells into the extracellular matrix. Wolf [14] first discovered in 1967 that platelets can release large numbers of vesicles, which were thought to be nonfunctional cell fragments at the time. With the deepening of research, EVs were found to be derived from almost all mammalian cells, such as mesenchymal stem cells (MSCs) [15], cancer cells [16], neuron cells [17], endothelial cells [18], and immune cells [19]. Cells can communicate with each other by secreting and absorbing EVs. EVs bring their endogenous cargo to target cells through body fluids, promote communication between cells, and regulate cell functions [20]. There are two main groups of EVs: ectosomes and exosomes (exos). Ectosomes are small outgrowth vesicles that are detached from the plasma membrane and include microvesicles, microparticles, and macrovesicles with a diameter of 50 nm to 1 μm. Exos are vesicles originating from the endosome pathway in the nucleus with a diameter of 40 to 160 nm (average 100 nm) [20, 21]. Since no consensus has been reached regarding specific markers for EV subtypes, in the following sections, we will use the term "EV" to describe the extracellular vesicles in the literature, as recommended by the International Extracellular Vesicular Society in its position statement of 2018 [22] and the joint consensus of multiple societies of 2019 [23]. Among them, exos originate from endosomes in cells, and the occurrence process is shown in Figure 2.

3.1. Biological Function of EVs. The study of EVs has long been plagued by conceptual and technical problems [24]. The heterogeneity of EVs includes mainly their size, composition, biological function, and cells of origin, and the heterogeneity of EVs will affect their downstream applications. EVs contain a large number of MHC class I and/or class II molecules [25, 26], nucleic acids, and lipids of various proteins (such as HSPs, cytoskeletal proteins, transmembrane proteins, and metabolic enzymes) [27]. After secreting into ECM, EVs reached other tissue cells through the circulatory system, and the N-ethyl maleimide sensitive factor attachment protein receptor complex and endosomal sorting complex required for transport (ESCRT) [28, 29]. Subsequently, EVs enter the recipient cells through vesicle incorporation and endocytosis [30] and play the biological role. To put it simply, after the EVs from different sources entering the recipient cell, some of them release the macromolecular components and reform MVBs; ligands on the

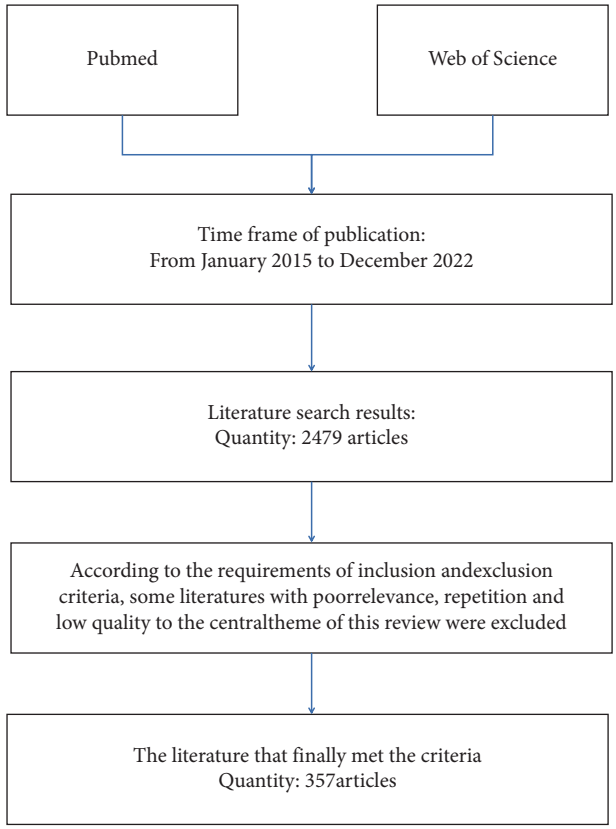

Figure 1: Literature screening flowchart.

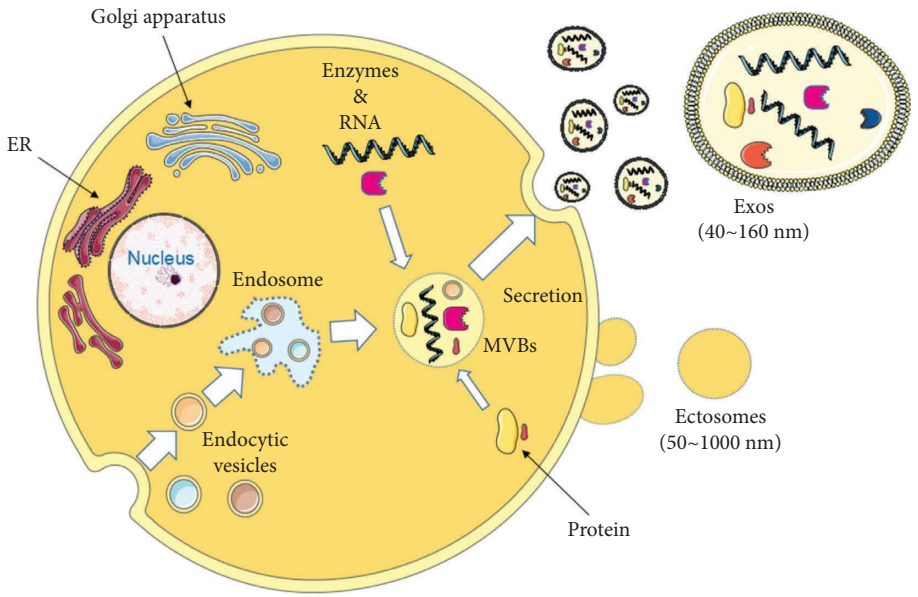

Figure 2: The occurrence process of exos: first, cells produce vesicles through endocytosis, also known as endocytotic vesicles. Then, vesicles will fuse to form the early endosomes, which will gradually evolve into the late endosomes and form the multivesicular body (MVB) in the interior. MVBs will selectively bind miRNA and heat shock proteins in the cellular matrix. HSPs, enzyme molecules, and other substances, fused with the plasma membrane; finally, the vesicles are released and enter the extracellular matrix (ECM) to form the lipid bilayer exos. ER: endoplasmic reticulum.

surface of some EVs bind to receptors on the cell membranes of recipient cells, transmitting signals, and transporting goods. Others transport internal cargo into the cytoplasm and fuse themselves with the cytoplasmic membrane. Either way, EVs regulate the cellular activity and pathological changes, such as the regulation of inflammatory response, tumorigenesis, and angiogenesis [31]. It is worth noting that EVs have different effects on receptor cells due to different expression of receptors on the cell surface. This functional heterogeneity can lead to different biological reactions (such as apoptosis, immune modulation, and microenvironmental regulation) by EVs in different target cell types. Therefore, the application of EVs in clinical therapy needs to take into account the heterogeneity of function and origin of EVs.

MSCs are multifunctional stem cells that can be obtained from a variety of tissues, including bone marrow, adipose tissues, umbilical cord, tendon, amniotic fluid, and placenta. MSCs have become the main source of cells for stem cell therapy due to their reproducibility, immune regulatory characteristics, low risk of inducing tumors, and no ethical constraints. It has been widely used in various types of tissue repair and regeneration therapy such as skin wound healing, osteoarthritis, cerebral ischemic injury, and myocardial injury [22, 32, 33]. However, tumorigenesis, chromosomal aberration, immunological incompatibility, and other disadvantages limit the clinical potential of MSCs to repair tissue injury [34, 35]. In recent years, studies have found that MSCs do not exert their tissue repair effects through direct transplantation or replacement of damaged tissues, but through the paracrine pathway. EVs are a key functional element in the paracrine pathway [36, 37]. In addition, EVs have many advantages compared with MSCs: (i) EVs are more stable and more resilient to damage from freezing and thawing, making them easier to stockpile for transport [38]. (ii) The generation of EVs is a physiological process that is closely regulated. EVs do not self-replicate, without potential tumorigenesis [39, 40]. (iii) EVs have a smaller volume and are capable of accommodating the structural apertures of a variety of delivery systems. (iv) EVs' membrane surface tissue antigen is almost not expressed or shows low expression, and the immunogenicity is lower [37]. Therefore, the use of MSC-EVs instead of MSCs can effectively avoid many disadvantages of applying MSCs. In addition, combined with biological materials such as hydrogels, they can achieve long-term release of EVs and promote the long-term repair of tissue, which has shown significant therapeutic effects in a variety of diseases [12, 41–45].

With the further study of EVs, their biological role in musculoskeletal disorders is becoming more clearly understood. The microenvironment and intrinsic biological characteristics of the parent cells may affect the levels of EVs and their biological markers, and EVs contain a lot of nucleic acids [46, 47] (including DNA, RNA, miRNA, and noncoding RNA) and proteins. Through the fusion with the receptor cells, the exchange of proteins and lipids is realized, which is involved in many physiological and pathological processes in the body. Based on this property, EVs have been designed as a therapeutic vector capable of mediating cell communication and cargo transport by transferring the desired miRNA to target cells and have very low immunogenicity and negligible tumorigenicity, which is essential for clinical transformation. In addition, EVs have a regulatory effect on ECM, controlling its composition and function, and thereby affecting cell proliferation and migration. It is well known that changes like ECM are the core mechanism of many musculoskeletal degenerative diseases. Studies have shown that EVs can promote the synthesis of ECM through the regulation of ADAMTS5, MMP13, and MMP3 and realize the regulation of the microenvironment of injured tissues to promote the healing of injured tissues [12, 48, 49]. However, the clinical application of EVs still faces many challenges due to its short half-life, rapid clearance by phagocytes when administered intravenously, and its accumulation in the liver and spleen. The low retention rate will significantly limit the local application of the injured site. To deal with this situation, researchers have made many attempts, such as repeated systemic or local injection directly into the defect site and increasing the amount of delivery [50]. However, these methods did not achieve satisfactory results but led to inflammation and increased tumorigenesis rate [51, 52]. Therefore, we need better drug delivery strategies that can achieve a high degree of specificity, deliver EVs to the target region, and achieve long-term retention and controlled release.

3.2. Isolation and Purification of EVs. The occurrence of EVs is a precisely regulated physiological behaviour. Various combinations of EVs and their mediators are colloids [53]. Therefore, as with many other biologics, complete purification or complete separation of EVs from other entities is an unrealistic goal. In addition, isolation of EVs, whether from body fluids or culture medium supernatants, is often hindered by nonvesicular biomolecules, due to the fact that EVs are heterogeneous in size, origin, and molecular composition [54]. More importantly, the distribution of these biomolecules in different liquids is also different, which means that even if the same separation strategy is used to separate EVs from different sources, different results will be obtained [55]. According to the global survey conducted by ISEV in 2016 [56], the most widely used EVs isolation technology is ultracentrifugation (including differential centrifugation) [57–62], which utilizes the difference in buoyant density of particles and increases the centrifugal speed by stages to enable different particles to settle in batches from the mixture to the bottom of the tube for separation. In general, differential centrifugation consists of three to four stages [63, 64]. The purer EVs formulation can be obtained through repeated centrifugation, washing, and suspension microfiltration. However, the difference in centrifugation conditions and the number of stages can affect the separation result [65]. Moreover, this method has obvious defects. Expensive equipment is required. The extraction time of this method is long, and EVs may be damaged during centrifugation [66]. Therefore, the selection of isolation strategy should be based on the application scenario. For example, when EVs are applied to the treatment of clinical diseases, it is necessary to ensure its

structural integrity and to fully exert its biological function to achieve the therapeutic effect. In Table 1, we provide a detailed description of the current mainstream EVs isolation strategies and compare the advantages and disadvantages of the various isolation strategies and their clinical applicability.

In order to obtain a better specificity for the isolation of EVs or EVs subtypes, many scholars often use one or more additional techniques after the main isolation steps, such as alternating current electrophoresis technology [80, 81], field-free viscoelastic flow control technology [73, 82], and lipid affinity technology [83, 84]. Under certain research conditions (such as the use of fluorescence activation sorting to obtain larger-diameter apoptotic bodies [85]), a combination of multiple extraction strategies may be more effective than a single extraction strategy. However, more studies are needed to prove that these combinations of extraction strategies can achieve better recovery and EVs specificity than traditional methods. Therefore, EVs extraction strategies with lower development cost, higher yield, no impact on immune recognition, and sufficiently pure extract will also become a research focus in the future.

In conclusion, exos are a kind of EVs that can carry a large amount of cellular cargo and deliver it to target cells to play its biological role, playing an important role in cell proliferation, inflammatory response regulation, and ECM remodeling. In addition, exos have low immunogenicity and tumorigenicity, which means that it has a high potential for clinical application. Therefore, most current studies have focused on exploring the therapeutic mechanism of exos therapy in musculoskeletal disorders. However, the short half-life of exos and its inability to be prepared on a large-scale limit the clinical transformation of this therapy. Further investigation of more affordable isolation techniques is required. The ideal EVs isolation method should be relatively simple and inexpensive. It should also allow for rapid isolation of relative EVs even in larger samples and with minimal compromise of the therapeutic efficiency of EVs. The novel EVs isolation method combined with a suitable release control system, such as hydrogels, will eliminate the dilemma that EVs cannot be released in damaged tissues for a long time and ensure the clinical efficacy of the therapy.

4. Hydrogels Were Used to Prepare the EVs Delivery System

Since the early 1980s, when Langer and Vacanti first proposed the idea of implanting cells into biodegradable biomaterials to construct tissues, to the success of numerous applications of tissue engineering techniques to repair clinical defects in recent years [86–88], tissue engineering techniques have proven to be one of the effective ways to address tissue trauma repair and functional reconstruction. The four basic elements of tissue engineering technology include biological scaffold materials, seed cells, signal molecules, and bioreactors [89]. Among them, biological scaffold materials are to provide the necessary space for seed cells to attach and survive before they form tissues. Common biological scaffold materials include coral [90], bioactive glass [91, 92], biological ceramics [93, 94], metals [95–98], hydroxyapatite [99], and collagen [100, 101]. These substances can be broadly categorized into natural polymer materials, synthetic materials, bioceramic materials, metallic materials, and nanomaterials. Based on their qualities, each of these materials has its own advantages and disadvantages. Among these materials, hydrogels have unique advantages. Due to the high water content, great biocompatibility, minimal cytotoxicity, and several other benefits, hydrogels are frequently employed in the fields of tissue engineering and drug delivery [102].

Hydrogels were first reported in 1894 [103], which are a class of elastic semisolid polymers with a three-dimensional network structure formed by linear polymers through covalent, ionic, hydrogen, and electrostatic interactions. The structural integrity of the hydrogel network is not dissolved by high concentrations of water due to inherent crosslinking [104]. Based on their excellent degradability, hydrophilicity, biocompatibility, water content, and excellent viscosity and elasticity, hydrogels are now widely used in regenerative medicine and various tissue engineering studies [105–107], showing good potential for clinical applications. Previous studies on hydrogels have focused on chemically cross-linked polymer networks to investigate their fundamental characteristics such as swelling/dissolution kinetics and equilibrium [108], diffusion of solute [109], sliding friction [110], and volumetric phase transition [111]. In recent years, the focus of hydrogel research has gradually shifted from simple networks to "responsive" networks. Currently, a variety of hydrogels have been developed that can respond to changes in environmental conditions such as pH [112], temperature [113], and electric and magnetic fields [114]. On this basis, a variety of mechanical properties have been imparted to the hydrogels by physical or chemical means, further expanding the range of applications for the material. Hydrogels have been shown to promote tissue repair and effectively enhance the retention time of drugs in the treatment of musculoskeletal injuries [42, 115, 116].

4.1. Hydrogel Bioscaffolds vs. Other Biomaterial Scaffolds. Although tissue-engineered scaffolds for tissue damage repair can be made from a variety of materials and methods, the effectiveness of treatment depends on the ability of the material to effectively promote normal fresh tissue production and replace the original tissue after implantation. Biodegradability, biocompatibility, suitable degradation rates, and a certain mechanical strength are the key points to consider when selecting materials. Additionally, the biomechanical requirements, topologies, and compositions of various tissues vary greatly. For instance, skin tissue has low tissue density and low compressive strength but high elasticity, while bone tissue has high tissue density and has certain requirements for the mechanical properties of biomaterial scaffolds. Therefore, the requirements for the scaffold materials used to build various tissues are very different. The advantages and disadvantages of various bioprosthesis materials are summarized in Table 2. Compared with other bio-scaffold materials, hydrogels have their unique advantages.

Table 1: Current mainstream EVs isolation strategies and a comparison of their advantages and disadvantages.

Extraction techniques of exosomes	The principle of extraction	Advantage	Disadvantage	Clinical applicability	References
Differential centrifugal (DC)	Due to the differences in particle size, shape, and density, the particle settlement coefficient in the solvent is also different. By increasing the centrifugal speed by grading, different particles are settled from the mixed liquid to the bottom of the tube in batches to achieve separation	It can satisfy the extraction of a large sample size Low pollution risk Saving reagent cost	The operation is difficult, and the cost is high Long extraction time Exos are damaged during centrifugation It has an impact on the therapeutic effect	No	[57–62]
Density gradient centrifugation (DGC)	Using gradient media to remove vesicle particles	Good separation effect Having a higher concentration Maintaining certain particle activity Preventing the effect of convective mixing on purity	It takes a long time The operation is difficult	No	[67]
Ultrafiltration (UF)	Exosomes and other macromolecules are trapped in the filtration membrane by pressurizing the solvent and small molecules through the filtration membrane	Easy to operate High enrichment rate Low cost	Filter membrane blockage Low purity There is contamination Difficult to recover during elution	No	[68, 69]
Size exclusion chromatography (SEC)	According to the size difference between exos and other particles, the protein was purified by similar chromatography	It can ensure the activity and integrity of exos Higher yield Short time	Special instruments are needed There is a minimum exos load limit	Yes	[70, 71]
Immune capture (IC)	Isolation is performed by binding specific antibodies to antigen receptors on the surface of exos	Higher purity Suitable for subtype isolation The effect on the structure of the exos membrane is small	Cost prohibitive May affect immune recognition Cannot be prepared in large quantities	Suitable for specific diseases, but not large-scale preparation	[72]
Precip	The solubility and dispersibility of exos were changed by hydrophobic polymer, and exos were extracted by sedimentation under low centrifugal force	Easy to operate Low requirements for equipment Suitable for high-dose treatment	Mixed with some pollutants (protein)	Yes	[70]
Microfluidic technology	The isolation and extraction of exos were dependent on the immunoaffinity, size, and density of EXOS	High purity and recovery Short time Good biocompatibility	Low flux High requirements for equipment	Yes	[73–76]
Two-phase isolation	Incubation in a polyethylene glycol-dextran mixture	Low cost Simple process No deformation of EVs High purity	Repeated replacement of the polyethylene glycol phase is required	Yes	[77]
Binding of heat shock proteins	Isolation of heat shock protein-modified EVs by polymerization using the heat shock protein (HSP)-binding peptide Vn96	High EVs integrity	Costly	No	[78, 79]

4.1.1. Hydrogels Are Easy to Modify. Traditional hydrogels have performance issues such as slow response times, poor mechanical strength, quick degradation, and insufficient antibacterial properties. As a result, hydrogels must be modified to meet the application requirements of various tissues if they are to find widespread use. For instance, gelatin, a partial hydrolyzate of collagen with a triple helix structure and 18 amino acids distributed unevenly, has a molecular weight of tens to hundreds of thousands. Gelatin is used in tissue repair applications without causing the protein rejection reactions that collagen does [158]. As shown in Figure 3, R1–R5 are amino acid residues of gelatine. The abundance of residues is also the basis of the molecular structure of gelatine that can be easily modified. Modification of the functional groups on the collagen polypeptide chains can result in modified gelatins with significantly improved gelation, water resistance, thermal stability, and mechanical properties. Vuocolo et al. [159] synthesized catecholamine-modified gelatin and phenol-modified gelatin by EDC/NHS grafting of amino and carboxyl groups and photochemical crosslinking using ruthenium groups. Through this technique, hydrogels for wound closure were developed in the form of photocrosslinking, which greatly enhanced the surface adhesion of the tissue. Moreover, Rajzer et al. [160] and his colleagues prepared a three-dimensional bilayer scaffold, the calcium phosphate-modified gelatin/PCL scaffold (Gel/SG5/PCL), which has good mechanical properties and has an in vivo degradation rate comparable to the bone growth rate, successfully compensating for the disadvantages of gelatin's rapid in vivo degradation rate and poor mechanical properties. In addition, the application of chitosan modification is also very representative. Chitosan is a product of chitin N-deacetylation. However, the clinical promotion application is limited by the problems of poor water solubility and low mechanical strength. As shown in Figure 4, the basic unit of the chitosan molecule is glucose with an amino group. As the molecular structure of chitosan contains amino, acetylamino, and hydroxyl groups, it is chemically active. In clinical applications, quaternization, carboxymethyl, phosphorylation, and alkylation [161] are often used to improve the physical and chemical properties and biological functions of chitosan. Xu et al. [162] grafted quaternary ammonium groups on the outside of the chitosan backbone. By optimizing the reaction conditions, N-[(2-hydroxy-3-trimethylammonium) propyl] chitosan and 3-chloro-2-hydroxypropyltrimethylammonium chloride could be produced from cyclopentane-trimethylammonium chloride and chitosan in neutral and alkaline environments, respectively. The mild reaction conditions allowed the acetamide group of chitosan to remain intact. The introduction of quaternary ammonium groups on the primary hydroxyl group at the C-6 position of the chitosan backbone allowed the quaternized chitosan to easily form micelles and exhibit good antibacterial activity, providing a new idea for the preparation of regenerative dressings for infected wounds. Therefore, hydrogels are easy to modify, which provides a potential for the extensive clinical application of musculoskeletal injuries (including cartilage, muscle, tendon, and bone). The local microenvironment and mechanical properties of these tissues are very different, so the development of hydrogels that match these tissue properties is the key to the clinical transformation of this therapy.

4.1.2. The Conductivity of Hydrogels. Bioelectrical signals have an important role in regulating cell behaviour, promoting cytokine secretion, and improving the microenvironment of tissue damage. Therefore, in regenerative medicine, nanomaterials with electrical conductivity (graphene [163] and carbon nanotubes [164]) and conductive polymers (polyaniline and polypyrrole [165]) are usually doped in hydrogel networks to construct composite conductive hydrogels. Conductive hydrogels have appropriate electronic, mechanical, and chemical properties, which are widely used in neural tissue repair [166–168], myocardial repair, and conductive bioink preparation in 3D printing technology (as shown in Figure 5) [169, 170]. Compared to materials such as bioceramics and metals, the electrical conductivity of hydrogels plays an irreplaceable role. Cai et al. [166] developed a graphene-based nerve-guided catheter by combining a natural dual-network hydrogel and a neurotrophic concentration gradient with a noninvasive treatment for diabetic patients. The results showed that embedding a graphene mesh in the catheter not only enhanced the mechanical strength of the catheter but also promoted the regeneration of muscle and peripheral nerve tissue. In addition, moderate bioelectrical stimulation enhances signaling to cardiomyocytes and promotes rapid cell maturation, facilitating the repair or regeneration of more uniform and mature beating myocardial microstructures. Li et al. [171] prepared polydopamine-reduced graphene oxide (PDA-rGO) and doped it into methacrylate-based gelatin (GelMA, EFL-GM-60) hydrogels to obtain the conductive hydrogels with enhanced electrical conductivity and rigidity. The conductive hydrogels had a controlled Young's modulus equivalent to that of heart tissue. Through electrical stimulation, the contraction rate of myocardial cells is enhanced, the expression of Cx 43 protein is upregulated, and the synergistic effect of myocardial cell functionalization and maturation is realized. In addition, Wei et al. [170] built a conductive 3D cell culture system using carbon nanofibers and hydrogels through 3D printing technology, which can determine the damage to cells after toxin stimulation by measuring the impedance value with electrochemical alternating current impedance. This method is simple and effective, providing a new direction for the preparation of cytotoxicity batch evaluation tools. Moreover, Wang et al. [172] demonstrated the 3D bioprinting-assisted fabrication of conductive hydrogel scaffolds based on poly(3,4-ethylenedioxythiophene) (PEDOT) deposited in hydrogels. The results showed that electrical stimulation of the scaffolds promoted muscle-forming differentiation of mouse myogenic cells and facilitated the formation of myotubes in vitro. The conductive hydrogels can be used as bioink, and a repair scaffold or a 3D cell sensor of specific tissues can be customized through a 3D bioprinting technology to promote tissue repair with its excellent conductivity and controllable Young's modulus, or to detect the cell state and the damage by electrochemical method.

Table 2: Comparison of advantages and disadvantages of various biological scaffold materials.

Material types	Further classification	Rohstoffe	Ingredients	Advantages	Disadvantages	References
Hydrogel scaffold	Natural hydrogels	Alginate salt	Linear polysaccharide copolymer	Low cost can be made from algae and bacteria; low biological toxicity; low immunogenicity	Part of the molecular chain dissociates during the gelation process; low mechanical stiffness; weak cell-matrix interactions resulting in poor cell adhesion	[117–119]
		Chondroitin sulfate	N-Acetylgalactosamine and glucose Glucuronide chain polymer	Good for restoring stiffness to damaged cartilage; pro-proliferative; low cytotoxicity	Insufficient mechanical strength; degrades too quickly; and needs to be used in combination with other materials	[120]
		Hyaluronic acid (HA)	Glycosaminoglycan	An important component of the ECM and can simulate the microenvironment of cell survival Immunogenicity is extremely low Strong stability Easy to modify in order to meet a variety of application scenarios	Natural HA molecules have poor cell adhesion in organisms and are easily degraded and diffused; too rapid a degradation rate will result in insufficient residence time in the body	[121–125]
		Chitosan	Linear polysaccharide	Easily modified for a variety of applications; chemically modifiable; antibacterial properties; easy to manufacture; some pain relief Good water solubility	Poor self-mechanical properties; sensitive to pH and temperature; low cell adhesion	[126, 127]
		Gelatin	Triple helix broken collagen	Numerous RGD peptide sequences can enhance cell attachment Excellent physical properties Easy to modify	Poor thermal stability; blocky degradation rate; poor mechanical properties	[127, 128]
		Fibrous protein	Polypeptide chain	Very low antigenicity Can be made from blood Easy to prepare directional structures	Requires modification; the original structure aperture is too small Low stiffness	[129–131]
		Collagen	Collagen	Low antigenicity; low inflammatory response; degradable; high biocompatibility	Potential immunogenic risk; insufficient mechanical properties; excessive degradation rate	[132]
		Silk fibroin	Multilayer protein polymer	Long degradation time; can provide long-term support for tissue; nontoxic in the body; excellent physical performance	Slow gelation rate; insufficient inductivity of seed cells; difficulty binding to surrounding tissue	[133, 134]
		GelMA	Gelatin derivative	Can form a three-dimensional structure similar to normal tissue Excellent biocompatibility Fast crosslinking The physical and chemical properties are flexible and adjustable	Curing conditions require high requirements, light intensity, light distance, and light time need to be strictly controlled	[13, 135–138]
	Synthetic hydrogels	PHEMA	The polymer of vinyl	Adjustable mechanical properties, suitable for different parts of the tissue	Different from the natural tissue structure	[139]
		PVA		Excellent elasticity and material endurance Can be used to repair cartilage tissue	Interaction with cells is poor; oligopeptide coupling modification is required	[140]
		PEG	Glycol polymer	Responsiveness of stimulus; controlled degradation time	It is different from the natural tissue structure	[141, 142]
	Mixed hydrogels	GelMA-dHAMMA	Mixed material	Porous structure with two-component polymer network; promotes skin healing; can stably simulate the extracellular micro-environment; convenient and fast preparation	The degree of crosslinking is higher than GelMA, and the water absorption becomes weaker	[143]

Table 2: Continued.

Material types	Further classification	Rohstoffe	Ingredients	Advantages	Disadvantages	References
Metal biological scaffold	Non-degradable metal	Titanium/titanium alloy	Ti, Ti_6Al_4V	High mechanical strength; low density; nontoxic; high biocompatibility; corrosion resistance	Lack of biodegradability; microstructure has a great influence on mechanical properties	[144]
	Degradable metal	Magnesium, iron, and their alloys	Metal/alloy/coated metal	High mechanical strength; degradable; and the degradation product is nontoxic; metal ions promote tissue healing, especially bone healing	The degradation speed is too fast, which requires coating modification. The degradation rate is difficult to control, and mechanical properties will be affected along with degradation	[96, 145]
		Zinc (zinc oxide)	Metal/metal oxide	Antibacterial ability; good biocompatibility; good osteogenic effect	Need 3D printing technology support; high preparation difficulty	[146]
Bioceramic scaffolds	Bioceramics	Hydroxyapatite, tricalcium phosphate, biphase calcium phosphate, etc.	Hydroxyapatite is the main inorganic substance	Good biocompatibility and bioactivity; wide source, low cost; easy synthesis and preparation	Slow degradation rate; poor mechanical properties	[147, 148]
	Composite biological ceramics	Bioceramics combine metal and polymer materials	Composite material	The mechanical properties of composite bioceramic scaffolds were improved by integrating the advantages of other materials	The preparation is complex and needs to consider the organizational environment and other factors	[149]
	Biodegradable bioceramics	Calcium sulfate, tricalcium phosphate, etc.	Calcium compound	Degradation products such as Ca and P do not produce rejection reaction; good biocompatibility; good bone induction ability	Low strength and high brittleness; low flexural strength	[150]
Membrane	Nondegradable membrane	Polytetrafluoroethylene (PTFE)	Synthetic polymer material	Excellent mechanical properties; high biocompatibility; strong chemical and thermal stability; low surface friction	It is not degradable and needs to be removed by a second operation. Dehiscence affects tissue regeneration; the application scope is limited	[151]
	Degradable absorbent membrane	Natural collagen membrane	Natural collagen	Excellent shielding performance; degradable; low biotoxicity; no need for a second operation	Poor mechanical stability; fast degradation rate; tissue adhesion is low	[151, 152]
		A compound of polyester, polywater, and polyurethane	Synthetic polymer	Biodegradation is controllable; high mechanical strength; drug encapsulation	Low biological activity; low hydrophobicity	[153, 154]
		PCL/PLA/CA electrospinning nanofiber membrane	Nanofiber membrane	Antibacterial activity; strong ability to organize and combine; excellent bone conductivity	The cost is high; complex preparation	[155]
		Polyamide-6/chitosan fiber membrane	Nanofiber membrane	Strong biocompatibility; the double-layer structure allows the membrane to have different surface properties, acting as an insulator while allowing nutrients to penetrate	The preparation process is complex and requires a combination of electrospinning, solvent casting, and evaporation	[156]
Mesh	Synthetic compound	Hydroxyapatite, etc.	Inorganic compound	Controllable porosity; can adapt to a certain range of stress changes; to ensure the normal metabolism of tissues	The mechanical strength is poor, which needs to be toughened by adding sintering additives, whiskers, second particles, etc.	[157]

Note. GelMA: methacrylate gelatin; HEMA: 2-hydroxyethyl methacrylate; PVA: polyvinyl alcohol; PEG: polyethylene glycol; GelMA-dHAMMA: methylpropenyl acylated gelatin-methacrylate complex hydrogels; PCL/PLA/CA: electrospinning nanofiber membrane: electrospinning nanofiber membranes prepared by polycaprolactone/polylactic acid/cellulose acetate polymers.

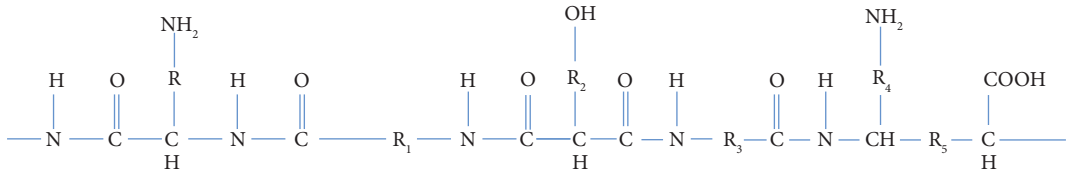

Figure 3: The molecular structure of gelatin.

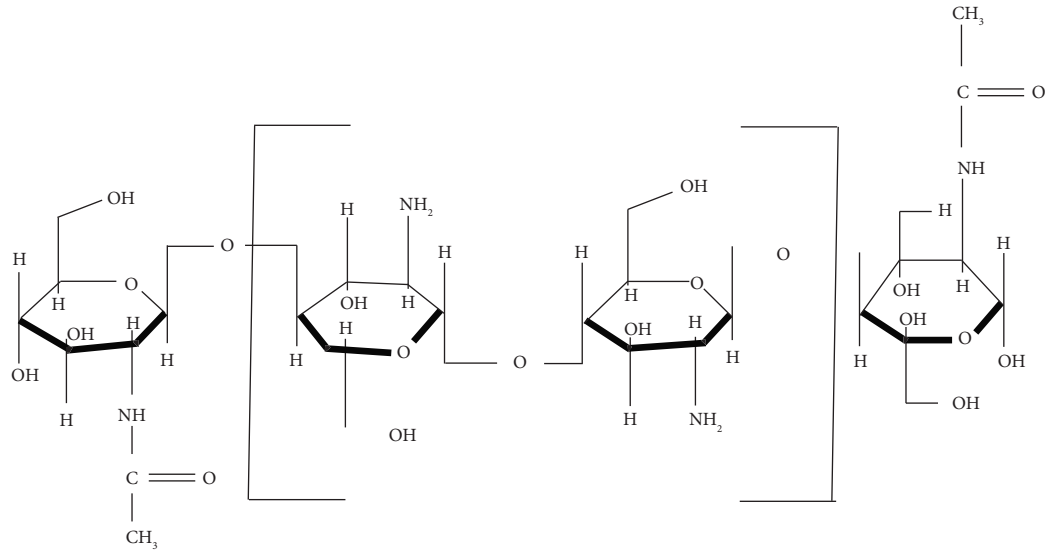

Figure 4: Molecular structure of chitosan.

4.1.3. Hydrogels Have Unique Antibacterial Properties. Asepsis is a fundamental requirement in surgery. However, in orthopaedic surgery, 10% of patients who undergo surgery are exposed to bacterial infections, despite strict sterilisation procedures [173]. Although most patients recover without experiencing long-term negative effects, surgical site infections are also the most prevalent type of hospital-acquired illness, which can lengthen hospital stays. Of the patients who die from surgical site infections, 77% of deaths are due to infection [174]. Traditional means of anti-infection include inorganic antimicrobial agents and antibiotics. Although inorganic antimicrobial agents such as Ag nanoparticles (Ag NPs) [175] have good antimicrobial properties, their biocompatibility and dosing dependence are suboptimal, limiting clinical application. In addition, the misuse of traditional antibiotics has led to the evolution of many drug-resistant bacteria [176], which has initiated a more serious challenge for clinical response to post-operative infections. In recent years, many studies have focused on how to improve the antimicrobial properties of biological scaffold materials, such as the coating of calcium peroxide over bioceramic materials [177] and antimicrobial metal ion composite scaffolds [178, 179]. However, these studies still have the problems of antimicrobial materials being cytotoxic and less testing in animal models that need to be addressed. The limitations of the materials themselves, such as metals and ceramics, have also limited their use in multiple. Some hydrogel materials (e.g., chitosan) have inherent antimicrobial effects [162], which can be beneficial for tissue repair and healing.

Currently, antimicrobial hydrogels can be classified into three categories based on their matrix and preparation methods: hydrogels with inorganic nanoparticles added, hydrogels with antibiotics added, and hydrogels with intrinsic antimicrobial properties. Inorganic antibacterial materials include Ag, Au, and Cu ions. Antibacterial effects can also be achieved by adding zinc oxide and nickel oxide. Kar et al. [180] prepared the guar gum-based macroporous hydrogels to which Ag NPs and epigallocatechin gallate (EGCG) were added as antimicrobial agents. The modified hydrogels with excellent antimicrobial properties promoted rapid wound healing and suppressed infection in type II diabetic C57BL/6 mice. Moreover, An et al. [181] achieved greater neural tissue regeneration in spinal cord-injured mice by using hydrogels loaded with EVs and Au nanoparticles in vivo, demonstrating good antibacterial properties. Metals and metal oxides have good antibacterial effects. Nanoparticles can damage the cell membranes of bacteria or have harmful effects on its organelles. However, antimicrobial ions, such as copper, have certain cytotoxicity. So it is very important to control the dose level of ions released from hydrogel scaffolds to reduce cytotoxicity to the lowest level. Antibiotics, the most commonly used and effective antimicrobial agents, are often added to hydrogels such as ciprofloxacin, gentamicin, vancomycin, and quinolones [182]. Some studies have shown that the toxicity of

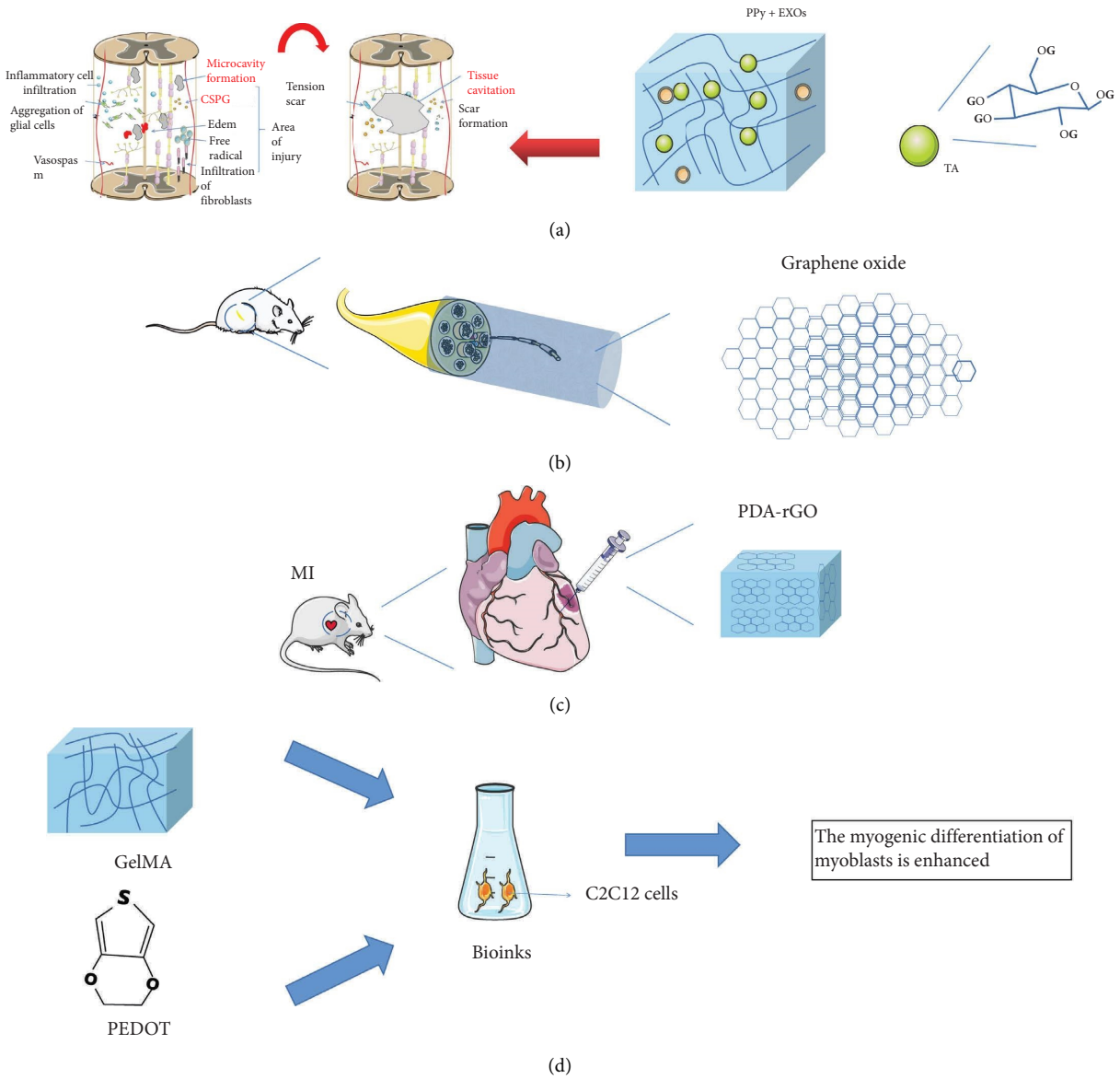

Figure 5: The application potential of conductive hydrogels. (a) Conductive scaffolds prepared by polypyrrole (PPy) and tannic acid (TA) for repairing spinal cord injury; (b) nerve catheter prepared by reduced GO/GelMA hydrogels promoted axon regeneration and myelination in rats; (c) polydopamine-reduced graphene oxide (PDA-rGO)/GelMA hydrogel conductive scaffolds promoted the maturation of cardiomyocytes and improved their structural and functional properties under electrical stimulation; (d) poly3, 4-vinyl dioxythiophene (PEDOT) hydrogel bioink has good electrical conductivity, and electrical signals stimulated by 3D printed scaffolds promoted myoblast differentiation and muscle tube formation in vitro.

ciprofloxacin is dose-dependent [183]. Hydrogels, as the local drug delivery system, can control the release rate of ciprofloxacin and gentamicin to reduce the influence of drug toxicity, solving the disadvantages of low blood concentration of gentamicin [184]. Antimicrobial peptides (AMPs), which have a unique inhibitory mechanism, offer a fresh approach to combating bacterial resistance in light of the widespread overuse of conventional antibiotics and other antibacterial medications. Broad-spectrum antibacterial activity, strong thermal stability, high antibacterial activity, high specificity, and little harmful side effects on animals are all benefits of AMPs [185]. It is believed that the mechanism of bacterial inhibition by AMPs is achieved mainly by binding to the cell membrane of bacteria and destroying them [186] with the specific biological mechanism of action as shown in Figure 6. Although some disadvantages of antimicrobial peptide hydrogels have also been reported, such as tissue toxicity and hemolysis [187], they have better biocompatibility than other biomaterial scaffolds. In a word, hydrogel antibacterial biological materials provide unique prospects and synergistic antibacterial effects, as an alternative to traditional antibiotic therapy. Hydrogel antibacterial biological scaffolds have the unique advantages of controlled release, stimulation switch release, good biocompatibility, low toxicity, and variable mechanical properties, which provide a bright prospect for clinical tissue repair and anti-infection treatment.

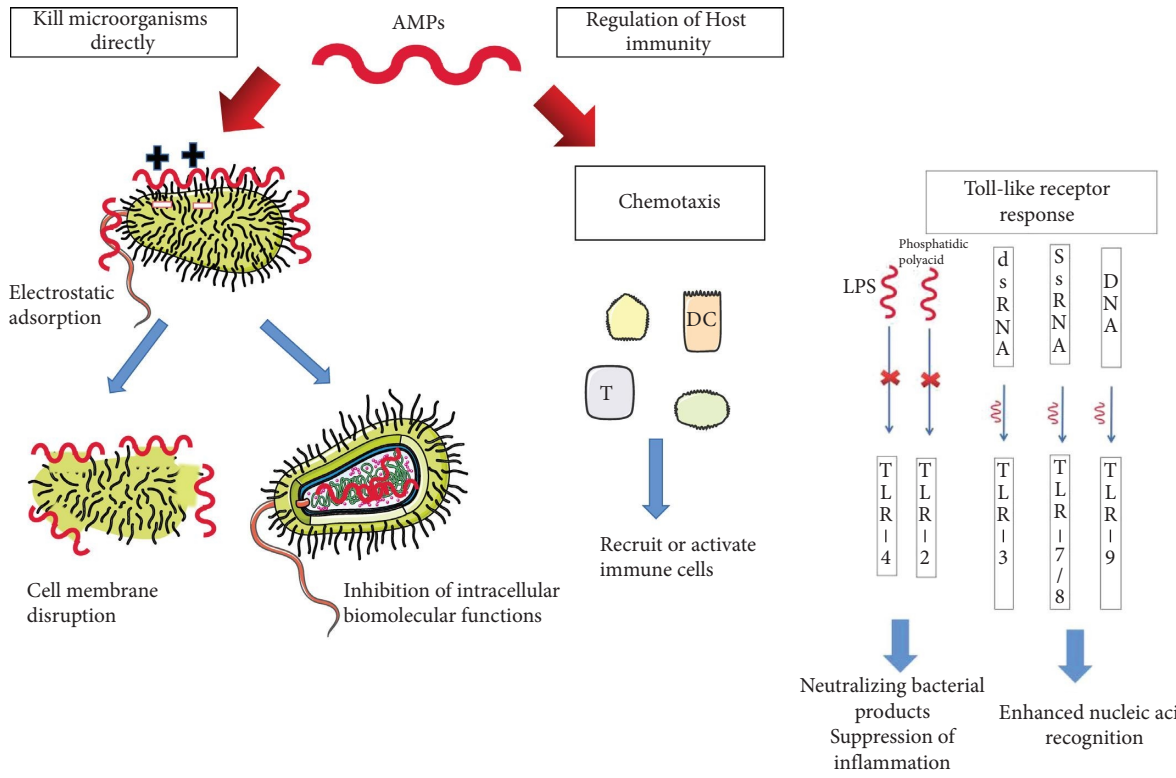

FIGURE 6: Antibacterial mechanism of bacteriostatic peptides. The left half of the image depicts the binding of the bacterial peptide to the bacterial cell membrane and the disruption of the bacterial structure by electrostatic adsorption, while the right half depicts the biological effect of bacterial inhibition by modulating host immunity. dsRNA: double-stranded RNA; ssRNA: single-stranded RNA; LPS: lipopolysaccharide; TLR: toll-like receptors.

4.1.4. Stimulation Responsiveness of Hydrogels. Hydrogels can be loaded with a variety of hydrophilic and hydrophobic drugs and cell goods. The loaded drugs can be released by diffusion according to the degradation properties of the basic material. However, this release cannot be controlled as needed according to the changes in the pathological environment. In clinical practice, excessive local drug concentrations often result in greater cytotoxicity [188]. In recent years, the development of smart responsive hydrogels has brought a solution to this problem. Through the design of hydrogel polymer molecular chains, a hydrogel with stimulus-response characteristics can be prepared, which can respond to stimuli and external environmental changes (such as pH and temperature) and can be divided into internal and external stimuli depending on the type of stimulus. Internal stimulation relies on the physicochemical properties within the diseased tissue to trigger a change in the shape of the hydrogel system, while external stimulation relies on temperature, light, magnetic fields, or acoustic waves to activate the activity of specific types of hydrogels for better tissue repair [189, 190].

Due to the different pH values in organisms, pH value has become an important response component in preparing responsive biomaterials. By constructing chemical bonds with different pH responses into hydrogel systems, conventional hydrogels can be endowed with pH responses, thus improving their applicability in living organisms. Some tissue injuries or local tissues are often accompanied by changes in pH (e.g., tumor cells have an external environment pH of about 6.5, while the internal environment pH is around 5.0 [191]). Therefore, pH-responsive hydrogels can be used as controlled release carriers for the loaded goods, which can be released through the changes in pH when the loaded goods reach the lesion tissue within a specific pH range. Hu et al. [192] prepared DNA hydrogels for insulin encapsulation by the polymerization of acrylate-modified nucleic acid chains and acrylamide with ammonium persulphate and tetramethylethylenediamine under ambient temperature for gelation. Under acidic conditions, the hydrogels could maintain a dense tissue structure at pH = 7.2 (approximately, the pH of intestinal fluid). The DNA hydrogels broke down into the liquid phase and completed the release of insulin. Moreover, He et al. [193] constructed a nanodelivery platform for andrographolide using modified mesoporous silica nanoparticles and ph-responsive polyacrylic acid to achieve slow release of the drug in an OA environment and overcome the dilemma of drug removal by synovial fluid. In addition to pH, researchers designed glucose-responsive hydrogels based on the glucose-sensitive mechanism of phenylboronic acid. The phenylboronic acid moiety is generally present in a hydrophobic undissociated form. When glucose is present, the combination of glucose and phenylboronic acid increases the ionisation of phenylboronic acid, leading to increased hydrophilicity of the phenylboronic acid-containing polymer [194], thereby releasing the internal drug. This property makes the hydrogels suitable for use in high-glucose

environments within the joint due to injury [195, 196] and in diabetic patients [197]. Moreover, Li et al. [198] prepared the multistimulus-responsive hydrogels for the integrated diagnosis and treatment of diabetic bone defects. The dual-network gel system interprets pathological cues (glucose fluctuations, ROS, and matrix metalloproteinases) with diagnostic logic that determines when to release drugs in the diabetic microenvironment. Matching the immune-bone cascade to better promote tissue regeneration provides new ideas for the promotion of repair of tissue damage under specific pathological conditions. Future studies can be based on the characteristics of the microenvironmental changes following different tissue injuries by identifying and responding to pathological cues to release cytokines or drugs to promote tissue healing and repair. The characteristics and potential application directions of temperature-triggered response hydrogels are summarized in Table 3.

4.1.5. Preparation Diversity of Hydrogels in Tissue Repair. Depending on the application conditions, hydrogels can be prepared in different sizes shapes and properties to meet the needs of the microenvironment and mechanical characteristics of specific tissues. Common forms of application include microspheres, microneedles, and 3D-printed scaffolds [218, 219]. Microspheres are monodisperse spherical systems in the micron or submicron size range. Compared with bulk hydrogels, gel microspheres have the advantages of simple structure, small size, large specific surface area, and easy self-assembly. Based on microfluidic technology, Zhao et al. [220] first coupled osteogenic and angiogenic signal peptides by solid-phase synthesis method to construct integrated biosignal peptide systems and, subsequently, cross-linked them by click reaction of sulfhydryl groups and double bonds to form biosignal integrated microsphere gels. The results showed that the microsphere gels significantly promoted bone tissue regeneration at bone defects by inducing osteogenic differentiation and neovascularization. Moreover, Zhao et al. [221] constructed a magnet-functional composite microsphere that traps Mg^{2+}, which enhances its ability to activate osteoblasts and endothelial cells and inhibit osteoclasts by loading and controlling the release of magnesium ions, ultimately enabling cancellous bone reconstruction through the integration of multipurpose microspheres. Because the porosity and pore size of the hydrogel microspheres can be adjusted, this structural design is more similar to the microenvironment of the extracellular matrix, which can enhance angiogenesis and promote new tissue remodeling, showing a promising application potential in musculoskeletal injury. In addition, with the continuous maturation of preparation techniques such as nonequilibrium microfluidics [222], the disadvantage of uneven particle size of microspheres in traditional preparation strategies has been solved, ensuring the bioactivity and membrane integrity of the internal EVs. Microneedles play an important role in tissue repair as an efficient means of transdermal drug delivery. A microneedle delivery system loaded with EVs is obtained by mixing the hydrogel precursor solution with EVs and curing it into shape in microneedle drug delivery systems [223]. After the microneedle is punctured into the damaged site, EVs are gradually released slowly as the hydrogels degrade, which in turn promotes tissue repair. Liu et al. [224] developed an array of microneedles loaded with EVs driven by nitric oxide nanomotors for promoting repair of Achilles tendinopathy in rats with superior efficacy to EVs injection. The loaded EVs were delivered transdermally via microneedles, which released anti-inflammatory NO gas and increased the depth of penetration of EVs. In addition, the tissue damage caused by microneedles is negligible. Hydrogel-based microneedle preparation has the advantages of easy administration, high permeability, and low toxicity. The preparation of microneedle does not affect the biological activity of EVs. In addition to microneedles and microspheres, hydrogel-loaded EVs can be used as 3D-printed bioinks to prepare stereotactically printed scaffolds with personalised structures due to their excellent rheological properties [169, 170] without affecting the biological activity of exosomes. Chen et al. [35] used bench top stereolithography to fabricate 3D-printed hydrogel-EV scaffolds with radially oriented channels that effectively repaired chondrocyte mitochondrial dysfunction and significantly promoted regeneration of patellar groove defects in rabbits. This therapeutic effect could be attributed to mitochondria-associated proteins in EVs. In addition to the above preparation methods, in Table 4, we also describe in detail the preparation strategies and advantages and disadvantages of the EV-hydrogel controlled release system, indicating that the selection of the preparation form needs to match as much as possible the characteristics of the application organization.

4.2. Hydrogels and Bioactivity of EVs. The release behaviour (e.g., amount and rate of release) of active substances in loaded hydrogels can directly affect the therapeutic efficacy. Unlike active substances such as drugs and growth factors, the biological activity of EVs depends on the integrity of the membrane [255]. Therefore, the ideal scaffold material should be able to ensure the integrity of the EVs membrane during loading and release to ensure therapeutic efficacy. It has been shown that −80°C is the optimal condition for storing biological fluids and isolating EVs, while isolated EVs can be stored for short periods at 4°C [256]. There is a risk of EVs deconstructing at 37°C, which is detrimental to the in vivo application of EVs. Therefore, it is necessary to discuss the release behaviour of EVs as hydrogel materials should be able to accommodate the long-lasting release of EVs with guaranteed bioactivity. Current methods used to detect EVs loaded in hydrogels include immunostaining [167], the BCA method for protein quantification [13], and enzyme-linked immunoassay (ELISA) [257]. Hu et al. [13]. prepared the hydrogel scaffolds using GelMA and nanoclay and loaded human umbilical cord MSC-derived EVs by physical mixing for treating cartilage defects in rat knee joints. The researchers used PBS as the release medium and quantified the release profile of the exosomes by the BCA method at regular intervals. The results showed that the GelMA-based hydrogels were able to sustain the release of

Table 3: Trigger conditions and examples of intelligent responsive hydrogels.

Type of stimulation	Examples	Features and mechanisms	Application directions	References
Enzyme	MMP Phosphatase Trypsin Tyrosinase	Under the catalysis of specific enzymes, the hydrogels show the characteristics of configuration change or fracture and promote the formation or degradation of hydrogels	Bone defect; myocardial infarction; diabetes mellitus; spinal cord injury; oral drug controlled release; cancer; wound healing	[199, 200] [201, 202] [203] [204]
Temperature	Poly (n-isopropylacrylamide)	It usually contains hydrophilic and hydrophobic groups, which can undergo phase transformation at an undetermined temperature so that the morphology of hydrogels changes with the change of swelling degree	Injectable hydrogels; drug delivery; breast cancer; perforation of digestive tract	[205, 206]
Light	Polymers containing acrylics/ coumarin functional groups Polymers containing the o-nitrobenzyl groups and their derivatives	Photocrosslinked hydrogels can undergo addition polymerization or cyclization under the combined action of photoinitiator Photodegradable hydrogels usually contain groups that break under light irradiation	Hydrophobic drug delivery; hydrogel crosslinking; development of far-infrared light-responsive hydrogel materials; tissue adhesion	[207, 208]
Magnetic	The composite hydrogels containing magnetic nanoparticles	When no magnetic field is applied, the drug is released through free diffusion. When the magnetic field is applied, the magnetic particles in the hydrogels aggregate and shrink the 3D network structure of the hydrogels, and the porosity decreases rapidly, resulting in the binding of drug molecules in the hydrogels, and the drug release rate decreases. When the field turns off again, the hydrogels return to original state, increasing the drug's release rate	Diabetes mellitus; cancer; bone defect; articular cartilage injury; arthritis	[209–211]
Shear force	Natural organic-based hydrogels	It is usually formed by high polymer with dynamic imine bond crosslinking. Spherical structure with layered fiber network, shear thinning, and self-healing properties	Injectable hydrogels; drug delivery; cancer; myocardial infarction; ligament rupture; osteoarthritis; articular cartilage injury; gastrointestinal perforation; limb ischemia	[212–216]
Ultrasonic	Hydrogels with Diels–Alder bonding bonds	When ultrasound is focused, these connections undergo a reverse reaction, reassembling the hydrogels, and releasing the model protein of internal load. Increasing the amplitude and time of focused ultrasound can improve the rate of protein release	Integration of diagnosis and treatment; synergistic therapeutic ultrasound improves therapeutic effect	[217]

EVs and had the ability to stimulate cartilage regeneration. Importantly, even after 21 days of implantation of the hydrogel loaded with EVs, there was a good therapeutic effect. In addition, hydrogels can provide the necessary protection for EVs in certain environments. It has been shown that sodium alginate hydrogel microspheres coated with gelatin layers loaded with EVs can protect EVs from degradation in acidic and enzymatic environments, maintaining the stability and biological activity of EVs, and protecting them from the adverse environment in the gastrointestinal tract [225]. The unique biological characteristics of hydrogel materials can keep the activity of EVs. The internal 3D network structure provides sufficient space for loading EVs, while gradual degradation can slowly release EVs to the target tissue and promote the repair of damaged tissue under physiological conditions or triggered by pathological clues, making it an ideal material for constructing EVs delivery systems. In addition, different forms

Table 4: The preparation strategy of the current mainstream exo-hydrogel controlled release system.

Form of preparation	Preparation method	Advantages	Disadvantages	Application	References
Hydrogel microbeads	Traditional methods Emulsion Microfluid flow Electrostatic droplet extrusion Coaxial air injection In situ polymerization	Low cost Fast preparation speed High mechanical strength	The bioactivity and release of exos were affected by the inability to obtain uniform small-size microbeads	Bone damage Colitis	[225, 226]
	Nonequilibrium microfluidic technology	Uniform in size The scale can be controlled below 100 μm Ensure exos activity	It is more complex than traditional methods	AF injury Bone damage Cartilage damage and so on	[222, 227]
Hydrogel fiber	Electric spinning yarn Microfluidic spinning Wet spinning Gel spinning Hydrodynamic spinning	Be able to inject Can stay longer to ensure exos' long-term release The properties are diversified after modification	Swelling is high, and sudden release of the drug may occur after injection The difference in mechanical strength Additional modification steps are required	Bone defect of weight-bearing bone of lower limb Myocardial tissue injury	[227–230]
Hydrogel nanoparticles	Physical or chemical crosslinking, such as polymerization of emulsion Distillation-precipitation polymerization	High expansibility High biocompatibility High mechanical properties Simple preparation High load drug Adjustable size	Crosslinks are difficult to control The controlled release performance is not good	Defect of bone Fracture Tendon injury Myocardial injury Cartilage damage (microtia)	[12, 13, 231, 232]
3D scaffolds were prepared by emulsion lyophilization	Hybrid Freeze drying Salt leaching Foaming of gas	Easy to store Application in laboratory Strong stability	There are limitations to interconnectivity Does not provide the structure of the native organization Time-consuming	Myocardial repair Repair of cartilage	[13, 229]
Spinning nanofiber scaffolds	Electrospinning	Biodegradation kinetics is adjustable High biocompatibility Adjustable porosity	High temperature may damage the structure and exos' activity during modification	Defect of bone Periodontitis AF injury	[233–235]

Table 4: Continued.

Form of preparation	Preparation method	Advantages	Disadvantages	Application	References
3D printing technology	Technique of extrusion	Fluid and slurry can be distributed in three dimensions, which is suitable for the treatment of various musculoskeletal disorders	Low accuracy Mechanical damage caused by shear force affects the therapeutic effect	Bone damage Tendon injury Cartilage damage	[236–240]
	Traditional bioink printing	Fast	Low strength		
	Drop ink as needed	High accuracy	Thermal/mechanical damage to exos Hydrogels that are cured may collapse	Bone damage OA	[241–244]
	Continuous inkjet	Low cost			
	Response to microporous molding	High strength Pore size is suitable for cell migration and proliferation Viscoelasticity can be matched to biological tissue	High cost The operation is difficult	No large-scale controlled trials have been conducted	[245]
	Photocuring-assisted printing Stereoscopic lithography Digital light processing	No nozzle can avoid exos damage Higher printing speed High precision The 3D structure is stacked smoothly Uncured hydrogels do not collapse	High cost The front-end design work is heavy	Muscle injury Defect of bone Cartilage damage Tendon injury	[246–254]

of crosslinking can have an impact on the biological activity of EVs. Depending on the nature of the polymer backbone and its functional groups, hydrogels can be crosslinked by various methods, including chemical [258], physical [259], and enzymatic [260] forms. Among them, ionic crosslinking relies mainly on the interaction between charged polymer chains and oppositely charged divalent or multivalent ions to trigger crosslinking. It is worth noting that although this method can provide uniform gelation across the droplet template and may cause the pH value to drop below physiological values, which impairs the biological activity of EVs. A more widely used method for loading EVs is photocrosslinking. The main biocompatible hydrogel materials commonly used to construct photocrosslinkers are natural or synthetic macromolecules, such as hyaluronic acid, gelatin, chitosan, and polyethylene glycol. Photocrosslinking is fast, easy to operate, and less damaging to cells and EVs, which ensures the biological activity of the carrier.

In summary, natural hydrogels have the advantages of easy preparation, low price, high biocompatibility, and low toxicity, which is an ideal raw material for the preparation of the EVs delivery system. However, the stability and cell adhesion are weak. The performance needs to be improved by additional chemical modification or crosslinking. In comparison to materials such as metals and ceramics, hydrogels have a special advantage. With appropriate modifications, hydrogels have controlled structural porosity, which means that their use as delivery systems for EVs can be personalised to obtain the desired EVs release rate and release trigger conditions to meet the needs of musculoskeletal injury treatment. The hydrogels also have special antibacterial properties that are helpful in treating wound infections, open fractures, and joint injuries while aiding repair and healing. In addition, the hydrogels ensure the biological activity of EVs in vivo. The hydrogel enables the controlled release of EVs in the target tissue area to avoid secondary damage from repeated injections or revision surgery. All these prove the application prospect of hydrogel-loaded EVs for the treatment of musculoskeletal injuries. However, current biomaterial preparation equipment is still unable to achieve the structural accuracy of normal human tissues and organs. Future research should support smart biomaterials, try to combine more composition ratios with different preparation strategies, and further test the effect of application in vivo, to provide a basis for selecting preparation strategies corresponding to exo-hydrogel delivery systems for specific types of musculoskeletal disorders.

5. Application of Exo-Hydrogel Delivery System in Musculoskeletal Disorders

Musculoskeletal injuries often heal poorly. Traditional treatment strategies are unable to fully restore the mechanical mechanics of the native tissue, which is detrimental to the patient's functional recovery. In recent years, decellularization therapy to promote recovery from musculoskeletal injuries has received widespread attention. In this

section, the application and therapeutic mechanism of hydrogels loaded with EVs in the treatment of various musculoskeletal disorders will be described to provide ideas and theoretical support for future research.

5.1. Application in the Treatment of Intervertebral Disc Injury. Intervertebral disc degeneration (IVDD) is a chronic degenerative disease and one of the main causes of low back pain [261, 262]. Surgery is the most widely used treatment for IVDD, which can partially improve the disease. However, invasive treatment can also cause greater pain and burden to patients. In addition, IVD is a nonvascular organ with poor internal healing ability. Deficient annulus fibrosus (AF) is often used to achieve compensatory repair in the form of the fibrous envelope on the outer part of the AF [263–265]. However, it increases the risk of recurrent nucleus pulposus herniation, which often leads to adverse outcomes. Therefore, current research is aimed at developing more minimally invasive treatment strategies that alleviate the core mechanisms of the disease rather than relieving symptoms. Under normal physiological conditions, anabolism and catabolism in IVD are closely coupled, and damage and exogenous stimuli will disrupt the metabolic balance of ECM, thus triggering a cascade reaction of degeneration, leading to the deterioration of its microenvironment [266]. Previous studies have confirmed that the decrease of type II collagen is related to the abnormal expression of matrix metalloproteinases (MMPs) and the imbalance of ECM, which changes the microenvironment of IVD [267, 268]. In addition, changes in the IVD microenvironment, activation of the inflammasome, and death of nucleus pulposus cells (NPCs) exacerbated the cascade of worsening reactions [269–271]. Therefore, improving the IVD microenvironment, remodeling the ECM metabolic balance, and protecting NPCs can slow down the process of IVDD. In addition, IVD was shown to be an immune-privileged organ. AF, together with cartilage endplate (CEP) and immunosuppressive molecules, and the nucleus pulposus (NP) barrier was constructed. This barrier is a physical and molecular barrier that separates NP from the host immune system [272, 273]. Therefore, emerging therapies for IVDD should ensure the improvement of the IVD microenvironment and anabolism without damaging the integrity of AF.

EVs have been shown to reduce the catabolism of ECM by inhibiting MMPs [274, 275], which is consistent with the therapeutic idea of alleviating IVDD by improving the IVD microenvironment. EVs are considered an attractive candidate for acellular therapy due to the low immunogenicity and carrying a variety of intrinsic cargo. To ensure the integrity of AF, intervertebral injection of EVs is the primary treatment option. However, direct injection of EVs cannot achieve a good therapeutic effect. The integrity of the EVs membrane may be damaged during injection due to the shear force of the syringe [276]. In addition, the rapid clearance and biological inactivation of EVs have become the biggest challenge in the treatment of IVDD by this therapy [277, 278]. In clinical application, repeated injections will bring great pain and risk of infection to patients. In recent years, to overcome these limitations, a variety of new hydrogels have been developed in intervertebral disc tissue engineering as new-generation EV carriers suitable for IVD. Compared with traditional EV vectors, hydrogels overcome the disadvantages of cytotoxicity, difficulty in degradation, and low biocompatibility and significantly improve the retention time of EVs around IVD [279]. Xing et al. [280] selected acellular porcine NP matrix-derived hydrogels combined with adipose-derived mesenchymal stem cell EVs to prepare a temperature-sensitive injectable EVs delivery system. Porcine NP matrix has been shown to have a regenerative effect, promoting the anabolic response of NPCs in an inflammatory environment [281]. This system can replace matrix loss due to metabolic disorders of ECM and provide structural support for the proliferation of NPCs. In addition, studies have shown that based on the mechanical properties of ECM, the relaxation stress enables EVs to overcome the constraints and regulate the transport of EVs under constrained conditions. The higher crosslinking density is conducive to the fluctuating transport of EVs through the polymer grid, thus triggering free diffusion and rapid transport [282]. This mechanical property also determines the superior delivery mode. Due to the presence of EVs surface channel proteins, EVs can deliver and target NPCs in the hydrogel drug delivery system, promote the anabolic metabolism of NPCs by down-regulating MMP-13, inactivate NLRP3 inflammasomes [280], and correct the changes in the early microenvironment of IVDD and ultimately the accumulation of ECM. In addition, EVs can be modified through lentivirus engineering and other ways to specifically improve the therapeutic effect. Luo et al. [48] constructed cartilage endplate stem cells (CESCs) overexpressing sphingomglycine kinase 2 (SPHK2). Sphk2-engineered EVs (Lenti-Sphk2-Exos) can be produced. Sufficient and functional SPHK2 can be transported to NP tissues by EVs and expressed mainly in the cytoplasm. Studies have shown that SPHK2 activates sphingine to form sphingine 1-phosphate (S1P), which regulates cell migration and invasion, angiogenesis, immune cell response, and other biological functions through p-AKT, p-ERK1/2, and autophagy signaling pathways [279, 283, 284]. Autophagy is a highly conserved catabolic activity that is involved in a variety of cellular biological activities, among which the AKT signaling pathway is considered to be an important regulator of autophagy. Studies have shown that there is a significant difference in AKT signaling activation between normal and degenerative IVDs [285]. In addition, different localization of SPHK2 in cells determines the difference in its function. SPHK2 is mainly expressed in the cytoplasm, which can inhibit cell senescence and promote proliferation [286]. Interestingly, when SPHK2 is expressed in the nucleus, it can inhibit cell growth [287, 288]. Since cell senescence is a pathological marker of IVDD [289], it may be a potential therapeutic target for IVDD by regulating autophagy to delay senescence in the process of degeneration. In addition, as IVDD progresses, AF may be structurally deficient, which challenges the modulus and stiffness requirements of exos carriers. DiStefano et al. [222]

integrated polylactic acid-glycolic acid copolymer microspheres (PLGAμSphere) into the interpenetrating network (PIN) hydrogels to prepare a new delivery system. PLGAμSphere can encapsulate exos and enhance endogenous AF repair response by injection for AF sealing. Polylactic acid-glycolic acid copolymer (PLGA) is a biodegradable and biocompatible synthetic copolymer approved by the US Food and Drug Administration (FDA), which is widely used in the preparation of PLGAμsphere controlled release carrier [290]. In vivo, PLGA will degrade into two acidic compounds, which will not significantly reduce the pH value of the environment and avoid having an impact on the IVD microenvironment. In addition, the rationality of cell infiltration should be taken into account when selecting the delivery system for hydrogel preparation [291]. AF cells are less than 20 μm in diameter. Under ideal conditions, to promote cell infiltration, PLGAμSphere must be degraded synchronously. Moreover, a moderate EVs load must be released into the therapeutic environment to establish a chemotactic gradient and create a repair site for AF.

In summary, compared with cell therapy, hydrogel-loaded EVs have very low immunogenicity and are suitable as cell-free treatment strategies for IVDD. Compared with direct injection, hydrogel-loaded EVs by injection can avoid the adverse consequences caused by the repeated injection and conform to the pathological process of IVDD, which is suitable for the IVD microenvironment and has no influence on the biological force of IVD. Therefore, hydrogels are an ideal controlled-release carrier. In vivo, EVs and engineered hydrogels have a synergistic effect to promote the anabolism of ECM and regulate the IVD microenvironment by down-regulating MMP-13. Lenti-Sphk2-Exos can regulate NPC autophagy and delay the aging process by activating the PI3K/AKT signaling pathway. In addition, the modulus and rigidity of the delivery system can be optimized by blending and integrating multicomponent materials. However, the differences in release dynamics due to the different proportions of formulations need to be further evaluated to find the optimal formulation of the delivery system to maximize the therapeutic effect by adjusting the release concentration.

5.2. Application in the Treatment of Tendinopathy. Tendinopathy is mainly divided into acute and chronic injuries, which is one of the most common musculoskeletal disorders worldwide [292, 293]. Cute tendon injury often occurs in sports. When the load of the tendon exceeds its maximum elastic variation, the tendon becomes plastic deformation, resulting in a partial tear or even rupture, which usually requires surgical reconstruction. However, the retear rate after tendon reconstruction is as high as 20%~90% [294], which will bring great pain to patients and seriously affect their quality of life. This high recurrence of retear is closely related to the healing process of the tendon. Tendon healing is a long and complex process [295]. In the early stage of tissue healing, a large number of inflammatory cells can be recruited at the injured site. Subsequently, a high level of proinflammatory factors is released. A large number of fibroblasts are recruited to gather at the injured site [296, 297], resulting in a strong inflammatory response that persists and stimulates fibroblasts to secrete excess ECM, promoting scar tissue formation [298, 299]. Excessive scar tissue weakens the tendon and increases the risk of tendon tears, especially tendon-bone interface (TBI). Normal TBI is a very complex heterogeneous structure composed of bone, mineralized and nonmineralized fibrochondrocytes, and tendons. This unique anatomical structure determines its important biomechanical properties, facilitating the transfer of tension from muscle contraction to bone, and preventing stress concentration, while scar tissue leads to the loss of normal mechanical transition. Disturbances in the biomechanical properties of TBI [298, 300, 301] increase the risk of tissue tearing under the load.

Chronic tendon injury, on the other hand, is associated with overuse of the tendons, mainly manifested as pain, dysfunction, and collagen fiber disorders. At present, the main clinical treatment methods include nonsteroidal anti-inflammatory drugs, local injection of corticosteroids, exercise therapy, and physical factor therapy [302, 303]. However, to achieve good therapeutic effects, these treatment methods are largely limited to controlling patients' pain, assisting tendon ECM reconstruction, and failing to restore the original state of the tendon. The pathological mechanism of tendinopathy has not been clearly defined. The current mainstream pathogenic hypothesis tends to be the dual effect of tissue degeneration and abnormal self-healing [304]. Like other tissues, the main characteristics of degeneration are apoptosis and abnormal degradation of ECM. It has been proved that the degree of apoptosis and tissue repair are closely related to the formation of normal fiber structure. Reducing the apoptosis of injured tissues is conducive to the recovery of the mechanical properties of tendon tissues [305, 306]. Therefore, the treatment of chronic tendon injury should be aimed at reducing the apoptosis of tendon cells and promoting the anabolic balance of tendon ECM. Although the tendon can heal itself, the biochemical and biomechanical properties of the healed tissue may not match that of the complete tendon [307]. Current treatment options for the repair of fractured or denatured tendons include sutures, prosthetic devices, autografts, and allografts. Up to now, none of these methods have succeeded in long-term tendon repair. After surgical reconstruction, the healing form of the tendon tissue with the anastomosis of the stump is scar healing. Moreover, the arrangement of collagen fibers in the tendon parenchyma is disordered, which seriously reduces the mechanical conductivity of the tendon tissue and increases the risk of fracture after surgery. Therefore, a new therapeutic method is urgently needed to repair the fiber arrangement of the injured tendon as much as possible, reduce the scar healing of the tendon, and restore the normal mechanical properties of the tendon tissue.

Tendon-derived stem cells (TDSCs) were first discovered in 2007 [308]. TDSCs are unique cells with common characteristics of stem cells, such as multifunctional differentiation, high self-regeneration capacity, and clonicity. Yu et al. [309] implanted EVs derived from BMSCs into

fibrin hydrogels. The controlled release of EVs was successfully internalized by TDSCs. The expression of Mohawk egg, type I collagen, and fibromodulin (Fmod) was enhanced. TDSCs are located in a unique ecological niche composed of ECM. EVs are one of the key components of this niche [308]. The loss of Fmod will regulate the bone morphogenetic protein signaling pathway, affect the differentiation of TDSCs, and hinder the endogenous repair of tendons. TDSCs play a role in cell proliferation, migration, and tissue repair by internalizing BMSC-derived EVs. There is potential communication between the two. Similarly, Fu et al. [310] combined adipose-derived stem cell EVs (ADSC-EVs) with hydrogels to prepare an exosome-hydrogel complex (ADSC-EV-hydrogel complex). EHC significantly promoted osteogenic and lipid-forming differentiation and up-regulated the expressions of TNMD, TNC, Runx2, Sox-9, and Scx, which was highly consistent with the molecular changes during the repair of injured tendons. The repair of the tendon after injury begins with inflammation. Subsequently, the tendon and the surrounding tissues release a large number of growth factors and cytokines [299]. Interleukin produced by proinflammatory M1 macrophages and growth factors produced by anti-inflammatory M2 macrophages participate in the important molecular reaction during the repair process [311]. In addition, coding TNMD, Scx, and TNC were also up-regulated in injured tendons [311, 312]. Scx plays an important role in tendon development and repair, participating in the aggregation of TDSCs and affecting tendon differentiation. Mice lacking the Scx allele showed reduced tendon ECM, disordered fiber arrangement, and even disintegration of tendon cells [313, 314]. Interestingly, it has been shown that Scx exerts different effects on different types of tendons. Scx allele deletion has little effect on tendons that only serve as anchors [314]. In addition, Scx has a positive regulatory effect on the expression of TNMD [313]. In early development, Scx is expressed earlier than TNMD, showing a promoting effect on its expression. Scx is also involved in the composition of the TGF-β signaling pathway, which is crucial for the formation of tendons and ligaments [315]. TGF-β phosphorylates Smad2 and Smad3 and activates the Smad signaling pathway to upregulate the expression of Scx and TNMD, thus promoting tendon repair and regeneration.

In addition to the above-mentioned involvement of EVs in the repair of damaged tissues through internalization, TDSCs can also be excellent EV donors in tendon repair. Previous studies have shown that allogeneic TDSCs can promote tendon repair. TDSCs show a stronger ability for tendon proliferation than MSCs from other sources [316]. Wang et al. [115] found that EVs from tendon-derived mesenchymal stem cells (TDSCs) can decrease MMP-3 expression and increase the expression of TIMP3, Col-1a1, and TNMD in injured tendons to improve the biomechanical properties of injured tissues. MMPs play a crucial role in the remodeling of almost all ECM, participating in the modification of collagen and fibronectin, as well as the regulation of signaling molecule activity [317–319]. TIMPs, an inhibitor of MMPs, play an important role in regulating cell growth, apoptosis, and angiogenesis [317, 320, 321]. The dynamic balance control between MMPs and TIMPs is an important prerequisite for tendon healing. When the balance change is broken, tendon healing can be affected. Therefore, maintaining tendon homeostasis is essential for the repair of injured tendons. Moreover, Song et al. [322] built a pHA-TDSC-EVs sustained release therapy system by embedding TDSC-EVs into photopolymerizable hyaluronic acid (p-HA) scaffold in a rat model of tendon defect. miR-144-3p targeting AT-rich interaction domain 1A (ARID1A) enriched in TDSC-EVs promoted the proliferation of tendon cells. Studies have shown that the overexpression of miR-144-3p can enhance cell proliferation and migration in nasopharyngeal carcinoma [323], epithelial cell repair [324], renal clear cell carcinoma [325], and other diseases. ARID1A, as the target of miR-144-3p [325], is a key component in the SWI/SNF chromatin remodeling complex and plays an important role in regulating the cell cycle by affecting DNA damage responses in an ATP- and complex-dependent manner [326, 327]. Downregulation of ARID1A will inhibit the access of transcription factors C/EBP-α and E2F4 to promoters [328], thereby interfering with the process of inhibiting cell cycle re-entry and terminal forced differentiation and inhibiting tissue regeneration. Therefore, conditional ablation of ARID1A can promote cell proliferation and migration, thus promoting tissue repair. Numerous studies have also shown that EVs can promote tissue repair by activating phosphorylation of cell survival pathways, among which phosphorylation of PI3K/Akt and MAPK/ERK1/2 pathways has been verified by numerous experiments [329–331], which are involved in the proliferation and migration of various cells. Zhang et al. [295] attempted to use methyl acryloyl (GelMA) and TDSC-EVs to prepare a gel-controlled release system to repair damaged tendons. The results showed that TDSC-EVs could induce the rapid phosphorylation of AKT and ERK and activate the prosurvival signals in cells to promote cell proliferation and migration. Thanks to the excellent physical and biological properties of GelMA [332], it is able to form a stable gel state at the damage gap and provide space for tissue regeneration after photo-crosslinking, demonstrating the strong potential of this method for clinical applications.

In summary, the delivery system constructed by hydrogels and EVs has strong clinical application potential in the repair of tendon injuries. Tendon repair is special. The mechanical structure repair of TBI and the orderliness of fiber arrangement are the key points to be paid attention to when selecting clinically applicable therapy. TDSCs play an important role in cell proliferation and migration to promote tendon repair through communication with EVs. In addition, TDSC-EVs may be more suitable for the application of tendon injury, by enriching miR-144-3p conditions to ablate ARID1A, to reduce the promoter access inhibition of C/EBP-α and E2F4, and to promote tissue repair. It promotes cell proliferation and migration by activating the PI3K/Akt and MAPK/ERK1/2 pathways. Finally, the use of ideal hydrogels to prepare the controllable shape of the delivery framework and simulate the structural and functional complexity of the native tissue is also an important point to pay attention to when designing the delivery system.

5.3. Application in Promoting Bone Healing. In clinical practice, bone-related diseases include fractures, bone defects, periodontitis, bone nonunion, and other diseases. The bone injury and large-area bone defect caused by these diseases [332, 333] have brought severe challenges for orthopaedic surgeons. In addition, about 7%–10% of patients experience delayed or even nonunion of bone due to mechanical or infectious causes [334]. Regeneration of lost bone tissue is the only way to achieve patient recovery. The response of bone to injury is a continuous process with overlapping time, starting from the initial inflammation, followed by the gradual activation of the repair mechanism, and entering the tightly coupled stage of tissue remodeling [335, 336]. Moreover, in this process, angiogenesis is essential for bone tissue regeneration [337], which is an important potential mechanism for promoting bone regeneration. Traditional clinical treatment strategies include the following: the "gold standard" treatment is autologous bone graft [338]. However, the parts that can be collected are very limited and cannot adapt to various types of bone injury and defects. In addition, biomaterials [339] and allogeneic bone grafts [340] are also expensive and prone to immune rejection and even infection at the transplant site. Recently, bone tissue engineering (BTE) has become a promising alternative therapy. BTE combines biological scaffolds with natural or synthetic molecules to promote bone regeneration [341, 342]. However, the direct use of MSCs has some problems, such as abnormal cell phenotype, low homing efficiency of transplanted cells, and changes in differentiation and proliferation ability. Recent studies have shown that the function of MSCs is mainly to play the role of paracrine, rather than directly differentiating into target tissues. Therefore, the hydrogel-loaded MSC-EVs are a solution to this dilemma. As a kind of "cell-free therapy," EVs derived from MSCs can help to replace their parent cells and to bypass the conventional restrictions of MSCs transplantation. However, the angiogenesis in tissue engineering scaffolds is not ideal, which also directly leads to low regeneration efficiency, as the regeneration of bone tissue requires timely delivery of nutrients and metabolic waste. Therefore, the development of angiogenic BTE technology is the key to replacing the traditional treatment. Wu et al. [343] used chitosan (CS)/β-glycerophosphate (β-GP) hydrogels loaded with small EVs (sEVs), which were able to form gels in situ at body temperature after injection and sustained release of sEVs, showing excellent angiogenic effects in in vivo and in vitro assays. In addition, Zhang et al. [344] prepared the injectable hydrogel materials that showed good sustained exosome release. After 14 days of injection into the body, approximately 71.2% of the EVs were released from the material and continuously repaired cranial defects in rats. Previous studies have shown that miR-21 is expressed at high levels in BMSC-EVs [345]. miR-21 induces angiogenic differentiation of MSCs and promotes angiogenesis and bone formation through upregulation of SPRY2 expression [346]. Furthermore, the culture environment of EVs can also affect their therapeutic utility. Yu et al. [347] prepared a temperature-induced autologous loaded collagen hydrogel 3D culture model for culturing and loading human periodontal membrane stem cell-derived EVs to promote in situ bone regeneration in rats. Interestingly, the results showed that culturing in 3D conditions resulted in high yields of EVs, greatly improving the efficiency of EVs collection. In addition, 3D-EVs were able to activate the YAP signaling pathway for more effective osteoinductive effects. Previous studies have mostly used 2D culture systems (e.g., multiwell plates and Petri dishes), which only provide cellular interactions under unnatural conditions, whereas 3D culture systems can provide a more realistic in vivo microenvironment through more primitive cellular communication and cell-matrix interactions, and precisely regulate the downstream responses of MSCs through dynamic feedback mechanisms. In addition, engineered EVs that add specific ligands to the surface of exos can selectively enhance affinity for specific cells and have a certain targeted capture effect, which is critical for the efficiency of healing. EVs have a small negative zeta potential, which can meet the requirements of a long cycle. This high potential to prevent immune system clearance makes EVs an ideal load cargo choice. Liu et al. [88] constructed an EVs delivering overexpressed miR-181 to regulate macrophage polarization. These engineered EVs suppressed inflammatory responses by regulating the PRKCD/AKT signaling pathway and promoting M2 polarization, which in turn promoted new bone formation in vivo, providing a new idea to suppress bone injury with excessive inflammatory responses locally in the graft. It is exciting that BTE technology has also achieved a good therapeutic effect in patients with metabolic diseases. Jing et al. [348] prepared an exos delivery system that can be triggered by the diabetic pathological clue MMP-9 and improved the alveolar bone loss in diabetic rats through the high expression of miR-126-5p and miR-150-5p. Diabetes patients are characterized by chronic inflammation and oxidative stress. This pathological state will lead to the expression of proinflammatory markers such as MMPs in the defect site, inhibition of growth factors, increased tissue degradation, and influence the healing of the defect tissue [349, 350]. Conversely, MMPs can also express the severity of dynamic inflammatory response in diabetic conditions. Dynamic response hydrogels realize the identification of pathological clues markers. DNA aptamers can be used as antibody-like antagonists of MMP-9 to neutralize its negative effects and protect the defect [351, 352].

To sum up, the above studies show that hydrogel-loaded EVs have a good application prospect in repairing bone injury. EVs mixed with hydrogels can significantly improve the retention rate of EVs at the injured site and improve the therapeutic effect. In addition, researchers should pay attention to the fact that simulating the behaviour of cells in their natural niche is of fundamental importance for cell-free therapy based on EVs. Studies have also shown that functional miRNAs can be selectively loaded into EVs. Hydrogel-loaded engineered exos involved in osteogenic differentiation (such as miR-885-5p, miR-181a, and miR-148a) may achieve better therapeutic effects.

Table 5: Summary of the efficacy and mechanism of cartilage repair by EVs from different sources of MSCs.

EVs source	Main mechanism	Proliferative effect	Ease of preparation	References
Human embryonic stem cells MSC (hESC-MSC)	Inducing phosphorylation of AKT and ERK and activates the AKT/ERK signaling pathway via CD73/ecto-50 nuclease activity	+++	√	[364, 365]
Bone marrow MSCs (BMSCs)	Inducing phosphorylation of AKT and ERK and activates the AKT/ERK signaling pathway via CD73/ecto-50 nuclease activity Inducing the expression of chondrocyte markers (col II, aggregated proteoglycans) while inhibiting catabolism (MMP-13, AD-AMTS5) and inflammatory markers (iNOS)	++	×	[366, 367]
Syno vial-derived MSC (SMSC)	Carrying Wnt5a and Wnt5b that activate transcriptional coactivator-associated protein (yes-associated protein, YAP) via alternative Wnt-signaling pathways and enhancing proliferation and migration of articular chondrocytes. But extracellular matrix secretion is significantly reduced and miR-140-5p needs to be packaged to suppress this side effect via RalA	++	×	[368, 369]
Infrapatellar fat pad-derived MSC (MSCIPFP)	Protecting cartilage from OA by inhibiting chondrocyte apoptosis and balancing anabolic and catabolic processes, possibly associated with miR-100-5p-mediated inhibition of the mTOR autophagic pathway	++++	√	[370, 371]
Adipose-derived MSC (AD-MSC)	Reducing the production of the inflammatory factors TNF-α, IL-6, and IL-10 in chondrocytes, thereby reducing ACD and preventing the development of OA Reducing c-jun, which further reduces the DNA binding affinity of AP-1 and NF-κB and thus decreases MMP-13 expression Prdx6 mediates cartilage antioxidant and protective effects	++	√	[372, 373]

5.4. Application in Articular Cartilage Injury. Cartilage injury caused by acute trauma, chronic strain, degenerative diseases, and other reasons is very common in clinic. However, the cartilage tissue structure is complex and contains no blood vessels, which leads to its limited self-repair ability [353] and tends to more extensive cartilage degeneration. The final outcome of patients may develop into osteoarthritis (OA). OA is a degenerative joint disease characterized by cartilage degeneration subchondral bone remodeling, synovial inflammation, and osteophyte formation, which leads to joint pain, swelling, and limited movement of patients, bringing a heavy burden to the social health system [354]. Currently, the main clinical strategies for treating cartilage injury are accelerated tissue repair and regeneration through cartilage simulation implants or microfracture surgery. However, the above methods are invasive. The process of tissue repair is mostly uncontrolled and associated with serious side effects. Importantly, the newly generated cartilage tissue is fibrocartilage, not hyaline cartilage, resulting in a mismatch between the mechanical properties of fibrocartilage and the healthy autogenous cartilage tissue, which cannot prevent the progression of degeneration [355]. There is no effective treatment to repair articular cartilage degeneration and reverse the progression of OA [356].

Recent evidence suggests that MSCs can play a therapeutic role in OA through the paracrine mechanism, especially the secretion of EVs, which is mainly reflected in the fact that MSCs can promote chondrocyte proliferation, reduce cell apoptosis, and maintain chondrocyte autophagy [357–359]. Therefore, MSC-derived EVs are considered a promising acellular tissue engineering therapy for OA. Hu et al. [13] explored the efficacy of this therapy by injecting hydrogel-loaded EVs derived from human umbilical cord mesenchymal stem cells into the joint. With the degradation of hydrogels, EVs are continuously released and are internalized by chondrocytes and hBMSCs to enhance their migration, proliferation, and differentiation, thus promoting the synthesis of ECM and type II collagen, and delaying the progression of OA. The therapeutic effect is still obvious after 21 days. Sang et al. [41] prepared the temperature-sensitive hydrogels of Pluronic F-127 in situ cross-linked HA for the inclusion of EVs derived from primary chondrocytes and injected it into the knee joint to delay the progression of OA. In the pathological process of OA, MMP-13 and proinflammatory factors are rapidly upregulated, leading to an excessive inflammatory response, which further leads to chondrocyte apoptosis, accelerates the degradation of ECM, and aggravates the severity of OA [360, 361]. The

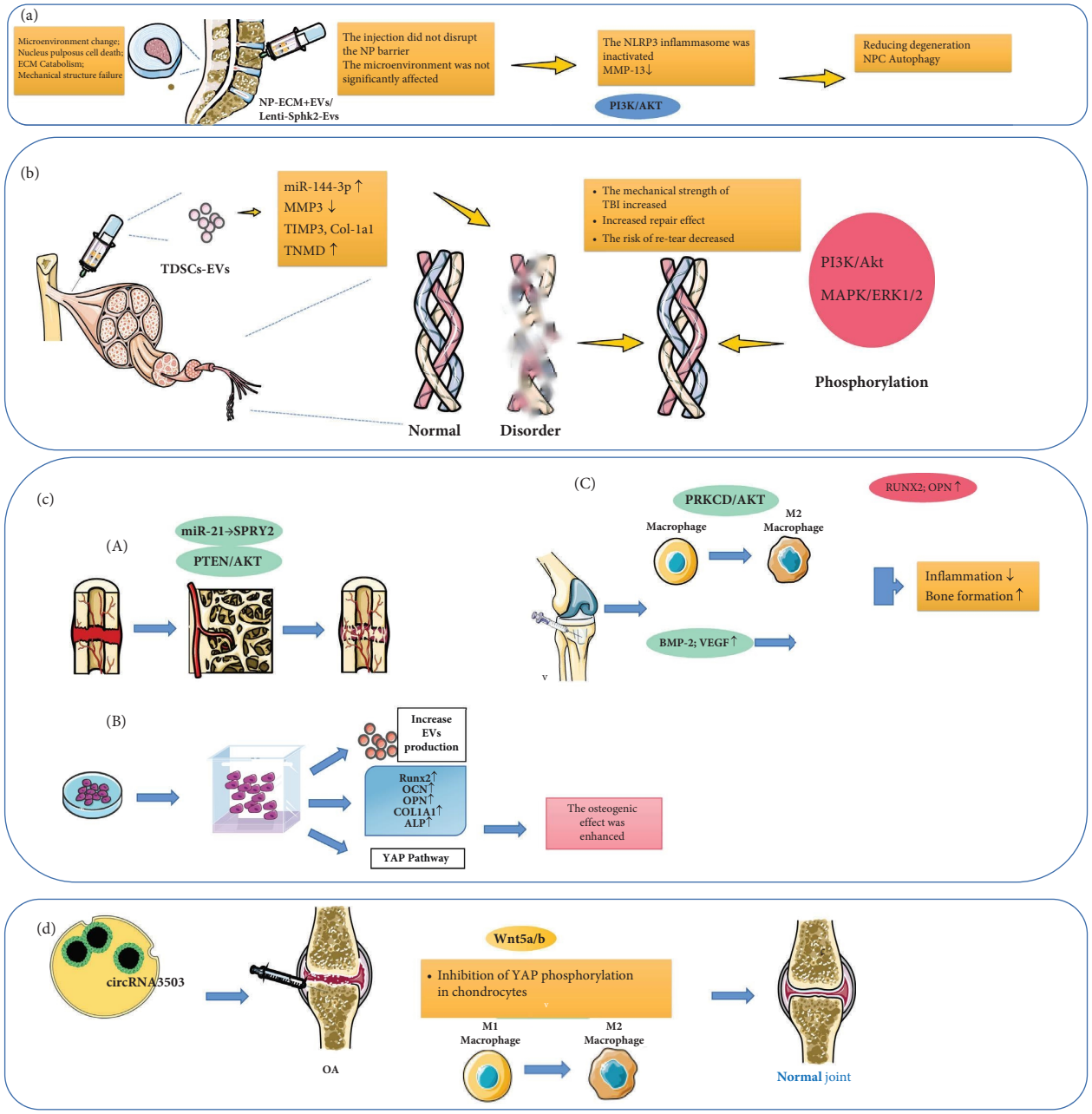

FIGURE 7: Application and therapeutic mechanism of EV-loaded hydrogels in the treatment of intervertebral disc, tendon, bone, and cartilage injury.

inflammatory response is related to M1 and M2 macrophages in tissues. Studies have shown that M1 macrophages in OA synovial tissue secrete IL-6 to inhibit chondrogenic differentiation, while M2 macrophages secrete IL-10 to inhibit inflammation, providing a microenvironment for chondrocytes to survive and easing the progression of OA. Moreover, patients' pain levels were negatively correlated with dysfunction and IL-10 levels [361–363]. Some studies have reported that EVs loaded with hydrogels can improve the inflammatory response of OA tissue and reverse the cartilage destruction of OA by promoting the polarization of M1-type macrophages to M2-type macrophages. Different sources of MSC-EVs have different repair capacities for cartilage tissue. Among them, human umbilical cord MSC-derived EVs have a strong repair and proliferative capacity, despite potential ethical controversies; although adipose-derived MSCs are readily available, they produce fibrocartilage rather than articular hyaline cartilage when used for cartilage injury repair, and the proliferative capacity of BMSC-EVs is affected by the age of the donor patient. Therefore, determining the optimal parental cell source for EVs plays an important role in cartilage regeneration strategies (as shown in Table 5).

In addition, to give natural EVs more functionality, researchers have tried to engineer EVs. Common engineering modification strategies include loading drugs,

conditional pretreatment of parental cells, and surface modification. Tao et al. [374] generated circRNA3503-carrying EVs through circRNA3503 overexpression in MSCs and loaded them into hydrogels. Engineered EVs mitigate ECM degradation by competitively adsorbing hsa-miR-181c-3p and hsa-let-7b-3p to regulate SOX9 expression, thereby inhibiting chondrocyte death and maintaining cartilage homeostasis. circRNA3503 is associated with sleep activity. Melatonin (MT) can help to raise the level of circRNA3503. It has been reported that MT can induce cell sleep [375] and regulate cartilage regeneration and degradation by regulating transcriptional activators [376, 377]. Therefore, circRNA3503 is a potential therapeutic target for OA. In addition, EVs derived from MSCs are rich in Wnt5a/b. Studies have shown that Wnt5a/b can inhibit YAP phosphorylation in chondrocytes [369]. Studies have reported that Wnt5a is able to induce macrophage polarization from M1 to M2 type and promote chondrocyte proliferation and migration by regulating the macrophage phenotype [365, 378]. Therefore, EVs and circRNA3503 play a synergistic therapeutic role.

In summary, hydrogel-loaded EVs derived from multiple MSCs have a good therapeutic effect on OA. Hydrogels have the properties of bionic cartilage, such as high water content, elasticity, and resistance to friction. They also have a 3D structure suitable for cell growth and differentiation, excellent biocompatibility, and cellular response properties, which may be a potential alternative to artificial basement membrane materials. In addition, MSC-derived exos inhibit YAP phosphorylation through Wnt5a/b, inducing macrophages to polarization from M1 type to M2 type to improve OA. Modification of exos can amplify the therapeutic effect. Loading specific circRNAs can slow the progression of OA by acting as miRNA sponges. In future studies, more therapeutic microRNAs, proteins, and target molecules can be loaded into exos for better therapeutic effects. Researchers should also consider the therapeutic mechanisms of action of different sources of MSC-EVs in their design, select the most suitable source of EVs, and load it with bioactive factors (e.g., extracellular matrix, diclofenac sodium, tretinoin [379], and other drug molecules) to achieve anti-inflammatory and stimulate chondrocyte proliferation and differentiation at the site of cartilage injury by slow release to obtain the most suitable combination of materials for cartilage repair.

6. Conclusions and Perspectives

In this review, we discuss the dilemmas encountered in the clinical practice of repairing difficult-to-heal injuries to the musculoskeletal system. Potential therapies are also offered depending on the available data. EVs offer new hope for a variety of musculoskeletal disorders that are difficult to heal. MSC-derived EVs have shown promising therapeutic effects in a variety of disease models. Recent studies have been devoted to solving the disadvantages of the short half-life of EVs. It is of great importance to select efficient, low cost, and highly biocompatible materials to construct drug delivery systems for exosome therapy. Hydrogels and other composite biomaterials have excellent degradability, hydrophilicity, biocompatibility, water content, and excellent viscosity and elasticity, which are highly similar to ECM. Accumulating evidence shows that EVs compounded with hydrogels can improve the stability of EVs and provide a continuous therapeutic environment for damaged tissues. In addition, it also helps to maintain the levels of EVs proteins and miRNAs in vivo, making it an ideal material for preparing controlled-release drug delivery systems. These findings show that hydrogel-loaded EVs have a good application prospect in the treatment of musculoskeletal injuries (as shown in Figure 7). There is no doubt that the key properties of hydrogel composite materials (such as biocompatibility, release ability of loaded EVs, and the activity of EVs) are essential. In addition, musculoskeletal injury is a broad concept. Various tissues have different local microenvironment and biomechanical characteristics. Therefore, the interaction between these materials and the surrounding tissues is also an important factor to consider. As the core of tissue repair, seed cells are widely used in various fields of regenerative medicine. EVs produced by different parent cells have different repair abilities in different tissues. For example, MSC-EVs derived from infrapatellar fat pad show more advantages than other tissues in amplification and cartilage differentiation [370]. In addition, factors such as the EVs isolation strategy and the specific form of hydrogel preparation can affect the therapeutic outcome, which also needs to be considered. Our study examined and explored the numerous aspects that may influence the therapeutic impact, as well as the distinct benefits of hydrogel as a carrier, with the goal of boosting the clinical implementation of hydrogel-EVS therapy.

However, many of the current therapeutic strategies to promote musculoskeletal injury repair are also deficient. Through more in-depth research, a standardized selection system can be established to provide a basis for selecting the therapy with the best composition ratio, the most economical and time-saving preparation strategy, and the highest specificity of engineering exos in clinical practice. Although there have been many studies of exos coated with hydrogels, there are still many challenges in achieving the clinical translation of this therapy, such as (1) can biointelligent hydrogels be developed To respond to a wider range of pathological markers and achieve drug release, or to achieve interactions between material and cellular responses to improve their microenvironment? (2) Different types of hydrogels have different advantages and characteristics. However, in the face of complex clinical needs, such as tissue microenvironment in the damaged area, tissue damage degree, different etiology, whether there are metabolic or immune complications, individualized differences, and other factors, how to choose? There is no best recommendation and sufficient evidence to support it. (3) Are hydrogels suitable for use as exos vectors in disease models of other systems? (4) How to define the most appropriate dose and the best time window of administration? Therefore, further studies are needed to explore relevant issues to continuously promote the clinical transformation of this therapy. We believe that with the comprehensive disclosure

of the biological functions of EVs, hydrogel-loaded EVs will have more applications in the clinical treatment of musculoskeletal injuries that cannot be ignored.

Data Availability

No data were used to support this study.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

Chunyu Zhang designed the methodology, prepared the original draft, and reviewed the manuscript. Xuchang Zhou conceptualized the study, designed the methodology, and performed supervision. Dongxue Wang visualized the study and reviewed and edited the manuscript. Li Hao performed visualization and reviewed the manuscript. Zhipeng Zeng performed supervision and visualized the manuscript. Lei Su visualized the manuscript and reviewed and edited the manuscript. Chunyu Zhang, Xuchang Zhou, and Dongxue Wang contributed equally to this work.

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