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Review article

Hydrogel-exosome system in tissue engineering: A promising therapeutic strategy

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ABSTRACT

Characterized by their pivotal roles in cell-to-cell communication, cell proliferation, and immune regulation during tissue repair, exosomes have emerged as a promising avenue for "cell-free therapy" in clinical applications. Hydrogels, possessing commendable biocompatibility, degradability, adjustability, and physical properties akin to biological tissues, have also found extensive utility in tissue engineering and regenerative repair. The synergistic combination of exosomes and hydrogels holds the potential not only to enhance the efficiency of exosomes but also to collaboratively advance the tissue repair process. This review has summarized the advancements made over the past decade in the research of hydrogel-exosome systems for regenerating various tissues including skin, bone, cartilage, nerves and tendons, with a focus on the methods for encapsulating and releasing exosomes within the hydrogels. It has also critically examined the gaps and limitations in current research, whilst proposed future directions and potential applications of this innovative approach.

1. Introduction

In the contemporary landscape of regenerative medicine, exosomes, as a subtype of extracellular vesicles (EVs) with a diameter ranging from 40 to 160 nm, have shown substantial potential for applications across diverse tissue repair processes [1–3]. Originating from multivesicular bodies (MVBs), these cup-shaped structures are released into the extracellular space through membrane fusion [4]. Laden with rich protein, lipid, and nucleic acid components, exosomes can selectively deliver these biomolecules to recipient cells through various mechanisms including ligand-receptor-mediated interactions, membrane infusion, and endocytosis, thereby eliciting tailored responses [5–7]. These distinctive features have positioned exosomes as pivotal mediators in the intercellular communication, exerting influence over the tissue microenvironment and playing a crucial role in both cellular communication and tissue repair [8].

However, despite the notable effectiveness of the exosomes as carriers for drug delivery in treating various diseases, their application has still encountered several challenges [9,10]. With conventional drug delivery systems, technical difficulties may arise with the isolation, loading, and release of exosomes, posing obstacles to clinical applications. Current research has predominantly focused on three primary strategies: i) to isolate exosomes and load them into drug-treated cells; ii) to load drugs into parent cells and subsequently release exosomes; iii) to transfect parent cells with DNA encoding drugs and then release the exosomes [11–13]. While some success has been attained with such strategies in laboratory settings, the challenges of high production cost, time-consuming processes, and the low retention and instability of exosomes in the body have made scaling up for large-scale application in human patients a formidable task [14].

To address the aforementioned challenges, hydrogels have surfaced as a dependable material for exosome carriers. The outstanding biocompatibility and adjustable physicochemical properties have rendered them as an ideal platform for exosome delivery [15–17].

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Utilizing hydrogels may allow for a more precise control over the release of exosomes, thereby augmenting the efficacy of treatment. Furthermore, certain hydrogels inherently have the potential to regulate cell adhesion, proliferation, and differentiation. This synergistic interplay with the exosomes may further amplify their therapeutic effects, positioning them as a promising avenue for improving the treatment outcomes [18,19]. Consequently, since the year 2015, there has been a rapid surge in both the interest and advancements in hydrogel-exosome systems. These systems have been predominantly utilized in the field of tissue engineering, particularly for the regeneration and repair of skin, bones, cartilage, nerves and tendons (Fig. 1).

This article has aimed to comprehensively review the recent developments in the hydrogel-exosome system within the field of tissue engineering and investigate its potential as a promising therapeutic strategy (Fig. 2). Initially, we have examined the biogenesis of exosomes and their sources by summarizing their methods of extraction and isolation, along with their respective advantages and disadvantages. Subsequently, we have underscored the potential applications of exosomes in regenerative medicine by exploring their mechanisms of action in tissue repair and methods of engineering modification. Thereafter, we have delved into the detailed advantages of the hydrogels and elucidated how hydrogels may facilitate precise control for the release of exosomes as a carrier. Finally, we have furnished a detailed account of the therapeutic effects and mechanisms of the hydrogel-exosome system in animal models for skin, bone and cartilage, nerve, and tendon tissue injuries over the past decade, whilst summarizing the current limitations in preclinical research.

Through an in-depth exploration of the hydrogel-exosome system, this article may provide insights and solutions for the future advancement in tissue engineering and regenerative medicine. Based on our exploration, we hold the belief that this system has the potential to evolve into a viable therapeutic strategy and instill new hope for clinical applications.

2. Overview of the exosomes

2.1. Biogenesis of exosomes

Exosomes are membrane-bound vesicles with a diameter of 40–160 nm, which are encapsulated by a phospholipid bilayer [4]. They are secreted by most cells and present in nearly all biological fluids. In 1985, researchers exploring the transformation of reticulocytes into mature red blood cells had discovered that multivesicular bodies, upon fusion with the cell membrane, could release small vesicles carrying transferrin and its receptors into the extracellular space. In 1987, Johnstone had coined the term “exosome” for such vesicles [20]. Initially, the exosomes were thought to be “garbage bin” which may aid the cells with discarding unwanted cellular components. It was until 1996 when Raposo had first observed that exosomes released by immune cells, such as B lymphocytes, had carried membrane-bound molecules crucial for triggering immune responses [21]. Two years later, Zivogel and others had discovered that another cell type, dendritic cells, could secret exosomes

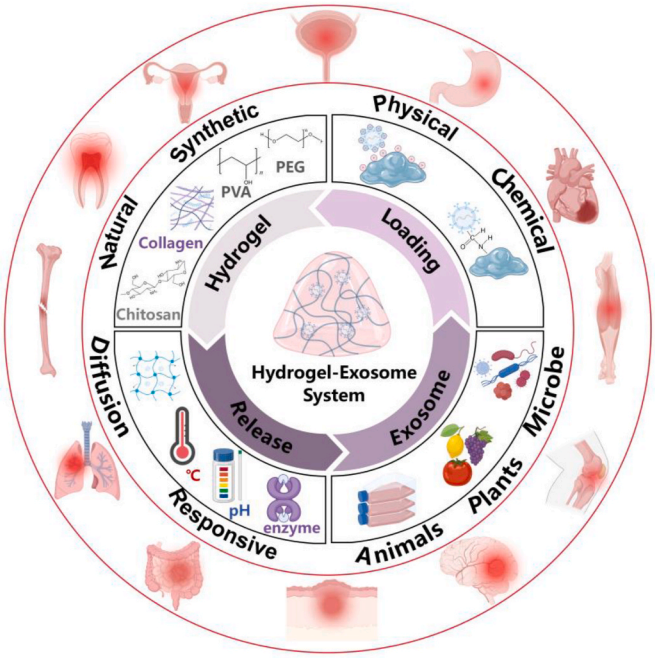

Fig. 2. Schematic illustration of the hydrogel-exosome system for tissue engineering and regenerative medicine.

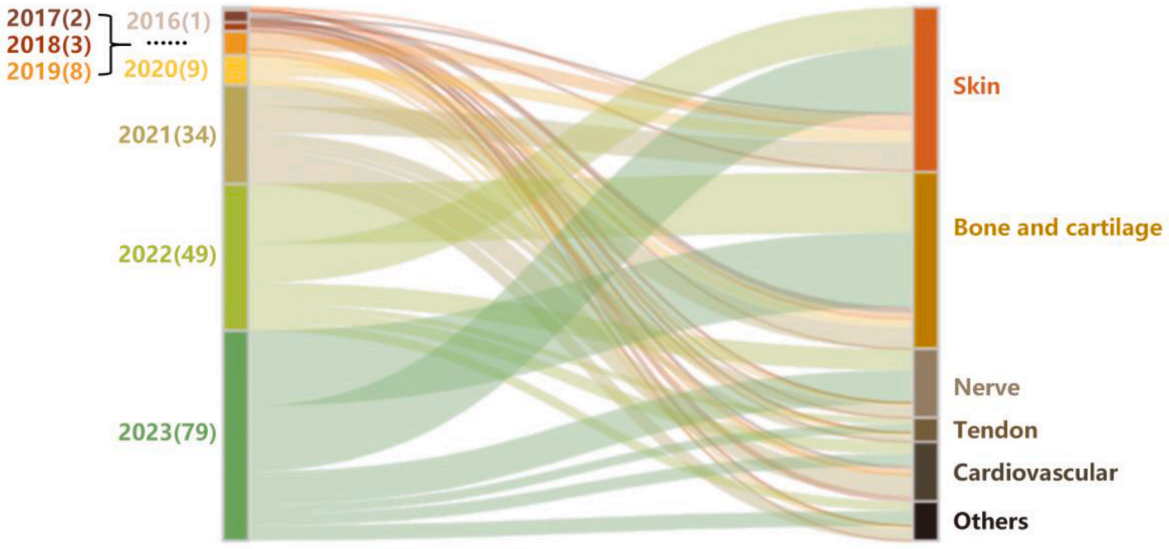

Fig. 1. Hydrogel-Exosome Systems. The Sankey diagram, leveraging bibliometric analysis, visually represents the distribution of published articles over the years (left) and categorizes them according to their application in various tissues or entire organs (right). The fields of bone and cartilage, skin, and neural tissue engineering emerge as the predominant areas of application for hydrogel-exosome systems. This diagram was developed using a comprehensive dataset of 175 published articles.

carrying functional immune molecules which could promote anti-tumor responses in mice [22]. These milestone studies had underscored the indispensable role of exosomes in intercellular communication and fueled exploration into their clinical applications.

Exosomes originate from the endosomal system, and their biogenesis involves a series of intricate steps including biosynthesis, endoplasmic reticulum secretion, cell membrane vesicle formation, reverse fusion of intravesicular membranes, and exocytosis [23,24]: (1) The generation of exosomes initiates intracellularly, where specific proteins, nucleic acids, and lipids are synthesized in the cytoplasm, serving as the foundational elements for exosome composition. (2) Synthesized proteins, nucleic acids, and lipids will traverse the endoplasmic reticulum (ER) to reach the Golgi apparatus, where these molecules will undergo modification and packaging, culminating in the formation of membrane-enveloped vesicles known as MVBs. (3) The MVBs will encapsulate membrane vesicles containing the internal contents, created through inward protrusions of the cell membrane. At this stage, internal proteins, nucleic acids, and lipids may find refuge within the small vesicles. (4) Some intravesicular contents of the MVBs are retained, and through the process of reverse fusion, they may amalgamate with the MVBs, resulting in the formation of larger vesicles containing intravesicular vesicles. (5) Ultimately, these sizable vesicles (exosomes) are released into the extracellular space through fusion with the cell membrane, a process typically accomplished by exocytosis.

2.2. Source of exosomes

Exosomes can be derived from a variety of organisms, ranging from mammalian cells such as immune cells, tumor cells, and stem cells, to plant cells, as well as various microorganisms including bacteria and fungi. They mediate intercellular communication by encapsulating and transporting proteins, lipids, RNA, and other molecules, playing pivotal roles in disease progression, immune modulation, tissue regeneration, and numerous other biological processes. Given their diverse origins and intricate functionalities, exosomes hold tremendous promise in the realm of biomedical research (Table 1).

2.2.1. Exosomes derived from mammalian organisms

Mammalian cells, which span a wide array of types, possess the ability to produce and release exosomes. These include cells like neutrophils, reticulocytes, dendritic cells, B cells, T cells, mast cells, epithelial cells, tumor cells, etc. [47]. Exosomes are naturally present in body fluids including blood, saliva, urine, and breast milk. Upon release, they will enter the circulatory system, travel to other cells and tissues, and exert regulatory effects from a distance. Exosomes derived from mesenchymal stem cells have functions akin to those of mesenchymal stem cells but with lower immunogenicity. They have shown remarkable therapeutic efficacy for conditions such as chronic wound healing, intervertebral disc degeneration, kidney diseases, and gastrointestinal cancers [48–51].

Currently, exosomes derived from mammalian organisms remain the most promising therapeutic tool, given their advanced research status and high resemblance to human cells. They contain a rich array of biological molecules, including proteins, lipids, mRNA, and miRNA, which serve as direct indicators of the biological status and functions of the source cells. Exosomes play pivotal roles in intercellular communication, immune modulation, disease progression (such as cancer advancement and metastasis), as well as tissue repair.

2.2.2. Exosomes derived from mammalian plants

Plant cells can release exosomes in response to diverse biotic and non-biotic environmental stresses including infections and attacks. Researchers have isolated exosomes from edible plants including ginger, lemon, grapefruit, ginseng, and wheat, which revealed their involvement in the regulation of gut microbiota, modulation of oxidative stress, and prevention of vascular calcification [37,52,53]. While plant-derived

Table 1
The biological functions of exosomes from various sources compared.

Type	Sources	Functions/Applications	Reference
Mammalian-derived Exosomes	M2 Macrophages	Improved the Bone Immune Microenvironment in Diabetes.	[25]
	$CD4^+$ T Cells	Promoted B cell activation, proliferation, and antibody production.	[26]
	Neutrophils	Activated apoptosis and inhibited tumor cell viability.	[27]
	Platelet-Rich Plasma	Increased MSC proliferation, viability, and activity, and reduced MSC apoptosis under stress.	[28]
	Urine	Regulated stone formation through the influence on focal adhesions.	[29]
	Adipose-Derived Mesenchymal Stem Cells	Improved neuronal function and neurotrophic effects.	[30]
	Bone Marrow-Derived Mesenchymal Stem Cells	Promoted aging osteogenesis by enhancing vascularization.	[31]
	Umbilical Cord-Derived Mesenchymal Stem Cells	Weakened macrophage infiltration and localized liver injury.	[32]
	Amniotic Epithelial Cells	Mediates signal transduction between the fetus and the mother.	[33]
Edible plant-derived Exosomes	Ginseng	Penetrating the blood-brain barrier and modulating the tumor microenvironment.	[34]
	Wheat	Having proliferative and migratory characteristics, promoting wound healing.	[35]
	Ginger	Inhibiting levels of inflammatory cytokines.	[36]
		Targeting digestive organs, regulating intestinal flora.	[37]
		Inhibiting virus-mediated lung inflammation.	[38]
	Grapefruit	Inhibiting the growth of bone marrow progenitor cells derived from leukemia patients.	[39]
		Quelling the activation of Th17 cells and inducing Treg cell infiltration.	[40]
	Garlic	Inhibiting the activation of the NLRP3 inflammasome.	[41]
Microbial-derived Exosomes	*Lactobacillus rhamnosus* GG	Promotes osteogenesis and inhibits osteoclast formation.	[42]
	Escherichia coli Nissle 1917	Increases beneficial microbiota, inhibits pathogenic bacterial phyla, regulates gut-liver homeostasis, and improves obesity.	[43]
	Lactobacillus casei Hy2782	Promotes neural growth, maturation, and synaptic plasticity.	[44]
	Lactobacillus gasseri	Modulates host cell responses and pathogen behavior.	[45]
	Streptococcus pneumoniae	Interacts and co-localizes within immune cells, facilitating antigen presentation and induction of immune responses.	[46]

exosomes can also transport genetic material, proteins, lipids, and other molecules, their composition and functions differ from those of mammalian exosomes. Typically, they are involved in biological processes such as inter-plant signaling and stress response [54]. Although some studies have reported their interaction with mammalian cells and their potential roles in functions like anti-tumor, anti-inflammatory, antiviral, and antifibrotic activities, such interactions are generally less specific or complex compared to mammalian exosomes. Therefore, engineering approaches may be necessary to enhance their functionality and targeting in the human body [55–58]. Zhang et al. extracted nanoparticles from ginger and reassembled their lipids into ginger-derived nanocarriers. Following modification with the targeting ligand folic acid (FA), they successfully achieved active and specific targeting inhibition of tumors in vivo. Experimental data suggest that the antitumor effect is attributed not only to the delivery of doxorubicin by the nanocarrier but also, in part, to the intrinsic property of ginger-derived nanoparticles in inhibiting the levels of inflammatory cytokines [36]. Exosomes derived from ginseng has showcased augmented targeting abilities for the blood-brain barrier (BBB) and glioblastomas, leading to significant therapeutic effects. They also displayed robust efficacy in recruiting M1 macrophages within the tumor microenvironment [34]. The exploration of the distinctive biological functions of plant exosomes not only offered a safe and cost-effective therapeutic option for various diseases but also provided a targeted strategy for individualized biological treatments. Furthermore, it has also paved a way for the development of innovative drug delivery systems in the future.

2.2.3. Exosomes derived from microbes

Researches on microbial exosomes have primarily focused on bacterial extracellular vesicles (BEVs), which may influence various biological processes such as virulence factor transport, resistance transfer, biofilm formation, pathogenic factor transfer, autophagy, and cell death regulation [59]. Outer membrane vesicles (OMVs) purified from *Xanthomonas campestris pv*, a Gram-negative plant pathogenic bacterium, could induce significant transcriptional changes in Arabidopsis. This activation has involved the immune system and upregulation of related pathways including multiple immune receptors [60]. Compared to mammalian exosomes, microbial-derived exosomes offer greater convenience for engineering modifications. Researchers have developed a synthetic bacterial vesicle by treating *Escherichia coli* with lysozyme and high pH. This engineered vesicle exhibits a 40-fold higher yield compared to natural vesicles, with minimal cellular content and devoid of RNA or DNA. As a result, it avoids eliciting systemic pro-inflammatory cytokine responses in mice [61]. Su and colleagues designed and constructed a bioengineered delivery system based on BEVs, enabling the delivery of miRNA or siRNA to the bone microenvironment. This system promotes osteogenic differentiation and alleviates osteoporosis [42,62]. The BEVs have also offered advantages over their parent bacteria, featuring better biocompatibility and lower risk for malignancy. However, to achieve an optimal balance between the low toxicity and high immunogenicity may still require substantial efforts [63].

2.3. Extraction and isolation of exosomes

A method capable of efficiently providing complete and pure exosome samples forms the foundational step for cell-free exosome therapy. Understanding existing isolation techniques is crucial for developing more efficient and rational methods for exosome separation (Table 2). Traditional methods like differential ultracentrifugation (DUC) represent the earliest and most frequently reported strategies for exosome isolation, often heralded as the “gold standard” in exosome separation techniques. However, these methods necessitate the use of costly equipment such as ultracentrifuges and entail lengthy processing times (typically >4 h). Additionally, the excessively high centrifugal forces involved inevitably lead to the disruption of exosome membrane structures, thereby impacting functional analysis. Density gradient ultracentrifugation is an enhanced technique derived from DUC, which typically utilizes two or more separation media with varying densities, such as sucrose and iodixanol [64]. This method allows for the isolation of exosomes with higher purity. However, the preparation steps for the separation media are intricate, rendering it impractical for large-volume exosome extraction [65]. Ultrafiltration is a size-based isolation technique. When a sample passes through an ultrafiltration membrane with specific pore sizes, substances smaller than the membrane pore size (such as exosomes) can pass through, while larger substances (like cell debris and large protein complexes) are retained. This method is relatively straightforward, suitable for rapidly processing large quantities of samples, and yields high purity. However, membrane clogging during use can occur, leading to higher consumable costs [66,67]. Size exclusion chromatography operates based on the ability of molecules to permeate the pores of a gel filler. Larger molecules are unable to enter these pores and thus travel along the shortest path through the chromatographic column, eluting before smaller molecules. Conversely, smaller molecules can penetrate the pores, resulting in a longer path and delayed elution. This method facilitates the separation of various sample components based on their sizes. Size exclusion chromatography effectively isolates exosomes and other cellular particles, such as microvesicles and protein aggregates, yielding exosome samples with higher purity. However, it may introduce nonspecific protein contamination [68]. The principle behind polymer precipitation relies on the capability of polymers, like polyethylene glycol (PEG), to induce changes in the ionic strength and solubility of a solution as their concentration increases. This phenomenon causes nanoparticles, such as exosomes, to aggregate and precipitate. Once the polymer concentration reaches a certain threshold, exosome particles are efficiently separated from the solution. Polymer precipitation, which is independent of centrifugal force, can yield higher quantities, but the presence of organic solvents

Table 2
Comparison of the advantages and disadvantages of the commonly used exosome extraction methods.

Method	Advantages	Disadvantages	Reference
Differential Centrifugation	Simple to operate and yields high efficiency, free from non-specific protein contamination	Low purity and time-consuming, requiring special equipment	[64]
Density Gradient Centrifugation	High yield and purity	Tedious operation, suitable for small volume samples	[65]
Ultrafiltration	Simple operation and short time-consuming	High consumable costs and prone to blockage	[66,67]
Size Exclusion Chromatography	High purity and yield, and short processing time. Preserving the integrity of the membrane	Contamination from non-specific proteins	[68]
Affinity Nanoparticle-based Isolation	High yield and purity	Antibodies are expensive and not suitable for large-scale production	[70,71]
Polymer Precipitation	No need for special equipment, high yield	Non-specific protein contamination affects downstream analysis	[69]
Commercial Kits	Simple and quick, high purity	High cost, limited yield	[70,71]
Microfluidic technologies	Automated processing, high production efficiency	Expensive equipment, high requirements for operators' professional skills	[78,79]

like PEG, lectins, and fish gelatin can introduce contamination to the exosome proteins [69]. The core principle of affinity nanoparticle-based methods involves modifying the surfaces of nanoparticles with ligands capable of specifically binding to molecules on the surface of exosomes. These ligands, which can be antibodies, proteins, peptides, or other highly-affine molecules, recognize specific markers on the exosome surface. When samples, such as body fluids or cell culture supernatants, pass through a medium containing these affinity nanoparticles, exosomes are selectively captured while other components are washed away. Subsequently, exosomes can be released and collected by adjusting conditions such as pH or ionic strength, allowing for their separation and enrichment. Affinity nanoparticle-based methods offer high selectivity and sensitivity while preserving the integrity of exosomes. Both commercial kits and affinity nanoparticle methods provide simple and convenient means to rapidly extract high concentrations of exosomes from biological samples. However, the high cost associated with commercial kits and affinity nanoparticle methods hinders the large-scale production of exosomes [70,71].

In summary, to obtain satisfactory extraction and purification of exosomes is challenging with a single isolation method. Researchers have often resorted to the combination of various methods. In 2018, a method termed Cushioned-Density Gradient Ultracentrifugation (C-DGUC) was developed, which utilized an iodixanol liquid cushion during the ultracentrifugation. This approach had led to a threefold increase in the yield of concentrated exosomes from the conditioned media of J774.1 mouse macrophage cells [72]. In a separate study, the combination of tangential flow filtration and subsequent size-exclusion chromatography has attained an over tenfold efficiency compared with the combined ultracentrifugation and size-exclusion chromatography [73]. In recent years, the development of efficient and reliable techniques for extracting exosomes has emerged as an active area of research. Microfluidic technology stands out for its unique advantages in exosome extraction. Firstly, it enables precise control over fluid movement, mixing, and separation, which is crucial for maintaining the integrity and activity of exosomes [74,75]. Secondly, microfluidic platforms can integrate multiple functions such as sample pretreatment, exosome enrichment, separation, and analysis, all on a single chip. This integrated approach reduces potential contamination and sample loss during processing, thereby improving the repeatability and reliability of experiments. Moreover, microfluidic devices require minimal amounts of reagents and samples, making them particularly valuable for handling precious or difficult-to-obtain biological samples [76]. Recently, researchers have developed a zigzag-patterned nanochip, which enhances the contact and interaction between structures and exosomes by inducing strong fluid mixing and reducing the fluid boundary layer. The nanochip incorporates anti-human epidermal growth factor receptor 2 (HER2) antibodies into its nanostructures, enabling the capture and detection of exosomes derived from HER2-positive cancers, with a capture efficiency as high as 97.7 % [77].

2.4. The potential of exosomes for regenerative medicine

2.4.1. Tissue repair mechanisms

Growth factors, cytokines, and genetic information within the extracellular vesicles, including exosomes, derived from the secretions of surrounding cells can produce a diverse range of therapeutic effects including regulation of inflammation, cell proliferation, migration, and angiogenesis. However, the capacity to achieve such functions may vary between the exosomes, primarily depending on their source cells. For example, monocyte-derived exosomes may contain anti-inflammatory proteins and miRNA, which can contribute to the alleviation of inflammatory responses [80]. Exosomes isolated from myocardial cells may play a crucial role in mediating pathological fibrotic remodeling, promoting cardiac vascularization, and inhibiting myocardial infarction [81–83].

Mesenchymal stem cell-derived exosomes (MSC-Exos) represent one of the most widely used exosomes clinically. As non-hematopoietic multipotent stem cells with self-renewal and multi-lineage differentiation capabilities, the MSCs stand among the most crucial types of adult stem cells and have found application in cell therapy for various diseases [84,85]. The MSC-Exos carry proteins, lipids, DNA, and RNA originated from the MSCs, which have formed the basis of their therapeutic effects. The MSC-Exos have shown biological functions similar to the MSCs, whilst their smaller size has allowed penetration of biological membranes, low immunogenicity, and the ability to mitigate risks such as immune rejection, embolism, and tumorigenesis. The lipid bilayer of exosomes not only can shield their internal components but also effectively protect the nucleic acids against damage by RNase. The exosomes can transport various bioactive components reflecting the physiological and pathological states of the source cells, convey information, clear intracellular components, and possess drug transport capabilities [86].

The MSC-Exos can also inhibit cell apoptosis, promote cell regeneration and migration, regulate immune and inflammatory responses, and stimulate vascular and neural regeneration. Researchers have intervened in seven critically ill COVID-19 patients by administering aerosolized exosomes derived from allogeneic adipose mesenchymal stem cells, which attained significant improvement in their lung damage [87].

2.4.2. Exosome engineering transformation

In recent years, researchers have undertaken the engineering transformation of the exosomes to better meet the clinical demands for enhanced therapeutic efficacy and facilitate their widespread applications in the biomedical field. The key directions of such modifications can be summarized as below. i) Surface protein modification: To alter the membrane proteins on the surface of the exosomes may achieve specific targeting and/or increase their affinity for target cells. These may enhance the enrichment and uptake efficiency of the exosomes by specific tissues and/or cell types. For example, compared with non-engineered cardiosphere-derived cells (CDC) controls, primary cardiomyocytes have shown a 3.8-fold increase in the uptake of CDC exosomes with targeted cell membrane peptide [88]. ii) Cargo engineering: To load the exosomes with drugs, nucleic acids, proteins, and other bioactive molecules can render them as drug delivery vehicles. This may be achieved by modifying the gene expression of source cells or modification of the exosome shell. For instance, by electrostatically loading the anti-proliferative drug doxorubicin (Dox) onto the surface of amine-modified intraocular lenses (IOL), researchers have significantly inhibited the development of posterior capsule opacification (PCO) [89]. iii) Engineering for miRNA or mRNA: To modify the exosomes to carry specific miRNA or mRNA may regulate gene expression in target cells. Engineered miR-31 exosomes, for instance, have promoted the angiogenesis, fibrogenesis, and re-epithelialization by inhibiting factor inhibiting HIF-1 (HIF1AN, also known as FIH) and epithelial membrane protein 1 (EMP-1) [90]. iv) Lipid composition modification: To alter the lipid composition of the exosomes may enhance their stability, biodistribution, and biological activity in vivo. This can be achieved by modifying the lipid metabolism of the source cells or through post-processing methods. Short-term lipopolysaccharide (LPS)-stimulated macrophages, for example, could alter the abundance of phosphatidylinositol-4-phosphate (PI4P) on the MVB, thereby promote the release of small extracellular vesicles (sEV) [91]. v) Surface engineering with antigens or antibodies: To modifying the surface of the exosomes to carry antigens or antibodies may confer them with a role in inducing immune responses. Modification of the four-transmembrane protein fusion protein on antigen-presenting exosomes (AP-Exos) using single-chain MHCI trimer (scMHCI) has amplified endogenous tumor-specific $CD8^+$ T cells and induced an anti-tumor effect [92].

However, due to various factors involving biological distribution, metabolism, immune recognition, and others, extracellular vesicles face challenges in retention and stability within the body. Firstly, owing to their small size, extracellular vesicles are susceptible to filtration by the kidneys and subsequent excretion in urine, resulting in a short

circulation time in the body. Moreover, as foreign entities, they may be recognized and engulfed by phagocytic cells (such as macrophages) in the immune system, especially upon entering the bloodstream. Additionally, abundant enzymes in body fluids, such as proteases and nucleases, can degrade the proteins and RNA within extracellular vesicles, thereby reducing their stability and functionality. Changes in pH and ionic strength in body fluids may also affect the physical and chemical stability of extracellular vesicles, such as membrane lipid fluidity and membrane protein conformation [93]. PEG modification of extracellular vesicles offers an effective strategy to enhance their stability, prolong circulation time in the body, and reduce immune clearance. Selecting PEG with active functional groups (such as NHS-PEG) enables the formation of stable covalent bonds with amino acid residues on the surface of extracellular vesicles (mainly amino groups of lysine or thiol groups of cysteine) [94]. Alternatively, PEG with lipophilic anchoring groups (such as DSPE-PEG) can be non-covalently modified onto extracellular vesicles by insertion into the lipid bilayer, with the lipophilic portion embedded within the vesicle's lipid bilayer and the PEG chain extending to the outer surface of the vesicle [95]. Additionally, PEGylation compounds can be added during the cell culture process of extracellular vesicle production, indirectly resulting in PEGylation on the surface of newly generated extracellular vesicles [96]. There are various methods for PEG modification of extracellular vesicles, and selecting the most suitable method depends on specific application requirements, the desired degree of PEGylation, and the physicochemical properties of the extracellular vesicles. Proper execution of PEG modification can significantly enhance the clinical potential of extracellular vesicles.

3. Advantages of hydrogel-exosome systems for tissue engineering

3.1. Overview of hydrogels

Beginning in the 1980s, scholarly research has increasingly delved into the intricate aspects of exosomes and hydrogels (Fig. 3). As a highly hydrophilic gel with a distinctive three -dimensional network structure, hydrogels can rapidly absorb and retain a substantial volume of water without dissolving [97]. The presence of a cross-linked network will allow the hydrogels to swell and maintain a significant water content, with the extent of water absorption closely associated with the degree of cross-linking [98]. The combined application with hydrogels can prevent premature clearance of extracellular vesicles. By directly placing hydrogels containing extracellular vesicles at or near the target site, it ensures a more concentrated dose, providing sustained and more pronounced therapeutic effects. Hydrogels can be classified into natural hydrogels (such as protein hydrogels, polysaccharide hydrogels, DNA hydrogels, decellularized matrix hydrogels, etc.) and synthetic hydrogels (such as polyvinyl alcohol, polyacrylic acid, acrylate-based hydrogels, etc.) based on their source materials. Water-based gels derived from various monomers exhibit distinctive physicochemical properties and biological traits, rendering them adaptable for diverse applications as drug carriers (Table 3).

3.1.1. Protein hydrogels

3.1.1.1. Collagen-based hydrogels. Collagen, the most abundant protein in both the extracellular matrix (ECM) and mammals, can be directly extracted from various animal sources including skin and bones. Collagen molecules contain specific amino acid sequences, such as the

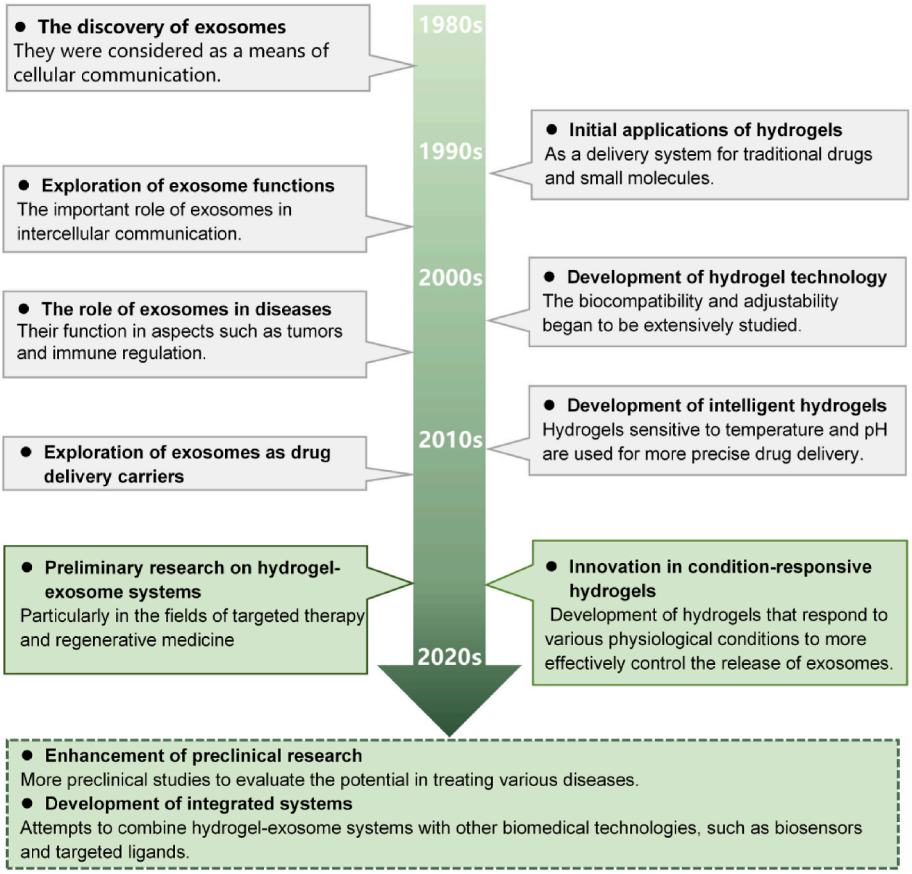

Fig. 3. Developmental Timeline of hydrogel-exosome systems.

Table 3
Hydrogels for drug delivery.

Composition of hydrogels		Drug	Disease	Modification methods	Reference
Proteins hydrogels	Collagen-based	Dexamethasone	Corneal inflammation	Combining nanofibrillated cellulose with dual chemical-photocrosslinking to enhance scaffold mechanical stability and enzymatic resistance.	[102]
	Gelatin-based	Curcumin	Diabetic ulcer	Self-crosslinking with stable mechanical properties, self-healing, and injectability.	[167]
	Silk protein-based	Amorphous calcium phosphate	Critical-sized bone defect	Oxidized silk fibroin.	[168]
Polysaccharides hydrogels	Hyaluronic acid-based	Stromal cell-derived factor-1 alpha	Fracture	Two chemically modified hyaluronic acid blends.	[169]
		Metal-polyphenol nanoparticles	Rotator cuff tear	Dopamine modification to enhance adhesion and plasticity.	[120]
	Alginate-based	Mg^{2+}	Sciatic nerve transection	Highly dynamic interaction between bisphosphonate-modified alginate and Mg^{2+}.	[170]
		Bifidobacterium	Inflammatory bowel disease	Formation of hydrogel microspheres via electrostatic droplet generator for oral administration.	[171]
	Chitosan-based	Rosemary acid	Abdominal wall defect	Methacrylic anhydride-modified chitosan to enhance water solubility.	[129]
		Tartaric acid brompheniramine	Ocular diseases	Formation of films by dissolving in ionic liquids.	[131]
DNA hydrogels	/	Fusion protein C2IN-C3li	Osteoporosis	Rapid gelation via DNA hybridization.	[172]
Extracellular matrix hydrogels	SIS	Exosomes	Diabetic ulcer	Combination with mesoporous bioactive glass.	[173]
Synthetic hydrogels	PVA-based	Magnesium nanoparticles	Diabetic ulcer	Dual-network hydrogel composed of sodium alginate.	[174]
		Tannic acid	Hypertrophic scar	Dual-network hydrogel composed of agarose.	[157]
	PAM-based	Sulfamethoxazole	Full-thickness skin defect	Chitosan semi-interpenetrating polymer network.	[162]
	PEG-based	Bone Morphogenetic Protein-2	Skull defect	Dual-network structure composed of thiolated chitosan.	[166]

arginine-glycine-aspartic acid (RGD) sequence, which play a crucial role in cell adhesion and recognition within the ECM. These sequences are recognized by integrins on cell surfaces, facilitating cell adhesion [99]. Additionally, collagen serves as a key component of the natural ECM, providing a three-dimensional fibrous network structure akin to that found in the ECM. This structure not only provides physical support for cells but also creates a conducive three-dimensional growth environment, promoting cell-cell interactions and signal transduction, essential for cell proliferation and differentiation. In the realm of tissue engineering and regenerative medicine, collagen-based hydrogels find extensive use in treating burns, chronic wounds, and other skin injuries [100,101]. However, collagen hydrogels often exhibit relatively low mechanical strength, particularly when they have high water content, limiting their utility in load-bearing tissue engineering applications such as bone and cartilage repair. To address these limitations, researchers are exploring various strategies including improvements in chemical crosslinking methods and compositing with other materials (such as synthetic polymers and inorganic nanoparticles) to enhance mechanical properties. In one notable study, researchers mechanically reinforced collagen with high-purity cellulose nanofibers and subsequently constructed a dexamethasone-loaded collagen scaffold using double crosslinking methods. This approach enabled successful implantation for the treatment of corneal stromal diseases [102]. In recent years, the development of recombinant collagen has emerged as a more efficient, controllable, and biologically safe alternative. Recombinant collagen, produced by expressing human collagen genes in microbial or other host systems, exhibits significantly reduced immunogenicity. Moreover, through gene engineering techniques, specific sequences or functional domains, such as the cell adhesion-promoting RGD sequence, can be introduced into recombinant collagen, thereby altering its mechanical properties and biodegradation rate to meet specific application requirements [103,104].

3.1.1.2. Gelatin-based hydrogels. Gelatin is a versatile protein derived from the partial hydrolysis of animal collagen. Physically and chemically akin to collagen, gelatin undergoes a process of molecular chain shortening during hydrolysis, rendering it more amenable to interactions with water molecules. One of its notable characteristics is its unique gel-forming ability. Upon heating and subsequent cooling, gelatin molecules re-establish certain three-dimensional structures, entangling water molecules within to form a gel network. Unlike collagen, which typically requires processing to gel, gelatin's properties make it more adaptable and practical across various industries, including food, pharmaceuticals, tissue engineering, and beyond. Its adjustable solubility and capacity for gentle gelation make it a favored material in drug delivery systems and an excellent scaffold for cell culture, particularly for delicate cell types [105]. However, the thermal reversibility of gelatin necessitates careful handling during preparation and storage to prevent unintended dissolution or structural alterations, which could compromise its stability and functionality in biological applications. Chemical modifications can also significantly broaden the applications for protein hydrogels, endowing them with more intricate physiological functionalities. As an example, a gelatin-methacryloyl (GelMA) hydrogel loaded with extracellular vesicles derived from tenocytes (TSC-sEV) has been administered in liquid form and subsequently solidified through UV irradiation, which has enabled effective coverage of irregular surfaces of injured Achilles tendon tissue [106]. In another study, within infected wounds, bacteria secrete gelatinase, leading to the degradation of the gelatin hydrogel structure. This process releases the photosensitizer Ce6 that was encapsulated within the hydrogel. Upon laser irradiation, reactive oxygen species (ROS) are produced, effectively achieving bactericidal effects [107].

3.1.1.3. Silk fibroin-based hydrogel. Silk fibroin is a natural protein primarily extracted from silkworm silk but can also be sourced from other insects like spiders. Compared to the aforementioned biological materials derived from proteins, silk fibroin-based hydrogels exhibit significantly high mechanical strength, mainly due to their unique molecular structure and assembly. Silk fibroin molecules contain numerous β-sheet structures, which are stabilized by strong hydrogen bonds. These highly organized β-sheet structures can withstand high tensile forces without breaking, imparting silk fibroin with exceptional mechanical

strength [108]. Additionally, the structure of silk fibroin comprises alternating highly ordered crystalline regions and loosely packed non-crystalline regions. The crystalline regions, mainly composed of β-sheets, provide strength and rigidity, while the non-crystalline regions, containing random coils and α-helices, contribute to the material's ductility and elasticity. This combination of microstructural features endows silk fibroin with outstanding mechanical properties, allowing it to withstand substantial forces without fracturing and recover its original shape after the removal of external forces. At the macroscopic level, silk fibroin exhibits highly organized hierarchical structures, ranging from individual protein molecules to fibers and fiber bundles. This hierarchical assembly further enhances the material's mechanical strength and toughness [109,110]. Therefore, silk fibroin-based hydrogels have emerged as ideal scaffold materials in bone and cartilage tissue engineering [111,112]. They can also be used in cardiovascular tissue engineering to fabricate vascular scaffolds, promoting the repair of damaged blood vessels and the formation of new ones [113,114].

3.1.2. Polysaccharide hydrogels

3.1.2.1. Hyaluronic acid-based hydrogel. Hyaluronic Acid (HA) is a naturally occurring linear polysaccharide belonging to the glycosaminoglycan (GAG) class, widely distributed in the human body, particularly in the skin, eyes, and synovial fluid [115]. Comprised of repeating disaccharide units, HA typically ranges in molecular weight from several hundred thousand to several million Daltons (Da), with high molecular weight imparting high viscosity and excellent hydration capabilities. Its viscosity increases with concentration and molecular weight, enabling its role as a lubricant and shock absorber within the body, particularly in joint fluid for joint protection and in maintaining intraocular pressure. HA has the capacity to absorb and retain several times or even thousands of times its own weight in water, playing a crucial role in maintaining tissue hydration balance and providing physical support to the extracellular matrix [116]. Unlike most other glycosaminoglycans, HA is non-sulfated, reducing its specificity in protein interactions but also granting it broad biocompatibility. Furthermore, HA is a major natural ligand for CD44, and its specific binding to certain structural domains on the CD44 receptor can activate intracellular signaling pathways, influencing cell behavior. Leveraging the specific affinity between HA and CD44, drugs, nanoparticles, or other therapeutic molecules can be combined with HA or its derivatives to form complexes. These complexes can be targeted for drug delivery through CD44 to specific cells, such as cancer cells overexpressing CD44 [117–119]. HA-based hydrogels typically exhibit lower mechanical strength and stability, limiting their use in tissue engineering applications subjected to mechanical stress, such as skeletal or cartilage tissue engineering. Researchers have addressed this limitation by chemical modification or compositing with other high-molecular-weight materials. One study reported a dopamine-modified HA hydrogel, which synthesized magnesium-procyanidin (Mg-PC) coordination metal polyphenol nanoparticles through a self-assembly process, integrated into a dual-component hydrogel. The sustained release of magnesium and procyanidin not only alleviates inflammation but also enhances collagen synthesis and mineralization, promoting the repair of the tendon-bone interface [120].

3.1.2.2. Alginate-based hydrogel. Alginate is a naturally occurring linear polysaccharide primarily derived from the cell walls of algae. It consists of repeating units of β-D-mannuronic acid (M units) and α-L-guluronic acid (G units) linked alternately by 1–4 glycosidic bonds, forming its fundamental structure. Regions rich in G units, known as G blocks, exhibit a higher gel-forming capability due to the formation of strong hydrogen bonds, while regions rich in M units, termed M blocks, play a minor role in gel formation. In the presence of divalent metal ions such as calcium ions (Ca^{2+}), specific "egg-box" interactions occur between G blocks, leading to the crosslinking of alginate molecules and the formation of a three-dimensional network structure, resulting in gelation [121–123]. Despite alginate-based hydrogels possessing favorable attributes such as biocompatibility, low toxicity, and strong adsorption capacity, they undergo uncontrolled degradation when exposed to calcium chelators. Control over gel formation and dissolution can be achieved by adjusting the concentration of metal ions or utilizing specific ion exchange reactions [124,125]. Previous studies have involved chemical modification or surface coating of alginate hydrogels to modulate their surface properties or degradation rates, thereby regulating the release kinetics of drugs. Researchers have developed a nanoengineered injection system by incorporating two-dimensional layered double hydroxide (LDH) clay materials with high surface area into alginate hydrogels. This not only enhances the mechanical properties of the hydrogel by 5–30 times but also exhibits a high binding affinity with proteins [126].

3.1.2.3. Chitosan-based hydrogel. Chitosan is derived from the deacetylation of chitin, containing numerous hydroxyl and amino groups along its molecular chain. These functional groups can form hydrogen bonds, but under neutral and alkaline conditions, the amino groups in chitosan molecules are not sufficiently protonated, leading to difficulties in forming stable solutions in water. This poses a significant challenge for utilizing chitosan in hydrogel formation and its application in tissue engineering. Researchers have addressed this challenge by reducing the molecular weight of chitosan through chemical or enzymatic degradation, facilitating its dispersion and solubility in water. Alternatively, adjusting the pH of the solution to acidic conditions allows for protonation of the amino groups in chitosan, enhancing its solubility. Another commonly employed approach involves chemically modifying chitosan to introduce hydrophilic functional groups (such as carboxyl or sulfonic acid groups), significantly improving its water solubility [127,128]. For instance, Zhao et al. synthesized chitosan methacrylate (CS-MA) through acylation reaction with methacrylic anhydride, enhancing water solubility and conferring photoresponsiveness. Furthermore, by incorporating rosmarinic acid, CS-MA demonstrated efficacy in alleviating local inflammation, reducing oxidative stress, and regulating the fibrinolytic system, effectively mitigating abdominal adhesions resulting from conventional polypropylene mesh implantation in the abdominal wall [129]. Nonetheless, the use of acidic conditions may pose risks of skin and mucosal tissue corrosion. In this regard, ionic liquids (ILs) have emerged as promising solvents for chitosan dissolution due to their non-volatility, high thermal stability, and chemical inertness [130]. Li et al. developed a method for chitosan dissolution in pure water by dissolving it in a mixture of IL and the anti-glaucoma drug brimonidine tartrate (BT). Subsequently, drug-loaded chitosan-based hydrogel films were prepared through casting and air-drying, showing potential for ocular drug delivery applications [131].

3.1.3. DNA hydrogels

DNA-based hydrogels are materials with a three-dimensional network structure formed by DNA molecules through self-assembly or cross-linking reactions with other molecules. By leveraging the principles of complementary base pairing in DNA, precise DNA sequences can be designed to achieve programmability of the hydrogel. This design flexibility enables precise control of DNA hydrogels at the molecular level. By adjusting parameters such as DNA concentration, chain length, and the type and concentration of cross-linking agents, the cross-linking density and pore size of DNA hydrogels can be finely tuned, thereby influencing their mechanical properties and substance transport characteristics. Moreover, since DNA molecules are naturally occurring substances in the body, DNA-based hydrogels typically exhibit excellent biocompatibility and biodegradability, rendering them suitable for tissue repair applications such as skin, bone, and cartilage repair

[132–135]. DNA hydrogel is a material which forms through the self-assembly of DNA molecules, possessing the attributes of programmability, responsiveness, and biocompatibility [136–139]. This versatile substance not only has extended the applications of DNA within genetics but also proved to be valuable in diverse fields such as gene delivery, biosensing, and nanotechnology. The interaction between DNA hydrogels and exosomes is prominently evident in three key aspects: i) Enzymatic amplification for the synthesis of ultra-long DNA chains, along with DNA hydrogels formed through complementary base pairing, can facilitate the specific and non-destructive isolation of exosomes from within complex biological matrices [140]. ii)By adjusting the amplification cycles of DNA hydrogels, a wide range of exosomal miRNAs may be detected, which can offer highly precise disease information and enhance diagnostic accuracy [141]. iii)DNA hydrogels can exhibit exceptional programmability, enabling precise tailoring of their structure and functionality [142]. For instance, a bioresponsive PEG/DNA hybrid hydrogel has achieved controlled release of stem cell-derived exosomes (SCAP-Exos), which demonstrated substantial therapeutic effectiveness for the treatment of diabetes-related bone defects [143].

3.1.4. ECM hydrogels

ECM derived from tissues and/or organs has found extensive use in various applications in tissue engineering and regenerative medicine. Surgical procedures have utilized porcine decellularized dermis, submucosa of small intestine, and decellularized bladder, among other sources. However, such materials, whether in the form of membrane sheets or powdered materials, are ill-suited for minimally invasive procedures such as those requiring injection or external catheterization [144]. In preclinical models, hydrogels based on decellularized heart, kidney, liver, bladder, bone, have been developed, either through direct preparation or modification via chemical and physical methods [145–149]. Such hydrogels have offered a wider range of applications, exceptional versatility, and can be customized for specific animal models using various combinations and approaches. Leveraging low-temperature 3D printing technology, a hydrogel derived from decellularized submucosa of the small intestine (SIS) has been engineered. It has integrated mesoporous bioactive glass (MBG) and exos to create a 3D scaffold dressing known as SIS/MBG@Exos. This hydrogel scaffold has boasted impressive 3D structural integrity, ideal porosity, biocompatibility, and hemostatic properties, and has expedited the healing process of diabetic wounds by enhancing blood circulation and triggering the vascular regeneration mechanism [150]. However, despite the ideal environment for cell growth and tissue regeneration provided by natural ECM, the direct use of natural ECM faces challenges such as limited sources, immunogenicity, difficulty in customization, and complex processing in practical applications. The development of biomaterials that mimic the extracellular matrix allows researchers to precisely control the composition, cross-linking density, mechanical properties, and biological activity signals of the materials. Chen et al. achieved this by constructing a dynamic biomimetic hydrogel similar to ECM through controlled polymerization and click chemistry. Besides its inherent antibacterial properties, this hydrogel demonstrated high adaptability to wound geometry and effectively resisted stress-induced support for skin wound repair [151]. He et al. conducted an intriguing study where they utilized reversible receptor-ligand interactions to construct dynamic biological interfaces and 3D hydrogel networks. These structures exhibited specificity and reversibility in molecular recognition, along with reshaping properties akin to ECM. Moreover, they were easy to manipulate, offering fresh insights into the design of biomaterial scaffolds in tissue engineering and regenerative medicine [152].

3.1.5. Synthetic hydrogels

3.1.5.1. Polyvinyl alcohol-based hydrogels. Polyvinyl alcohol (PVA) is a water-soluble synthetic polymer formed through the polymerization of vinyl alcohol monomers, containing numerous hydroxyl groups (-OH). These hydroxyl groups contribute to its excellent water solubility and ability to form hydrogen bonds, endowing PVA hydrogels with good biocompatibility and mechanical properties. They find wide applications in the biomedical field, such as wound dressings, artificial cartilage, and drug delivery systems [153–155]. Constructing a double network structure is a common method to enhance the toughness and strength of PVA-based hydrogels [156]. The dual-network structure, as designed by Liu et al., based on PVA and gelatin, provides excellent mechanical support to the hydrogel. Loading with hyperbranched poly-L-lysine (HBPL) and tannic acid (TA) enhances the hydrogel's antibacterial and antioxidant functions. This multifunctional hydrogel demonstrates excellent therapeutic effects in hypertrophic scar models. Moreover, due to its common raw materials and simple preparation methods, this hydrogel is highly suitable for large-scale production, with broad prospects for translation [157]. However, in the field of cartilage regeneration, PVA-based hydrogels often face challenges in simultaneously achieving lubrication performance and mechanical strength. Feng et al. proposed a combination of double network and nano-additive strategies. The hydrogen bonding interaction between polyacrylic acid (PAA) and PVA chains increases the network density of the hydrogel. The uniformly dispersed modified graphene oxide (GO) in the PVA hydrogel network increases its hydration strength and mechanical properties, enabling it to maintain excellent lubrication even after 500, 000 cycles under high contact pressure. This approach holds promise as an alternative material for artificial joints [158].

3.1.5.2. Polyacrylamide-based hydrogels. Polyacrylamide (PAM) is a long-chain polymer formed through the free radical polymerization of acrylamide monomers. The amide groups along the main chain have the capability to interact with water molecules or neighboring polyacrylamide chains via hydrogen bonding. This imparts excellent hydration and water absorption properties to PAM hydrogels, with the ability to absorb water up to hundreds of times its own weight. Consequently, PAM finds extensive utility across various domains including water treatment and soft catalysts [159–161]. However, due to acrylamide's toxicity, its biomedical applications remain restricted unless subjected to specific treatments to mitigate toxicity. Presently, chemical modification is employed to introduce biocompatible functional groups (e.g., hydroxyl, carboxyl, polyethylene glycol chains, etc.), or non-toxic or low-toxic crosslinkers are utilized to substitute traditional chemical crosslinkers. Biocompatible crosslinkers like biological enzymes or biodegradable crosslinkers are preferred to minimize additional toxicity during the crosslinking process. Peng et al. devised a method where functionalized polyacrylamide microgels were combined with chitosan solution to create a microgel-embedded hydrogel exhibiting high biocompatibility and reversible adhesion. This hybrid hydrogel was used to deliver the antibacterial drug sulfamethoxazole (SMZ), resulting in a significant enhancement of wound healing [162].

3.1.5.3. Polyethylene glycol-based hydrogels. Polyethylene glycol is a synthetic polymer that readily dissolves in water at room temperature. Its repetitive ether chain segments grant it high water solubility. Chemical crosslinking of PEG-based hydrogels is typically achieved by adding crosslinkers such as diisocyanates, dialdehydes, or click chemistry reagents [163,164]. These crosslinking reactions can occur under mild conditions without the need for extreme pH or high temperatures, which is advantageous for preserving sensitive biomolecules and cells. Therefore, PEG-based hydrogels exhibit good biocompatibility and non-immunogenicity, making them suitable for biomedical applications. Importantly, PEG chains possess excellent non-adhesive properties, meaning that PEG-based hydrogels are not prone to nonspecific adsorption of proteins or cells, which is an important advantage when used as drug delivery vehicles and cell culture matrices [165]. However,

this may also affect cell proliferation and differentiation in PEG-based hydrogels, limiting their effectiveness as cell scaffolds. Sun et al. designed a double network hydrogel composed of physically/chemically crosslinked polyethylene glycol-polyacrylate covalent network and ionically crosslinked chitosan network. This DN hydrogel, consisting of two asymmetric networks, exhibits excellent mechanical properties while providing cell adhesion sites, demonstrating good osteogenic activity in a rat calvarial defect model [166].

3.2. Exosome loading strategies in hydrogels

3.2.1. Physical methods

This approach involves integrating exosomes into hydrogels through physical adsorption, which has been a favored method in tissue engineering and drug delivery for encapsulating and releasing exosomes. The process is simple by utilizing non-covalent bonds for the attachment, and typically involves immersing the hydrogels in exosome-containing solutions. The hydrogel's porosity and swelling abilities will facilitate exosome absorption whilst maintaining their biological activity. Key factors affecting their adsorption have included: i) The porosity of the hydrogels will play a crucial role in dictating both the quantity of exosomes adsorbed and their rate of release. ii) External environmental factors such as pH levels and temperature may significantly influence the process of physical adsorption. iii) Characteristics such as the surface charge and the hydrophilic or hydrophobic nature of both exosomes and hydrogels are critical determinators for the efficiency and stability of their adsorption. An example of this interaction is seen in the reversible binding between phosphate groups on the exosomal membranes and polyphenolic groups, like dopamine or tannic acid, through non-covalent bonds. This reversible binding has granted the exosomes with superior sustained-release properties, allowing for a prolonged release period of up to 14 days (Fig. 4a and b) [175]. Nevertheless, compared with chemical crosslinking, physical adsorption will require specific conditions for effective adsorption and optimal release.

3.2.2. Chemical methods

3.2.2.1. Covalent crosslinking. Covalent crosslinking involves the formation of stable chemical bonds between the hydrogel matrix and exosomes, achieved through chemical reactions. Using crosslinkers is a common approach to establish these covalent bonds. For example, EDC/NHS can activate carboxylic acid groups to form amide bonds with amino groups [176]. This allows proteins on the surface of exosomes to be covalently attached to amino acid side chains or other amine-containing compounds introduced into the hydrogel. Employing biocompatible crosslinkers facilitates the stable integration of exosomes with the hydrogel matrix while preserving their biological activity. Genipin, a natural compound extracted from the fruits of plants in the Gardenia genus, reacts with amino-containing biomacromolecules like gelatin and polysaccharides to create crosslinks through the formation of covalent bonds. Its in vivo toxicity is much lower than that of traditional chemical crosslinkers such as glutaraldehyde [177]. Platelet-rich plasma-derived exosomes (PRP-Exos) can be encapsulated in hydrogels through covalent crosslinking with genipin. Scanning electron microscopy images demonstrate that this method effectively embeds PRP-Exo nanoparticles into hydrogel films(Fig. 5a) [178].

3.2.2.2. Self-assembling peptide crosslinking. Self-assembling peptide technology harnesses the intrinsic ability of short peptide sequences to spontaneously form nanofibers and establish a three-dimensional hydrogel network. By engineering peptides with specific recognition sequences, extracellular vesicles (EVs) can be effectively encapsulated within the hydrogel matrix through either covalent or non-covalent interactions (such as electrostatic attractions or hydrogen bonding) during or post gelation [179,180]. However, the encapsulation of EVs by self-assembling peptides is significantly influenced by spatial constraints due to the size distribution range of EVs, which typically ranges from 40 to 160 nm, enveloped within lipid bilayers. Sun et al. elucidated a mechanism involving silk protein self-assembly below 0 °C. During this process, unfrozen silk protein chains transitioned from random coils to nanospheres, eventually fusing into smooth blocks, thereby entrapping a minor fraction of hydrating water molecules within. Meanwhile, EVs aggregated at the hydrophilic core of the peptide. Upon phase separation, silk proteins self-assembled into scaffolds, effectively encapsulating EVs within the resultant frozen sponge-like structure. This innovative approach not only preserves the integrity of EV membranes but also facilitates their controlled release through enzymatic degradation of the scaffold(Fig. 5b) [181].

3.2.2.3. Surface functionalization. Surface functionalization involves the introduction of specific functional groups (such as antibodies,

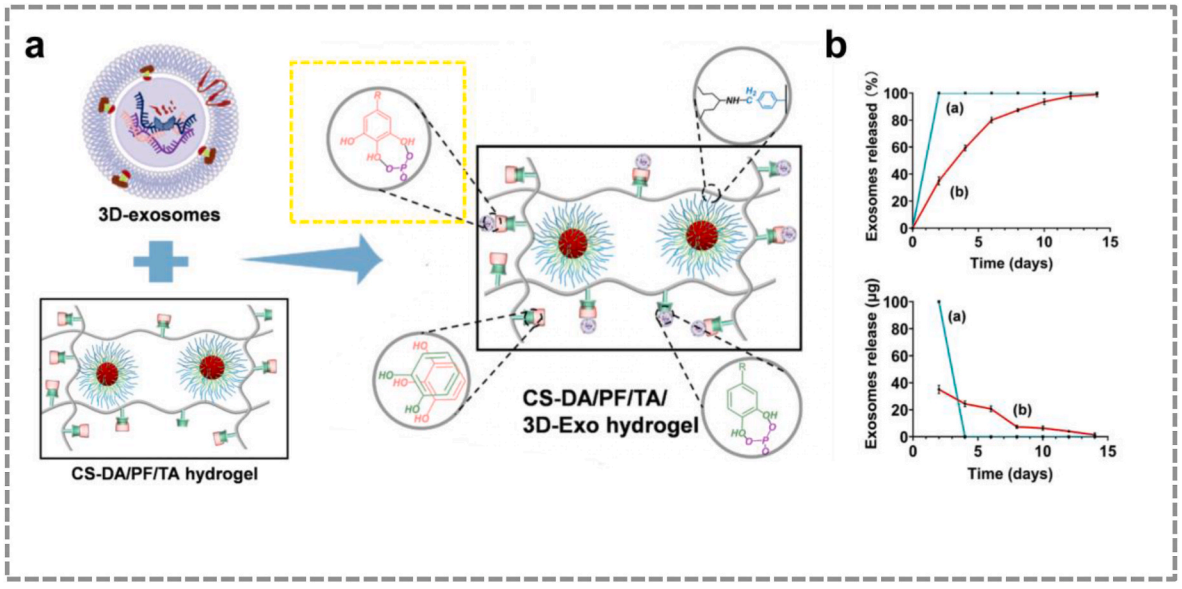

Fig. 4. (a) Schematic illustration of exosomes physically binding to the hydrogel and (b) release curves. Reproduced with permission from Ref. [175]. Copyright 2023, Elsevier.

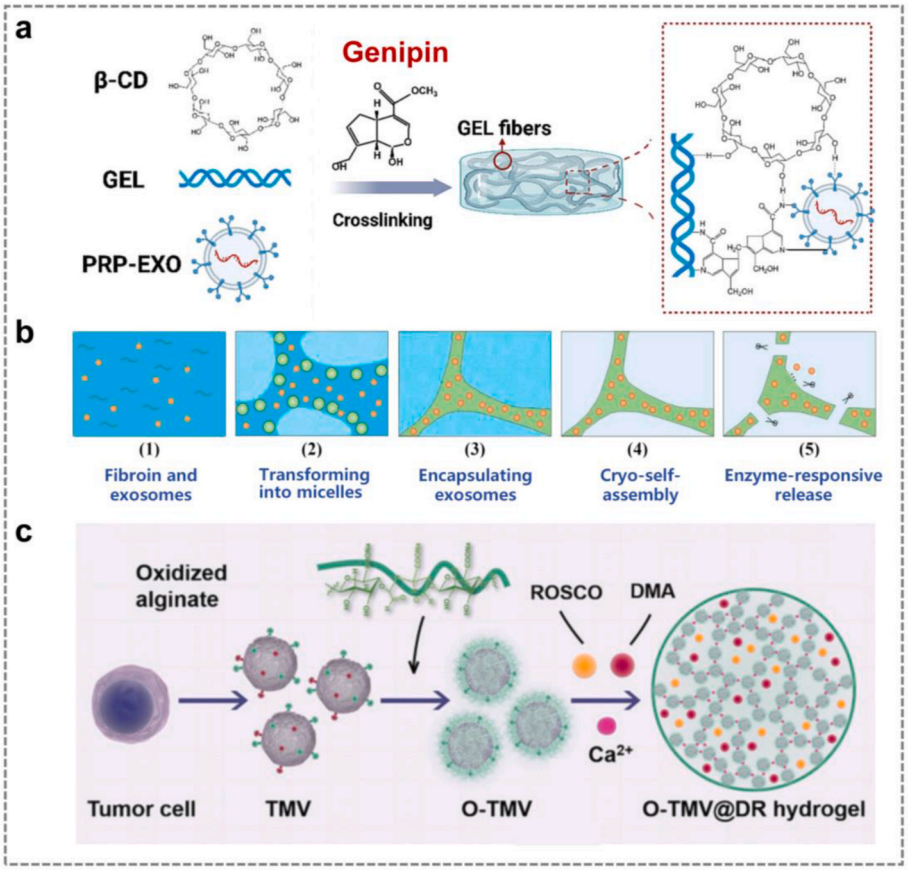

Fig. 5. The chemical strategy for loading exosomes into hydrogels. (a) The method based on covalent crosslinking (with genipin as the crosslinker). Reproduced with permission from Ref. [178]. Copyright 2023, Elsevier. (b) Self-assembling peptide crosslinking. Reproduced with permission from Ref. [181]. Copyright 2022, Elsevier. (c) Surface functionalization. Reproduced with permission from Ref. [182]. Copyright 2021, Wiley.

affinity ligands, etc.) onto the surface of hydrogels, enhancing the specific binding between exosomes and hydrogels. This approach not only improves the loading efficiency of exosomes but also enables selective encapsulation and release of specific exosome types. Li et al. devised a sophisticated exosome-controlled release platform, utilizing sodium alginate-loaded tumor membrane vesicles (O-TMV) as the hydrogel base, along with the Ca2+ channel blocker diltiazem (DMA) and the cell cycle protein-dependent kinase 5 (Cdk5) inhibitor roscovitine, to form a hydrogel in vivo. This composite can act as an antigen reservoir to establish an immune niche. In the tumor environment, DMA continuously inhibits Ca2+ influx into cells, suppressing Ca2+-controlled exosome release. Simultaneously, roscovitine reduces exosome PD-L1 expression inherited from parent tumor cells, offering an innovative approach for cancer immunotherapy with modulated immune checkpoints(Fig. 5c) [182].

3.3. Exosome release strategies in hydrogels

Exosome release from hydrogels typically initiates with a rapid phase due to easier liberation of surface-bound exosomes. This phase will then transits into a slower, steady-state “plateau phase”, driven by internal diffusion dynamics and hydrogel degradation. The hydrogel’s crosslink density, pore size, and degradation rate can significantly impact the kinetics of exosome release and plateau formation. The spatial distribution and load of exosomes within the hydrogel may further determine the onset and duration of the plateau, underscoring the importance of such factors in the regulation of exosome release.

3.3.1. Passive diffusion

Passive diffusion of exosomes in hydrogels is typically driven by the hydrogel’s swelling or hydrolytic degradation. The figure depicts the hydrolysis process of a biodegradable hydrogel with an interpenetrating polymer network (IPN) structure. This structure comprises a covalently crosslinked network and a peptide self-assembled network, exhibiting overall degradation due to the hydrolysis of the poly(DL-lactide) domain (Fig. 6a) [183]. Generally speaking, the release rate of exosomes may be influenced by controlling the hydrogel’s crosslink density, which affects its pore structure. Higher density may result in smaller pores and slower release rates. Another approach has considered tuning the molecular weight of the hydrogel’s crosslinking components, along with crosslink density, to achieve more precise control for the duration of exosome release, adapting to various timelines for tissue repair. Researchers have used hydrogel components of 20 kDa and 10 kDa and varied the crosslink density to regulate the exosome release between 6 and 27 days (Fig. 6b) [184].

3.3.2. Environmentally responsive release

The environmentally responsive release of exosomes in hydrogels was an intelligent drug delivery strategy which allowed exosome release in response to particular biological or physicochemical environmental changes. This method employed specially designed hydrogel materials to ensure effective exosome release at the right time and targeted location. Hydrogels used for exosome delivery can respond to environmental changes such as temperature, photothermal effects, pH, H_2O_2, enzymes, and glucose (Fig. 7)(Table 4).

3.3.2.1. Temperature-responsive release. Temperature-responsive

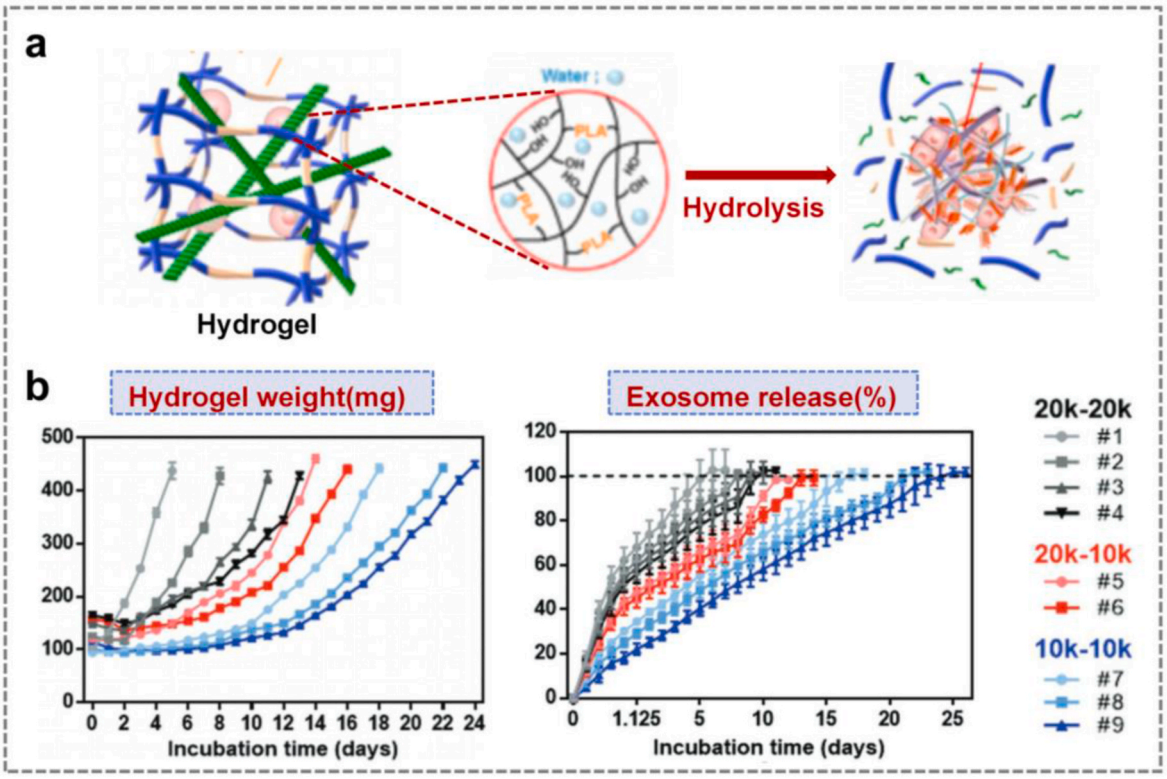

Fig. 6. (a) Passive diffusion of exosomes based on hydrolytic degradation of hydrogel. Reproduced with permission from Ref. [183]. Copyright 2021, Elsevier. (b) Release curves of exosomes in hydrogels with varying molecular weights and cross-linking concentrations. Reproduced with permission from Ref. [184]. Copyright 2022, Wiley.

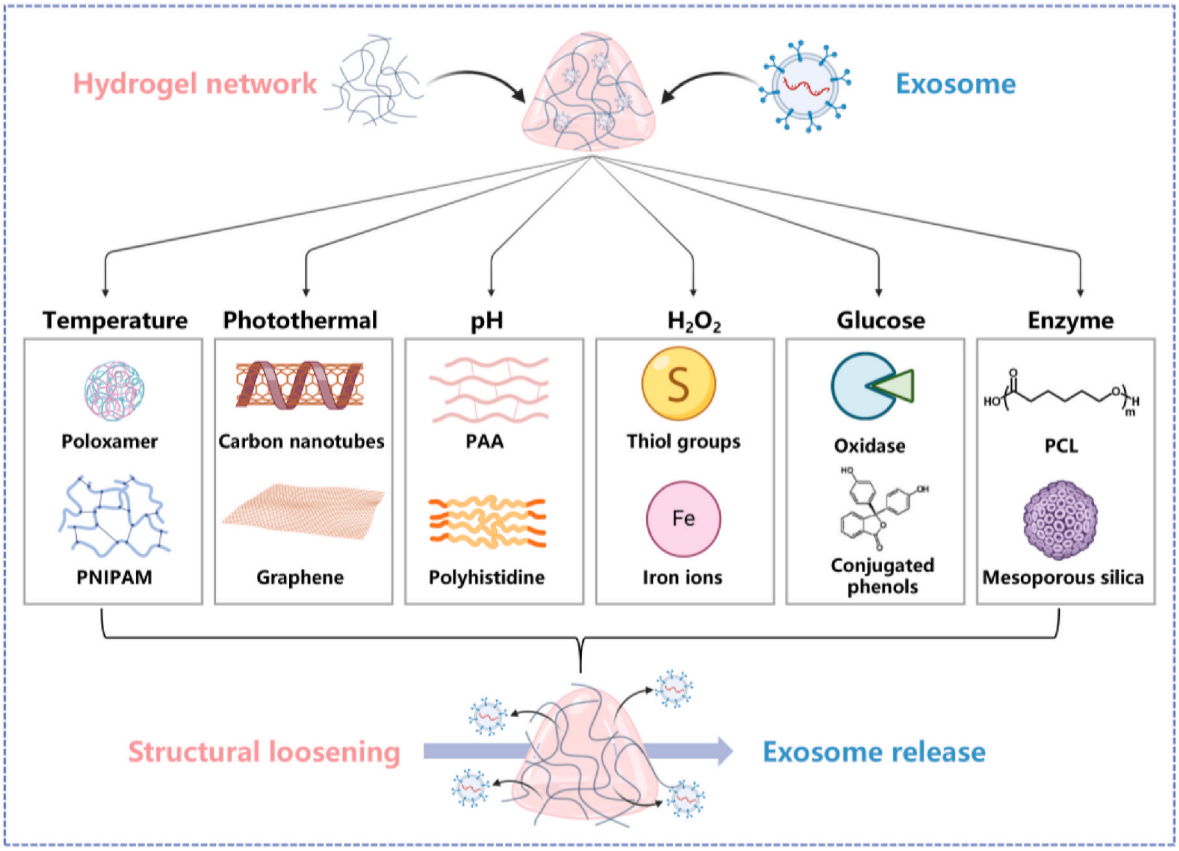

Fig. 7. Schematic diagram of exosome release in environmentally responsive hydrogels.

Table 4
Exosome release strategies in hydrogels.

Release strategy	Initial loading concentration	Release kinetics	Reference
Diffusion	100 μg/mL	95.1 % in 72 h	[185]
	1500 μg/mL	92.5 % in 14 days	[186]
	100 μg/mL	The release time was up to 14 days	[175]
Temperature response	Not mentioned	T = 25 °C, 90 % in 4 days T = 37 °C, 90 % in 28 days	[187]
	Not mentioned	T = 25 °C, 75 % in 200 h T = 37 °C, 40 % in 200 h	[188]
Photothermal response	0.55×10^8 Evts/μL	regular released under near-infrared radiation	[189]
	Not mentioned	80 % in 9 days	[190]
pH response	10 μg/mL	pH = 5.5, about 95 % in 21 days pH = 7.5, about 85 % in 21 days	[191]
	50 μg/mL	pH = 9, exhibited threefold increase	[192]
H_2O_2 response	Not mentioned	90 % in 28 days	[193]
	50 μg/mL	exhibited threefold increase	[192]
Glucose response	50 μg/mL	exhibited threefold increase	[192]
Enzyme response	1×10^6 Evts/μL	the release time was up to 10 days	[181]
	Not mentioned	80 % in 28 days	[193]
	0.5–2 mg/mL	92.29–96.29 % in 9 days	[194]
	50 μg/mL	90 % in 20 days	[195]
	Not mentioned	92.2 % in 14 days	[143]

release in the hydrogel-exosome systems often used synthetic materials like PEG or poly(N-isopropylacrylamide) (PNIPAM) for injectable gels with controllable biochemical properties [187,196]. Poloxamers, as non-ionic triblock copolymers comprising hydrophilic polyethylene oxide (PEO) and hydrophobic polypropylene oxide (PPO), are biocompatible and widely used in pharmaceuticals and biomedicine for drug delivery and tissue engineering. For example, Poloxamer 188, known for reducing cell damage [197], and Poloxamer 407 (commercially Pluronic F127), which can spontaneously form gel at body temperature, have demonstrated reversible sol-gel transitions based on temperature [198]. Studies have shown that addition of Poloxamer to thermosensitive hydrogels could control the release of PRP-exos, with most exosomes released within a day at 25 °C and continuously over a month at 37 °C [187]. Nevertheless, Poloxamer gels' lack of transparency and poor cell adhesion properties may limit their use in fields like ophthalmology and require surface modifications for tissue engineering applications [199,200]. Integrating temperature-sensitive PNIPAM polymer chains into hydrogel networks could allow exosome diffusion below the lower critical solution temperature (LCST) and restricts it above LCST through hydrophobic interactions and network contraction [188].

3.3.2.2. Photothermal-responsive release. The design of photothermal-responsive hydrogel systems for drug delivery primarily has utilized the capability of materials to absorb and convert near-infrared (NIR) light into heat, thereby triggering drug release. Researchers have chosen efficient photothermal converters such as metal nanoparticles, carbon nanomaterials, and polymer nanocomposites [201–203]. Key considerations have included localizing the photothermal effect to minimize the thermal damage, ensuring high conversion efficiency for rapid heat generation, and selecting suitable light source for controlled exposure. Temperature-sensitive hydrogels, shrinking at their LCST, can facilitate the release. A notable development has involved graphene GC modified for compatibility with the thermoresponsive polymer PHOM, which has achieved controlled exosome release through rapid photothermal conversion in response to NIR radiation [189].

3.3.2.3. pH-responsive release. pH-responsive mechanisms in hydrogel systems for exosome release have exploited their ability to change states like swelling, shrinking, or dissolving at specific pH levels. Key polymers like polyacrylic acid (PAA), polymethacrylic acid (PMAA), and polyhistidine have been employed for this purpose [204,205]. The design of such hydrogels will necessitate ensuring exosome's compatibility and stability across pH changes and tailoring the system to the pH of targeted body sites. A notable design has involved a hydrogel formed by reversible Schiff base reactions between poly-ε-L-lysine (EPL) and oxidized hyaluronic acid, which enabled exosome release in slightly acidic conditions due to the breakage of Schiff base bonds [191].

3.3.2.4. H_2O_2-responsive release. Hydrogen peroxide (H_2O_2) plays a crucial role in various biological contexts, particularly in inflammation, infection, and tumor environments where its concentration tends to be higher [206]. For designing H_2O_2-responsive hydrogel drug delivery systems, the objective is to harness H_2O_2's oxidative properties for controlled drug release. This involves using materials that can chemically react with H_2O_2, such as polymers with thiol groups, iron-containing complexes, or other oxidation-sensitive substances [207–209]. One innovative approach has involved creating a composite hydrogel using hyperbranched polymer monomers and thiolated hyaluronic acid, crosslinked via Michael addition. This system has enabled prolonged exosome release, extending up to 28 days in the presence of H_2O_2, demonstrating its potential for targeted therapeutic applications [193].

3.3.2.5. Glucose-responsive release. The goal for designing the glucose-responsive hydrogel systems was to capitalize on variations in glucose levels for targeted drug release, a concept highly relevant for diabetes management [210]. Such systems have typically incorporated materials which can react to specific glucose concentrations, employing elements like hydrogels containing glucose oxidase (GOx) or polymers sensitive to glucose levels, such as those with conjugated phenolic compounds [211,212]. A notable development has been a soluble thioctic acid-modified chitosan hydrogel. The redox-sensitive thioctic acid could alter its binding with chitosan in high glucose environments, triggering hydrogel disintegration and thereby facilitating accelerated exosome release [192].

3.3.2.6. Enzyme-responsive release. The design of enzyme-responsive hydrogel systems has focused on exploiting specific enzymatic activities in the body to initiate and/or accelerate the release of drugs. Such systems often use polymers that are degradable by targeted enzymes, such as polyesters, polypeptides, or enzyme-sensitive crosslinkers [213]. A significant innovation in this field has been the development of a self-assembling silk fibroin scaffold, which can undergo continuous degradation in the presence of protease K *in vitro*. The degradation is accompanied by the release of exosomes, whereas in non-enzymatic environments, the release from such scaffolds is significantly reduced [181]. Matrix metalloproteinases (MMPs) as zinc-dependent endopeptidases play a crucial role in many biological processes, notably in tumor microenvironments and inflammation. They have shown diverse activities and expressions under various pathophysiological conditions. For example, MMP-1 is more active in arthritis and cancer, MMP-2 is involved in tumor invasion by degrading gelatin and collagen, and MMP-7, which can degrade multiple extracellular matrix proteins, is overexpressed in certain cancers like colon cancer [214]. For designing MMP-responsive hydrogel systems for drug delivery, the primary goal is to harness the specific cleavage activity of MMPs to trigger drug release, using peptides cleavable by specific MMPs as crosslinkers or embedded in the hydrogel. Materials like polycaprolactone (PCL), self-assembling peptides (SAP), mesoporous silica nanoparticles (MSNs), and liposomes with MMP substrate peptides have been employed in the MMP-responsive materials [215–217]. Researchers have used

microfluidic techniques to develop an MMP1-sensitive KLDL hydrogel, which could enable significant exosome release in the presence of MMP1, especially in the first three days [194].

Enzyme-responsive hydrogels are designed to respond to specific enzymes which are often overexpressed or uniquely active in certain diseases such as cancer or inflammation, offering a level of specificity beyond that of temperature, pH, or H_2O_2-responsive materials [218,219]. Due to the localized enzyme activity in diseased tissues, such hydrogels may facilitate precise drug targeting whilst minimize their impact on healthy tissues. The efficacy of such hydrogels is highly contingent upon the activity and expression levels of specific enzymes in the target area. Variability in enzyme activity across disease states or individuals can impact the consistency and predictability of drug release. Moreover, interactions between hydrogel components and bodily enzymes or unintentional degradation by other enzymes can occur. Consequently, the development of enzyme-responsive hydrogels has often involved complex chemical synthesis and meticulous material processing, potentially increasing the production cost and manufacturing complexity.

3.4. Characterization of exosome distribution in hydrogels

The characterization of exosome distribution in hydrogels is a pivotal process to ensure uniform dispersion and maintain their functionality within the hydrogel system.

3.4.1. Microscopic imaging

For microscopic imaging, exosomes are labeled with fluorescent dyes such as PKH26, PKH67, DiI, DiO, allowing them to be observed in the hydrogels under a fluorescence microscope [220–222]. This method is straightforward and typically involves incubation of exosomes with the fluorescent dye, followed by the removal of excess dye. Confocal microscopy offers high-resolution three-dimensional imaging, providing detailed insights into the precise positioning and vertical distribution of exosomes within different hydrogel layers (Fig. 8a and b) [143,223].

3.4.2. Scanning electron microscopy

Scanning electron microscopy (SEM) is a vital tool for examining the surface and pore structures of hydrogels, thereby providing indirect insights into the distribution of exosomes (Fig. 8c) [143]. The process entails intricate sample preparations including chemical fixation, stepwise dehydration, drying, and conductive material coating. Analyzing the acquired images is crucial for identifying exosome distribution as well as quantifying their size and density within the hydrogel. This detailed analysis is instrumental for understanding the release dynamics of exosomes and optimizing the design of hydrogel carriers [224].

3.4.3. Fourier transform infrared spectroscopy

Fourier Transform Infrared Spectroscopy (FTIR) is an essential analytical method for determining the chemical composition and molecular structure of materials. When applied to hydrogel-exosome systems, FTIR may provide critical insights into their interactions and chemical structures by detecting the types of bonds between the exosomes and hydrogels, including covalent and non-covalent bonds like hydrogen bonds and electrostatic interactions [225,226]. FTIR spectra comparison of the exosomes, pre- and post-hydrogel binding, are key for evaluating the stability and structural changes (Fig. 8d) [227].

4. Application of the hydrogel-exosome system for tissue repair and regeneration

By synergizing hydrogel's biocompatibility and structural support with exosomes' pivotal role in cellular communication, the integration of hydrogel-exosome systems in tissue engineering and regenerative medicine is swiftly gaining momentum. Notable advancements in tissue regeneration encompassing the skin, bone, cartilage, nerves, and

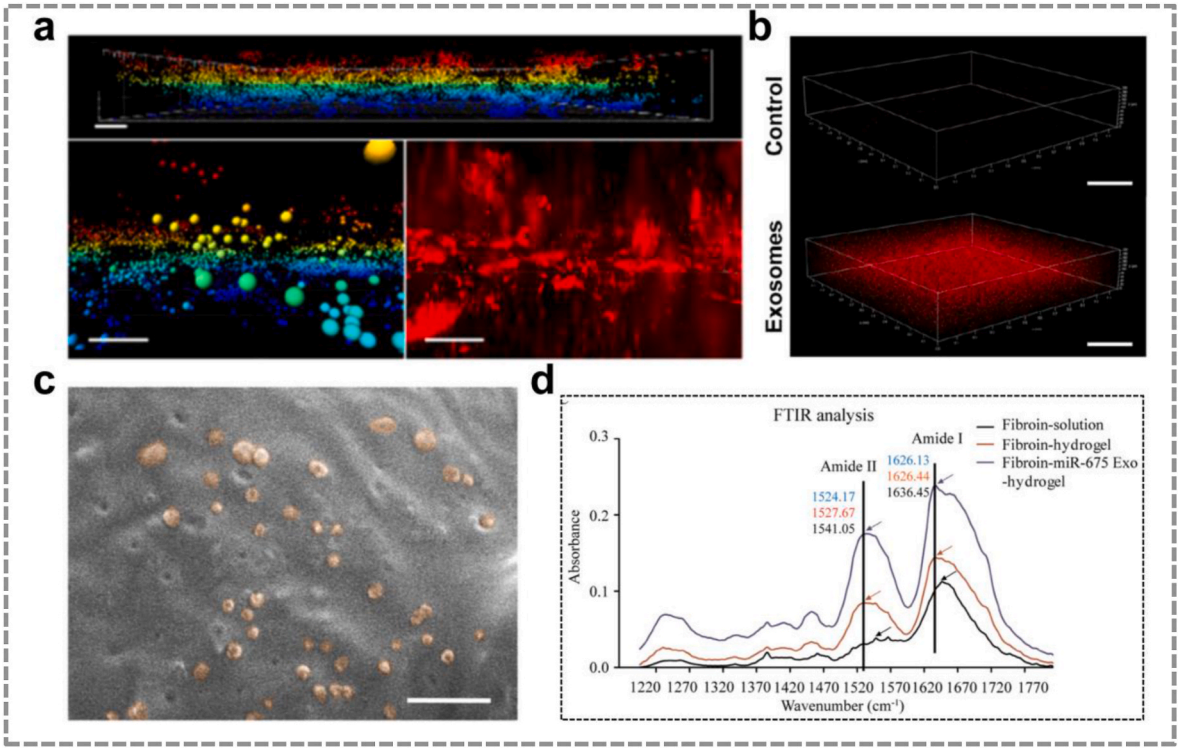

Fig. 8. Stacked confocal images of exosomes labeled with (a) CM-DiI, Reproduced with permission from Ref. [223]. Copyright 2020, American Chemical Society, and (b) PKH26 distributed in hydrogel. (c) SEM image of exosomes adhering to the internal surface of the hydrogel. Reproduced with permission from Ref. [143]. Copyright 2022, American Chemical Society. (d) FTIR of exosomes encapsulated within the hydrogel. Reproduced with permission from Ref. [227]. Copyright 2019, Elsevier.

tendons have demonstrated the system's efficacy (Table 5). For instance, for skin regeneration, the exosomes could promote cell proliferation and angiogenesis and accelerate the healing [175,228,229]. For bone and cartilage regeneration, such systems could facilitate tissue repair by providing osteogenic and chondrogenic factors [230]. For neural tissue engineering, the exosomes have shown potential in promoting axonal regeneration and mitigating inflammation [231,232]. However, further exploration is still needed before clinical application, especially understanding exosomes' mechanisms and refining the delivery strategies. The choice of exosome source, crucial for particular regenerative purposes, and cautious translation from animal models to human therapies, particularly considering the biosafety and long-term outcomes, has remained critical.

4.1. Skin tissue regeneration

As the body's largest organ, the skin serves as the primary interface with the environment and plays a crucial role in protecting the body from environmental challenges. However, extensive skin damage, particularly in conditions like diabetes leading to chronic wounds and/or ulcers, can hinder the normal healing process. Hence, the focus in the realm of skin tissue regeneration and repair has been on expediting the regeneration of skin tissue and promoting the restoration of impaired skin function. The healing of skin wounds has involved four stages: hemostasis, inflammation, proliferation, and remodeling, each requiring closely coordinated biological processes to ensure the essential functions of the skin barrier. Hydrogels loaded with exosomes derived from various tissues and/or cell sources have shown promising therapeutic effects on animal models, particularly those of diabetic wounds and full-thickness skin defects.

Owing to their extensive research history, broad applicability, and stable sources, adipose-derived mesenchymal stem cells (ADSCs), bone marrow-derived mesenchymal stem cells (BMSCs), and umbilical cord-derived mesenchymal stem cells (UC-MSCs) have been recognized as the most reliable sources of exosomes for preclinical research in skin tissue regeneration. The applications of exosomes in skin repair can be summarized as follows:

i) Regulation of immune response and inflammation: Inflammation serves as the body's self-defense mechanism against harmful stimuli. However, chronic and excessive inflammation may often hamper the wound healing process. BMSC-Exos can mitigate the polarization of LPS-stimulated macrophages towards the M1 phenotype, which is characterized by lower expression of iNOS. Simultaneously, they can enhance polarization towards the M2 phenotype, marked by higher expression of CD206, thereby inhibiting the release of various proinflammatory cytokines such as IL-1β and TNF-α [238].
ii) Regulation of fibroblast proliferation and differentiation: Fibroblasts, as the primary cellular component of loose connective tissue, originate from mesenchymal cells during embryonic development and play pivotal roles in hair follicle initiation and scar formation during wound healing [239]. ADSC-Exos can boost the proliferation capacity of fibroblasts *in vitro* and within the wound area, increasing the expression of ki67 [228]. Additionally, they may increase the mRNA levels of genes encoding α-smooth muscle actin (α-SMA) and fibroblast growth factor-2 (FGF-2) [240].
iii) Promotion of angiogenesis: Following skin injury, the damaged area will release various cytokines or growth factors which can activate endothelial cells in surrounding capillaries. The activated endothelial cells will proliferate, bud, and penetrate the basement membrane, migrate, and converge with adjacent endothelial cells at the front end of blood vessels to form new capillary loops [175,229,241]. Exosomes from the MSCs are rich in various miRNAs related to angiogenesis such as miR-423-5p, miR-146a, miR-21-5p, and let-7c-5p, which can target multiple signaling pathways associated with angiogenesis and tissue repair including PI3K/AKT, Ras/MAPK, VEGF/VEGFR, and others [242–244].

In recent years, researchers have shifted their focus to new cell source for exosomes whilst maintained the distinct biological functions of the donor cells. Such exosomes are explored for specific applications in skin tissue regeneration and repair. The exosomes derived from neural stem cells carry a rich content of neurotrophic factors such as neuron-derived neurotrophic factor (NDNF) and immunomodulatory proteins [245], which can facilitate the migration of human dermal fibroblast (HDF) cells and tube formation in human umbilical vein endothelial cells (HUVECs), offering potential avenues for skin injury treatment. Since 2017, researchers have investigated the impact of platelet-rich plasma-derived exosomes (PRP-Exos) on chronic skin wounds. The regenerative potential of PRP is often ascribed to the supra-physiological concentrations of growth factors released from activated platelets, including platelet-derived growth factor (PDGF), transforming growth factor-beta (TGF-β), and VEGF. The proliferative and re-epithelialization responses induced by PRP-Exos involve RhoA/YAP signaling transduction [246,247]. With with BMSCs, gingival mesenchymal stem cells (GMSCs) are abundant, easily accessible, and demonstrated enhanced proliferation, stable morphology, and notable immunomodulatory properties [248]. Synovial membrane-derived mesenchymal stem cells (SMSCs) have attracted attention due to their "tissue-specific" regenerative capabilities in connective tissues. They are readily expandable in culture whilst maintain a stable molecular profile and retain multipotency through at least 10 passages [249,250].

To tackle the limited yield of exosomes, researchers have recently explored the development of exosome mimetics. Utilizing an extrusion process, they have crafted exosome mimetics from polymorphonuclear neutrophils (PMNs), which resulted in a tenfold increase compared with PMN-Exos. Leveraging the inherent pathogen-engulfing ability of neutrophils as a primary defense line against invasive pathogens, such exosome mimetics had shown excellent antibacterial capabilities,

Table 5
Animal experiments on Hydrogel-Exosome system in tissue engineering.

Animal model	Hydrogel carrier	Exosome dose	Usage Method	References
Diabetic wounds	Decellularized Matrix of Porcine Heart	100 μg/mL	Treat with 100 μL every three days	[185]
Osteoarthritis	GelMA microspheres	800 μg/mL	Starting day 3 post-surgery, inject 10 μL biweekly for 8 weeks	[233]
Intervertbal disc degeneration	RGD-Complexed Decellularized Nucleus Pulposus Hydrogel	1 mg/mL	Inject 2 μL into nucleus pulposus, repeat after 4 weeks	[234]
Achilles tendon rupture	GelMA	10 mg/mL	50 μL hydrogel, single-dose, *in situ* photo-crosslinked	[106]
Anterior cruciate ligament	Composed of GelMA and N-Butyrylated Hyaluronic Acid	10 mg/mL	Single injection of 300 μL	[235]
Stress urinary incontinence	PEP collagen-1 hydrogel	1×10^{12} Evts/mL	Administer 50 mg/kg weekly for 6 weeks	[236]
Cavernous nerve injury	Hydroxyethyl Chitosan Composite with Sodium β-Glycerophosphate	1 mg/mL	Single injection of 100 μL	[237]
Sciatic nerve injury	HAMA	500 μg/mL	Use 100 μL once, with *in situ* photo-crosslinking	[15]

showcasing promising antimicrobial effects for chronic diabetic wounds [251,252].

In the hydrogel-exosome system for skin tissue repair, the hydrogels are not only required to act as effective carriers for exosomes, but are also expected to possess particular functions including promoting blood clotting, tissue adhesion, antimicrobial activity, anti-inflammatory properties, and antioxidation. Such functions are crucial for synergistically promoting the regeneration and repair of skin tissues in conjunction with exosomes. Researchers have expedited the repair process by altering the hydrogel's mode of action, the composition of hydrogel matrix materials, specific responses to the environment during the repair period, and the loading of bioactive factors necessary for repair. Serving as a minimally invasive tool, microneedles may offer advantages such as minimal trauma, low risk, enhanced skin permeability, and the stimulation of endogenous repair compared to conventional hydrogels, which may enable skin penetration for drug delivery. A hydrogel microneedle patch based on GelMA and PEGDA and loaded with Tazarotene and HUVECs-Exos has achieved targeted drug release and effectively expedited cell proliferation, migration, and blood vessel formation [253]. In another study, microneedles with tips made of a porous methacrylate gelatin hydrogel had continuously delivered anti-inflammatory and angiogenesis-promoting MSC-Exos to the wound area. The back of the microneedles, composed of silk fibroin, had adhered well to the skin and stabilizes at the injured site, eliminating the need for secondary dressings. Additionally, the incorporation of antibacterial silver nanoparticles (AgNPs) had inhibited bacterial infection in the wound, synergistically promoting wound healing in diabetic rat models(Fig. 9a) [254]. The extracellular matrix can degrade to form a temperature-sensitive and injectable hydrogel. ECM hydrogel loaded with ADSC-Exos derived from decellularized porcine left ventricular myocardium was injected into the wound site and effectively promoted wound healing(Fig. 9b) [185].

Although studies on the hydrogel-exosome systems have highlighted their role in promoting granulation tissue formation, re-epithelialization, angiogenesis, and collagen deposition, such investigations could only offer a superficial analysis and fell short in delving more profoundly into the mechanisms, particularly with regard to the crucial components and functions of exosomes in the healing process of skin wound. Furthermore, the use of mice and rats as model organisms has presented a common practice. However, rodent skin an undergoes contraction during the healing process, which has diverged from human skin and may introduce bias into the experimental outcomes.

4.2. Bone and cartilage tissue regeneration

4.2.1. Osteoarthritis

Osteoarthritis (OA) is a chronic, progressive joint disease characterized by the deterioration of joint cartilage, periarticular osteophyte formation, and inflammatory reactions [255]. Whilst commonly emerges with age, this condition can also result from joint injury, genetic predisposition, and metabolic abnormalities. The OA necessitates prolonged management and treatment, significantly impacting the patient's quality of life [256]. Novel approaches for cell-free osteoarthritis treatment have involved targeting cartilage and employing exosome-mediated drug delivery [257]. In a recent study, researchers have used cartilage-affine peptides to modify the surface of exosomes loaded with LRRK2-IN-1. The modification could effectively prevent local cytotoxicity and potential adverse effects induced by small molecule inhibitors, thereby enhancing the targeting capability. Additionally, a photocrosslinkable spherical hydrogel was employed for encapsulation to address the challenges related to the rapid clearance and low retention of exosomes [258]. Furthermore, recognizing the dynamic nature of the joint region during treatment, researchers have

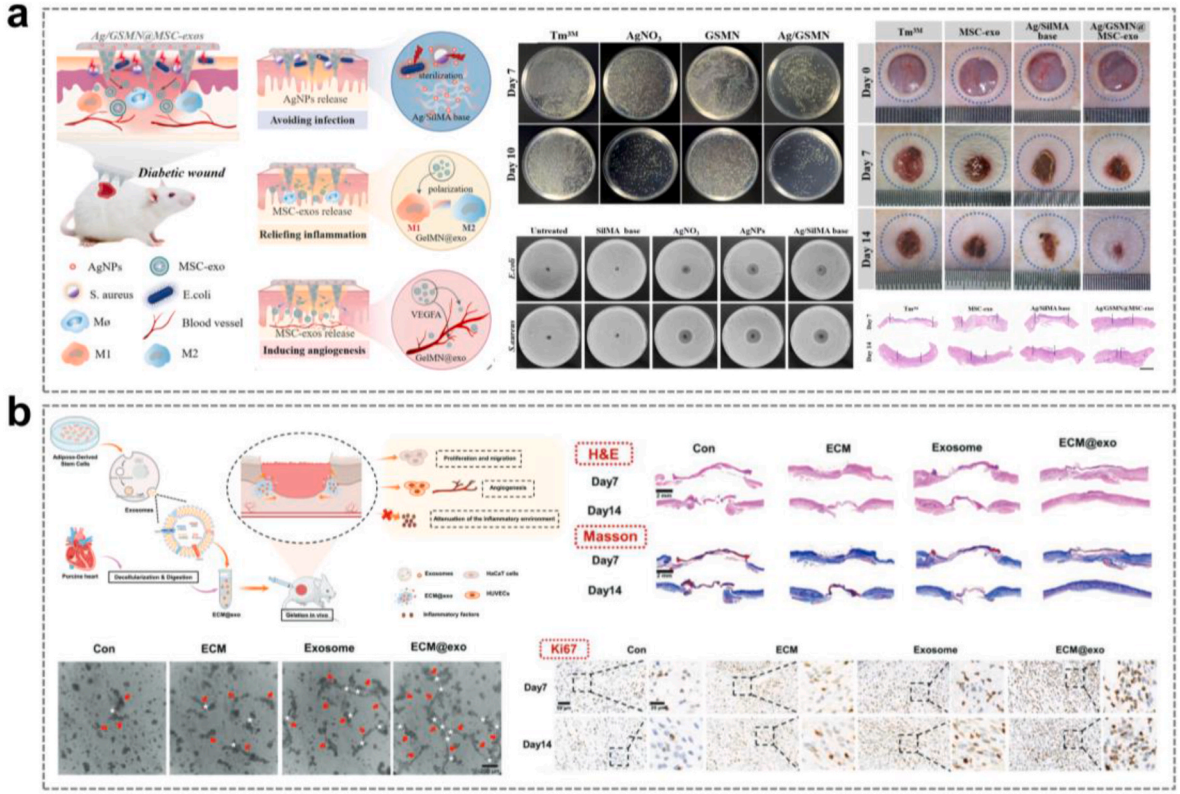

Fig. 9. Design and application of hydrogel-exosome systems in skin tissue engineering. (a) Hydrogel microneedles loaded with exosomes and possessing antimicrobial properties. Reproduced with permission from Ref. [254]. Copyright 2022, Elsevier. (b) Extracellular matrix hydrogel loaded with ADSC-Exos promotes wound healing. Reproduced with permission from Ref. [185]. Copyright 20123, Wiley.

developed a biomimetic hydrogel inspired by “mussel adhesion”. This hydrogel has shown self-healing and adhesive properties, which could facilitate adhesion to the wet cartilage surface and enhance the efficiency of drug utilization [259]. In a recent study, researchers have introduced a gene-editing-based method where a hydrogel is loaded with both FGF18-targeting chondrocyte-affinity peptide (CAP) and exosomes, effectively activating the FGF18 gene of OA chondrocytes at the genomic level in vivo. Additionally, the hydrogel provides long-lasting lubrication functionality(Fig. 10a) [260].

4.2.2. Bone defects

Bone defects resulting from trauma, infection, bone tumor, congenital deformity, and other causes have presented a significant challenge in clinical practice [261]. Autologous and allogeneic bone grafts have been commonly employed for bone regeneration [262]. The use of a hydrogel-exosome system to fill bone defect areas has represented a novel therapeutic approach for its adaptability. On the one hand, appropriate pretreatment of exosome donor cells, such as 3D cultivation, lentiviral engineering, overexpression or knockdown of function-related miRNAs, and hypoxic stimulation, can enhance the exosome’s production, targeting capability, and functions related to bone regeneration [263–266]. On the other hand, various biomaterials can produce distinct chemical signals, influencing cell communication and cellular microenvironment, thereby regulating exosome secretion [267]. BMSCs induced by PEGMC combined with β-TCP has shown dual functionality in osteogenesis and angiogenesis [268]. Recently, 3D printing has emerged as an ideal strategy for accurately constructing tissues and organs with complex spatial structures. Through 3D printing, researchers have developed a novel spatial hydrogel scaffold which has provided a tissue-specific microenvironment with a dual network comprised of a rigid and brittle first network, as well as a soft and extensible second network. This achievement has enabled efficient regeneration of cartilage and subchondral bones (Fig. 10b) [269].

Alveolar bone defects can increase the risk for periodontal diseases such as periodontitis. Dental stem cells, including dental pulp stem cells (DPSCs), human shedding deciduous teeth stem cells (SHEDs), stem cells from the apical papilla (SCAP), and periodontal ligament stem cells (PDLSCs), have been used as seed cells for tissue engineering [270,271]. The combination of exosomes derived from such cells with alginate-gelatin or collagen hydrogels has yielded promising results in a rat model for alveolar bone defect [272,273]. Nevertheless, there is still a lack of evidence for the efficacy of such composite scaffolds in large animal models.

4.2.3. Intervertebral disc degeneration

Abnormal intervertebral disc degeneration (IVDD) can result in pain, restricted movement, and other adverse conditions. Non-invasive surgical approaches using the hydrogel-exosome system may offer a promising avenue for treating the IVDD. Researchers have developed functional materials targeting the primary pathogenic mechanism of the

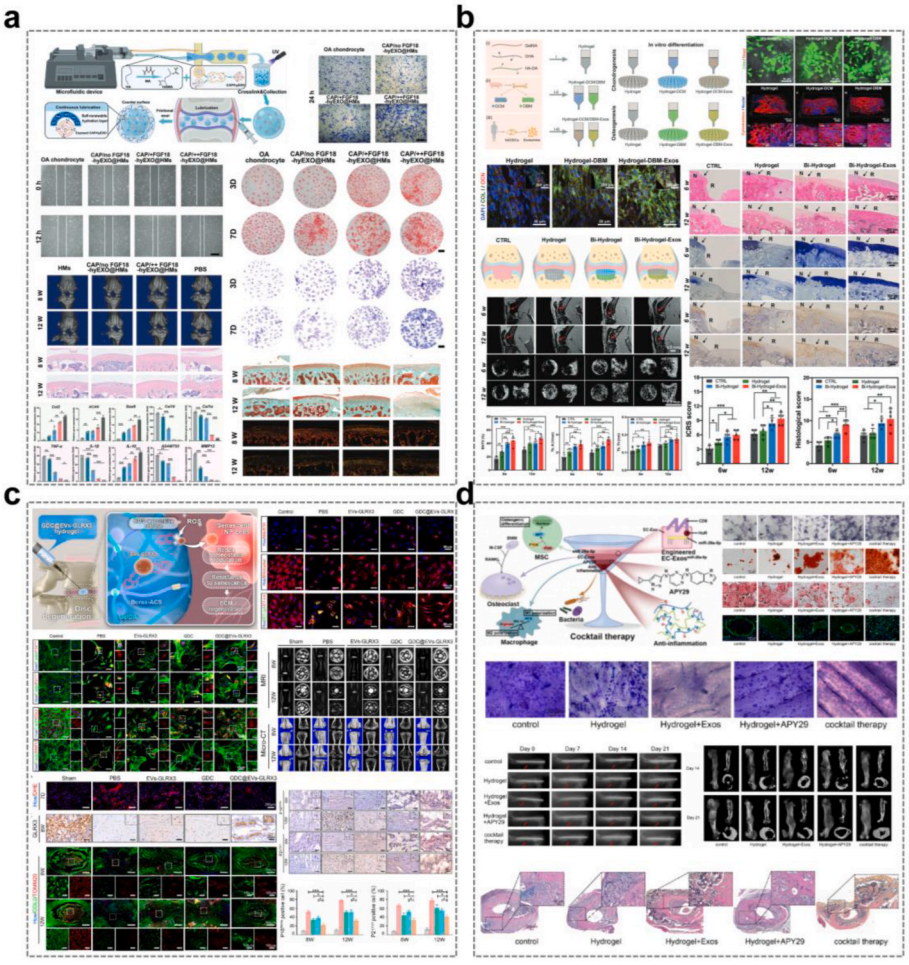

Fig. 10. Design and application of hydrogel-exosome systems in bone and cartilage tissue engineering. (a) Osteoarthritis. Reproduced with permission from Ref. [260]. Copyright 2024, Wiley. (b) Bone defect. Reproduced with permission from Ref. [269]. Copyright 2023, Wiley. (c) Intervertebral disc degeneration. Reproduced with permission from Ref. [275]. Copyright 2023, Wiley. (d) Fracture. Reproduced with permission from Ref. [279]. Copyright 2022, American Chemical Society.

IVDD, which has involved the aging of nucleus pulposus cells (NPCs). Extracellular vesicles from chondrocyte endplate stem cells (CESCs) overexpressing Sphk2 were loaded onto rib cartilage ECM hydrogels for the injection. This process has activated the PI3K/p-AKT pathway and intracellular autophagy in NPCs, which ultimately improved the IVDD [274]. In another study, injectable and self-healing hydrogels with ROS-clearing capabilities and characteristic disc-responsive properties were employed to load MSC-Exos overexpressing GLRX3. This strategy has effectively alleviated mitochondrial damage, mitigated the aging state of nucleus pulposus cells, and restored the ECM deposition by modulating redox homeostasis (Fig. 10c) [275].

4.2.4. Fracture

Fractures are typically accompanied by intense pain and discomfort. Open fractures, where the bone has penetrated the skin, can expose the injured area to infections, leading to serious complications such as osteomyelitis. Despite the development of various therapeutic strategies for

nonunion fractures such as fracture reduction, joint replacement, bone grafting, and bone stimulation, the clinical outcomes may vary [276]. In the process of fracture healing, the dynamic changes in the ratio of osteoblasts/osteoclasts and M1/M2 macrophages have played a crucial role, and can influence the fracture healing in real-time. Therefore, to regulate the inflammatory environment is essential [277,278].

In recent years, the design of hydrogel-exosome systems in animal models of fractures has predominantly focused on the immune regulation. In one study, researchers had obtained exosomes enriched with PD-L1 from genetically engineered HUVECs and applied them during the inflammatory phase of fracture healing. The sustained release of exosomal PD-L1 from the hydrogel has attained immunosuppressive effects, leading to a reduction in $CD8^+$ T lymphocytes in the nearby peripheral lymph nodes, which provided sufficient immune homeostasis during bone remodeling [230]. Through a similar approach, researchers have created a cocktail therapy by using hyaluronic acid to load EC-Exos overexpressing miR-26a-5p along with APY29. This has effectively suppressed pro-inflammatory cytokines whilst promoted M2 polarization and osteogenic differentiation, thereby accelerated fracture repair (Fig. 10d) [279]. Unfortunately, the simple fracture models used in such studies may not be entirely applicable to complex types of fractures clinically.

In addition to the four frequently used animal models for bone and cartilage tissue injury repair, researchers have also explored the effect of hydrogel-exosome systems on the models for more intricate bone injuries such as subtalar osteoarthritis [187], mandibular defect [143], femoral condyle defect [280] and posterolateral spinal fusions [268].

4.3. Nerve tissue regeneration

4.3.1. Spinal cord injury

Spinal cord injury (SCI) is a devastating trauma to the central nervous system, which will typically result in disruptions to motor, sensory, and autonomic nervous functions. GelMA has emerged as a promising biomaterial for neural tissue repair, with an aim to enhance the therapeutic effect through the reconstruction of microvascular structures, immune modulation, and mitigation of oxidative stress [281–283]. GelMA microneedle patches loaded with exosomes derived from

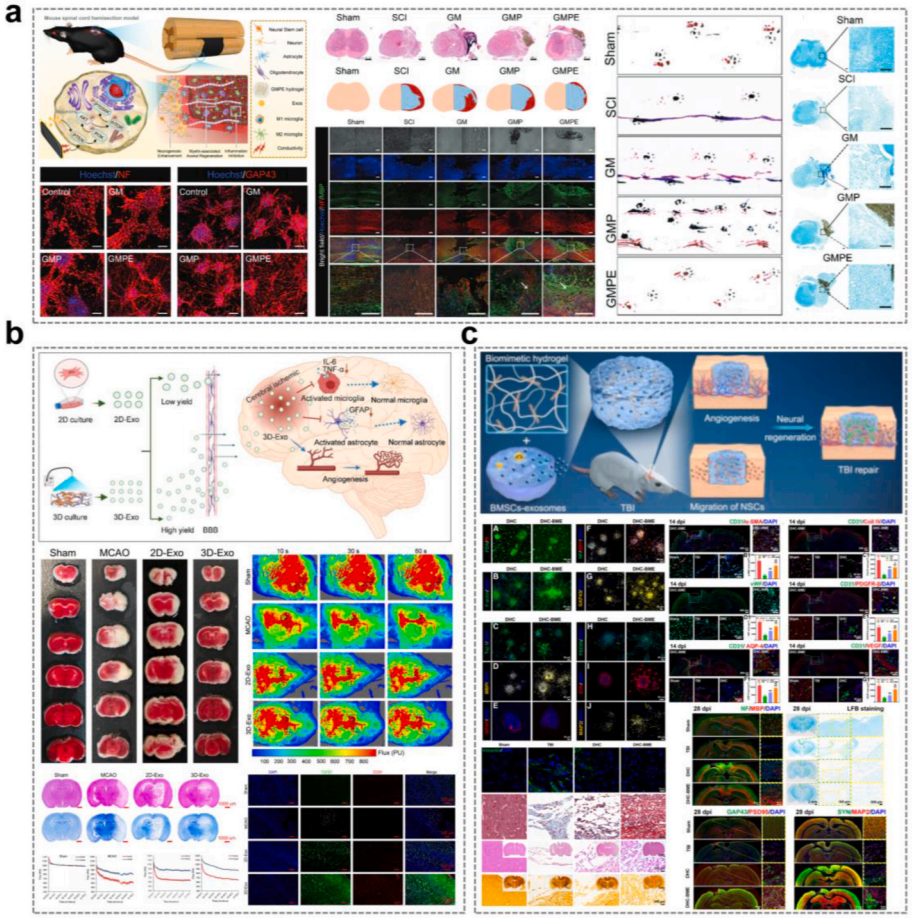

Fig. 11. Design and application of hydrogel-exosome systems in nerve tissue engineering. (a) Spinal cord injury. Reproduced with permission from Ref. [232]. Copyright 2022, American Chemical Society. (b) Middle cerebral artery occlusion. Reproduced with permission from Ref. [285]. Copyright 2023, Elsevier. (c) Traumatic brain injury. Reproduced with permission from Ref. [293]. Copyright 2023, Elsevier.

three-dimensional cultured MSCs could facilitate the transition of microglial cells from the M1 to M2 phenotype in the post-SCI microenvironment, which has in turn led to a reduction in neuroinflammatory reactions, showcasing a remarkable neuroprotective effect (Fig. 11a) [232].

Hydrogels based on HA have shown excellent compatibility with spinal cord tissue, although their capacity to fuse with the exosomes seems to be limited [284]. To bolster the affinity between the material and exosomes, researchers have developed a HA hydrogel modified with the peptide PPFLMLLKGSTR, thereby increasing the loading efficiency of the exosomes derived from the HUC-MSCs [223,281]. Additionally, the material's compatibility with the electrical and mechanical properties of the natural neural tissue must be considered. Fan et al. have crafted an exosome-loaded double-network conductive hydrogel (GM/PPy) which was comprised of photopolymerized GelMA and polypyrrole (PPy) hydrogel. This adjustment has influenced the fate of mechanically sensitive NSCs and promoted axon regeneration and outcomes related to neurons and myelin [231].

4.3.2. Middle cerebral artery occlusion

Cerebral artery occlusion refers to obstruction of middle cerebral artery (MCA) or its branches, resulting in interruption of blood supply to the corresponding brain regions. This condition may arise through thrombus formation, atherosclerosis, and vascular spasms, and cause cognitive impairment, neurological dysfunctions, and ischemic stroke. Researchers have commonly used tail vein injection to directly administer the exosomes into animals with middle cerebral artery occlusion (MCAO). The 3D culture of the donor cells within the bioactive hydrogels may enhance their paracrine capability, boost exosome production and biological functions, and stimulate neovascularization in the ischemic area(Fig. 11b) [285].

An effort has also been made to explore the mechanism of action of exosomes in ischemic brain injury, focusing on their protein and miRNA components. It has been confirmed that the decreased levels of miR-206-3p in BMSC-Exos can activate the PI3K/AKT signaling pathway, thereby alleviate ischemic brain injury in MCAO rats [286]. Engineered biomaterial scaffolds loaded with stem cells have emerged as a novel and promising strategy for treating ischemic strokes [287,288]. In the ischemic microenvironment, such scaffolds could retain the stem cells in the brain and enhance their paracrine capabilities. Studies have indicated that the hydrogel/nanofiber composite scaffolds, closely resembling the brain's endogenous ECM, loaded with BMSCs, have shown superior therapeutic potential in terms of neuroprotection and vascular regeneration [286]. Nevertheless, as admitted by the authors, this approach may lead to increased intracranial pressure and tissue swelling. Additionally, the biocompatibility of the hydrogel/nanofiber materials also warranted further consideration.

4.3.3. Traumatic brain injury

Traumatic brain injury (TBI) refers to brain dysfunction caused by external forces to the head, resulting in conditions such as coma, impaired consciousness, intracranial bleeding, brain edema, and even seizures [289,290]. Vascular system damage, activation of microglial cell inflammation, and excessive release of ROS during the traumatic process are major obstacles to the patient recovery, and the researchers' strategies have largely revolved around addressing such challenges. A multifunctional hydrogel based on poly(citric acid-substituted fumaric acid) has shown potent antioxidant capabilities. Loaded with SHED-Exos, this hydrogel could eliminate excess ROS from the microenvironment, rescuing cortical lesions in TBI rats and alleviating brain edema [291].

As a naturally derived ECM-based hydrogel, hyaluronic acid has also shown a promising role in maintaining neural development and inhibiting neural-glial scar formation. However, as aforementioned, the hyaluronic acid lacks cell adhesive domains [284]. Collagen, known for its ability to construct biomimetic neural microenvironments with specific functional peptide sequences for cell adhesion, growth, and differentiation, is utilized [292]. Liu et al. have constructed a hyaluronic acid-collagen hydrogel, simulating the brain ECM environment. When combined with BMSC-Exo, this hydrogel could promote lesion vascularization and achieve axon regeneration, myelin sheath regeneration, synaptic formation, and even remodeling of brain structures, attaining robust neural functional recovery(Fig. 11c) [293].

In addition to the aforementioned animal models for nerve injury, the hydrogel-exosome system has also been employed in more intricate nerve injury models, including sciatic nerve compression injury (SNCI) [15,237], bilateral cavernous nerve injury (BCNI) [237], and erectile dysfunction [186]. In all such models, it has consistently exhibited excellent abilities in suppressing microglial cell inflammation, fostering vascular neogenesis, and facilitating functional neural regeneration.

4.4. Tendon tissue regeneration

Tendons are dense connective tissues that connect muscles with the bones, which plays a crucial role in transmitting the force generated by the muscles to the skeletal structure [294]. Both acute trauma and chronic overuse can lead to tendon injuries, which are often accompanied by intense pain, restricted movement, and an increased risk for recurring injuries, and can significantly affect the quality of life. Researchers have developed animal models for conditions such as rotator cuff tears, Achilles tendon injuries, urinary incontinence, and reconstructed the anterior cruciate ligament in the right knee, with an aim to explore the treatment strategies comprehensively and achieve the perfect reshaping of tendon structure and function. Natural tendons possess a hierarchical arrangement of collagen fibers and exhibit high modulus at gigapascal levels, characteristics that are difficult to replicate using conventional synthetic hydrogels. Sun et al. have developed a multifunctional hydrogel mimicking tendons, which is constructed from an opic assembly of aramid nanofibers composite material. This hydrogel not only matches the mechanical properties of natural tendons but also regulates cellular behavior [295].

Since the introduction and widespread application of cell-free therapies based on exosomes, extracellular vesicles derived from ADSCs, BMSCs, and urine-derived stem cells (USCs) have been proven to promote tendon repair by modulating the biological characteristics of TSCs and the extracellular microenvironment. Preconditioning such stem cells under hypoxic conditions has shown to be advantageous in promoting vascular generation and accelerating tendon-to-bone healing [235,296,297]. In another study, extracellular vesicle products derived from human platelets expressing CD41a and CD9 demonstrated extensive skeletal muscle regeneration in a rat model for volumetric muscle loss (VML) defect. Additionally, to closely mimic the human anatomy, researchers have investigated the functionality of clinical-grade fibrous protein gel-loaded PEP in a large animal model of stress urinary incontinence (SUI) using pigs, and observed a significant restoration of urethral pressure after 37 days(Fig. 12a) [236]. Biomimetic scaffolds, designed to simulate the tendon environment, include GelMA scaffolds with parallel arranged ECM structures. Loaded with exosomes delivering Yap1, such scaffolds could promote the rejuvenation of tendon stem/progenitor cells (TSPCs) and functional regeneration of aged-related tendon damage(Fig. 12b) [298]. However, most animal studies on tendon injuries have employed acute modeling using drugs or mechanical trauma, and there is still a challenge to accurately simulate chronic tendon tissue damage caused by aging or prolonged misuse in clinical scenarios.

5. Challenges and future prospects

Over the last decade, synergistic use of hydrogels and exosomes has emerged as a highly effective duo in the foundational research of tissue engineering and regenerative medicine. While numerous studies have demonstrated the efficacy of the hydrogel-exosome system for repairing

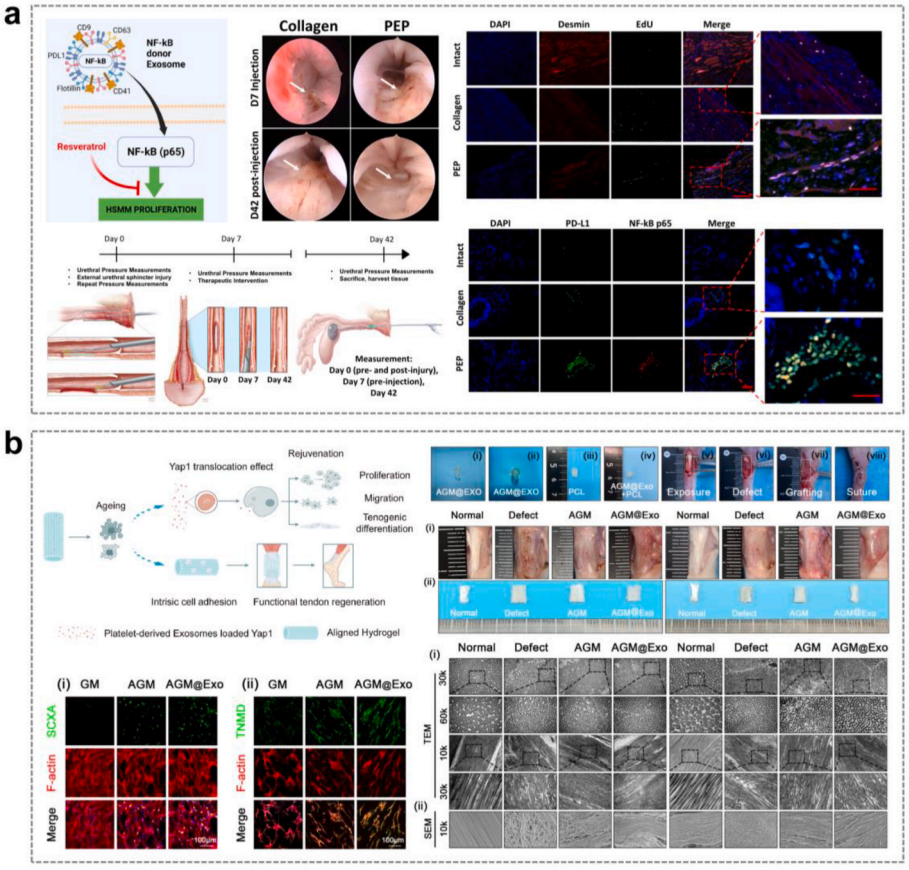

Fig. 12. Design and application of hydrogel-exosome systems in tendon tissue engineering. (a) Application in a large animal pig model of urinary incontinence. Reproduced with permission from Ref. [236]. Copyright 2022, Springer Nature. (b) Simulating tendon environment with biomimetic biodegradable scaffolds. Reproduced with permission from Ref. [298]. Copyright 2023, Elsevier.

various tissue injuries, it is important to acknowledge and address the limitations with such research endeavors (Fig. 13).

5.1. Development of novel techniques for exosome extraction and purification

Conventional methods like ultracentrifugation, while widely used, are time-intensive and may damage the exosomal membranes [64]. On the other hand, commercially made kits, though efficient, are too expensive for large-scale extraction [70,71]. Researchers are therefore exploring new, more cost-effective, and less invasive techniques for exosome separation. Chen et al. have developed an EXODUS system, a rapid separation method using negative pressure oscillation and dual coupled resonators for membrane vibration, significantly enhancing the efficiency and speed of exosome purification [299]. Liu et al. employed a biotinylated Supported Lipid Membrane (SLM) array to selectively capture specific extracellular vesicles based on their RNA and protein biomarkers [300]. Bathini et al. introduced an approach using Vn96-bound magnetic particles in a liquid biopsy chip for isolating EVs, providing a more efficient and streamlined process [301]. These innovations offer various advantages and can be tailored according to the specific requirements of the exosome separation, including the source, desired purity, and intended applications. Their implementation is vital for advancing exosome-based research, particularly in disease diagnosis and treatment.

5.2. Expansion of exosome sources

Plant cells, more easily cultivated and multiplied than animal cells, and their more readily available materials make them economically viable sources of exosomes. Plant-derived exosomes, rich in bioactive molecules like secondary metabolites, proteins, and lipids, have shown potential in anti-inflammatory, anti-cancer, and antioxidant therapies. Their high biocompatibility and low toxicity are due to the evolutionary divergence of plant cells from human pathways, reducing the likelihood of triggering immune responses [302–304]. The burgeoning field of plant exosome research holds great promise, highlighted by the innovative work of Pan et al. They developed a novel plant-derived exosome-like nanovesicle-hydrogel formulation, leveraging exosome-like nanovesicles from aloe vera's outer skin as the active ingredient. The inner pulp of aloe vera was processed into a hydrogel matrix, and the combination of these two components demonstrated remarkable therapeutic effectiveness in mouse models of atopic dermatitis and diabetic wounds [305]. Microbial exosomes possess characteristics that make them advantageous for future applications in tissue engineering, such as easy scalability and the ability for facile functionalization through genetic engineering. However, addressing the challenges related to the biosafety and biocompatibility of microbial exosomes is crucial for advancing their medical and biotechnological applications. One essential strategy is to select microbial strains with known high biosafety, such as Generally Recognized As Safe (GRAS) strains, as sources of exosomes. Alternatively, genetically modifying microbes to remove potential pathogenic factors or enhance specific safety features, such as encoding human-tolerable proteins and peptides, can also improve their safety profile [306]. In addition to strain selection and genetic modification, developing and optimizing purification methods for exosomes is vital. These methods should efficiently remove endotoxins and other potentially harmful components from microbial cultures. Sensitive

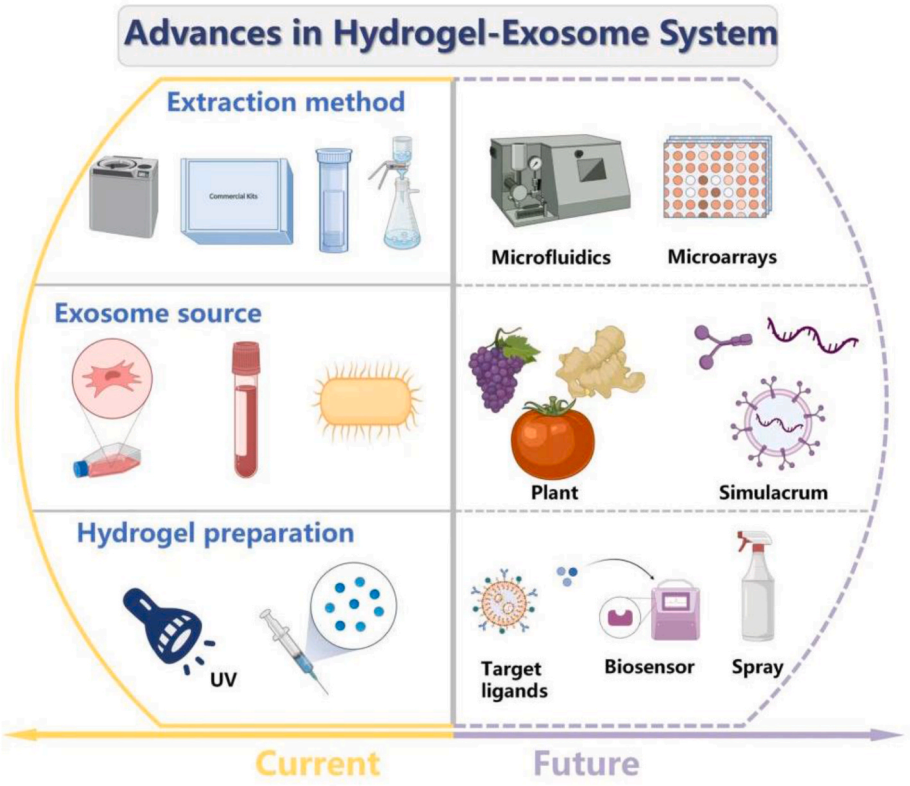

Fig. 13. Schematic illustration of perspectives of Hydrogel-Exosome System in regenerative medicine.

detection methods like Limulus Amebocyte Lysate (LAL) can be employed to assess endotoxin levels in exosome preparations, and appropriate removal techniques should be applied to ensure their safety. By implementing these strategies, we can maximize the safety and biocompatibility of microbial exosomes in clinical applications while retaining their unique functionalities and potential therapeutic value.

5.3. *Designing biologically safe and tissue-adaptive hydrogels*

There is a critical need to develop hydrogels that are not only simple, quick, and reproducible but also align with the stringent standards of biocompatibility and safety, especially designed for the diverse characteristics and pathologies of tissues encountered in clinical settings. Drawing inspiration from the natural composite material derived from mussel byssus cuticles, Bao et al. have innovated an unique process that merges photo-crosslinking with interfacial bonding. This technique rapidly produces hydrogels with exceptional toughness and tensile strength within mere seconds. These hydrogels demonstrate a structural stability that rivals or surpasses that of conventional elastomers, rendering them exceptionally suited for repairing soft tissues, such as skin. This novel method, known as the PTPC reaction, represents a significant advancement in hydrogel technology [307]. In another groundbreaking development, Zhang et al. have designed a sprayable hydrogel sealant [308]. Based on the homogeneous network principle, this sealant is adept at managing wounds that are moist, dynamic, and not easily accessible, offering a practical solution in clinical treatment scenarios. Additionally, injectable drug delivery systems (DDS) have emerged as a game-changer in repairing internal tissues like bone, cartilage, brain, and teeth. These systems enable precise drug targeting, thereby minimizing the need for invasive procedures and effectively eliminating the necessity of open surgeries. This approach significantly lowers the risk of tissue damage and infection [309,310]. The advent of injectable hydrogels has revolutionized drug delivery, allowing for accurate targeting of drugs to specific internal locations, particularly beneficial for surgically challenging areas. Their capability for *in situ* gelation ensures an exact fit even in irregularly shaped cavities. Moreover, Liu et al. have engineered an injectable hydrogel that is both self-lubricating and responsive to friction, perfectly emulating the meniscus. This innovation is key in protecting joint cartilage and enhancing movement, marking a significant leap forward in the field of biomedical engineering [311].

Encapsulating and releasing exosomes requires intricate techniques and precise handling. Hydrogels, serving as carriers, must fulfill diverse criteria including stability, release kinetics, and biocompatibility concerning exosomes. This complexity may lead to instability during preparation and challenges in reproducibility. Encapsulation methods may suffer from low efficiency, where only a fraction of exosomes are successfully encapsulated, potentially impacting subsequent release and therapeutic efficacy. Controlling the release kinetics of exosomes may be influenced by various factors such as hydrogel structure, environmental conditions, and interactions with exosomes, posing challenges in precise control and affecting therapeutic outcomes and safety. Moreover, hydrogels must exhibit excellent biocompatibility and stability to safeguard exosomes from environmental influences. However, certain hydrogels may elicit immune or toxic responses, or fail to maintain structural stability over time, thereby limiting their clinical feasibility. As the encapsulation and release techniques for exosomes using hydrogels are relatively novel, long-term clinical data and comprehensive evaluations are essential to ensure their safety and effectiveness.

Furthermore, areas of tissue repair that have received less attention in current research warrant more focus from researchers. For instance, in 2021, Yang et al. developed a bladder decellularized matrix material loaded with exosomes derived from adipose-derived stem cells to enhance bladder tissue regeneration and functional recovery in a rat bladder substitution model. While the feasibility of this approach was demonstrated, questions remain about whether the scaffold can withstand the strength of repeated urine flushing in the bladder, and whether the material can be cross-linked or modified using physical or chemical

methods to better match the specific environment of bladder tissue. These are issues that future research needs to address [312]. In another study focusing on endometrial injury, Lin et al. designed a dynamic PEG hydrogel produced by Ag–S coordination, which dynamically coordinates and merges with ADSC-exos, simulating the endometrial microenvironment to promote neovascularization and tissue regeneration [313]. There have been few reports on the hydrogel-exosome system in the treatment of myocardial infarction, enteritis, and periodontitis [314–316]. Therefore, we encourage further exploration of tissues that are currently less researched to fill existing knowledge gaps.

5.4. Understanding the behavior mechanisms of exosomes within hydrogels

The design of a hydrogel-exosome system will typically involve embedding the exosomes within the hydrogel matrix to regulate their release. This has presented a challenge, as exosomes, being protein-based cargoes, may undergo denaturation should they be incompatible with the hydrogel's crosslinking chemistry. The incorporation of nanotechnology can significantly augment the drug-carrying efficiency and the release precision of the hydrogels. Nanoparticles can act as effective drug carriers, either by encapsulating the therapeutic agents or adsorbing them onto their surfaces. This not only shields the drugs from environmental degradation but also boosts their concentration and efficacy at the target site. One such advancement has been the use of Laponite, a synthetic layered silicate. The nanoparticles of Laponite are characterized by their extensive surface area and unique layered structure. Their adjustable interlayer gaps have rendered them an optimal template for controlled release systems, enabling the execution of sophisticated drug release mechanisms [317–319]. Additionally, Laponite has endowed the hydrogels with specialized rheological properties, such as shear-thinning behavior, which is particularly advantageous in the context of injectable hydrogels [320,321].

Furthermore, exosomes are complex biological entities containing a myriad of proteins, lipids, and RNA molecules, each exhibiting distinct behaviors under various physiological and pathological conditions. This complexity and diversity present challenges in comprehensively predicting exosomal behavior within different types of hydrogels using a single model. Presently, the techniques and methods employed to study exosome behavior may be limited by factors such as resolution constraints, potential exosome loss or alteration during sample preparation, and challenges in quantitative analysis, all of which may hinder our ability to deeply understand exosomal behavior. Interactions between exosomes and hydrogels involve various forces, including electrostatic interactions, hydrogen bonding, and hydrophobic interactions, among others, which are influenced by factors like hydrogel chemical composition, cross-linking density, and pore size. The intricacy of these interactions complicates the prediction of exosomal behavior within hydrogels. Additionally, the mechanisms underlying exosomal uptake by target cells are diverse and complex, encompassing processes such as endocytosis and fusion, with limited understanding of how these mechanisms are affected by the hydrogel microenvironment.

6. Conclusions

The hydrogel-exosome system, as an innovative biomedical engineering strategy, exhibits immense potential in the fields of tissue engineering and regenerative medicine. This system combines the high water content and excellent biocompatibility of hydrogels with the tunable physicochemical properties and natural bioactivity of exosomes, offering a novel approach for efficient and targeted therapies. In the future, it will be important to implement quality control measures in the production process of exosomes, aiming to enhance both the efficiency and quality of extraction through appropriate purification methods. Additionally, exploring alternative sources for obtaining exosomes and improving their biological functions through suitable pretreatment methods will be crucial. Concurrently, a deep understanding of hydrogel's physicochemical characteristics is vital. Tailoring and refining hydrogel materials to fit different clinical contexts and requirements may lead to more precise and controlled exosome release. By simplifying their application, these advancements will expedite the clinical adoption of the hydrogel-exosome system.

Data availability

Data will be made available on request.

Ethics approval and consent to participate

There are no human and animal subjects in this review and informed consent is not applicable.

CRediT authorship contribution statement

Ming-Hui Fan: Writing – original draft. **Jin-Kui Pi:** Investigation. **Chen-Yu Zou:** Project administration. **Yan-Lin Jiang:** Investigation. **Qian-Jin Li:** Writing – review & editing. **Xiu-Zhen Zhang:** Software. **Fei Xing:** Funding acquisition. **Rong Nie:** Supervision. **Chen Han:** Investigation. **Hui-Qi Xie:** Supervision.

Declaration of competing interest

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

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