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Review article

Extracellular Vesicles-in-Hydrogel (EViH) targeting pathophysiology for tissue repair

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ABSTRACT

Regenerative medicine endeavors to restore damaged tissues and organs utilizing biological approaches. Utilizing biomaterials to target and regulate the pathophysiological processes of injured tissues stands as a crucial method in propelling this field forward. The Extracellular Vesicles-in-Hydrogel (EViH) system amalgamates the advantages of extracellular vesicles (EVs) and hydrogels, rendering it a prominent biomaterial in regenerative medicine with substantial potential for clinical translation. This review elucidates the development and benefits of the EViH system in tissue regeneration, emphasizing the interaction and impact of EVs and hydrogels. Furthermore, it succinctly outlines the pathophysiological characteristics of various types of tissue injuries such as wounds, bone and cartilage injuries, cardiovascular diseases, nerve injuries, as well as liver and kidney injuries, underscoring how EViH systems target these processes to address related tissue damage. Lastly, it explores the challenges and prospects in further advancing EViH-based tissue regeneration, aiming to impart a comprehensive understanding of EViH. The objective is to furnish a thorough overview of EViH in enhancing regenerative medicine applications and to inspire researchers to devise innovative tissue engineering materials for regenerative medicine.

1. Introduction

Regenerative medicine has garnered significant interest in recent decades due to its potential to address challenges related to donor scarcity and immune-related complications in direct transplantations. This field focuses on restoring diseased or damaged tissues and organs by integrating biological principles with engineering science, encompassing areas like bone, nerves, and blood vessels. Presently, research in regenerative medicine, particularly cell therapy, is thriving, utilizing a diverse array of cell types. Among these, stem cells are widely recognized as the optimal cell source for regenerative medicine due to their capability for self-renewal and multi-directional differentiation [1]. Numerous studies have shown that mobilizing endogenous stem cells or directly administering exogenous stem cells to injured tissues leads to significant improvements in both structural and functional regeneration [2,3]. Nonetheless, the clinical application of cell therapy encounters several challenges, such as the necessity for obtaining autologous or allogeneic cells [4], the variability associated with isolating and amplifying cell populations in a laboratory setting [5], the unpredictable differentiation patterns *in vivo* [6], and the immunogenicity of cells. Additionally, concerns regarding storage, transportation, ethical considerations, and the risks associated with tumorigenesis and fibrotic complications must be carefully addressed before the implementation of direct stem cell transplants. Recent studies have indicated that the therapeutic effects of cellular therapies, like mesenchymal stem cell (MSC)-based therapy, can be attributed to the secretion of extracellular vesicles (EVs) by the cells themselves [7,8]. These EVs offer a promising alternative to using whole cells in the field of regenerative medicine.

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EVs are lipid bilayer spherical nanostructures (50–200 nm) released by cells [9,10], categorized as exosomes, microvesicles (MVs), and apoptotic bodies based on their biogenesis mechanism [11,12]. EVs can transfer biomolecules like proteins, lipids, and nucleic acids, providing them with unique properties such as anti-apoptotic effects, regulation of cellular proliferation and differentiation, promotion of angiogenesis, and immune modulation [13]. Compared to biotic cells, abiotic EVs offer advantages in terms of storage, transportation, and biosafety. Given these benefits, research on therapeutic EVs presents new possibilities in regenerative medicine, including applications in areas such as wound healing [14], bone and cartilage regeneration [15], nerve tissue repair [16], myocardial regeneration [17], as well as reduction of liver and kidney damage [18]. However, challenges exist in the clinical use of EVs in regenerative medicine, with one major obstacle being the inefficient delivery of EVs to specific tissues or organs. Following systemic administration, EVs are typically eliminated from circulation and accumulate in organs like the liver within 24 h [19,20]. Even when administered subcutaneously, most EVs are cleared by the reticuloendothelial system or lymphatic transportation [21,22], necessitating multiple doses for sustained effects. Moreover, increased EVs administration may result in off-target effects in peripheral organs due to abnormal vesicle levels in tissue or plasma. Localized delivery of EVs to target tissues emerges as a promising strategy to address these challenges and ensure optimal concentration for enhanced tissue regeneration efficacy.

Hydrogels play a crucial role as a promising three-dimensional (3D) hydrophilic scaffold, enhancing the transport and retention of EVs within lesions and shielding them from external factors [23]. These hydrogels closely mimic the native extracellular matrix (ECM), exhibiting exceptional bioactivity, biocompatibility, and physiochemical/biophysical properties [24]. The combined use of hydrogels and EVs not only ensures consistent EVs delivery but also stimulates the recruitment of endogenous cells for proliferation, creating a favorable environment for cell growth and enhancing tissue regeneration synergistically. To offer a comprehensive overview of the application of EVs in conjunction with hydrogels, a thorough search of pertinent articles and reviews was conducted through the Web of Science Core Collection (WOSCC). The retrieved documents were visually examined using

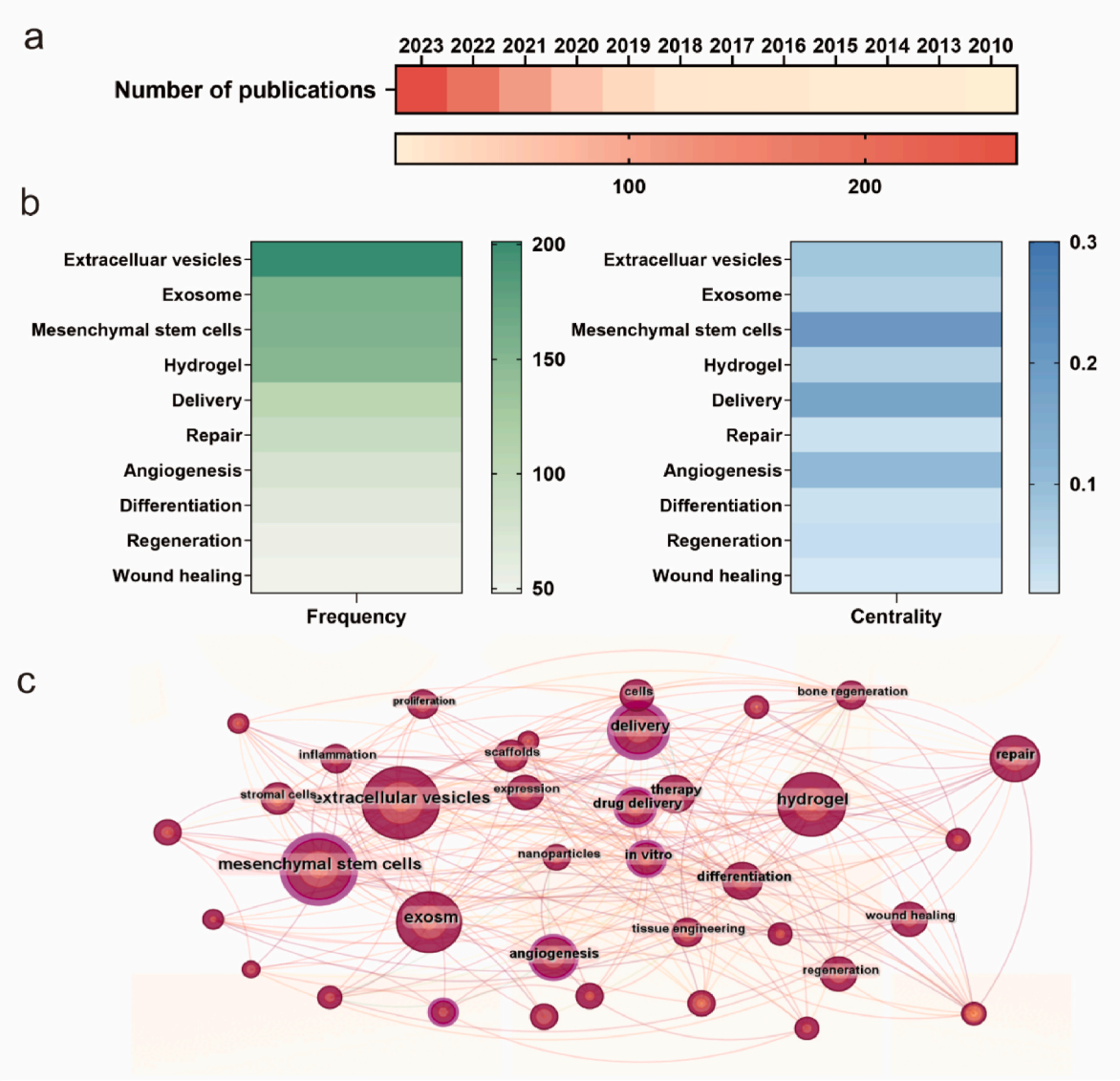

Fig. 1. The comprehensive overview of EVs and hydrogels research from 2010 to 2024. (a) The annual number of publications worldwide related to EVs and hydrogels from 2010 to 2024. (b) The top 10 keywords ranked by co-occurrence frequency with frequency ranking or centrality ranking. (c) A visual representation of the cooperative network map of keywords related to EVs and hydrogels from 2010 to 2024. The size of each node corresponds to the frequency of output, while the thickness of the pink outer ring signifies the centrality. The connecting lines between nodes signify the collaborative relationships between keywords.

CiteSpace software, and the data was graphed using GraphPad Prism. The search query employed was "*((TS = (extracellular vesicles)) OR TS=(exosomes)) AND TS=(hydrogels)*", resulting in a total of 643 reviews and articles published before August 31, 2024. Notably, the co-occurrence of the keywords EVs and hydrogels was first documented in an article in 2010, signifying the recent emergence of this field. The number of publications has been steadily increasing annually, with 219 relevant articles published in 2024 alone (Fig. 1a). Furthermore, an analysis of co-occurring keywords revealed that the top 10 most frequently mentioned keywords are displayed in Fig. 1b. It is evident that "repair" and "regeneration" are the prominent areas of focus for the application of hydrogels and EVs. Additionally, "tissue engineering", "bone regeneration", and "wound healing" are closely associated with the utilization of EVs and hydrogels (Fig. 1c).

The EViH system has garnered considerable attention in research, with various reviews outlining its key aspects. While existing reviews focus on the application of EViH in tissue repair [25,26], a comprehensive discussion on the features, advantages, and shortcomings of the EViH system is lacking, hindering its further development. Notably, they also do not delve into the pathophysiology of diseases or the biological processes involved in tissue regeneration. In our view, tissue engineering materials should align closely with the physiological processes of tissue repair. In this review, we compile the pathophysiological characteristics of different tissue injuries, such as skin wounds, bone injuries, cardiovascular diseases, nerve injuries, as well as liver and kidney injuries. It is important to highlight that exosomes and MVs are the primary types of EVs loaded in hydrogels for regenerative purposes. Our subsequent discussion will primarily focus on exosomes and MVs. This review aims to offer a comprehensive overview of advancements in EViH systems in regenerative medicine (Fig. 2). We begin by summarizing the history of EVs, their generation process, and acquisition methods, and exploring their potential functions in regeneration medicine. Subsequently, we provide a brief introduction to the construction of EViH, followed by a detailed discussion of its advantages and features, including biocompatibility, multifunctionality, protective properties, and controlled release. We then concentrate on the varied regenerative applications of EViH, including a section that delves into the pathophysiological processes in disease development and organ repair. Highlighted are specific applications of EViH under different pathological conditions, covering the source of EVs, types of hydrogels, controlled release effects, and biological functions. Finally, we conduct a comprehensive analysis of the current status of EViH systems, assessing the pros and cons related to tissue regeneration. The objective is to enhance knowledge and understanding of EViH, present a thorough overview of EViH's role in advancing regenerative medicine applications, and motivate researchers to innovate in the development of tissue engineering materials for regenerative medicine purposes.

2. Extracellular vesicles

EVs play a crucial role in intercellular communication within living organisms, being naturally released by cells [27]. Because of their significant involvement in various physiological processes like aging, cancer, and obesity [9,28], EVs have become a focal point in current research efforts. This section provides a succinct overview of the history, biogenesis mechanisms, and functions of EVs.

2.1. The history of EVs

The history of EVs dates back to the 1940s when Chargaff and West initially observed small vesicles released by blood platelets during blood clotting. Through high-speed centrifugation, they isolated coagulation

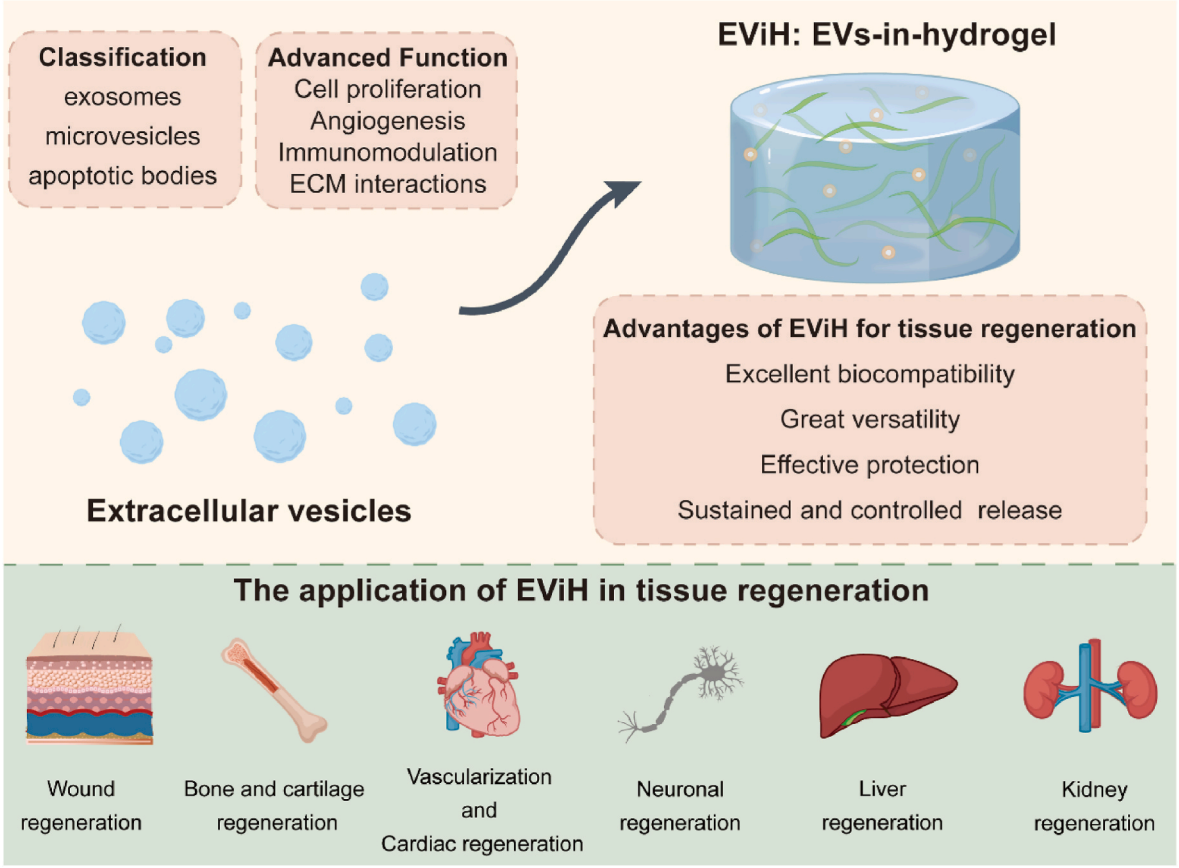

Fig. 2. The combination of EVs (exosomes and MVs) and hydrogels in regeneration application.

factors from cells and noted that precipitates obtained significantly shortened the clotting time of the plasma supernatant [29]. In 1967, Peter Wolf referred to these platelet-free plasma deposits as "platelet dust" [30]. Further investigations in 1971 by Crawford involved the examination of these deposits, which were termed "microparticles" and were found to contain lipids capable of carrying adenosine triphosphate (ATP) and contractile proteins [31]. Later that year, Aaronson identified vesicles produced by Ochromonas danica, which were released into the extracellular space and coined the term "extracellular vesicles" [32]. In 1974, Nunez discovered the presence of multivesicular bodies (MVBs) under an electron microscope [33]. The term "exosome" was first introduced in 1981 to describe EVs shed from the cell surface [34]. Subsequently, in 1983, Cliff Harding et al. captured images of internal vesicles released after the fusion of conductive MVB with the plasma membrane and theorized about an intracellular sorting and translocation pathway termed the "exosome secretion pathway" [35]. By 1987, RM Johnstone et al. unveiled that sheep reticulocytes released exosomes containing transferrin receptors during maturation, leading to the redefinition of "exosome" as vesicles released from the inner MVB lumen after fusion with the plasma membrane, a definition still widely acknowledged today [36]. However, in subsequent years, EVs were initially perceived as cellular waste products.

The late 1990s and early 2000s witnessed a significant expansion in EVs research with the advancement of techniques for isolating and characterizing these vesicles. This progress deepened the understanding of the diverse functions of EVs, specifically their involvement in immune responses, cell-to-cell communication, and disease pathogenesis. In 1996, Raposo identified vesicles originating from the endocytic compartments of B lymphocytes that played a crucial role in presenting major histocompatibility complex class II (MHC II) molecules and stimulating immune responses [37]. This discovery underscored the potential significance of EVs in intercellular communication and immune modulation. Presently, EVs are acknowledged as vital mediators of intercellular communication, transporting various cargoes like proteins, lipids, nucleic acids, and metabolites. Their roles in physiological processes and disease advancement have made them a prominent subject of study across diverse fields such as cancer biology, immunology, neuroscience, and regenerative medicine.

2.2. The biogenesis, isolation, and enrichment of EVs

EVs can be classified into two main types based on their biogenesis: "exosomes", which originate from endosomes, and "ectosomes" (microparticles/MVs), which are derived from the plasma membrane.

The release of exosomes occurs through the invagination of the endosomal membrane, leading to the formation of early endosomes through the inward folding of the plasma membrane. As the endosomal membrane buds inward, MVBs containing intracellular vesicles form, incorporating cytoplasmic inclusions and proteins. Exosomes are eventually secreted extracellularly when the MVBs fuse with the plasma membrane, enabling their functional roles. Various proteins, including CD82, CD81, CD63, CD9, ALIX, and TSG101, serve as markers for exosome identification. In contrast, ectosomes form through outward blebbing of the plasma membrane, influenced by the asymmetric distribution of phospholipids and calcium ion (Ca^{2+}) influx that disrupts bilayer asymmetry, initiating ectosome formation (Fig. 3).

EVs are abundantly found in various bodily fluids, such as blood [38], urine [39], cerebral spinal fluid [40], breast milk [41], ascites [42], saliva [43], bile, and semen [44]. The isolation and enrichment of EVs form the basis for further biological and medical investigations. Currently, conventional methods like ultracentrifugation and density gradient centrifugation are commonly utilized for initial separation [45]. Moreover, co-precipitation [46,47], size-exclusion chromatography [48], and field flow fractionation [49,50] are alternative separation techniques. In recent years, high-resolution density gradient fractionation and direct immunoaffinity capture have emerged as more precise methods for characterizing exosomes and other non-vesicular components, such as RNA, DNA, and proteins [12]. Numerous innovative approaches for enriching EVs have been developed to enhance isolation efficiency and specificity from diverse bodily fluids, including microfluidic filtering [51], immunoaffinity enrichment [52], and contact-free sorting [53]. In conclusion, exploring precise separation methods tailored to specific EVs types is crucial.

Despite real-time imaging techniques enabling the reliable capture of release pathways, attributing EVs to specific biogenesis pathways remains challenging. Therefore, terms like exosomes and MVs are advised against. It is essential to differentiate EVs based on rational and accurate characteristics, including (1) size, categorizing EVs into small EVs (sEVs) ranging from 100 to 200 nm, and medium/large EVs (m/l EVs) larger than 200 nm; (2) biochemical composition, like CD63+/CD81+-EVs or Annexin A5-stained EVs; and (3) descriptions of conditions or cell of origin (*e.g.*, podocyte EVs, hypoxic EVs, large oncosomes, or apoptotic bodies). In conclusion, researchers should select precise terms to describe and define EVs based on their characteristics for a more

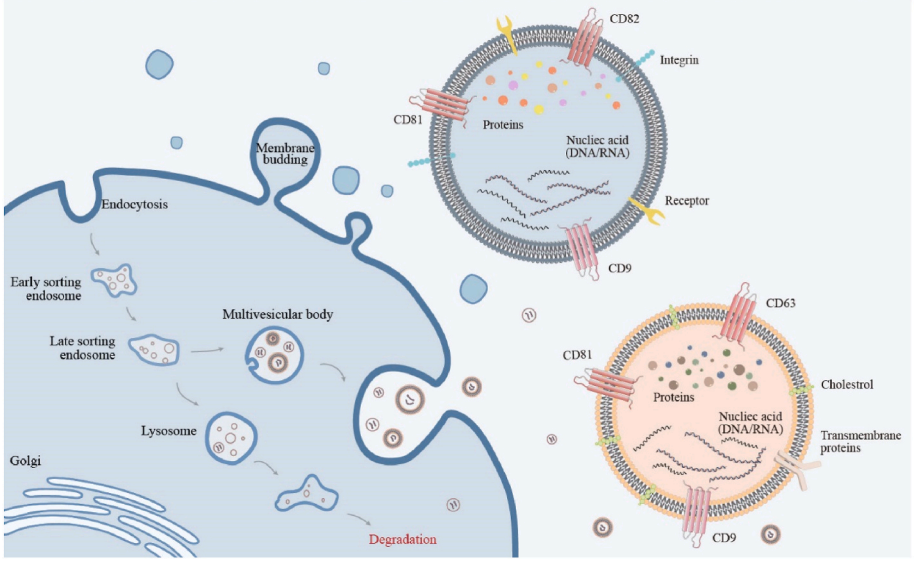

Fig. 3. The schematic diagram of the generation and structure of MVs and exosomes.

accurate understanding of the application outcomes. When specific EVs characteristics cannot be identified, using the term "extracellular particle" (EP) as a generalization, as recommended by the Minimal Information for Studies of Extracellular Vesicles 2018 (MISEV2018) publication [27], is suggested.

2.3. The therapeutic mechanisms of EVs in regenerative medicine

The size, cargo, and membrane composition of EVs are significantly heterogeneous, influenced by the cellular source, cellular state, and environmental factors, all of which impact the biological functions of EVs. EVs function as carriers for a variety of substances derived from parent cells [54], demonstrating diverse biological capabilities. These functions include regulating cell senescence, enhancing cell viability and modulating cell proliferation, promoting angiogenesis, immunomodulation, and interacting with the ECM. The significant role that EVs play in these processes underscores their potential as essential mediators in tissue regeneration and repair. This section provides a concise overview of the pivotal role EVs have in facilitating tissue regeneration.

2.3.1. Regulation of stem cell senescence

Cellular senescence is a state of permanent cell cycle arrest that occurs in diploid cells, limiting their proliferative lifespan. It involves the irreversible loss of growth and proliferative capacity triggered by various factors, leading to the secretion of signaling molecules related to senescence. While senescent cells play a role in tissue repair and organ growth regulation, their accumulation can disrupt the internal environment, causing organ dysfunction and contributing to diseases such as neurodegenerative and cardiovascular conditions.

The decline in stem cell numbers and function with age has been well-documented. Research has shown a notable decline in the proliferative ability of MSCs from a natural model of aging or the Ercc1−/− premature aging mouse model, accompanied by increased expression of senescence markers [55]. EVs have been identified as key regulators of stem cell senescence, transferring specific cargo molecules to modulate the senescence process. Embryonic stem cells (ESCs), being pluripotent, have the capacity for indefinite self-renewal and differentiation into various tissue types. EVs derived from ESCs (ESC-EVs) have been shown to rejuvenate aging MSCs by enhancing their regenerative potential through the transfer of specific factors [56]. Notable rejuvenation factors in ESC-EVs include transforming growth factor-β (TGF-β), Smad2, platelet-derived growth factor-BB (PDGF-BB), miR-291a-3p, miR-294, and miR-200a. *In vivo* studies have demonstrated the ability of ESC-EVs to ameliorate the senescent state of aged organs by transducing specific microRNAs into senescent cells and suppressing the AKT/mammalian target of the rapamycin (mTOR) pathway [57]. Additionally, through the modulation of signaling pathway-related targets, such as Src phosphorylation, ESC-EVs containing miR-146a can mitigate oxidative stress-induced endothelial cell senescence, restoring angiogenic and migratory capacities effectively [58].

In the context of idiopathic pulmonary fibrosis (IPF) treatment, human bronchial epithelial cell-derived EVs (HBEC-EVs) have exhibited therapeutic potential by inhibiting TGF-β-mediated myofibroblast differentiation and lung epithelial cell senescence induction more effectively than MSC-derived EVs (MSC-EVs). This therapeutic mechanism is attributed to the downregulation of WNT-associated proteins facilitated by miR-16, miR-26a, miR-26b, miR-141, miR-148a, and miR-200a enriched in HBEC-EVs [59].

2.3.2. Enhancement of cell viability and modulation of cell proliferation

Cell viability and proliferation are fundamental for tissue and organ repair and regeneration. EVs play a pivotal role in regulating cell proliferation by transporting growth factors, signaling molecules, and nucleic acids that can either stimulate or inhibit cell growth. Xiao et al. demonstrated a significant enhancement in the proliferation of human umbilical vein endothelial cells (HUVECs) through the targeted delivery of growth differentiation factor 15 (GDF 15) *via* EVs. GDF 15, a member of the TGF-β superfamily, is associated with various biological regulatory processes and energy homeostasis balance [60]. Moreover, EVs derived from M2-type macrophages have shown the capability to protect oligodendrocyte precursor cells (OPCs) from injury due to oxygen-glucose deprivation (OGD) and enhance OPC cell viability in a dose-dependent manner by carrying miR-23a-5p [61]. In a cardiac repair study, it was observed that hypoxia induced the expression of circRNA-Whsc1 in endothelial cells, which were then delivered to cardiomyocytes (CM) through EVs. This delivery mechanism activated the pTRIM59/pSTAT3/cyclin B2 pathway, ultimately promoting CM proliferation and facilitating heart regeneration [62].

2.3.3. Promotion of angiogenesis

Neovascularization, the process of forming new blood vessels, is crucial in tissue engineering as it enables the transportation of nutrients and oxygen while eliminating metabolic wastes. Recent studies have highlighted the potential of EVs to stimulate angiogenesis. Administering MSC-EVs to non-human primates with myocardial infarction (MI) resulted in enhanced angiogenesis and cardiac recovery, without raising the risk of arrhythmic complications, thus confirming the safety and effectiveness of EV-based therapy [63]. In models of bronchopulmonary dysplasia, treatment with EVs derived from human umbilical cord MSCs (hUCSCs) restored peripheral pulmonary blood vessel loss and reduced pulmonary vascular muscle thickening [64].

As research advanced, it was discovered that EVs' pro-neovascularization effects were mediated through activating signaling pathways and transporting relevant molecules. For instance, MSC-EVs promoted angiogenesis in brain microvascular endothelial cells by upregulating intercellular adhesion molecule (ICAM)-1 expression, contributing to recovery from Parkinson's disease [65].

Similarly, the administration of EVs from hypoxia-preconditioned microglia in rodent stroke models stimulated angiogenesis and reduced neuronal cell apoptosis by modulating the TGF-β/Smad2/3 signaling pathway [66]. The activation or inhibition of signaling pathways is likely facilitated by signaling molecules carried by EVs, such as microRNAs (miRNAs), messenger RNAs (mRNAs), proteins, and mitochondria. EVs derived from bone marrow mesenchymal stem cells (BMSC-EVs, BMSCs) inhibited the phosphatase and tensin homolog (PTEN) signaling pathway and activated the AKT signaling pathway, promoting angiogenesis and suppressing neuronal apoptosis to alleviate ischemic brain injury, with miR-29b-3p in BMSC-EVs playing a crucial role [67]. Furthermore, studies identified EMMPRIN [68] and Nidogen-1 [69] in BMSC-EVs as potentially active components that regulate endothelial cell migration and angiogenic capacity, respectively.

2.3.4. Immunomodulation

The significance of immunomodulation in tissue regeneration is paramount. On one hand, an exaggerated or improper immune response in tissue engineering involving implants can result in failure, with factors such as surgical trauma, graft material type, graft location, and the patient's physical condition playing crucial roles. On the other hand, the immune response serves as a key stimulant for regenerative processes like cell recruitment, proliferation, and angiogenesis, which are essential for the success of *in situ* tissue engineering. Subsequently, we delve into the involvement of EVs in immunomodulatory mechanisms, encompassing innate and adaptive immunomodulation.

The innate immune response denotes a swift reaction to pathogens or danger signals. During this course, immune cells are prompted to release innate immune factors that combat invading pathogens and impaired host cells. Nevertheless, immune dysregulation can lead to prolonged pathogenic infections, immune suppression, and uncontrolled inflammation. Uncontrolled inflammation can trigger adverse effects such as hindered wound healing, progressive alveolar bone loss, and potentially lethal sepsis. MSC-EVs were found to alleviate mitochondrial DNA

(mtDNA) damage and inflammation following acute kidney injury (AKI). Specifically, MSC-EVs were effective in restoring the stability of mitochondrial transcription factor A (TFAM) proteins and TFAM-mtDNA complexes, thereby rectifying mtDNA deletion and mitochondrial oxidative phosphorylation (OXPHOS) dysfunction in injured renal tubular cells. *In vivo* experiments showcased that MSC-EVs mitigated renal lesion formation, mitochondrial impairment, and inflammation in AKI mice. This study underscores the potential of MSC-EVs in controlling inflammation and managing associated conditions by modulating OXPHOS and ameliorating mitochondrial impairments. Exosomes derived from human gingival-derived mesenchymal stem cells (hGMSCs-Exos) have demonstrated effectiveness in alleviating lipopolysaccharide (LPS)-induced inflammation and promoting the proliferation of Periodontal Ligament Stem Cells (PDLSCs) in inflammatory settings [70]. These exosomes were also shown to inhibit the NF-κB pathway to enhance the suppression of the Wnt/β-catenin signaling pathway caused by inflammation [71].

Numerous molecules associated with adaptive immunomodulation, such as immune checkpoint molecules like cytotoxic T lymphocyte antigen 4 (CTLA-4) and programmed death ligand-1 (PDL-1), along with ectoenzymes CD39 and CD73, which generate immunosuppressive adenoids from ATP, have been identified on EVs surfaces [72]. For instance, Regulatory T (Treg) cells release EVs that contribute to immunosuppression by surface expression of CD73 and related miRNAs like miR155, Let7b, and Let7d. EVs from dendritic cells (DCs) have been effective in treating tumors in mice by eliciting $CD8^+$ T cell responses [73]. Apart from EVs produced by immune cells, MSC-EVs exhibit immunomodulatory functions by regulating the activities of various immune cells like T cells, B cells, Natural Killer (NK) cells, DCs, monocytes, and macrophages. The modulatory actions of MSC-EVs likely involve inducing T cell apoptosis through adenosine A2A receptor, transferring miR-155–5p to B cells to inhibit the PI3K/AKT pathway, suppressing NK cells *via* TGF-β-mediated signaling involving Smad2 and Smad3, upregulating miR-146 expression and downregulating FAS (CD95) expression to inhibit DCs, and inducing macrophage suppression *via* miR-182-mediated TLR4-NF-κB-PI3K-AKT pathway targeting [74]. Interestingly, a study isolated EVs from milk and found their immunomodulatory properties to be achieved by blocking the TLR4-NF-κB and NLRP3 pathways, thereby restoring a balance between Treg cells and inflammatory T helper 17 (Th17) cells [75].

2.3.5. Extracellular matrix interactions

The structural and molecular composition of the ECM influences cell recruitment, retention, differentiation, and the final local cellular phenotype. In tissue engineering strategies utilizing biodegradable scaffolds, the ECM plays a crucial role in providing load-bearing support to the scaffold and maintaining cell retention as the scaffold degrades. EVs can impact the ECM composition by directly interacting with the ECM or ECM-producing cells [76].

Adhesion molecules, including members of the immunoglobulin superfamily and integrins, are expressed in EVs. Through these adhesion molecules, EVs can interact not only with cells but also with various ECM components. For instance, integrin α4β1 facilitates the binding of reticulocyte-derived exosomes to fibronectin [77], while β1 and β2 integrins enable B-cell-derived exosomes to bind to collagen 1, fibronectin, and TNF-α-activated fibroblasts [78]. EVs adhere to the ECM, forming gradients or potential reservoirs, allowing them to be released to exert therapeutic effects in the presence of inflammation or ECM degradation.

ECM remodeling is intricately linked to developmental processes like cell differentiation, stemness, morphogenesis, cell migration, and apoptosis, and interacts with growth factors and cytokines [79]. Matrix metalloproteinases (MMPs), crucial proteins in ECM remodeling, and tissue inhibitors of matrix metalloproteinases (TIMPs) play key roles in the multi-directional differentiation of stem cells [80]. Knockdown of MMP-13 inhibits alkaline phosphatase (ALP) activity and mineralization during osteogenic differentiation in adipose-derived mesenchymal stem cells (ADSCs) [81], while MMP-14 deficiency disrupts collagen renewal capacity, leading to abnormal skeletal development in mice [82]. Microparticles derived from monocytes and T-cells promote the production of MMP-1, MMP-3, MMP-9, and MMP-13 in fibroblasts [83]. Notably, MMPs are present in EVs, as evidenced by enzymatically active MMP-14 in human fibrosarcoma and melanoma cell-derived exosomes, which activate pro-MMP-2, ultimately degrading collagen-1 and gelatin. Moreover, EVs stimulate MMP production in target cells; for instance, keratinocyte-like cells prompt dermal fibroblasts to express MMP-1 *via* EV-mediated transfer of several 14-3-3 isoforms [84].

In conclusion, EVs inherit various cell components and functionalities from their parent cells. Proper selection of the cell source can enhance specific stages of tissue repair using EVs. The combined use of EVs derived from diverse sources has the potential to amplify the effectiveness of tissue repair mechanisms.

3. EViH

The EViH system has emerged as a promising approach in tissue regeneration applications, utilizing the unique properties of EVs and hydrogels to enhance tissue repair and regeneration. The interactions and synergies between EVs and hydrogels are pivotal in the development and implementation of EViH systems. A comprehensive examination of these interactions is essential for the advancement of novel EViH systems and the promotion of innovative applications. This section presents a concise overview of the construction and benefits of the EViH system, with a particular focus on the influence of EVs and hydrogel interactions on these aspects.

3.1. The construction of EViH

The construction of EViH systems mainly involves encapsulating EVs within a hydrogel matrix. Hydrogels, by replicating the water-rich composition and intricate 3D network structure of the ECM, offer an effective platform for the encapsulation and delivery of EVs. This biomimetic environment enhances the stability and bioavailability of EVs, while also providing a versatile mechanism for controlled release and targeted delivery. By integrating various physical and chemical properties such as self-healing capabilities and conductivity, hydrogels can be tailored to mimic the dynamic characteristics of the native ECM, thereby elevating the therapeutic potential of EVs. Current methods for constructing EViH systems include pre-gel loading and post-gel loading, depending on the timing of EVs loading [85].

Pre-gel loading entails directly encapsulating EVs within the hydrogel precursor solution before gelation occurs. The precursor solutions used in this method typically consist of macromolecular monomer substances known for their biocompatibility. Adjusting the concentration of the monomer and/or the final cross-linker added enables manipulation of the mechanical properties and degradation rate of the hydrogel [86]. This method, widely employed and convenient, has led to the development of numerous EViH systems. It offers several advantages: high loading of EVs, crucial for improving therapeutic efficacy; optimal distribution of EVs within the hydrogel matrix, achieved, for instance, through a layer-by-layer self-assembly approach to establish EViH systems with an EVs concentration gradient or different EVs species. An innovative EViH system was designed to regulate initial wound healing precisely and inhibit scar formation in later stages by leveraging different degradation rates of a bilayer hydrogel for sequential EVs release with distinct functions [87].

While this method allows regulation of EVs release by manipulating their distribution within the EViH system, a notable drawback is the potential impact of the gelation process on the structure and function of EVs. Interactions between hydrogel molecules and EVs, whether through chemical or physical cross-linking, may compromise EVs components like surface proteins, lipids, and polysaccharides. Thus, careful

consideration of the cross-linking method is crucial to prevent adverse effects on EVs when employing pre-gel loading for constructing EViH systems. Furthermore, EViH systems developed using the pre-gel loading technique are well-suited for filling critical-sized defects with intricate geometries. Hydrogel polymerization can be initiated by the local microenvironment or external stimuli to enable *in situ* gel formation [88], while the precursor hydrogel solution can also be combined with pre-fabricated scaffold materials tailored based on the injury site and shape using methods like 3D bioprinting. For instance, combining a HA precursor solution with UCMC-Exos in 3D printed nano-hydroxyapatite/poly-ε-caprolactone (nHP) scaffolds proved effective for cranial bone defects, showcasing excellent bone regeneration results [89].

Post-gel loading involves encapsulating EVs into a pre-formed hydrogel matrix, wherein achieving an effective EVs payload is crucial for success. The encapsulation process typically involves the simple diffusion of EVs into the hydrogel [90]. However, this straightforward method presents challenges in achieving controlled release of EVs within the EViH system. A more intricate approach utilizing covalent interactions can be employed to develop EViH systems capable of controlled EVs release. For instance, Huang et al. used integrins present on the surface of MSC-EVs to bind to RGD peptide hydrogels with methacrylate-esterified alginate *via* photopolymerization [91]. The designed hydrogel successfully encapsulated and retained EVs for over 7 days while preserving their structural integrity and osteoinductive function. During the preparation of EViH through post-gel loading, the hydrogel may require immersion in an EVs-containing solution. Prolonged immersion could cause hydrogel swelling or alterations in mechanical properties, highlighting the importance of optimizing EVs loading time. In our opinion, the post-gel loading method is better suited for constructing patch-type EViH systems, offering more efficient loading of EVs compared to other methods.

Recently, novel techniques have emerged in the development of EViH systems, such as leveraging microfluidic technologies to precisely encapsulate EVs within hydrogels, leading to the creation of microscale EViH particles or capsules. Nie et al. prepared MSC-EVs and embedded them in dopamine methacrylamide (DMA)-modified gelatin methacryloyl (GelMA) solution, employing microfluidics to produce EVs microcarriers with bioadhesive properties, demonstrating promising efficacy in treating inflammatory bowel disease (IBD) [92]. This technology showcases the potential to achieve a slow-release effect and enhanced adhesion properties.

In summary, these methods offer diverse approaches for developing EViH systems with tailored properties, suitable for applications in regenerative medicine, drug delivery, and bioengineering. Each method presents unique advantages and limitations in EViH system development, allowing researchers to select the most suitable approach based on their specific requirements and the intended functionality of the EViH system.

3.2. The advantages of EViH

The advancement of EViH aims to synergistically integrate the features of hydrogels and EVs, thereby surpassing individual contributions and yielding an outcome that exceeds the mere combination of its components. It is determined that EViH offers advantages such as outstanding biocompatibility, versatility, efficient protection, and sustainable and controlled release.

3.2.1. Excellent biocompatibility

Biocompatibility refers to the ability of a material or system to operate effectively within a specific biological environment without causing harm to living organisms. Both EVs and hydrogels in the EViH system demonstrate remarkable biocompatibility. Numerous studies have consistently affirmed the biological safety of various hydrogels in both *in vitro* and *in vivo* settings, yielding positive experimental outcomes. Clinical trials utilizing hydrogel-based approaches have exhibited promising results in diverse fields, including oral-maxillofacial and orthopedic trauma surgery, advanced heart failure, and chronic kidney disease, underscoring the biological safety of hydrogels [93]. Moreover, certain hydrogel polymers, such as Pluronic F-127 (PF-127), have obtained approval from the Food and Drug Administration (FDA) [94].

EVs are natural nanovesicles released by cells through the paracrine pathway, transporting various functional components from the parent cell. They demonstrate low immunogenicity and high biocompatibility, devoid of the tumorigenic and senescence vulnerability linked to cellular therapies. Over 300 EVs-related clinical trials have been conducted to date, revealing satisfactory outcomes in diverse fields, including prostate cancer diagnosis, refractory focal epilepsy treatment, atopic dermatitis management, and even COVID-19 treatment [95]. Noteworthy is that 5 % of these clinical studies focus on engineered EVs as therapeutic agents. When utilizing EVs (both native and engineered) in clinical settings, adherence to the consensus guidelines published by ISEV and COST is crucial [96].

Understanding from the introduction in **Section 3.1**, selecting appropriate preparation methods can minimize excessive damage to EVs and hydrogels during the construction of the EViH system. Therefore, one can infer that the EViH system, comprising EVs and hydrogels with outstanding biocompatibility, also demonstrates good biocompatibility, supported by numerous recent *in vitro* and *in vivo* studies [89,97,98].

3.2.2. Great versatility

The versatility of EViH is attributed to the diverse range of hydrogels and EVs available. Hydrogels can be customized with various properties, and the selection of EVs from different sources or engineered EVs can be made to meet specific requirements.

Designing hydrogels to be responsive to stimuli is a crucial strategy in enhancing the versatility of EViH. Responsive hydrogels can be categorized based on the type of stimulus into internally responsive hydrogels (*e.g.*, enzyme, pH, glucose, and electric-responsive hydrogels) and externally responsive hydrogels (*e.g.*, light, electricity, magnetism, ultrasound, and temperature-responsive hydrogels) [99]. pH-responsive hydrogels contain polymers that hydrolyze at specific pH levels, thereby altering the pore size of the molecular network in different environments and facilitating the controlled release of internal contents. For example, pH-sensitive hydrogels have been tailored to manage inflammation by releasing drugs at the inflamed site in response to the acidic conditions generally present [100,101]. Biomolecule-sensitive hydrogels undergo conformational changes in response to specific biomolecular concentrations, enabling applications such as insulin release triggered by glucose levels through a glucose oxidase and pH-sensitive domain [23]. Temperature-responsive hydrogels change in structure and function based on temperature variations, with biomedical applications that involve transitioning between gel and sol-gel states to adapt to environmental conditions during treatment.

Apart from responsiveness to stimuli, other distinct hydrogel properties such as self-healing and conductivity contribute to their adaptability in complex pathological environments, thereby enhancing the versatility of EViH. Self-healing hydrogels, utilizing non-covalent or dynamic covalent interactions, exhibit fluid-like behavior under stress and rapidly recover their original properties upon stress release [102]. These hydrogels offer advantages for tissue regeneration, including precise drug delivery, cell protection, adaptability to defect sites, stable drug release, and suitability for bioprinting [103]. For instance, a catechol-conjugated chitosan (CHI-C) self-healing multifunctional hydrogel based on dynamic Schiff base bonding formed a gel within minutes after being injected into irregular bone defects and acted as a hemostatic and regenerative agent [104]. Conductive hydrogels, incorporating materials like graphene and carbon nanotubes, have shown potential in biomedicine for applications such as tissue engineering, drug delivery, and biosensing [105]. Researchers are exploring

multifunctional conductive hydrogels to effectively address complex biomedical challenges. A study detailed the development of an electroconductive hydrogel (HASPy) for peripheral nerve repair, which directly targeted IL-17 receptor A (IL-17RA) and facilitated Schwann cell myelination through the IL-17 signaling pathway [106]. *In vivo* experiments demonstrated that the HASPy hydrogel promotes functional recovery and remyelination after sciatic nerve-crush injury.

Furthermore, hydrogels possess unique physical and chemical properties that enhance their utility. They can be engineered to exhibit stimulus responsiveness and self-healing capabilities. Wang et al. addressed challenging diabetic wounds by developing a hydrogel named PC (PEGS-PBA-BA/CS-DA-LAG) that displayed self-healing properties, dual responsiveness to pH and glucose, and released metformin through Schiff base and phenylboronate ester dynamic bonding [107].

EVs provide a diverse source that boosts the versatility of EViH due to the varied biological functions of EVs derived from different cells. Engineering of EVs can be customized to meet specific disease-related requirements, as evidence suggests that engineered EVs offer superior loading efficiency, targeting specificity, and therapeutic effectiveness. The engineering strategies for EVs can be broadly categorized into two main approaches: (i) indirect manipulation of donor cells; and (ii) direct engineering of EVs.

Common methods for modifying donor cells include co-incubation and gene transduction to introduce therapeutic cargo. Co-incubation involves introducing compounds, particularly small molecules, into donor cells and subsequently into EVs. For example, paclitaxel (PTX) can be loaded into MSC-EVs by co-culturing PTX with MSCs at 37 °C for 1 h [108]. Alternatively, gene drugs can be transfected into donor cells, and EVs containing the gene drugs can be isolated and purified thereafter. This natural biosynthetic process facilitates the packaging of exogenous nucleic acids such as DNA plasmid vectors and noncoding RNAs into EVs. While this method is simple and minimally impacts EV structure and yield, the efficiency of drug loading is dependent on the properties of the drug and the duration of incubation.

Direct methods for drug loading into EVs interiors include passive mixing, electroporation, and mechanical techniques. Passive mixing involves encapsulating cargo within EVs through direct blending, with encapsulation efficiency being boosted by adjusting experimental conditions. Saponin permeabilization is commonly applied to enhance efficiency [109]. This method is particularly suitable for hydrophobic drugs as they can easily interact with and pass through the hydrophobic EVs membranes. Electroporation, another popular technique, involves mixing EVs with the target cargo in a conductive solution, creating transporter holes on the EVs membrane's surface under the influence of an electric field to facilitate cargo translocation. However, electroporation can lead to the aggregation of EVs or cargoes, affecting the EVs' structure and therapeutic efficacy [110]. Mechanical engineering techniques for EVs include freeze and thaw cycles, sonication, and extrusion. Freeze-thaw cycles entail co-incubating cargo with EVs at room temperature, followed by rapid freezing and thawing cycles [111]. Sonication involves subjecting EVs and drugs to sonic disintegration, while extrusion requires forcing EVs and cargoes through polycarbonate membranes [112]. Mechanical methods offer a higher probability of drug encapsulation compared to passive mixing and electroporation but can compromise EVs' structural integrity if improperly applied [111, 113].

Furthermore, beyond loading target cargoes, modifying the EVs membrane surface is commonly practiced to enhance EVs functionality. Click chemistry, specifically copper-catalyzed azide-alkyne cycloaddition reaction, is utilized to attach specific molecules to EVs membranes. For example, Jia et al. successfully targeted gliomas by attaching the neuropilin-1-targeted peptide (RGERPPR, RGE) to EVs using click chemistry [114]. Similarly, Tian et al. conjugated the c(RGDyK) peptide to EVs surfaces for efficient delivery of curcumin targeting the ischemic brain [115].

In conclusion, the remarkable versatility of EViH is heightened by the functionality and diversity of hydrogels and EVs. Customizing hydrogels with specific properties allows them to serve efficiently as carriers, while harnessing the diverse functions of EVs can greatly boost tissue repair mechanisms.

3.2.3. Effective protection

The optimal protection of EViH primarily involves shielding the EVs from rapid clearance by the circulatory system following administration *via* various routes including intravenous, intraperitoneal, or subcutaneous, and preventing their accumulation in off-target organs such as the liver, spleen, kidneys, and lungs [116]. Wiklander et al. revealed that a considerable portion of exosomes administered intravenously was promptly engulfed by macrophages in the reticuloendothelial system [22]. The protective effect of hydrogels lies in shielding EVs from immune reactions and rapid clearance, mainly through the sustained release of EVs, ensuring their continued and stable functionality at the injury site. The retaining capacity of hydrogels for EVs has been confirmed across different tissues and organs, often using fluorescent dyes such as Dil, FITC, and PKH 26 to monitor *in vivo* retention. While free exosomes were predominantly cleared by day 14 post-administration, hydrogel-loaded exosomes were still detectable at brain injury sites up to day 28, emphasizing the sustained release efficiency [116].

Moreover, the hydrogel facilitated the prolonged preservation of EVs without significantly compromising their bioactivity. Analytical techniques like transmission electron microscopy (TEM), nanoparticle tracking analysis (NTA), and Western blot (WB) are valuable for assessing the viability of EVs released from hydrogels, showing maintained structural integrity, consistent size, and unchanged surface protein expression compared to directly isolated EVs [117]. The protective role of hydrogels in preserving EVs activity can be attributed to the maintenance of molecular structures or the incorporation of components such as peptides, biofactors, or drugs in the hydrogel matrix, serving as a physical barrier against environmental stresses like temperature fluctuations, pH changes, and mechanical pressures, thus enhancing EVs' integrity and stability.

In conclusion, employing diverse preparation methods effectively preserves the structural and functional integrity of EVs when integrating them into hydrogels, with the favorable physicochemical characteristics of hydrogels supporting long-term EVs activity.

3.2.4. Sustainable and controlled release

The interactions between EVs and hydrogels in the EViH system are governed by their respective physicochemical properties, significantly impacting the release dynamics of EVs within the hydrogel matrix.

Weak interactions between EVs and hydrogels in the EViH system may lead to the release of EVs from hydrogels primarily depending on EVs diffusion. When the polymer networks' mesh size exceeds that of the EVs, a high initial EVs concentration results in a burst release followed by gradual release, influenced by the hydrogel nature and degradation rate, lasting for hours to days. If the size of EVs is comparable to the mesh size, frictional resistance may impede diffusion. Variances in mesh sizes, where smaller pores increase path length, facilitate sustained release. When the mesh size is smaller than the EVs, EVs are trapped inside the gel. Therefore, hydrogels with high porosity and small pore sizes achieved through increased crosslink density and polymer concentration are effective in prolonging EVs retention [118].

Additionally, factors like hydrogel degradation and mechanical deformation affecting the mesh size of polymer networks also contribute to sustainable and controlled release. Degradation enlarges the mesh size, facilitating EVs diffusion, potentially induced by external stimuli like pH and ultraviolet (UV) light. For example, a pH-sensitive hydrogel containing hyaluronic acid (HA), PF-127, and poly-ε-L-lysine exhibited increased degradation in an acidic environment, leading to higher EVs release at pH 5.5 compared to pH 7.5 on day 12 [101]. Adjusting degradation rates by modifying hydrogel composition and cross-linking

conditions enables precise control of EVs release in specific environments. Mechanical stress-induced deformation causes convection, resulting in transient EVs release. Hydrogels with magnetic nanoparticles can deform rapidly under a magnetic field, promoting the swift release of loaded particles [119]. Furthermore, ultrasound can transiently disrupt the hydrogel network to enhance content release, improving drug penetration and absorption in tissues [120–123]. Nonetheless, external forces may irreversibly damage hydrogels, necessitating consideration of self-healing hydrogels as carriers.

It is worth mentioning that hydrogel swelling can significantly influence the mesh size of polymer networks. Research has shown that alginate hydrogels exhibit sensitivity to pH variations, contracting in acidic environments and expanding in neutral conditions, consequently influencing nanoparticle release. Although there is a lack of direct evidence on regulating EVs release through hydrogel swelling modulation, this avenue holds promise for further investigation.

In the EViH system, characterized by a strong interaction between EVs and hydrogels, achieving sustainable and controlled release is facilitated. EVs, possessing phospholipid bilayer membranes, transport various proteins, including transmembrane and surface proteins, from donor cells. The polar groups present in molecular polymers, such as carbonyl or hydroxyl groups, can interact with phosphate groups on phospholipids, as well as with amide, amine, hydroxyl, and carboxyl groups of proteins, through hydrogen bonding or van der Waals forces, thereby affecting the retention rate of EVs [124]. Ellagic acid (EA) has been employed as a cross-linking agent for the photocrosslinking of gelatin methacrylate (GM) and polypyrrole (PPy) to synthesize GMP hydrogels. The polyphenolic groups in TA effectively retain EVs within the hydrogel by establishing reversible hydrogen bonds with phosphate groups on the EV surface, leading to sustained *in vitro* release for over 14 days.

Moreover, charge-mediated interactions serve as an efficient method for retaining EVs within hydrogels. EVs acquire a negative charge due to the presence of anionic phosphatidylserine (PS) and glycocalyx residues on their surfaces. Hence, positively charged polymers have been investigated to improve EVs retention. For example, cationically charged chitosan (CS) hydrogels can prolong the *in vitro* release of EVs for more than 6 days [125]. Furthermore, polysaccharide-based pH-responsive fluorinated ethylene propylene (FEP) hydrogel-coated EVs demonstrate slow *in vitro* release under acidic conditions for up to 21 days [126].

EVs express adhesion molecules like integrins on their surfaces, which can bind to fibronectin and collagen. Li et al. enhanced EVs retention effectiveness by modifying the surface of an HA hydrogel with a laminin-derived peptide that interacts with integrins on EVs surfaces, resulting in sustained EVs release for 11 days *in vitro* [127]. Additionally, the establishment of chemical covalent bonds between EVs and the hydrogel molecular network immobilizes the EVs entirely. The release of EVs occurs only upon the rupture of these covalent bonds, making the control of covalent bond breaking an effective strategy for managing the release rate and timing of EVs.

In conclusion, the release behaviors of EViH can be effectively regulated by adjusting the specific characteristics of both EVs and hydrogels, while also carefully considering relevant pathophysiological processes.

4. Wound regeneration

4.1. Physiopathologic processes of wound healing

Wound healing is the complex process by which the body activates repair mechanisms to regenerate injured skin and tissues after an injury. This process consists of four key phases: hemostasis, inflammation, angiogenesis, epithelialization, and remodeling, illustrated chronologically in Fig. 4. It is crucial to understand that these phases are interconnected rather than entirely separate [128]. The following discussion provides a concise overview and detailed explanation of this intricate process.

The initial stage in the wound healing repair process involves vasoconstriction and platelet activation, followed by the formation of a fibrin

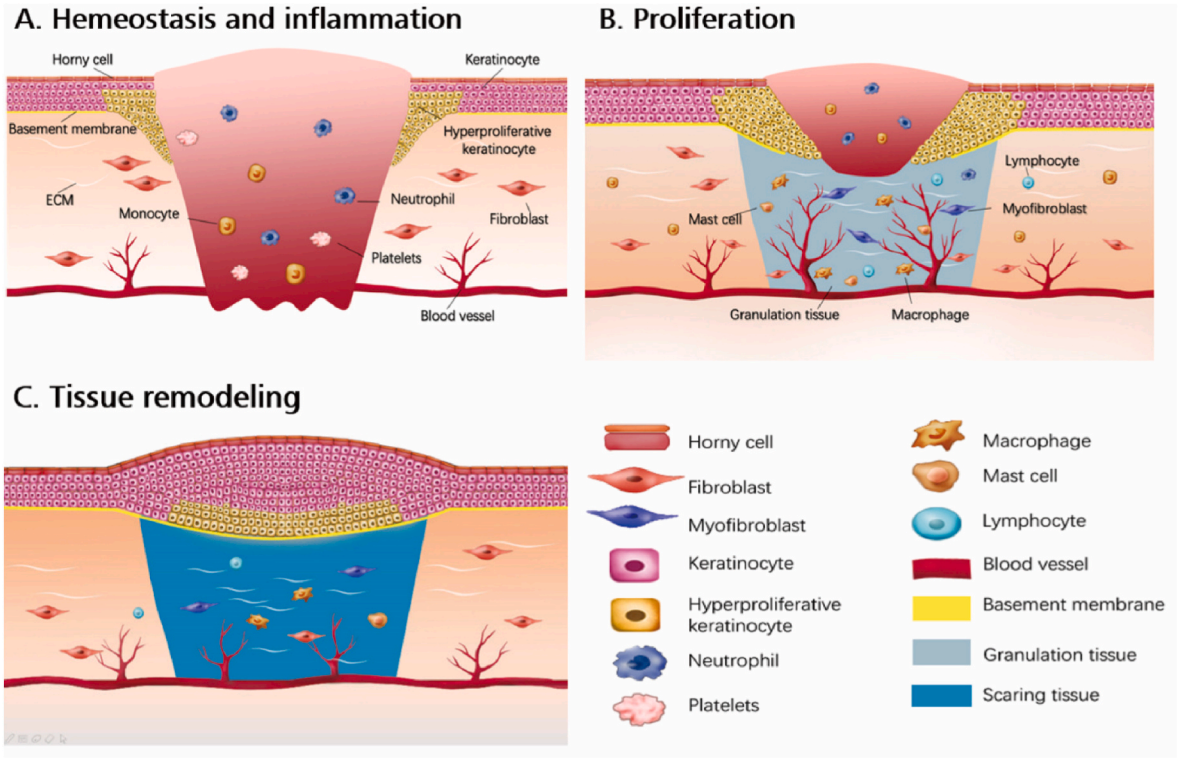

Fig. 4. The schematic diagram of four phases during wound healing [129]. © *2018 Published by Elsevier B.V.*

clot. Besides its hemostatic function, the fibrin clot acts as a scaffold for the subsequent recruitment of inflammatory cells. Neutrophils, the primary defense against microbial invasion, are the first immune cells to be recruited to the site of injury [130]. Subsequently, monocytes are recruited and transformed into tissue-activated macrophages within 48–96 h post-injury [131]. Furthermore, the adaptive immune system, including Langerhans cells, dermal DCs, and T cells, becomes activated. After the conclusion of the inflammatory phase, the angiogenic phase begins: (1) endothelial cells proliferate, migrate, and branch to form new blood vessels; (2) activated pericytes within the basement membrane provide structural support to the endothelial cells [132]; (3) circulating progenitor cells derived from the bone marrow play a crucial role in promoting the formation of new blood vessels during wound healing [133,134]. Following neovascularization, fibroblasts proliferate and migrate towards the fibrin clot, leading to the formation of granulation tissue. Fibroblasts differentiate into myofibroblasts to facilitate wound contraction [135]. In addition to the healing processes described above, the process of epithelial remodeling is crucial for skin wound healing, involving the proliferation of unipotent epidermal stem cells from the basement membrane and the de-differentiation of terminally differentiated epidermal cells [136]. Epidermal remodeling also includes the reconstruction of skin adnexa, such as sebaceous glands, sweat glands, and hair follicle-associated unipotent stem cells.

The complexity of wound healing presents challenges for clinicians and researchers, particularly in cases of large skin wounds, burns, chronic diabetic wounds, and the formation of abnormal scar tissue [137,138]. To achieve optimal wound healing, several prerequisites must be met: (1) prompt hemostasis; (2) controlled inflammation; (3) migration, proliferation, and differentiation of MSCs to the injured area; (4) sufficient neovascularization; (5) rapid epithelial re-formation; and

Table 1
The application of EViH for wound healing.

Disease	EVs species	Composite hydrogel type	Hydrogel loading	Crosslinking mode	Release Kinetics	Biological effects	References
Full-thickness skin wound	Exosomes of hUCSCs	P407/CMCS	Not mentioned	Thermo-crosslinking	85 % in 72 h	Reduces the inflammatory response and activates signaling pathways associated with collagen deposition, angiogenesis, and cell migration	[139]
	Exosomes of hEnSCs	Chitosan/Glycerol	Pre-gel loading	Electrostatic interaction and hydrogen-bonding interactions	Not mentioned	Promotes wound healing *via* improving angiogenesis and granulation formation	[140]
	Exosomes of hADSCs	PF-127	Pre-gel loading	Thermo-crosslinking	Over 96 h *in vivo*	Facilitates collagen synthesis and upregulates the expression of skin barrier proteins	[141]
	Exosomes of ADSCs	Alg	Pre-gel loading	Ionic crosslinking	Approximately 90 % in 168 h	Improves the migration ability of HUVECs and reduces healing time and scar formation	[142]
	Exosomes of HUVECs	GelMA	Pre-gel loading	Photo-crosslinking	Approximately 40 % in 7 days	Promotes angiogenesis and repairs cutaneous defects	[143]
	Exosomes of M2-Mϕs	Tren functionalized and SG functionalized 8-arm PEG	Pre-gel loading	Crosslinking of amine and ester groups	100 % in 11 days	Accelerates the quality and speed of wound healing *via* inducing reprogramming of M1-Mϕs toward M2-Mϕs	[144]
	B-sEVs; B-miR-29b-sEVs	BSSPD	Pre-gel loading	Photo-crosslinking	80 % in 9 days	Sequential release of B-sEVs (promote angiogenesis and collagen deposition) and B-miR-29b-sEVs (inhibit capillary proliferation and collagen deposition) to promote scar-free healing	[87]
Diabetic Wound	Exosomes of PMSCs	MC-CS	Pre-gel loading	Schiff base reaction	Over 168 h	Promotes diabetic wound healing through facilitating angiogenesis and regular and dense distribution of collagen	[145]
	Exosomes of hUCSCs	PF-127	Pre-gel loading	Thermo-crosslinking	Not mentioned	Promotes diabetic wound healing *via* accelerating angiogenesis	[146]
	Exosomes of PRP	Chitosan/Silk	Post-gel loading	Schiff base reaction	Not mentioned	Promotes diabetic wound healing by accelerating wound contraction, re-epithelialization, collagen deposition, and skin angiogenesis	[147]
	EVs of hUCSCs	rhCol III	Not mentioned	Crosslinking of amine and ester groups	80 % in 10 days	Promotes diabetic wound healing by regulating inflammation and macrophage polarization	[148]
	sEVs of UCSCs	Catechol-functionalized SIS/Fusion peptides	Post-gel loading	Amidation reaction	Over 7 days	Promotes diabetic wound healing and stimulates cell proliferation, migration, adhesion, and angiogenesis	[149]
	Exosomes of AMSCs	FHE	Post-gel loading	Schiff base reaction	Over 21 days	Promotes diabetic wound healing by modulating collagen deposition, epithelial regeneration, and angiogenesis	[101]
	Exosomes of BMSCs	CEC-DCMC	Pre-gel loading	Schiff base reaction	Not mentioned	Promotes diabetic wound healing and reduces the inflammatory response *via* facilitating macrophage conversion from M1 to M2	[100]
	Exosomes of M2-Mϕs	HA@MnO_2	Post-gel loading	Schiff base reaction	Over 21 days	Promotes diabetic wound healing by ameliorating oxidative stress and hypoxia	[150]

Abbreviations: EVs: extracellular vesicles; hUCSCs: human umbilical cord mesenchymal stem cells; P407: poloxamer 407; CMCS: carboxymethyl chitosan; hEnSCs: human endometrial stem cells; hADSCs: human adipose-derived mesenchymal stem cells; PF-127: pluronic F-127; HUVECs: human umbilical vein endothelial cells; Alg: alginate; GelMA: gelatin methacryloyl; M2-Mϕs: anti-inflammatory macrophages; M1-Mϕs: proinflammatory macrophages; Tren: Tris(2-aminoethyl)amine; SG: N-succinimidyl glutarate; PEG: poly(ethylene glycol); sEVs: small extracellular vesicles; BMSCs: bone marrow derived mesenchymal stem cells; B-miR-29b-sEVs: miR-29b-3p-enriched bone marrow derived mesenchymal stem cells; BSSPD: bilayered thiolated alginate/PEG diacrylate; PMSCs: placental mesenchymal stem cells; MC-CS: methylcellulose-chitosan hydrogel; PRP: platelet-rich plasma; rhCol III: recombinant human collagen III protein; SIS: small intestinal submucosa; FHE: oxidative hyaluronic acid (OHA) and poly-ε-L-lysine (EPL)/PF127; HA: hyaluronic acid.

(6) consistent, organized collagen production and arrangement. The EViH system shows significant potential for enhancing wound regeneration. We provide an overview of the most recent research on EViH-based interventions for wound healing, outlined in Table 1.

4.2. EViH for full-thickness skin wound regeneration

The skin, being the largest organ of the body, plays a critical role as a defense barrier, protecting internal tissues and organs from various types of physical, chemical, and biological damage. It consists of three main layers: the epidermis, which defends against external stimuli; the dermis, responsible for nourishment and immune responses; and the subcutaneous tissue, acting as an energy reservoir. When the skin is damaged, cells from different layers collaborate to initiate the healing process. However, the management of full-thickness skin wounds faces challenges such as non-healing, delayed healing, and the formation of abnormal scars like hypertrophic scars and keloids.

By targeting the physiological characteristics and enhancing the biological process of full-thickness skin wounds, strategies utilizing EViH show promise in addressing clinical challenges. Various hydrogels have been used as carriers to encapsulate MSC-EVs for promoting full-thickness skin wound regeneration. For instance, PF-127, an FDA-approved hydrogel for human use, has been employed to create a hydrogel for delivering exosomes derived from hADSCs (PF-127/hADSCs-Exos) in mouse models [141]. Notably, hADSCs-Exos labeled with red fluorescent PKH 126 accumulated at the wound site after 48 h and remained detectable even after 96 h post-administration of PF-127/hADSCs-Exos, while free hADSCs-Exos did not show a significant fluorescent signal within 48 h. This finding demonstrates that the hydrogel significantly prolongs the efficacy of the EVs. While PF-127 offers practical advantages as a wound-repair hydrogel scaffold, including injectability, biocompatibility, and thermo-sensitivity, it lacks inherent biological activity. Therefore, various biomacromolecules have been used to develop bioactive hydrogels capable of loading MSC-Exos.

Alginate, a naturally occurring acidic polysaccharide, is widely utilized in biomedical applications due to its excellent biocompatibility, low toxicity, and the ability to cross-link in the presence of divalent cations like Ca^{2+} to form hydrogels under mild conditions [151]. Moreover, alginate displays a range of biological activities, such as antioxidant, anti-inflammatory, and antibacterial properties. Furthermore, it can enhance cell proliferation and differentiation. The incorporation of ADSCs-Exos into alginate-based hydrogels (Alg-EXO) has emerged as a promising approach in skin tissue engineering [142]. The degradation of alginate hydrogel enables the sustained release of small bioactive molecules such as secreted exosome derivatives and growth factors. In rat models of full-thickness skin wounds, combining exosomes with alginate-based hydrogel significantly expedited the healing process, enhanced collagen synthesis and blood vessel formation, and inhibited scar formation, outperforming the effects of alginate-based hydrogel alone or traditional sterile gauze. Similarly, chitosan shows potential in regenerative medicine due to its biodegradability, low immunogenicity, antimicrobial activity, and cell-attracting properties [152]. The high presence of reactive amino and hydroxyl groups along the chitosan-based material backbone allows for easy modification to facilitate drug loading and achieve desired properties. Nooshabadi et al. successfully developed a hydrogel by utilizing the electrostatic interaction between chitosan and glycerol to effectively load exosomes derived from human endometrial stem cells (hEnSCs) [140]. Remarkably, the chitosan hydrogel, along with exosomes, significantly enhanced skin tissue regeneration and wound healing synergistically. In *in vivo* experiments, the chitosan-glycerol-exosome hydrogel notably promoted angiogenesis and granulation formation compared to other experimental groups, demonstrating superior wound-healing efficacy. However, the specific molecular mechanisms underlying the therapeutic role of exosomes and the potential synergistic mechanisms between exosomes and hydrogel remain unexplored, hindering their future clinical translation. The utilization of hydrogels formed through electrostatic interactions between polymers is an effective method for loading exosomes, as this gentle interaction minimizes potential exosome damage. Yet, it is essential to consider that the tissue microenvironment can influence the electrostatic interaction, leading to rapid hydrogel degradation and uncontrolled exosomes release. Therefore, strategies need development to enable spatiotemporal exosomes release for maximizing their regulatory functions.

While serving as a carrier for the sustained release of EVs, the hydrogel's stimulus responsiveness to the pathological environment is particularly intriguing for achieving controlled EVs release. Li et al. employed a dual-sensitive hydrogel, responsive to temperature and pH, to encapsulate exosomes extracted from hUCSCs' supernatant culture aiming at prolonged and controlled exosomes release in wound healing [139]. Comprising poloxamer 407 (P407) and a chitosan derivative, carboxymethyl chitosan (CMCS), with genipin (GP) as a natural and non-toxic cross-linking agent, this dual-sensitive hydrogel transitions from liquid to solid state at 37 °C, rendering it suitable for *in vivo* usage. Furthermore, the pH-responsive characteristic of the hydrogel exploits the pH contrast between healthy skin (pH 4–6) and wounded skin (pH 7–9) [153]. This dual-sensitive material facilitates intelligent exosome release by continuously monitoring wound status influenced by body temperature and local pH, thereby reducing infection risks and speeding up the healing process.

In addition to MSC-Exos, hydrogels have also been combined with other types of EVs for the regeneration of full-thickness skin wounds. Angiogenesis is a crucial step in the cutaneous wound healing process [154,155], where endothelial cells play a significant role in the formation of microvascular networks to initiate angiogenesis [156,157]. Thus, exosomes derived from endothelial cells have the potential to enhance skin wound repair. Zhao et al. utilized a gelatin methacryloyl (GelMA) hydrogel as a wound dressing to integrate exosomes derived from HUVECs (HUVEC-Exos) [143]. This system notably expedited re-epithelialization, improved collagen maturity, and facilitated angiogenesis, ultimately enhancing the efficacy of cutaneous wound healing. Furthermore,HUVEC-Exos promoted the proliferation and migration of both fibroblasts and keratinocytes, while the GelMA hydrogel enhanced cell adhesion using cell-binding motifs like arginyl-glycyl-aspartic acid (RGD). The GelMA hydrogel also integrated MMP degradable motifs, allowing enzyme-responsive degradation and sustained exosome release. It is essential to highlight that GelMA hydrogels with photocrosslinking capability offer convenience in application, but careful consideration is needed as residual photocrosslinking agents could potentially pose toxicity risks. Moreover, UV irradiation may impact exosome activity; therefore, radiation conditions must be thoughtfully selected.

In the wound healing process, macrophages play a pivotal role as effector cells of the innate immune system. Macrophages are categorized into the M1 phenotype, which has pro-inflammatory effects, and the M2 phenotype, which has anti-inflammatory effects [158]. The balance between M1 and M2 phenotypes is critical in the transition from inflammation to proliferation during skin regeneration, directly influencing the quality of wound healing. Studies have demonstrated that M2 macrophage-derived exosomes (M2-Exos) facilitate the shift from inflammation to the proliferative phase of wound healing by converting the M1 phenotype to the M2 phenotype [159]. This leads to the restored capability of reprogrammed M2 macrophages to produce MMP and vascular endothelial growth factors (VEGF). To pinpoint the key molecules involved in exosome-mediated macrophage reprogramming, researchers conducted analyses using proteomics and genomic sequencing techniques [144]. The proteomic analysis uncovered a noteworthy increase in arginase-1 (ARG1, Q61176) and the macrophage mannose receptor 1, also known as CD206 (Q61830, MRC1), in M2-Exo samples. Meanwhile, RNA sequencing revealed the overexpression of 74 miRNAs in M1-Exos and 66 miRNAs in M2-Exos. These findings suggest that exosome-mediated macrophage reprogramming is a result of the

synergistic effects of various components, including proteins, genes, and potentially lipids.

The primary objective of wound healing is to fully restore the skin to its original condition; however, this process often results in the development of scars [160]. Initially, highly active fibroblasts play a crucial role in promoting wound closure during the initial phases of wound healing [161]. Nevertheless, excessive production of collagen and ECM by these highly active fibroblasts can lead to the formation of undesirable scars, such as hypertrophic scars (HS) [162]. HS is associated with various complications including pruritus, compression, dysfunction, and local deformities [163,164]. Surgical excision is currently a popular treatment for scar removal [164,165], which necessitates the patient to undergo the entire wound healing process once again. To prevent unexpected scar formation and promote controlled wound repair, it is crucial to regulate the wound repair process in distinct stages. For example, fibroblasts should be activated during the initial phase of wound healing to facilitate wound closure; however, their activation should be carefully managed in the later stages of wound repair to prevent excessive collagen and ECM deposition. Achieving a controlled and staged approach requires the use of specific drugs at different points throughout the wound healing process. In addressing this need, Shen et al. developed a bilayer thiolated alginate/poly(ethylene glycol) (PEG) diacrylate (BSSPD) hydrogel (Fig. 5a) capable of sequentially releasing small extracellular vesicles (SR-sEVs) [87]. The ratio of thiolated alginate to PEG diacrylate was adjusted to create varying degrees of hydrophilicity, swelling, and degradation rates in the upper and lower hydrogel networks. The release of sEVs from the hydrogel occurs *via* physical diffusion. Notably, the sEVs release rate from the upper layer of the BSSPD hydrogel, comprised of the SA-SH-3/PEG-DA-700 hydrogel with a lower swelling rate (retaining 80 % of sEVs by day 9), was slower compared to the release rate from the lower layer of the BSSPD hydrogel, which consisted of the SA-SH-3/PEG-DA-250 hydrogel (releasing 80 % of sEVs by day 7). Degradation experiments indicated a complete degradation of the lower hydrogel by day 7, while the upper hydrogel underwent a slower degradation initially, accelerated after day 7, and reached 10 % degradation by day 14. The distinctive characteristics of the upper and lower hydrogel layers permit a gradual release of sEVs for optimized wound healing. The lower layer of the hydrogel facilitates the early-stage release of sEVs derived from BMSCs (B-sEVs). These B-sEVs promote angiogenesis and collagen deposition by enhancing the proliferation and migration of fibroblasts and endothelial cells involved in the inflammatory and proliferative phases of wound healing. Subsequently, in the later stages of wound healing, the upper layer of the hydrogel releases sEVs secreted by BMSCs enriched with miR-29b-3p (B-miR-29b-sEVs). These sEVs function to inhibit excessive capillary proliferation and collagen deposition by suppressing multiple signaling pathways, including PI3K/Akt, extracellular signal-regulated kinase (Erk) 1/2, and Smad3/TGF-β. As depicted in Fig. 5b, the groups treated with B-sEVs@BSSPD and SR-sEVs@BSSPD displayed significantly accelerated wound healing compared to the control groups. Furthermore, there was no significant difference between the B-sEVs@BSSPD and SR-sEVs@BSSPD groups on days 7 and 14, suggesting that the anti-angiogenic and anti-fibrotic effects of B-miR-29b-sEVs were not evident in the SR-sEVs@BSSPD group. The thickness of scars was quantified using Doppler ultrasound, revealing that the SR-sEVs@BSSPD group exhibited the lowest scar proliferation index (SEI), indicating a reduction in hypertrophic scar formation (Fig. 5c). In summary, the SR-sEVs@BSSPD group effectively mitigates hypertrophic scar formation without compromising wound closure.

Bilayer hydrogels or multilayer hydrogels have recently garnered significant attention due to their capacity for the sequential release of drugs tailored to pathophysiological characteristics, thereby enhancing disease treatment. It is crucial to emphasize that the functionalization of EVs can endow them with new functions, expanding their spectrum of applications. For instance, in the aforementioned illustration, BMSC-EVs can be engineered to carry exogenous genes for the modulation of signaling pathways, a functionality not intrinsic to natural BMSC-EVs. Nevertheless, ensuring the preservation of EVs activity during the functionalization process necessitates further exploration, as well as more in-depth investigation into the interplay between exogenous and endogenous components.

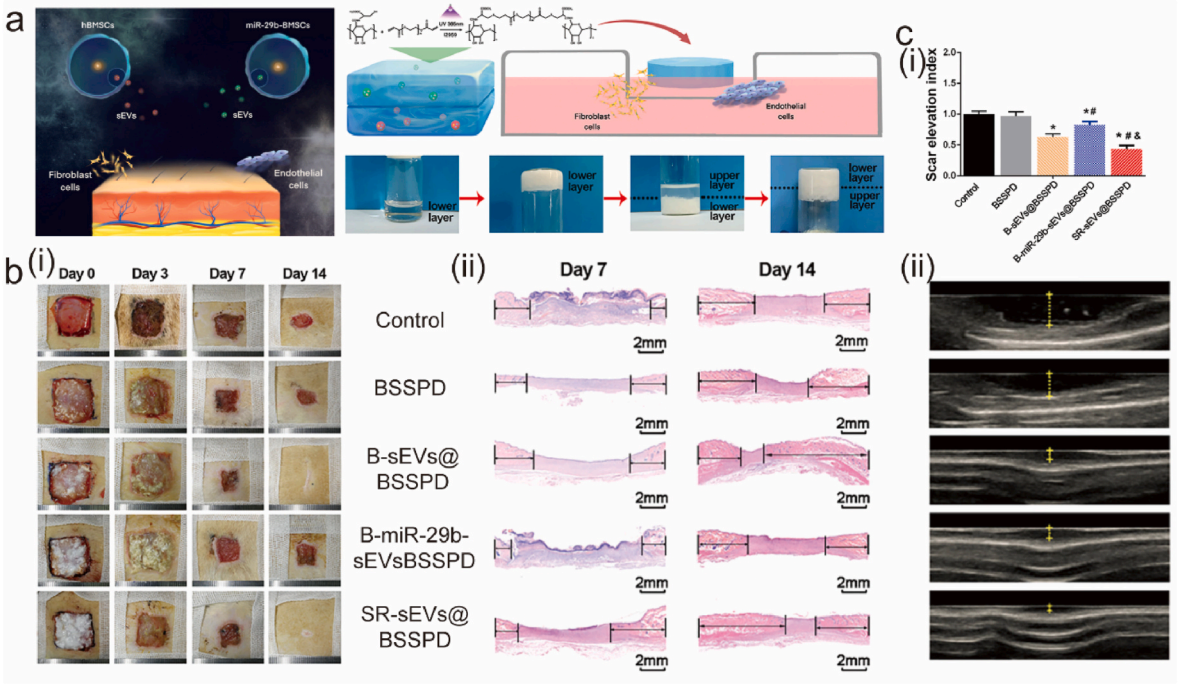

Fig. 5. (a) Patterns of the sequential release of B-sEVs and B-miR-29b-sEVs from the lower and upper layers of BSSPD. (b) Wound healing in a rat model. (i) Images in the wound closure areas. (ii) Images of hematoxylin-eosin staining (H&E) staining on days 7 and 14. (c) The results of the rabbit ear scar model. (i) Ultrasonography of wounds; (ii) Quantitative analysis of SEI. These figures have been reproduced from Ref. [87] with permission from *Copyright © 2021, American Chemical Society*.

4.3. EViH for diabetic wound healing

Diabetes mellitus (DM), a widespread chronic metabolic disease globally, is characterized primarily by a deficiency or reduced efficacy of endogenous insulin [166]. One of its significant manifestations is the abnormal elevation of blood glucose levels, leading to severe complications such as diabetic foot ulcers (DFUs) [167]. The treatment of chronic diabetic wounds, including DFUs, proves to be a substantial clinical challenge. While conventional therapies like surgical debridement are available, their outcomes often fall short due to the impaired function of cells in the damaged tissue [101,168]. To tackle these challenges, various strategies have been employed.

Numerous advancements have been achieved in the development of artificial barriers and biological scaffolds for shielding internal tissues from the outside environment during the healing process of diabetic wounds [169,170]. Among these materials, hydrogels have emerged as the most commonly used option. Hydrogels can fill the tissue around a fresh wound, absorb wound extract, and alleviate inflammation. However, current research indicates that hydrogels function primarily in the early stages of wound healing and show limited effectiveness during the remodeling phase. Yang et al. employed a temperature-sensitive PF-127 hydrogel to deliver hUCMSC-derived exosomes for diabetic wound healing [146]. The PF-127 hydrogel transitions from a liquid state at low temperatures to a semi-solid gel at 37 °C, enabling it to conform to the irregular spaces of diabetic wounds. In an animal model, both hUCMSC-exos and hUCMSC-exos/PF-127 displayed efficacy in reducing the wound defect area during the initial three days of treatment. However, hUCMSC-exos/PF-127 exhibited significantly higher healing efficiency in the subsequent two weeks. These promising findings underscore the role of the porous structure of the hydrogel, which prolongs the release time of exosomes and enhances their half-life in serum. Furthermore, the hydrogel exhibits potential therapeutic effects by reducing exudates. Immunohistochemical analysis and evaluation of microvascular density revealed that treatment with hUCMSC-exos/PF-127 markedly promoted angiogenesis compared to other treatment methodologies. *In vitro* experiments further supported these findings, illustrating that hUCMSC-exos/PF-127 facilitated the proliferation and migration of HUVECs, crucial steps in angiogenesis. This study presents compelling evidence for the efficacy of loading exosomes onto a hydrogel platform for the treatment of chronic diabetic wounds.

Innovative injectable self-healing hydrogel developed by Wang et al. utilizes a combination of methylcellulose and chitosan (MC-CS) through a Schiff base reaction [145]. Self-healing hydrogels offer distinct advantages due to the formation of Schiff base linkages within the hydrogel matrix, providing weak acid responsiveness and the ability to self-heal. These linkages also enhance adhesion between the hydrogel and surrounding tissue. By incorporating exosomes derived from placental mesenchymal stem cells (PMSCs), this bioactive composite system improves angiogenesis and results in well-organized and dense collagen distribution in injured tissue. Regenerated skin exhibited the presence of hair follicles and glands, demonstrating successful tissue regeneration. However, the precise molecular mechanisms behind the observed angiogenic effects remain unclear. Further exploration is needed to understand the potential formation of Schiff base linkages between the hydrogel and encapsulated EVs for controlled release. Investigating the impact of these interactions on the structural and functional integrity of the EVs is crucial.

Platelet-rich plasma (PRP) has shown promising results in chronic wound treatment by enhancing angiogenesis and epithelial regeneration [171]. This outcome is likely attributed to the abundance of growth factors, such as platelet-derived growth factor (PDGF), TGF-β, and VEGF, present in the plasma [172]. PRP-derived exosomes (PRP-Exos) are even more effective in promoting cell proliferation, migration, and angiogenesis [173], as they contain a higher concentration of growth factors compared to PRP with the same total protein content. The healing effects of PRP-Exos on chronic wounds are better understood, as they stimulate angiogenesis through the activation of Erk and Akt signal pathways and promote epithelial regeneration by activating Yes-associated protein (YAP). When combined with ZWP, a homogeneous polysaccharide, in a chitosan/silk hydrogel sponge as a carrier, PRP-Exos significantly accelerate wound contraction, re-epithelialization, collagen deposition, and skin angiogenesis in diabetic rat models [147].

In recent years, collagen has emerged as a widely used gel material due to its excellent biocompatibility, easy accessibility, and hemostatic properties. Recombinant collagen, produced through synthetic biology technology, is a novel biomaterial. Recombinant human collagen type III (rhCol III) is created by optimizing gene sequences based on the properties and major functional domains of human collagen. Xu et al. developed a hydrogel using rhCol III supplemented with EVs derived from hUCSCs (hUCSCs-EVs) to achieve sustained release [148]. The high expression of anti-inflammatory factors (Arg1 and TGF-β) and the increased number of CD206-positive cells in the rhCol III-EVs group indicate the hydrogel's ability to regulate macrophage conversion to the M2 phenotype. Moreover, the rhCol III-EVs hydrogel demonstrated a stimulating effect on the proliferation and migration of L929 cells and promoted the proliferation and angiogenesis of HUVECs *in vitro*. In a full-thickness skin defect model in diabetic rats, treatment with the rhCol III-EVs hydrogel significantly reduced the expression of the inflammatory factor IL-6, while increasing the expression of the cell proliferation factor Ki67, as well as vascularization factors CD31 and α-SMA, compared to treatment with rhCol III hydrogel or EVs alone. These results suggest that the rhCol III-EVs hydrogel accelerates the wound healing process by modulating the inflammatory response, promoting cell proliferation, and enhancing vascularization.

Compared to collagen, porcine small intestinal submucosa (SIS), a naturally occurring ECM material mainly composed of collagen and abundant in bioactive factors [174,175], shows potential for regenerating damaged tissues. Its favorable biocompatibility and low immunogenicity make it a promising candidate material. However, the mechanical strength of SIS hydrogel is insufficient. To overcome this limitation, catecholamine chemistry has been utilized to boost the biomechanical properties of SIS [149]. Subsequently, sEVs derived from UCSCs are incorporated into catechol-modified SIS-based hydrogels using fusion peptides (SC-Ps-sEVs) to prevent sEVs from diffusing into extracellular fluids and to prolong their activity. The release kinetics data revealed that the fusion peptide-treated hydrogels significantly increased the total sEVs loading and prolonged the release duration ($228.07 \pm 11.13 \times 10^8$ over 7 days) compared to the control group ($120.67 \pm 16.19 \times 10^8$ roughly in 3 days), illustrating the retention effect of the fusion peptide on sEVs. *In vitro* investigations have shown that SC-Ps-sEVs hydrogels stimulate cell proliferation, migration, adhesion, and angiogenesis. Moreover, *in vivo* experiments have validated the remarkable therapeutic benefits of these hydrogels in promoting wound healing.

Characteristics of diabetic wounds, such as drug-resistant bacterial infections, biofilm formation, impaired angiogenesis, compromised wound perfusion, and oxidative damage to the microenvironment, present significant challenges to effective wound healing. The restoration of skin integrity and ensuring adequate blood supply are vital for successful healing [176–178]. However, many current hydrogel-based therapies are inadequate for treating vascular diseases and skin lesions caused by multidrug-resistant bacteria [179,180]. Consequently, there is an increasing focus on developing multifunctional hydrogels with antimicrobial properties to enhance wound healing in refractory cases of diabetes mellitus. These advanced hydrogels typically possess the capability to perform multiple functions simultaneously, including antimicrobial, anti-inflammatory, and immunomodulatory effects. Wang et al. developed an injectable, self-healing hydrogel with superior antimicrobial efficacy and prolonged exosomes release in response to pH [101]. This hydrogel, named FHE hydrogel, consists of oxidative HA

(OHA) and poly-ε-L-lysine (EPL)/PF127 in an inter-penetrating network (IPN) structure. EPL, a natural cationic antimicrobial agent derived from *Streptomyces albulus* [181], is part of this composition. The hydrogel encapsulates negatively charged exosomes through electrostatic interactions [101]. In the context of diabetic wounds, known for their acidic environment, the hydrogel facilitates exosome release by breaking Schiff base bonds, thereby aiding in wound healing. *In vivo* tests showed that the FHE@exo hydrogel achieved an 88.67 ± 6.9 % healing rate by day 14, with complete wound closure and hair regrowth by day 21. Subsequent investigations delved into the pathways through which wound healing was promoted. Masson staining revealed a significant boost in type I and III collagen deposition on days 7 and 14 with the FHE@exo hydrogel. Immunohistochemical staining highlighted the hydrogel's role in granulation tissue formation and epithelial re-formation during the repair process. Immunofluorescence analysis visually displayed a higher vessel count (approximately 45) at the wound site with the FHE@exo hydrogel compared to other groups (around 20 vessels). In summary, the FHE@exo hydrogel demonstrated robust antibacterial properties and effectively enhanced wound healing in diabetic mice by increasing collagen deposition, encouraging epithelial regeneration, and promoting angiogenesis.

Moreover, maintaining a balance between M1 and M2 macrophages is essential for facilitating full-thickness wound healing, with particular significance in diabetic wound healing scenarios. The disruption of the shift from the M1 phenotype to the M2 phenotype during the later stages of healing can lead to an accumulation of pro-inflammatory mediators, hinder the production of anti-inflammatory mediators, and ultimately result in secondary tissue damage with impaired healing [182]. To address chronic wounds, a self-healing multifunctional hydrogel (MSC-Exos@CEC-DCMC HG) loaded with BMSC-Exo was developed to promote angiogenesis and facilitate the transition of macrophages from a pro-inflammatory M1 phenotype to a reparative M2 phenotype [100]. The utilization of carboxyethyl chitosan (CEC)-dialdehyde carboxymethyl cellulose (DCMC) hydrogels allowed for the sustained release of MSC-Exos with pH-responsive properties lasting over 168 h. This pH sensitivity was achieved by breaking the Schiff base bond between CEC and DCMC in acidic conditions. MSC-Exos trigger angiogenesis by activating VEGF-mediated signaling pathways and alleviate inflammation by modulating macrophage polarization from M1 to M2 phenotypes. Immunofluorescence and WB techniques were employed in this study to evaluate the influence of MSC-Exos on macrophage polarization under inflammatory conditions *in vitro*, revealing a reduction in the expression of inducible nitric oxide synthase (iNOS) associated with the M1 phenotype and an increase in CD206 expression indicative of the M2 phenotype transition. Furthermore, the MSC-Exos@CEC-DCMC HG demonstrated similar effects in *in vivo* models.

miRNAs, which are short non-coding RNAs approximately 20–24 nucleotides in length, have emerged as crucial regulators within cells. Recently, there has been considerable interest in the therapeutic potential of miRNA-based treatments due to their capacity to modulate gene expression [183–185]. Particularly, miR-223 has been discovered to have a vital role in facilitating the transition of macrophages from the pro-inflammatory M1 phenotype to the reparative M2 phenotype and in promoting angiogenesis *in vivo* [183–185]. Consequently, exosomes derived from M2 macrophages with overexpression of miR-223 ($Exos^{M2@miR-223}$) have been identified as a promising agent for skin repair. To enhance the effectiveness of ExosM2@miR-223 in treating diabetic wounds, Xiong et al. devised a hydrogel formulation (HA@MnO_2/FGF-2/Exos) that allows for controlled release, with fibroblast growth factor 2 (FGF-2) playing a critical role in tissue repair [150]. Analysis of release kinetics revealed that 34 % and 45 % of the encapsulated FGF-2 were released from the HA@MnO_2/FGF-2/Exos hydrogel at 12 and 24 h, with 75 % released by day 7, while $Exos^{M2@miR-223}$ were effectively enclosed within HA@MnO_2/FGF-2/Exos and gradually released over the subsequent 21 days. Additionally, manganese dioxide (MnO_2) nanoenzymes were integrated into the hydrogel to counteract the adverse effects of excessive production of reactive oxygen species (ROS) in the hyperglycemic wound microenvironment. These nanoenzymes facilitate the conversion of endogenous ROS into oxygen (O_2), thereby alleviating local oxidative stress and hypoxia [150,186,187]. In conclusion, the HA@MnO_2/FGF-2/Exos hydrogel represents a versatile and promising therapeutic strategy for enhancing the healing of diabetic wounds. The amalgamation of inorganic materials with the EViH system signifies a significant trend for future advancements in research and development.

5. Bone and cartilage regeneration

5.1. The mechanisms of bone development and repair

Bone, a crucial organ in the human body, serves multiple essential functions such as providing support and protection, enabling movement, storing minerals, and producing blood cells. Bone regeneration is a complex and well-coordinated biological process that consists of several distinct stages, including hematoma formation, soft callus formation, hard callus formation, and bone remodeling (Fig. 6) [188]. Initially, the coagulation cascade is triggered to create a blood clot at the bone injury site, which significantly influences the subsequent formation of new bone. Different types of cells, including inflammatory cells, fibroblasts, and stem cells, are attracted to the injury site along with the development of new blood vessels. Granulation tissue forms at the fractured bone ends and gradually gets replaced by fibrocartilage. Concurrently, the periosteum undergoes ossification, leading to the generation of an external bone scab. After these initial stages, the tissue undergoes further mineralization by depositing hydroxyapatite, promoting the formation of a more durable woven bone structure. As the healing process advances, the large fracture callus is substituted by secondary lamellar bone, and the blood supply to the area returns to normal, indicating the completion of the bone remodeling phases.

5.2. EViH for bone regeneration

Bone damage resulting from trauma, inflammatory infections, tumor resection, and congenital malformations presents significant challenges for clinicians [189]. Conventional approaches [190], such as autografts, allografts, and biological fillers, have been hindered by limitations including immune rejection [191], high cost [192], tissue infection [193], and limited donor sources [194]. In this context, bone tissue engineering (BTE) has emerged as a promising therapeutic strategy that leverages scaffolds and growth factors to facilitate bone regeneration.

Hydrogels can mimic the ECM and serve as scaffold materials for bone regeneration. For instance, researchers have developed hydrogels with integrated fibrous nanostructures and porous microstructures, known as microparticle annealed nanofibrous (MANF) hydrogels (Fig. 7a) [195]. The micron-scale structure of the natural ECM plays a critical role in cell migration and tissue formation [196,197], while the nanofibrous architecture of materials enhances serum protein adhesion [198], osteoblast attachment, cell differentiation, and tissue regeneration [199,200]. *In vitro* studies have demonstrated that MANF hydrogels effectively stimulate osteogenic differentiation of BMSCs. *In vivo* experiments have further revealed that MANF hydrogels promote cranial bone regeneration (Fig. 7b) and neovascularization (Fig. 7c) more effectively than smooth-surfaced gelatin microparticle annealed (MA) hydrogels. This precise manipulation of the physical structure of tissue engineering scaffolds has the potential to obviate the need for intricate chemical modifications and excessive use of growth factors, thus presenting significant clinical promise for regenerating various tissues. In summary, this study underscores the clinical potential of tissue regeneration through fine-tuning the physical structure of tissue-engineered scaffolds, offering a promising alternative to laborious chemical alterations and minimizing the reliance on excessive growth factors.

The utilization of scaffolds alone may only have a limited impact on

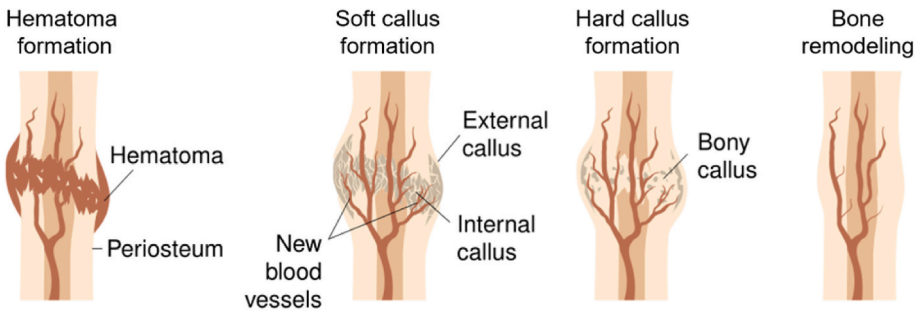

Fig. 6. Schematic representation of the four phases during bone repair. *Copyright © 2003 Elsevier Ltd. All rights reserved.*

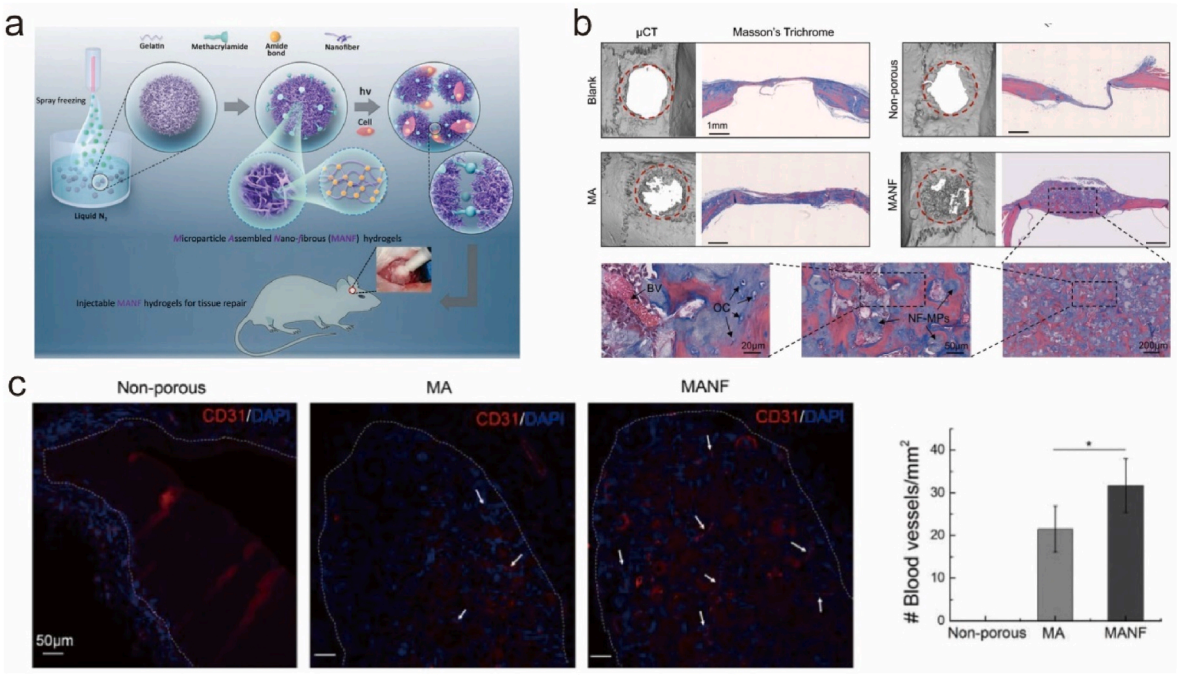

Fig. 7. (a) The strategy for the preparation of MANF hydrogels from gelatin nanofiber particles. (b) The μCT and histological images of bone defects treated by saline, non-porous hydrogel, MA hydrogel, and MANF hydrogel. (c) The analysis of the blood vessel density in non-porous, MA and MANF hydrogels. These figures have been reproduced from Ref. [195] with permission from © *2019 Elsevier Ltd. All rights reserved.*

enhancing bone regeneration, necessitating the incorporation of growth factors in cases of challenging bone injuries. EVs derived from cells involved in bone regeneration harbor a diverse range of components, such as growth factors and genes. Growing evidence underscores the significant role of MSC-EVs in tissue regeneration [201–203]. Therefore, a therapeutic approach that combines hydrogels with MSC-EVs is increasingly being utilized in cell-free BTE. Yang et al. successfully isolated hUCSCs-Exos and integrated them with injectable hydroxyapatite (HAP) embedded in a cross-linked HA-alginate (HA-ALG) hydrogel to achieve controlled exosome delivery and provide structural support for bone regeneration in defects (Fig. 8a) [204]. In this study, HAP was introduced into the hydrogel composite to enhance its mechanical properties, leveraging HAP's significant presence in natural bone tissue. The incorporation of nanosized HAP (nano-HAP) ensured its homogeneous dispersion within the material. Compared to the HA-ALG hydrogel, the composite hydrogel doped with HAP exhibited reduced swelling and degradation rates, forming the basis for the sustained release of EVs. Release kinetics confirmed this, as 71.20 ± 2.64 % of hUCSCs-Exos were released from the composite hydrogel after 14 days, in contrast to 84.81 ± 4.91 % from the pure hydrogel ($p < 0.05$). Furthermore, the composite hydrogel displayed enhanced mechanical strength, which is advantageous for employing EV-based therapy in bone defects. It is evident that an optimal scaffold, a key element in addressing substantial bone defects [205], should precisely correspond to the shape and size of the defect [206], serving as a guiding structure for tissue growth in BTE [207]. Consequently, 3D printing technology has been employed for bone regeneration. For instance, Zhang et al. fabricated nano--HAP/poly-ε-caprolactone (nHP) scaffolds using a 3D printing method, subsequently infilling the nHP scaffold structures with HA-coated hUCSCs-Exos (Fig. 8b(i)) [89]. The 3D porous nHP scaffolds were printed according to the volume and shape of the bone defects, then immersed in a mixed solution of HA containing the photoinitiator Irgacure-2959 and hUCSCs-Exos for 6 h, followed by UV light curing of the hydrogel. *In vitro* studies demonstrated that hUCSCs-Exos promoted the proliferation, migration, and vascular differentiation of endothelial progenitor cells (EPCs) without interfering with the osteogenic differentiation of BMSCs. Angiogenesis, critical for nutrient transportation and waste elimination [208,209], is imperative for bone regeneration [210]. It was observed that the regulation of miR-21 expression can influence angiogenesis, as illustrated in Fig. 8b(ii). Additionally, reverse transcription-polymerase chain reaction (RT-PCR) analysis revealed that this exosome-based strategy propels angiogenesis by upregulating the NOTCH1/Delta-like 4 (DLL4)/VEGFA pathway, as depicted in Fig. 8b (iii).

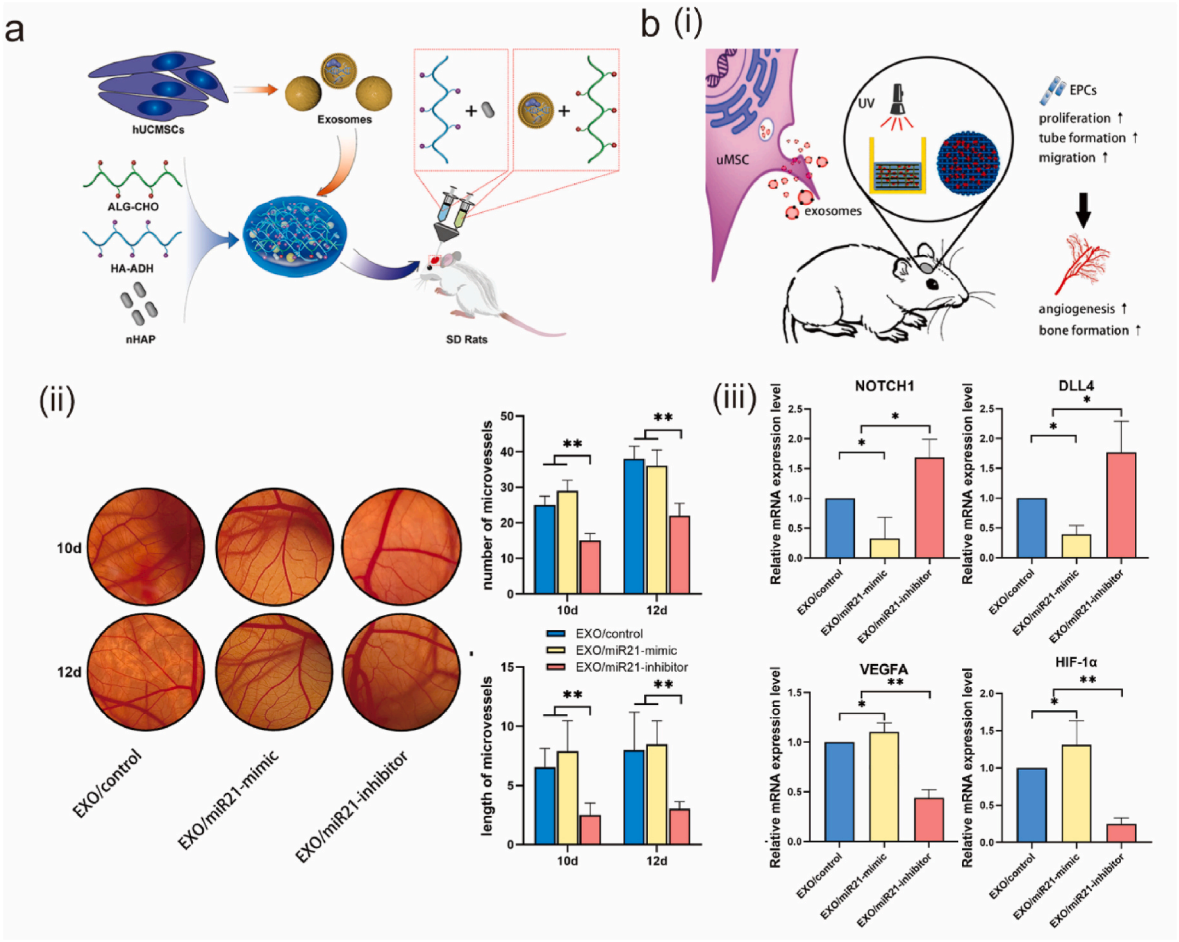

Fig. 8. (a) The system of exosomes combined with HA-ALG hydrogel to repair bone defects. *Copyright © 2020, American Chemical Society* (b) Exosome-encapsulated hydrogels accelerate bone repair by enhancing angiogenesis. (i) The scheme of exosomes combined with the HA-Gel and the nHP scaffold to repair bone defects; (ii) The picture of formed blood vessels on the chorioallantoic membrane and the number and length of newly formed micro-vessels; (iii) Expression of related genes exposed to different treatments. *Copyright © 2021, American Chemical Society*.

In cases of trauma or tumor-induced bone damage, inflammation, and immune imbalance are commonly observed, characterized by acute ischemia, hypoxia, the release of pro-inflammatory factors, and abnormal cellular metabolism. Therefore, modulating the immune response may be a promising approach to enhance bone regeneration. Li et al. found that exosomes derived from ADSCs suppressed inflammation and facilitated macrophage polarization towards an anti-inflammatory phenotype by targeting macrophage migration inhibitory factor (MIF) *via* its enriched miR-451a (Fig. 9a) [211]. Furthermore, the incorporation of gelatin nanoparticles (GNPs) hydrogels into critical exosomes provided improved physical and biological support, as depicted in Fig. 9b. Despite the compelling evidence supporting their regenerative potential, MSCs-EVs alone may not meet all treatment requirements. Therefore, engineering MSCs-EVs to carry cargo with specific functional roles may be necessary to satisfy therapeutic demands. Recent studies suggest that overexpressing bone morphogenetic protein-2 (BMP-2) in MSCs results in the production of functionally engineered EVs (FEEs) that effectively enhance BMP-2 signaling cascades in target cells and tissues, thereby promoting bone repair [212]. Moreover, it is worth noting that the uptake of MSCs-EVs by receptor cells typically follows a dose-dependent and saturable pattern [213,214], suggesting that an excessive abundance of EVs may not necessarily improve efficiency. Notably, *in vivo* experiments conducted on animal models with bone defects revealed that ALG hydrogels loaded with FEEs exhibited a rapid release of all EVs within a 4-week timeframe [91]. To enhance host cell attachment and proliferation, as well as retain EVs, RGD-containing scaffolds for BTE are commonly employed. The implantation of FEEs within a releasing hydrogel containing the RGD tether consistently stimulated osteogenesis between 4 and 8 weeks, thereby exemplifying the saturated nature of EVs activity and the advantages of prolonged EVs release (Fig. 9c) [212]. For their potential clinical applications, apart from the engineering strategies mentioned earlier, epigenetic modulation emerges as a promising approach to enhance the mineralization capacity of EVs. In particular, EVs derived from osteoblasts treated with Trichostatin A (TSA), a histone deacetylase inhibitor (HDACi), demonstrated a remarkable improvement due to their enrichment in bone-enabling miRNAs and transcriptional regulatory proteins [215]. Epigenetically activated osteoblast-derived EVs (TSA-EVs) were encapsulated in GelMA nanocomposite hydrogels to synergistically promote hBMSCs mineralization [216]. Furthermore, the incorporation of laponite (LAP), an FDA-approved synthetic montmorillonite nanomaterial [217], into the GelMA hydrogel provided precise control over the hydrogel's shear thinning behavior, stiffness, and shape retention. This controlled modulation proved instrumental in augmenting the clinical efficacy of the composite system for BTE applications. Although the study did not delve into the underlying mechanism or investigate its performance in animal models, these findings hold promise for future research and potential advancements in the field.

The engineered EVs mentioned above are currently under extensive investigation. Strategies to improve the loading efficiency of EVs by modifying producer cells or altering membranes are widely used in engineering [218]. Yet, numerous challenges hinder the clinical

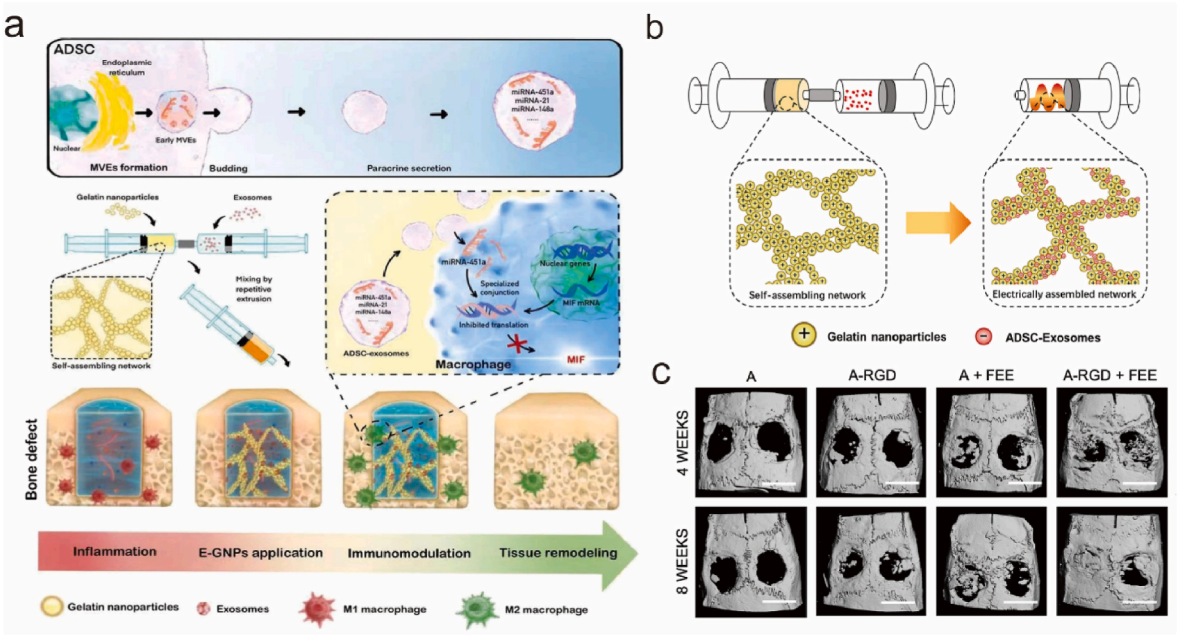

Fig. 9. (a) The schematic diagram of the mechanism: exosomes accelerate bone regeneration by regulating M1/M2 macrophage phenotypic polarization. (b) The electrostatic assembly of GNPs and Exos. *Copyright © 2022, The Author(s)* (c) Images of skull defect repair by different treatment groups. *© 2021 Acta Materialia Inc. Published by Elsevier Ltd. All rights reserved.*

application of engineered EVs, such as the necessity to enhance cargo loading efficiency and establish efficient tactics to evade long-term immune responses for sustained *in vivo* effectiveness. Moreover, further research is needed to determine how to effectively preserve the activity or function of engineered EVs.

In addition to the previous study on bone regeneration using EViH, there are several notable and important studies expected to be discussed in this context. The cargo composition of EVs is intricately linked to their parent cell type and the physiological or pathological environment in which the cells are located [22,219]. Recent research has revealed strong evidence showing that cells grown in two-dimensional (2D) conditions exhibit abnormal cellular behavior, such as abnormal cell polarization [220], expression of cancer-specific antigens [221], and reduced stem cell differentiation [222], which could be a significant factor contributing to the unsuccessful outcomes seen in various therapeutic EVs studies in animal models or humans [223,224]. Conversely, studies have demonstrated that the 3D culture of MSCs mimics the natural growth environment and significantly enhances the secretion efficiency of EVs compared to traditional 2D culture [225]. This is likely because 3D culture enhances cell signaling, supports cell proliferation, and preserves cells' differentiation capacity [226,227]. EVs released by cells cultured in 3D ALG hydrogels (3D-Exos) have been shown to notably increase the expression of osteogenesis-related genes/proteins (*e.g.*, Runx2, OCN, osteopontin (OPN), collagen type I alpha 1(COL1A1), and ALP) in hBMSCs, and greatly improve bone defect restoration in Sprague-Dawley (SD) rats [228]. Another study has isolated various types of EVs (MVs and exosomes) released by cells under different culture conditions and co-embedded these EVs with MT3T3 cells in hydrogels to evaluate their positive effects on the osteogenic mineralization of MT3T3 cells [229]. The findings indicated that MVs and exosomes could independently enhance osteogenesis, with a more significant effect observed when used in combination.

5.3. EViH for cartilage regeneration

Articular cartilage is an avascular connective tissue characterized by abundant stroma and sparse chondrocytes, making it susceptible to pathological damage and challenging to heal independently [230]. Various factors such as trauma, obesity, aging, immune system disorders, and among others, can lead to cartilage damage, with joint replacement as the final solution in the absence of prompt and aggressive intervention. While microfractures, autologous chondrocyte implantation, and autografting are common clinical treatments for cartilage injuries [231], treatment failures often occur due to fibrosis and inadequate integration of cambium into native tissue [232]. In recent years, EViH has emerged as a promising alternative.

Exosomes derived from human induced pluripotent stem cells (hiPSCs) combined with a hydrogel scaffold (EHG) have shown significant benefits for chondrogenesis both *in vitro* and *in vivo* [233]. The hydrogel scaffold (PIC) was created through a photoinduced imine crosslinking technique, utilizing o-nitrobenzyl alcohol-modified hyaluronic acid (HA-NB) and gelatin. This scaffold enables *in situ* gelation for seamless tissue attachment and integration, providing a conducive environment for cellular and exosomal interactions. Following a 14-day immersion in PBS, the hydrogel retained exosomes at a remarkable rate of 90 %, attributed to the smaller theoretical sieve size of EHG compared to the exosome diameter. Additionally, the daily release of exosomes (1×10^{10} mL^{-1}) was found to positively influence the adjacent damaged tissues and cells. HA-NB in the PIC hydrogel generates aldehyde groups under light exposure, which form imine bonds upon reacting with amino groups on the cartilage matrix surface, showcasing a strong affinity with cartilage tissue. Scanning electron microscopy (SEM) and H&E staining were utilized to visualize the hydrogel-cartilage interface. Results demonstrated complete attachment of the hydrogel to the lateral cartilage, infiltration into the subchondral bone, and establishment of a seamless interface, highlighting the firm bond between the hydrogel and cartilage. This seamless integration of the gel into cartilage tissue facilitates peripheral cell migration into the scaffold, enhancing the modulatory effects of exosomes. *In vivo* experiments conducted in this study revealed the presence of newly formed hyaline cartilage in the exosome-treated group, indicating a positive role of exosomes in tissue regeneration.

Previous research has highlighted the positive interaction between chondrocytes and BMSCs in preserving the articular cartilage phenotype [234]. BMSCs can produce cytokines and growth factors that support chondrocyte proliferation and the redifferentiation of articular cartilage

[235]. Thus, modulating the function of BMSCs to influence cartilage regeneration presents a promising approach. Zhang et al. found that PRP-Exos had significant effects on BMSCs by countering TGF-β1, leading to increased expression of ERK1/2 and p38 [236]. These signaling pathways are crucial for regulating cartilage differentiation in BMSCs. To enhance the therapeutic potential of PRP-Exos, a temperature-sensitive hydrogel comprising P407 and P408 was developed to extend the retention of exosomes at the injury site. The release kinetics indicated that PRP-Exos were consistently released from the hydrogel into the culture medium for approximately 1 month at 37 °C *in vitro*, and the hydrogel maintained the retention of PRP-Exos in affected joints *in vivo*. The hydrogel can achieve sustained and continuous release of PRP-Exos largely due to the high concentration of hydrogel polymers. Significantly, the exosome-loaded hydrogel effectively inhibited cartilage degeneration and the progression of osteoarthritis, as demonstrated by the experimental results.

Genes, particularly miRNAs, are a significant component of EV cargo. Research has shown that miRNAs found in EVs can play a crucial role in regulating chondrocyte function through exosomal delivery [237,238]. For example, exosomes derived from infrapatellar fat pad MSCs have been demonstrated to protect cartilage from damage by delivering miR-100–5p, which effectively inhibits the mTOR-autophagy pathway [237]. PTEN acts as a negative regulator of the AKT signaling pathway [239], known for its chondroprotective effects [239,240]. Moreover, studies by Hu and Dong et al. have underscored the importance of sEVs derived from hUCSCs (hUCSCs-sEVs) in promoting cartilage regeneration [241]. These sEVs were internalized by chondrocytes and hBMSCs, enhancing migration, proliferation, chondrogenic differentiation, glycosaminoglycan formation, ECM synthesis, and type II collagen expression. The observed effects were linked to the high levels of miR-23a-3p in hUCSCs-sEVs. Additionally, luciferase reporter gene assays confirmed that miR-23a-3p directly targets the 3′ untranslated region (UTR) of PTEN, leading to PTEN expression inhibition, subsequent upregulation of phosphorylated AKT (P-AKT), and activation of the PTEN/AKT signaling pathway (Fig. 10). To improve the *in vivo* delivery of hUCSCs-sEVs, GelMA with injectable properties and UV cross-linking were incorporated to fill defects, with laponite nanoclay added to augment mechanical and biological properties. Release kinetics indicated that sEVs in GelMA/nanoclay hydrogel could sustain release for up to 31 days *in vitro*, surpassing GelMA (13 days) and GelMA/Geltain (17 days). In addition, the sEVs released from the GelMA/nanoclay gel retained their spherical microbubble structure, suggesting that the sEVs within the hydrogel maintained their normal morphology without damage. Histological evaluation staining results indicated that treatment with hUCSCs-sEVs promoted the production of type II collagen and ECM.

We summarize the use of EViH for bone and cartilage regeneration applications (Table 2).

6. Vascularization and cardiac regeneration

In this section, we focus on the application of EViH in promoting cardiac function and vascular regeneration (Table 3).

6.1. Cardiac regeneration

Cardiovascular diseases (CVDs), such as hypertension and coronary atherosclerotic heart disease, are a significant global cause of mortality [251]. The Centers for Disease Control and Prevention in the United States report that approximately 655,000 people die from CVDs annually in the country, translating to an average of one death every 36 s [252]. Due to the substantial public health impact of CVDs, the imperative development of effective prevention and treatment strategies is highlighted.

6.1.1. The pathophysiology of myocardial infarction

MI is a common CVD characterized by the necrosis of the myocardium due to acute and persistent ischemia and hypoxia in the coronary arteries, ultimately leading to heart failure and premature death [253]. MI is typically classified as either ST-segment elevation myocardial infarction (STEMI) or non-ST-segment elevation myocardial infarction (NSTEMI) [254]. Unstable angina, which precedes MI, and NSTEMI share similar pathophysiological characteristics and are collectively referred to as non-ST-segment elevation acute coronary syndrome (NSTE-ACS). In MI patients, atheromatous plaques can be observed in the coronary arteries, leading to a progressive narrowing of the luminal diameter as plaque accumulates. However, severe luminal stenosis is not the primary cause of MI, as atheromatous plaques usually have a dense fibrous cap and collateral circulation is established in chronically stenotic lesions. Even with a luminal narrowing of ≥75 % at rest, the systemic oxygen and nutrient supply remain unaffected. Instead, MI is the result of a combination of atherosclerotic disease in the coronary arteries and thrombus formation [255]. Vulnerable plaques are characterized by thin and narrow fibrous caps (30–50 %), activated macrophages, and degradation of the ECM [256]. When these plaques rupture, thrombogenic components are released, leading to platelet activation, activation of the coagulation cascade, formation of thrombi, and embolization of atherosclerotic debris downstream. This process results in the necrosis of myocytes, with the extent of vascular occlusion, duration of occlusion, the establishment of collateral circulation, and the impact of reperfusion determining the severity of the lesion [257]. In essence, an imbalance between myocardial oxygen supply and demand is the underlying cause of MI, with the fundamental principles in the treatment of MI focusing on restoring normal oxygen balance and reducing myocardial cell apoptosis.

6.1.2. EViH for cardiac regeneration

Conventional therapies currently available lack the essential capability to efficiently repair and regenerate damaged cardiac muscle.

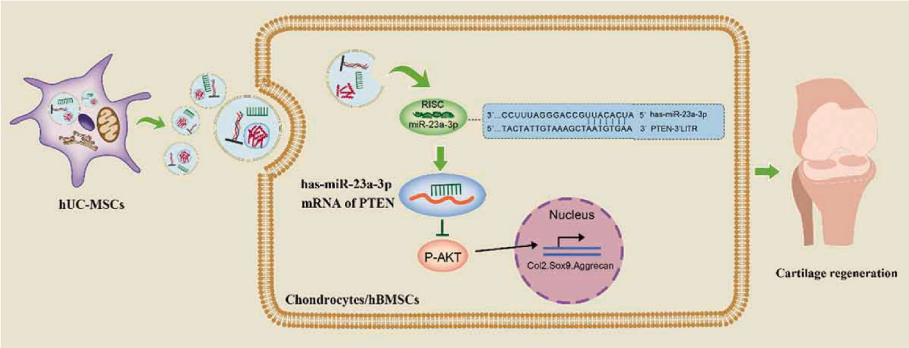

Fig. 10. Schematic representation of the mechanism by which hUCSCs-sEVs promote cartilage regeneration. © *2020 The Author(s).*

Table 2
The application of EViH for bone and cartilage regeneration.

Disease	EVs species	Composite hydrogel type	Hydrogel loading	Crosslinking mode	Release Kinetics	Biological effects	References
Bone defects	Exosomes of hUCSCs	HA-ALG	Pre-gel loading	Schiff base reaction	71.20 ± 2.64 % in 14 days	Repairs bone defects *via* promoting neovascularization	[204]
	Exosomes of UCSCs	HA	Pre-gel loading	Photo-crosslinking	Not mentioned	Promotes bone regeneration by upregulating the NOTCH1/DLL4/VEGFA pathway	[89]
	Exosomes of ADSCs	GNPs	Pre-gel loading	Schiff base reaction	Over 7 days	Promotes bone regeneration by promoting macrophage polarization towards the M2 phenotype	[211]
	FEEs of MSCs	Alginate	Pre-gel loading	Photo-crosslinking	Approximately 80 % of EVs were retained within the hydrogel for 1 week	Continuous osteoinduction by enhancing BMP2 signaling cascades	[91]
	TSA-EVs of osteoblasts treated by TSA	GelMA-LAP	Not mentioned	Photo-crosslinking	Over 7 days	Exhibits the potential for bone repair	[216]
Cartilage Defects	Exosomes of PRP	Poloxamer	Pre-gel loading	Thermo-crosslinking	Over 28 days	Promotes cartilage repair *via* inhibiting cartilage degeneration and osteoarthritis	[236]
	Exosomes of BMSCs	GelMA	Post-gel loading	Photo-crosslinking	Approximately 80 % in 48 h	Promotes cartilage repair by promoting ECM production and chondrocyte proliferation	[242]
	sEVs of hUCSCs	Gel-nano	Pre-gel loading	Photo-crosslinking	Over 31 days	Promotes cartilage repair based on the activation of the PTEN/AKT signaling pathway through the transfer of miR-23a-3p	[241]
	Exosomes of hiPSC-MSCs	PIC	Pre-gel loading	Photo-crosslinking	Retained inside the hydrogel (>90 %) in PBS for 14 days	Promotes cartilage repair	[233]

Abbreviations: EVs: extracellular vesicles; hUCSCs : human umbilical cord mesenchymal stem cells; HA-ALG: hyaluronic acid-alginate; HA: hyaluronic acid; DLL4: *Delta-like 4*; ADSCs: adipose-derived mesenchymal stem cells; GNPs: gelatine nano-particles; FEEs: functionally engineered EVs; MSCs: mesenchymal stem cells; BMP: bone morphogenetic protein; TSA: Trichostatin A; GelMA: gelatin methacryloyl; LAP: laponite; PRP: platelet-rich plasma; BMSCs: bone marrow derived mesenchymal stem cells; Gel-nano: GelMa/nanoclay hydrogel; sEVs: small extracellular vesicles; hiPSC: human induced pluripotent stem cell; PIC: photoinduced imine crosslinking.

Table 3
The application of EViH for vascularization and cardiac regeneration.

Disease	EVs species	Composite hydrogel type	Hydrogel loading	Crosslinking mode	Release Kinetics	Biological effects	References
MI	sEVs of MSCs	Alginate	Pre-gel loading	Ionic crosslinking	Over 10 days	Improves heart function by promoting macrophage polarization towards the M2 phenotype and increasing scar thickness and neovascularization	[243]
	EVs of BMSCs	GelMA	Pre-gel loading	Photo-crosslinking	71 ± 10.45 % in 72 h	Improves heart function without injection	[244]
	Exosomes of DC	Alginate	Pre-gel loading	Ionic crosslinking	Over 12 days *in vitro* and over 14 days *in vivo*	Improves heart function by activating Treg cells and by modifying the polarization of macrophages	[245]
	ISL1-MSCs	Ang-1 gel	Pre-gel loading	Entangled nanofibers	Not mentioned	Improves heart function through enhancing anti-apoptosis, proliferation, migration, and angiogenic capacity in HUVECs	[246]
Ischemic heart diseases	Exosomes of hUCSCs	PGN	Pre-gel loading	Schiff base reaction	Over 22 days	Improves heart function by decreasing inflammation, fibrosis, and apoptosis and by increasing angiogenesis	[247]
HF	Exosomes of MSCs	HA	Post-gel loading	No mentioned	Sustained retention over 48 h *in vivo*	Improves heart function *via* iPC injection without kidney and liver injury	[248]
CLI	Engineered-EVs of endothelial cells	MC	Pre-gel loading	Thermo-crosslinking	100 % in 8 days at 37 °C; 100 % in 5 days at 29 °C	Improves neovascularization, attenuates muscle injury, and recovers limb function by activating the VEGF/VEGFR pathway and autophagy-lysosomal pathway.	[249]
Hindlimb ischemia	Exosomes of hPMSCs	Chitosan	Pre-gel loading	No mentioned	Retained inside the hydrogel (>90 %) in PBS for 12 h	Improves hind limb ischemia *via* protecting endothelium and promoting angiogenesis	[250]

Abbreviations: EVs: extracellular vesicles; MI: myocardial infarction; sEVs: small extracellular vesicles; MSCs: mesenchymal stem cells; hUCSCs: human umbilical cord mesenchymal stem cells; PGN: peptide gelator-NapFF; BMSCs: bone marrow derived mesenchymal stem cells; GelMA: gelatin methacryloyl; DC: dendritic cell; HF: heart failure; HA: hyaluronic acid; iPC: intrapericardial; Ang-1: angiogenin-1; ISL1-MSCs: MSCs overexpressing Islet1; CLI: critical limb ischemia; Engineered-EVs: EVs engineered by VEGF (vascular endothelial growth factor) and TFEB (a pivotal regulator of autophagy); MC: methylcellulose; hPMSCs: human placental mesenchymal stem cell.

Recent research has emphasized the critical role of EVs in various processes, such as inhibiting post-infarction cardiomyocyte apoptosis [258], reducing scar formation [259], and promoting angiogenesis [260]. Moreover, the unique structure and importance of heart tissue present challenges in using specific scaffold materials for cardiac regeneration. Hydrogel emerges as the optimal choice for this purpose due to its resemblance to ECM properties. Consequently, EViH harnesses the advantageous properties of both hydrogels and EVs, leading to widespread adoption in the field of cardiac regeneration.

Alginate-based hydrogels serve as a notable example in this field.

Clinical trials have confirmed the safety and effectiveness of alginate in preventing ventricular remodeling, exhibiting favorable tolerability, a low incidence of adverse events, and the preservation of left ventricular function [261–264]. Lv et al. sought to boost the therapeutic potential of sEVs by incorporating them into alginate hydrogel to enhance their retention within cardiac tissue [243]. The release kinetics revealed that hydrogels treated with 0.5 % and 1 % calcium chloride solutions discharged sEVs within approximately 10 days, while those treated with a

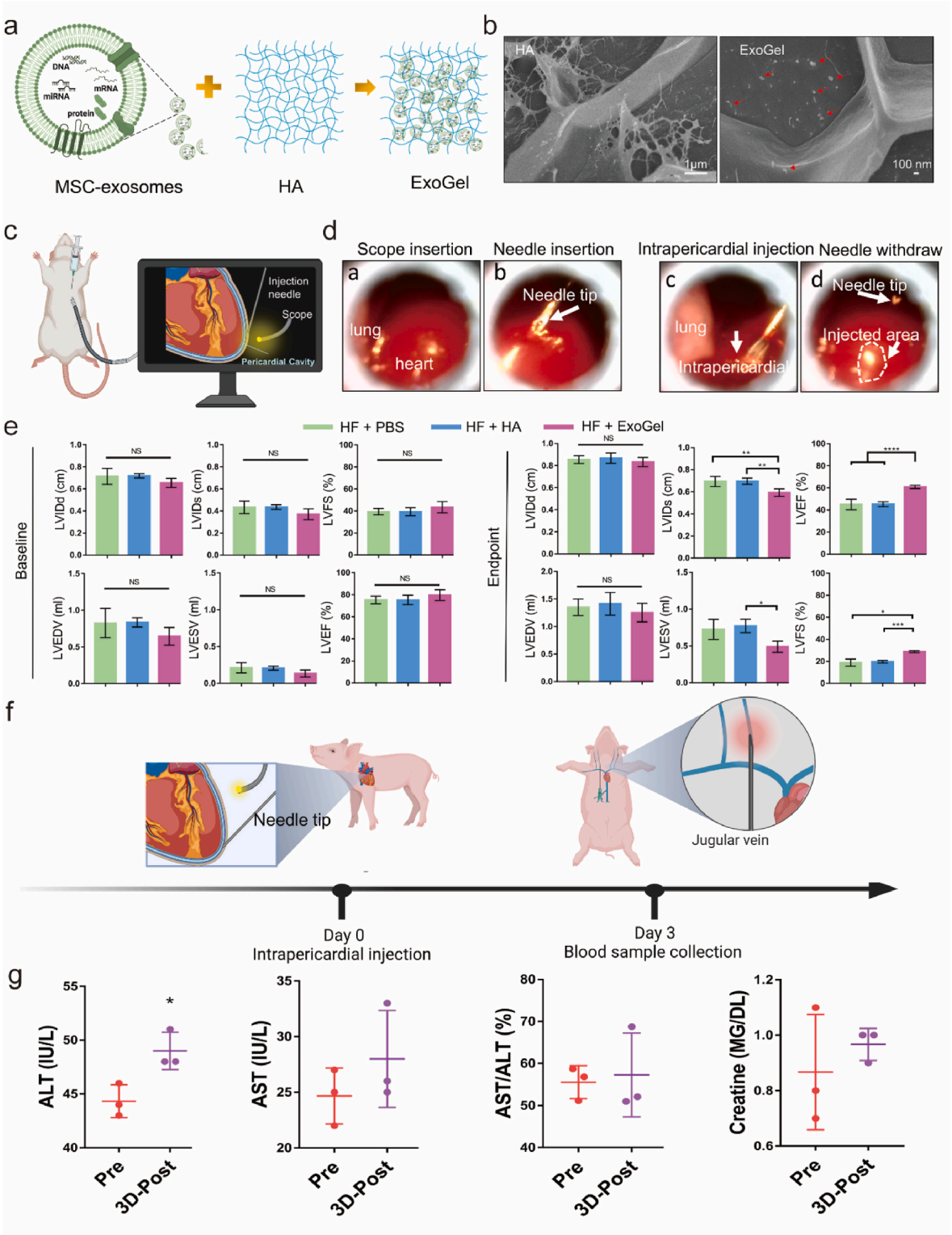

Fig. 11. Prevention of HF by iPC injection of ExoGel. a) Schematic diagram of the exosomes-loaded hydrogel. b) The SEM images of HA and ExoGel. c) The diagram of iPC injection to the rat model. d) Images of iPC injection steps. e) Echocardiography measurement of left ventricular internal diameter end diastole (LVIDd) and end-systole (LVIDs), left ventricular fractional shortening (LVFS), left ventricular end-diastolic volume (LVEDV), and LV end-systolic volume (LVESV), and left ventricular ejection fraction (LVEF) at baseline (pre-TAC) and endpoint (28 days after TAC). f) The diagram of iPC injection to the healthy pig. (G) Biomarker analysis of liver and kidney injury.

2 % calcium chloride solution discharged only about 60 % of the total sEVs in the same period. This disparity could be attributed to the increased concentration of calcium ions, which may impact the size of the pores within the hydrogel. Considering that MI results in immediate tissue damage and inflammatory infiltration within the first week, hydrogels treated with 0.5 % and 1 % calcium chloride may offer greater therapeutic benefits. Notably, the hydrogel treated with a 1 % calcium chloride solution displayed a G′ value of 1000 Pa, falling within the optimum range (400–1800 Pa) for cardiac tissue engineering [265]. Furthermore, the labeling of sEVs with lipophilic carbocyanine DiR facilitated the observation of sEVs retention *in vivo*, revealing higher retention of sEVs in the hearts treated with sEV-gel compared to free sEVs treatment. The combination of sEVs and hydrogel not only significantly reduced cardiomyocyte apoptosis but also promoted the shift of macrophages from the M1 to the M2 phenotype by the third-day post-MI. Additionally, the scar thickness and neovascularization notably increased in the fourth week following the infarction. This approach presented three key advantages: a sustained release of sEVs from the hydrogel for prolonged antiapoptotic effects, enhanced pro-angiogenic effects, and an improved healing response post-MI by reducing the overall number of CD 68+ macrophages and favoring the polarization of macrophages towards the M2 phenotype, which is critical for cardiac repair.

Peptide-based self-assembled hydrogels have garnered increasing attention in recent years due to their small pore sizes, capability to present epitopes, and the autonomous design potential of peptide chains to meet specific requirements. The PA-GHRPS peptides were synthesized by integrating cardiac protective peptides (GHRPS, Hisdtrp-Ala-Trp-Daph-Lys-NH2) along with MMP-2 biodegradable sequences (Glycine-Threonine-Alanine-Glycine-Leucine-Isoleucine-Glutamine, GTAGLIGQ) into a peptide amphiphile (PA). The combination of PA-GHRPS peptide and NapFF peptide led to the formation of a PGN hydrogel, in which the NapFF peptide exhibited remarkable self-assembly capabilities. The presence of the MMP-2 degradable sequence enables the hydrogels to degrade in the myocardial injury environment that contains high levels of MMP-2, thus ensuring environmentally responsive release of exosomes and GHRPS peptides. GHRPS, as part of the growth hormone-releasing peptide family, activates survival-promoting pathways while inhibiting cellular inflammation and fibrosis. The release kinetics indicated stable release of exosomes encapsulated in PGN hydrogels for up to 21 days, both *in vivo* and *in vitro*, with a higher release percentage observed in the presence of MMP in the environment. *In vivo* experiments demonstrated that the application of PGN hydrogels enhances the efficacy of exosomes in improving myocardial function [247].

The application of the cardiac patch approach described above is carried out through an invasive procedure that is frequently not well-tolerated by patients with severe heart disease. Utilizing EViH *via* minimally invasive thoracoscopically guided surgery presents a feasible alternative. As depicted in Fig. 11a, the study introduced an injectable HA hydrogel integrated with MSCs-EXOs (ExoGel) [248]. The structures and morphology of HA hydrogel and ExoGel were analyzed *via* SEM, as shown in Fig. 11b. ExoGel was administered through intrapericardial (iPC) injection into the pericardial cavity of rat models with transverse aortic constriction (TAC)-induced heart failure, with the injection process illustrated in Fig. 11c and d. Echocardiographic analysis conducted 28 days post-injection demonstrated significant protection against adverse cardiac remodeling in mouse hearts (Fig. 11e). Additionally, iPC injections were given to healthy pigs, and jugular vein blood samples were collected on the third day to evaluate the clinical feasibility of this approach (Fig. 11f). Levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), and creatinine were assessed before and after the injection, indicating normal liver and kidney function (Fig. 11g). The iPC procedure, guided by thoracoscopy and noted for its minimally invasive nature, is a surgeon-friendly approach associated with a low risk of post-surgical complications like suture breakage, bleeding, and respiratory distress. These findings offer valuable insights for managing diseases such as heart failure (HF), which often lead to frequent hospitalizations due to recurrent symptoms.

The contents of EVs play a vital role in the microenvironment of their originating cells. Specifically, studies have shown that MSCs exhibiting elevated levels of the transcription factor Islet1 (ISL1) demonstrate improved cell survival in models of MI. This benefit is likely due to the increased secretion of paracrine factors that aid in angiogenesis, both *in vitro* and *in vivo* [266]. To further explore the therapeutic potential of ISL1-MSCs, researchers isolated and assessed genetically modified exosomes from ISL1-MSCs (ISL1-MSCs-Exo) in MI models [246]. Results indicated that ISL1-MSCs-Exo possessed properties that protect endothelial cells and promote angiogenesis. Furthermore, the utilization of a thermosensitive hydrogel containing a short peptide derived from Angiogenin-1 (Ang-1) was investigated to enhance the retention of ISL1-MSCs-Exo within myocardial lesions, leading to significant enhancements in MI recovery. Ang-1 acts as a ligand for the endothelial cell membrane surface receptor Tie 2, influencing vessel maturation and supporting endothelial cell survival and adhesion. Treatment involving ISL1-MSCs-Exo within Ang-1 gels exhibited a more potent proangiogenic effect on HUVECs compared to ISL1-MSCs-Exo alone. However, it remains uncertain whether the observed therapeutic effects in the MI model were solely due to the increased exosome content or the synergistic interaction between exosomes and the Ang-1 hydrogel. Additionally, the authors noted that the high water content and large pore size of Ang-1 hydrogel continue to pose challenges in achieving sustained, stable, and controlled release of small molecules from its matrix.

The immune system plays a vital role in both the inflammatory and repair phases following MI [267]. Excessive or persistent inflammatory activation can result in maladaptive healing and subsequent left ventricular remodeling [268]. Exosomes derived from dendritic cells' culture supernatant (DEXs) have displayed positive associations with antigen presentation, immune activation, and inhibition [269]. Previous studies have indicated that DEXs enhance cardiac function by stimulating $CD4^+$ cells [270] and enhance angiogenesis *via* miR-494–3p [271]. Zhang et al. recently developed an injectable drug delivery system (DEXs-Gel) using an alginate hydrogel to investigate potential mechanisms for improving cardiac function after MI [245]. *In vitro* co-culture experiments were conducted to explore the interplay between DEXs, macrophages, and Treg cells. Flow cytometry analysis revealed that DEXs safeguarded cardiac function post-MI by activating Treg cells, which subsequently regulated macrophage polarization towards the M2 phenotype (Fig. 12a). Furthermore, the alginate hydrogel exhibited prolonged retention of DEXs. Results of release kinetics indicated that DEXs-Gel could be sustained for 10–12 days *in vitro*. Subsequent investigations by the authors focused on the clearance of DEX *in vivo* and demonstrated that DEX in the DEXs-Gel injected group entered the clearance phase on day 14, whereas DEX in the DEX-injected group was completely cleared by this point. Echocardiographic results illustrated that the gel injection treatment effectively prevented the deterioration of cardiac function compared to the MI group (Fig. 12b). Moreover, the application of DEXs-Gel improved cardiac function, underscoring the hydrogel's capability not only to extend DEXs retention but also to enhance treatment efficacy. Further *in vivo* studies showed that DEXs-Gel reduced infarct size and improved cardiac function. Nevertheless, the specific mechanisms through which DEXs activate Treg cells and how Treg cells modulate macrophage polarization still necessitate clarification.

Various hydrogels have been used as carriers to improve the retention of EVs in the lesion. However, administering them through multi-site intramyocardial injection can cause additional tissue damage and potential dysfunction of cardiac tissue [272,273]. Addressing this issue, Tang et al. proposed an innovative method for delivering EVs by utilizing a cardiac patch with *in situ* cross-linking ability, eliminating the need for injection [244]. They achieved EVs encapsulation within a cross-linked GelMA hydrogel network by spraying a mixture of GelMA precursor, EVs, and photoinitiators onto the target area and then

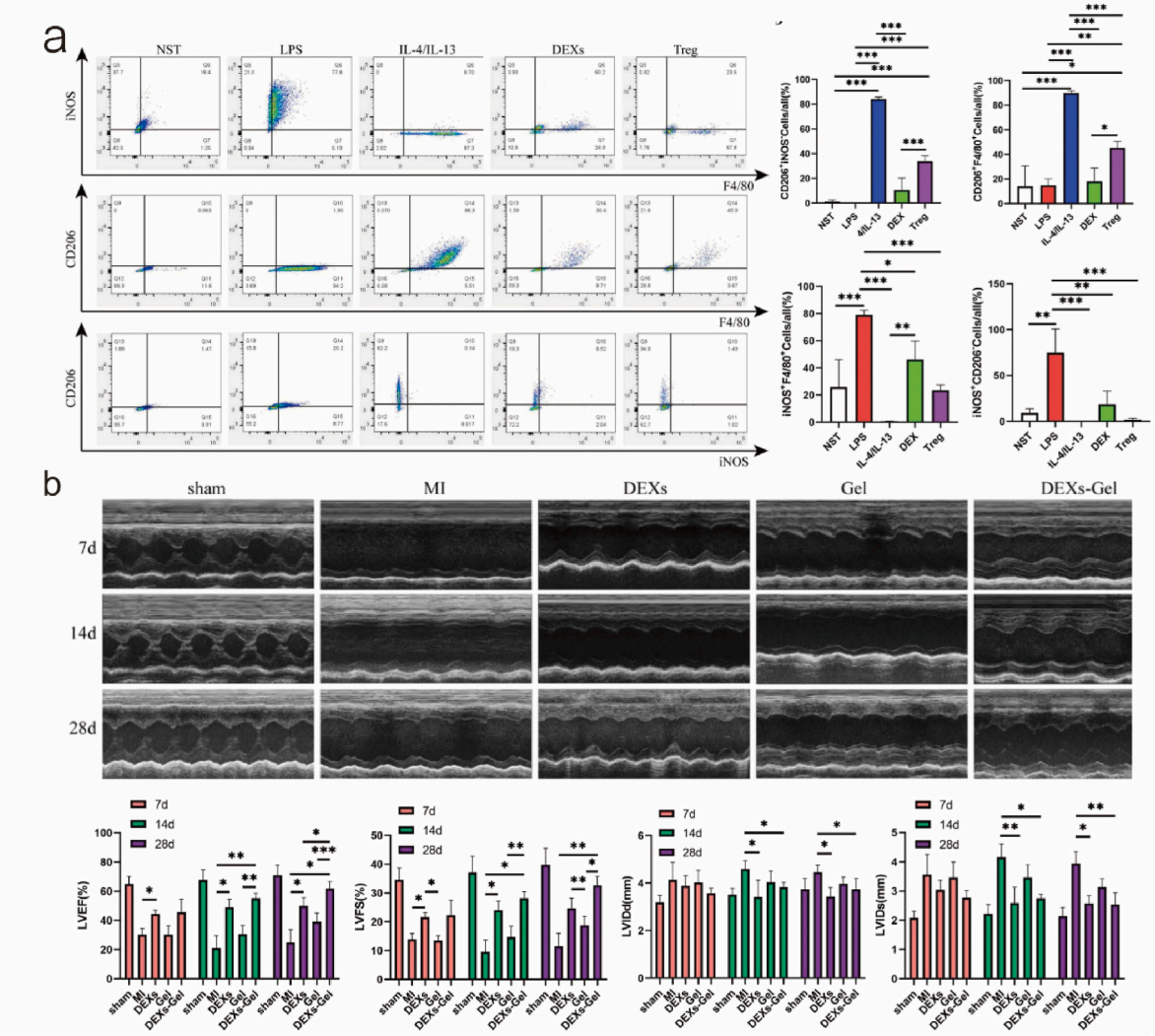

Fig. 12. The application of DEXs-Gel in the treatment of MI *via* activating Treg cells and regulating macrophage polarization. a) Flow cytometric images of different treatments and the proportions of $CD206^{+}iNOS^{-}$ cells, $CD206^{+}F4/80^{+}$ cells, $iNOS^{+}F4/80^{+}$ cells, $iNOS^{+}CD206^{-}$ cells in different groups: macrophages treated by IL-4/IL-13; macrophages co-cultured with DEXs; macrophages co-cultured with Treg cells. b) Echocardiographic images of MI animals with different treatments and the analysis of LVEF, LVFS, LVIDd, and LVIDs. These figures have been reproduced from Ref. [245] with permission.

exposing it to visible light for 30 s. The release kinetics indicated that 39.11 ± 9.27 % of EVs were released from the GelMA species within the first 6 h, gradually increasing to 71 ± 10.45 % at 72 h. This release pattern was attributed to early hydrogel degradation by enzymes in an enzymatic environment, with enzyme activity diminishing over time, thus delaying the release of the remaining EVs. Moreover, the use of visible light during hydrogel preparation helped prevent damage to EVs and cardiac tissue. Notably, the application of GelMA hydrogel loaded with EVs led to a substantial enhancement in heart function in mice with MI. In conclusion, this injection-free delivery strategy offers a promising and innovative approach to MI treatment.

6.2. EViH for peripheral arterial disease treatment

Peripheral arterial disease (PAD) is a consequence of chronic atherosclerosis, with a worldwide prevalence of up to 10 %, rising to almost 30 % in individuals aged 50 years and older. The clinical symptoms of PAD include reduced walking capacity, intermittent claudication, and critical limb ischemia (CLI). CLI represents the most severe form of the condition, capable of causing limb loss or even death if not promptly addressed. This section focuses on the utilization of EViH in managing CLI.

Traditional treatment methods, such as stent implantation, often fall short of meeting the needs of the majority of patients with CLI due to their invasiveness and associated surgical risks [274]. In response to this challenge, Zhang et al. explored a promising strategy to improve the retention and stability of exosomes isolated from PMSCs to enhance their therapeutic potency both *in vitro* and *in vivo* by combining these exosomes with chitosan hydrogel [250]. Through the use of Gaussia luciferase imaging, the study illustrated the integral role of chitosan hydrogels in enhancing the stability of proteins and microRNAs within exosomes and prolonging their retention within an *in vivo* environment. In the context of single-component hydrogels, the polymer concentration is frequently a critical determinant in controlling the rate at which EVs are released. This system demonstrated significant potential in safeguarding endothelial cells and fostering angiogenesis, ultimately facilitating improved recovery of ischemic hind limbs.

In addition to their therapeutic role, EVs serve as nanoscale carriers derived from natural tissues. For example, a study illustrated that miR-675 can combat aging by targeting the TGF-β/p21 pathway. Subsequently, miR-675 was integrated into exosomes encapsulated in silk fibroin to counteract aging-induced vascular dysfunction in the hindlimbs of mice [275]. However, the study did not account for the potential impact of exosomes themselves on the treatment efficacy for CLI.

Additionally, Zheng et al. successfully engineered a sophisticated hydrogel loaded with modified EVs expressing both VEGF and the transcription factor EB (TFEB). Release kinetics revealed that 99.9 % of the EVs were discharged from the hydrogel within 5 days at 29 °C, and 100 % were released within 7 days *in vitro* [249]. This temperature-responsive release behavior could be attributed to the disruption of hydrophobic bonds at lower temperatures, enhancing the contact surface between the hydrogel and the release medium to facilitate EVs release in CLI-related limb hypothermia lesions. This innovative system aimed to address CLI by leveraging the anti-inflammatory effects of endothelial cell-derived EVs (EC-EVs), the pro-angiogenic capabilities of VEGF, the autophagy-regulating functions of TFEB, and the temperature-sensitive properties of the methylcellulose-based hydrogel. The outcomes demonstrated that the combination of engineered EVs and temperature-responsive hydrogels effectively suppressed inflammation, promoted angiogenesis, and protected muscle tissue by activating the VEGF/VEGFR pathway and the autophagy-lysosomal pathway.

7. Neuronal regeneration

Neuronal regeneration is a complex process in which damaged or injured neurons in the central nervous system (CNS) or peripheral nervous system (PNS) attempt to self-repair and regain their functionality. Unlike many other cell types in the body, neurons have limited regenerative capabilities due to their intricate structure and the inhibitory microenvironment. CNS neurons, particularly, face significant challenges in regeneration due to factors like the presence of inhibitory molecules, lack of growth-promoting signals, and the formation of glial scars. This section will focus on the regeneration of CNS neurons, specifically addressing injuries caused by spinal cord injury (SCI) and ischemic stroke (IS).

7.1. Spinal cord injury

SCI is recognized as the primary cause of neurological damage within the CNS, impacting around 27 million individuals worldwide and leading to enduring disabilities such as urinary, digestive, and motor impairments [276]. The annual incidence of new SCI cases can reach up to 180,000 [277]. An overview of nerve injury repair after SCI will follow.

7.1.1. The pathophysiology of SCI

The pathological mechanisms underlying SCI can be categorized as primary damage caused by mechanical injury and secondary damage resulting from cellular and biological responses to the initial injury [278]. The primary injury triggers the accumulation of free radicals, ischemia, and hypoxia in the local microenvironment, leading to glutamate excitotoxicity, lipid peroxidation, Ca^{2+} influx, edema, and cellular damage. The secondary injury results in axonal demyelination, glial cell proliferation, and neuronal disconnection, hampering nerve regeneration and repair (Fig. 13). After SCI, the affected site can be divided into three main regions: a non-neural lesion core, an astrocytic scar surrounding the lesion core, and the remaining neural tissue with active metabolism. Cellular responses, which are crucial in SCI pathophysiology, exhibit temporal and spatial dimensions. The pathogenesis of SCI will be further elucidated at the cellular and molecular levels.

Immune cells, such as microglia, neutrophils, monocyte macrophages, and lymphocytes, impact the prognosis of SCI by releasing pro-inflammatory and immunomodulatory factors [278]. Nonetheless, the specific functions of these immune cells remain controversial. Microglia, inflammatory cells present in the spinal cord, demonstrate two primary phenotypes: M1 and M2. The M1 phenotype is linked to pro-inflammatory effects, while the M2 phenotype exerts anti-inflammatory effects. Upon SCI, microglia mostly switch to the pro-inflammatory phenotype, aiding in debris clearance and further contributing to secondary injury. However, recent studies have emphasized the crucial involvement of microglia in nerve regeneration. Notably, research using a neonatal mouse model of SCI has shown that the spinal cord can heal without scarring. Interestingly, in the absence of microglia, scar formation occurs, impeding axonal regeneration [279].

Astrocytes, as parenchymal cells in the CNS, play a critical role in maintaining CNS homeostasis [280]. After SCI, astrocytes undergo

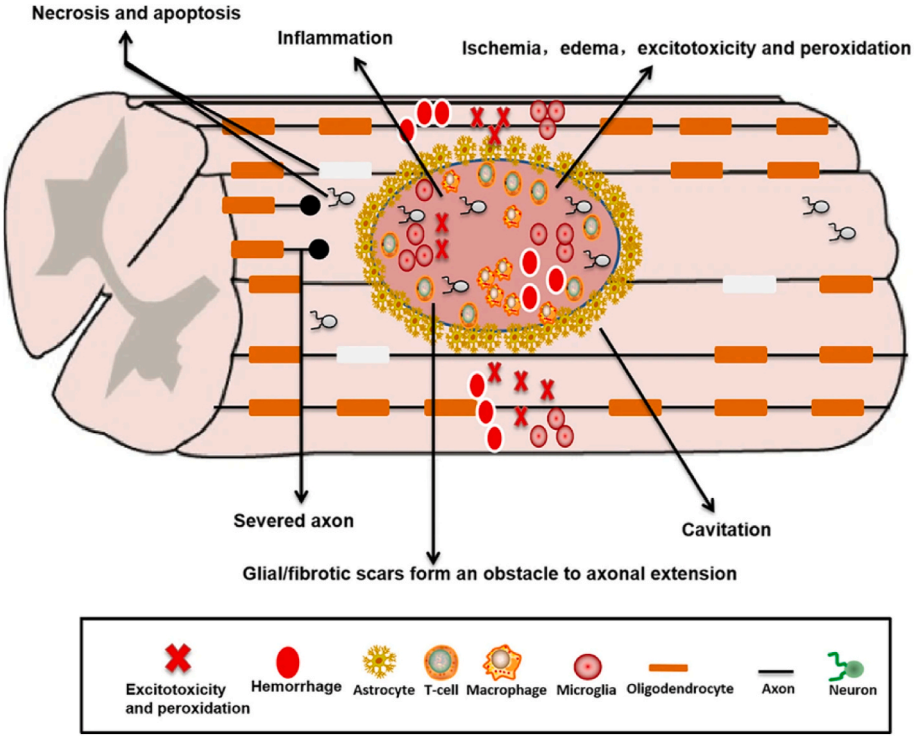

Fig. 13. The pathophysiological mechanism of SCI. *Copyright © 2020, The Author(s).*

significant morphological and functional changes, transforming into reactive astrocytes. These reactive astrocytes have a dual role in controlling excessive inflammatory cell infiltration and creating neuroglial scars that act as physical obstacles to nerve regeneration.

Oligodendrocytes, primarily found in the CNS, are localized near the perinuclear bodies in the cortex and intercalated among nerve fibers, forming myelin sheaths around axons in the white matter. After SCI, apoptosis of oligodendrocytes is detected within 24 h, peaking on the 8th day [281]. Oligodendrocyte precursor cells (OPCs), also referred to as NG2 cells, can differentiate into mature oligodendrocytes (MOL) [282]. Upon SCI, OPCs are activated to differentiate into oligodendrocytes and Schwann cells, promoting axonal regeneration and contributing to astrocytic scar formation [283]. Genetic fate mapping studies have shown the ability of endogenous OPCs to spontaneously repair myelin sheaths post-SCI [284]. However, the regenerative potential of OPCs is constrained by an unfavorable microenvironment characterized by scar-associated chondroitin sulfate proteoglycans (CSPGs), microglial activation, and neuregulin 1 (Nrg-1) [285].

In conclusion, the precise cellular and molecular modulation described above shows promising potential as a strategy for SCI treatment.

7.1.2. EViH for SCI treatment

Intrinsic regenerative processes naturally occur within nerves and surrounding tissues, but their capacity is limited. Hence, external interventions play a crucial role in enhancing nerve regeneration. Current standard clinical treatments include decompression surgery, aimed at restoring spinal stability, and high-dose intravenous methylprednisolone sodium succinate (MPSS) administration during the acute phase of injury (≤8 h) [286]. However, both approaches have shown inefficacy in promoting neuronal regeneration. Currently, EViH exhibits exceptional effectiveness in neuronal regeneration, excelling in both the method of administration and overall efficacy.

Effective modulation of unfavorable microenvironments is essential in facilitating neural regeneration. In a recent study, researchers investigated the synergistic effect of integrating human MSCs-Exos with adhesion peptide-modified HA hydrogels (pGel) for targeted therapy (Fig. 14a) [287]. Aldehyde-modified HA (HA-CHO) and hexanedihydrazide-modified HA (HA-ADH) were synthesized and used to create self-crosslinked hydrogels through Schiff base reactions. To

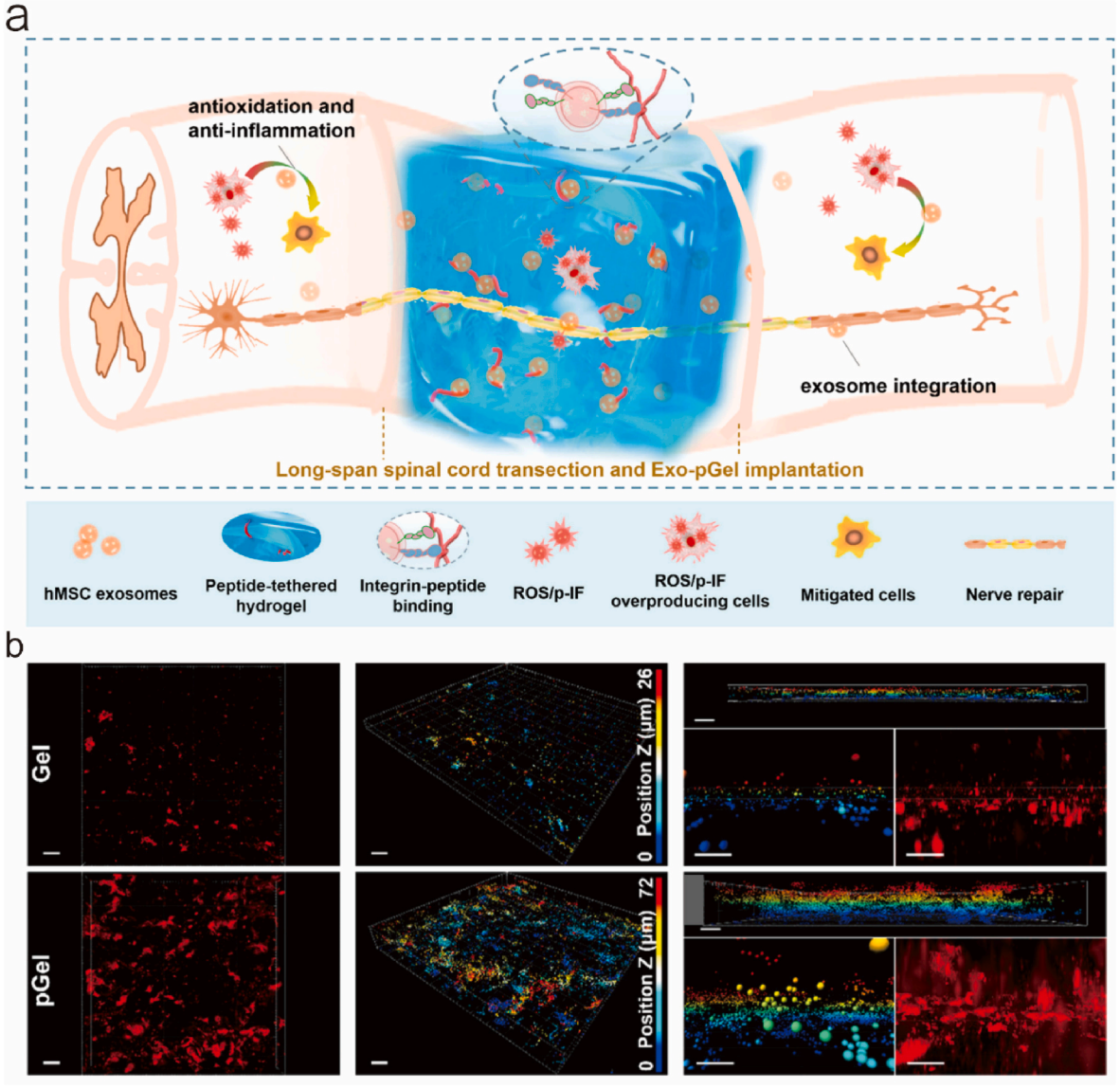

Fig. 14. a) The illustration of Exo combined with pGel therapy for SCI. b) The image of z-stack scanning by CLSM and the exosomes were labeled by CM-Dil (red). *Copyright © 2020, American Chemical Society.*

improve exosome adhesion, an adhesive peptide, PPFLMLLKGSTR, was incorporated into the HA hydrogels, resulting in a significant enhancement in adhesion efficacy, illustrated in Fig. 14b. Specifically, when labeled with CM-DiI (red), exosomes adhered to the porous structure within the pGel, rather than the HA hydrogel (Gel). Within the EViH system, controlled and sustained exosome release was achieved through the incorporation of Schiff bases and adhesion peptides. The release kinetics indicated that over 90 % of exosomes were consistently released from the 3D pGel for approximately 11 days, without aggregation or structural disruption within 3 days. This sophisticated approach effectively facilitated nerve regeneration and preserved urinary tissue by alleviating oxidative stress and inflammatory responses in the pathological microenvironment.

Ischemia presents a significant challenge in the pathological microenvironment of SCI, impeding neural repair within the damaged spinal cord. Previous studies have highlighted the critical role of functional neovascularization in this process [288,289]. ANGPTL3, a liver-specific secreted factor, has demonstrated potential in the modulation of blood vessel formation [290]. In a study by Cao et al., a novel approach was explored where exosomes encapsulating ANGPTL3 were incorporated into a hydrogel to facilitate neurological recovery [291]. *In vitro* experiments revealed that these exosomes effectively promoted angiogenesis by activating the PI3K/AKT pathway, underscoring the pivotal involvement of ANGPTL3 in this mechanism. Notably, the exosomes were observed to transport ANGPTL3 across the blood-brain barrier (BBB) to the site of injury, fostering neurological recovery through enhanced angiogenesis. The study further assessed the role of exosomes in restoring damaged spinal cord blood vessels post-SCI using 3D angiography *via* SRμCT and immunostaining. The findings demonstrated that USC-Exo, enriched with ANGPTL3, significantly contributed to the regeneration of blood vessels in the injured spinal cord. However, it is noteworthy that the specific type of hydrogel utilized in this study was not specified. The researchers primarily employed it as a delivery vehicle, without delving into its potential therapeutic effects.

The mechanical properties of biomaterials play a crucial role in tissue regeneration and repair [292,293]. Past research has suggested that hydrogels with slightly softer characteristics are more conducive to the differentiation of neural stem cells toward neuronal phenotypes [294]. In this study, HA methacrylate hydrogels with varying mechanical properties were synthesized to assess the release efficiency of exosomes and their consequent therapeutic impact on SCI [295]. The results demonstrated that softer hydrogels exhibited superior effects on nerve repair, attributed to the suppression of inflammatory cell expression and pro-inflammatory factor secretion facilitated by the rapid release of the hydrogel. However, the use of hydrogels alone is constrained by their lack of electrical conductivity, limiting their capacity to modulate the function of excitable cells [296]. To overcome this limitation, conductive hydrogels integrating hydrophilic properties with conductive components have been developed, showing promising outcomes in recruiting endogenous neural stem cells (NSCs) and fostering neuronal differentiation in SCI treatment. Nonetheless, the exclusive use of conductive hydrogels may trigger an immune rejection response from the host, potentially exacerbating early inflammation post-SCI. The incorporation of exosomes mitigates the unfavorable immune response from the host and synergistically enhances the therapeutic effect of the conductive hydrogel, thereby promoting neurological recovery. The recruitment of NSCs and the regeneration of neurons and axons were facilitated when a dual-network conductive hydrogel, composed of GelMA hydrogel (GM hydrogel) and polypyrrole (PPy) hydrogel (GMP hydrogel), was implanted at the injury site [297]. However, this hydrogel exacerbated inflammation, which hindered the regeneration of neurons. By introducing exosomes, the hybrid hydrogel (GMPE) was able to inhibit inflammation by modulating the M1/M2 phenotypic polarization through the NF-κB signaling pathways (Fig. 15a). In this

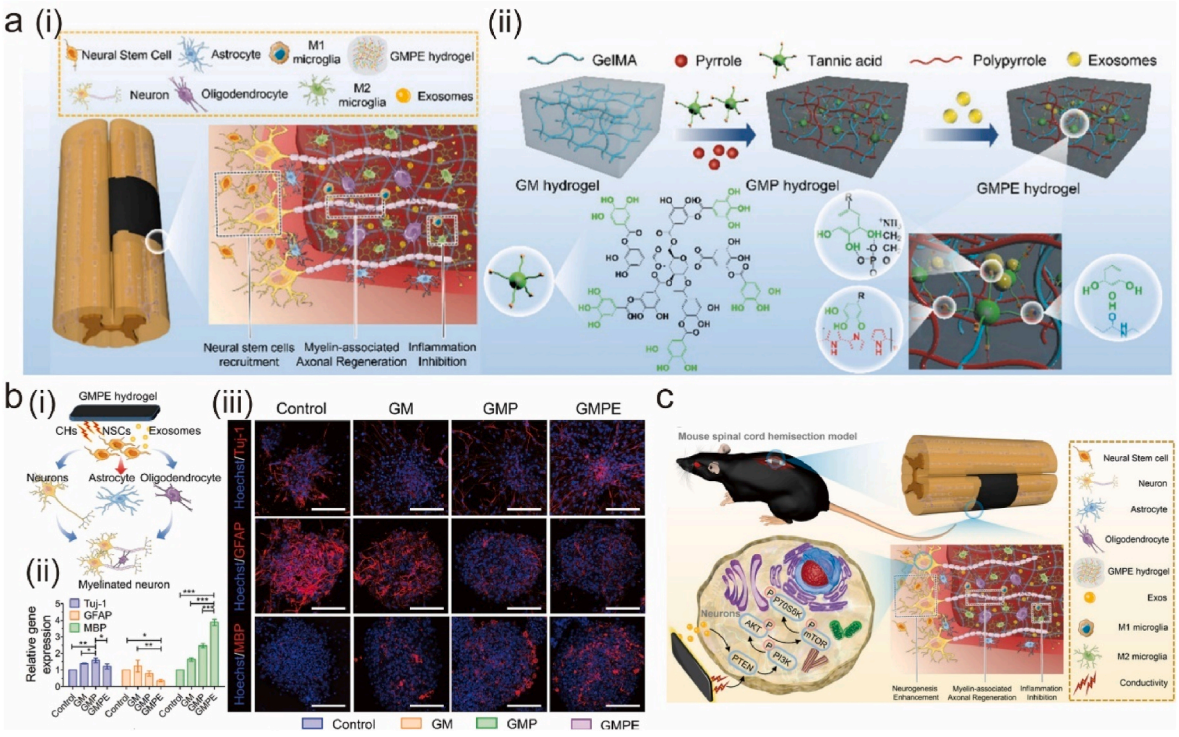

Fig. 15. a) (i) Mechanism of GMPE hydrogel to promote nerve regeneration diagram; (ii) Illustration of GMPE hydrogel synthesis steps. b) (i) Graphical representation of GMPE hydrogels affecting multidirectional differentiation of NSCs; (ii) RT-qPCR results of relative gene expression; (iii) Immunofluorescence images of the differentiation effect of NSCs after 7 days of hydrogel treatment. Red IF represents the neuron marker Tuj-1, astrocyte marker glial fibrillary acidic protein (GFAP), or oligodendrocyte marker myelin basic protein (MBP), respectively. Scale bars: 100 μm. c) GMPE hydrogel enhanced NSCs differentiation towards the neuronal and oligodendrocyte and promoted axonal growth through the activation of PTEN/PI3K/AKT/mTOR pathway.

study, GMPE hydrogels were observed to be well-suited for soft nerve tissue engineering, demonstrating an energy storage modulus of 1056.0 ± 133.1 Pa, closely matching the mechanical properties of neural tissue (600–3000 Pa). Additionally, the conductivity of GMPE hydrogel resembled that of normal tissue (ranging from 5×10^{-5} to 1.6×10^{-3} S cm−1), indicating its advantageous effects in promoting tissue regeneration. NSCs, being pluripotent stem cells capable of differentiating into astrocytes, oligodendrocytes, and neurons, play a crucial role in this process [298]. The regeneration of oligodendrocytes is particularly beneficial for facilitating rapid and efficient action potential propagation and functional recovery through their promotion of myelin regeneration [299,300]. The findings showed that both GMP and GMPE hydrogels supported NSC differentiation towards neurons and oligodendrocytes while suppressing astrocyte differentiation. Compared to GMP hydrogel, GMPE had a more pronounced effect on promoting oligodendrocyte differentiation due to the addition of exosomes (Fig. 15b). Moreover, the hydrogel enhanced neuronal and oligodendrocyte differentiation of NSCs and facilitated axonal growth by activating the PTEN/PI3K/AKT/mTOR pathway, as illustrated in Fig. 15c.

7.2. Ischemic stroke

Stroke is the primary cause of chronic disability globally and ranks as the second leading cause of death. Due to the aging global population, the incidence of stroke is rising, with IS being the most common type, accounting for over 85 % of cases.

7.2.1. The pathophysiology of IS

In the realm of IS, occlusion of cerebral arteries leads to a cessation of blood flow, leading to an insufficient delivery of oxygen and glucose to neurons and other brain cells. This insufficiency subsequently results in the disruption of ATP synthesis, energy deprivation, impaired ionic homeostasis, and acid-base imbalance. This discussion will elaborate on the diverse pathophysiological alterations linked to IS, encompassing glutamate excitotoxicity, neuroinflammation, BBB disruption, oxidative stress, and cell death [301].

Glutamate serves as a vital excitatory neurotransmitter within the CNS and plays a crucial role in regulating synaptic transmission. During IS, decreased ATP production in neurons results in energy depletion. This depletion impairs the functioning of Na^+/K^+-ATPase (NKA), Na^+/Ca^{2+} exchanger (NCX), and Ca^{2+}-ATPase in both the plasma membrane and intracellular organelle membranes. Consequently, the disrupted ionic gradient leads to increased glutamate release and inhibits glutamate reuptake, thereby disrupting glutamate metabolism. Additionally, this imbalance causes intracellular Ca^{2+} overload, intensifying glutamate release and activating Ca^{2+}-dependent enzymes. The accumulated glutamate in the synaptic space excessively stimulates the N-methyl-D-aspartic acid receptor (NMDAR) on the postsynaptic membrane, ultimately resulting in cell death. Furthermore, the heightened NMDAR excitation elevates intracellular Ca^{2+} levels, thereby exacerbating cell demise.

Oxidative stress arises from the disruption of intracellular redox reactions, leading to an overproduction of free radicals [302]. Ischemia/reperfusion (I/R)-induced oxidative stress can compromise the BBB integrity and prompt the release of inflammatory substances from neural and peripheral cells during an IS, thereby exacerbating reperfusion injury. Incidents like linear bursts of ROS, Ca^{2+} overload, excitotoxicity, and brain I/R injury can catalyze structural and functional flaws within mitochondria and spark off mitochondrial autophagy. The relationship between oxidative stress and mitochondria is profoundly interlinked in IS. Notably, oxidative stress has a crucial impact on mitochondrial function, subsequently impairing cell viability and operation. Conversely, mitochondrial dysfunction may also contribute to oxidative stress, as the oxidative phosphorylation process in mitochondria is a significant source of free radicals [303]. Therefore, a detrimental feedback loop emerges between oxidative stress and mitochondria that intensifies cellular damage and mortality, exacerbating the condition of IS.

The BBB structural framework comprises ECs with interconnected tight junctions (TJs), pericytes, astrocyte endfeet, and diverse ECM components [304]. During an IS event, the BBB undergoes significant compromise, as indicated by structural disorganization and increased permeability of TJs. This increased paracellular and intercellular permeability, along with substantial endothelial damage, allows various substances such as cells, chemicals, and fluids to breach the BBB and enter brain tissue, disrupting the water and ion balance and causing brain edema. Furthermore, the infiltration of leukocytes exacerbates the inflammatory response, further contributing to brain damage [301].

Overall, understanding the pathophysiology of IS is essential for the development of effective treatment strategies aimed at preserving brain tissue, restoring blood flow, and reducing neurological deficits in stroke patients.

7.2.2. EViH for IS treatment

The treatment of IS primarily involves mechanical thrombectomy or intravenous thrombolysis using recombinant tissue-type plasminogen activator to restore blood flow. However, the narrow time window for intervention limits the efficacy and safety of these treatments. Pharmacological approaches for IS, such as reperfusion and neuroprotective strategies, are crucial for neuroprotection and the restoration of cerebral function. Nevertheless, these treatments face challenges due to poor solubility, short half-life, high toxicity, and low bioavailability. Given the complex pathophysiology of IS, monotherapies are often inadequate, leading to the recognition of EViH as a potentially effective treatment option.

NSCs can differentiate into neurons or glial cells and release EVs that play a crucial role in post-ischemic healing by reducing apoptosis and oxidative stress, promoting neovascularization and neurogenesis. Research indicates that the usage of NSCs-derived exosomes (NSCs-Exos) is more effective in enhancing neural regeneration than NSCs themselves [305,306]. Challenges like low survival rates and rapid clearance hinder the efficient application of exosomes *via* the circulatory system. Gu et al. successfully isolated NSCs-Exos through ultracentrifugation and enhanced their bioactivity and retention efficiency by utilizing HA hydrogels [117]. The release profile demonstrated that Exos were consistently delivered from the hydrogel for 28 days *in vitro* and were still detectable at the injection site on day 28 *in vivo*. Tests including NTA, WB, and TEM confirmed that NSCs-Exos released from hydrogels preserved their biological activity without alterations in size, morphology, or surface proteins compared to pre-release NSCs-Exos. The integration of hydrogels reduces the frequency of administration compared to free NSCs-Exos application. *In vivo* experiments revealed that NSCs-Exos incorporated into hydrogels promoted cell proliferation in ischemic regions, facilitated angiogenesis, and mitigated inflammatory responses, consequently enhancing cerebral infarction recovery and neurological functions.

As outlined in **section 5.2**, 3D-EVs have been employed in the context of BTR with notable benefits, while hydrogels, due to their excellent biocompatibility and cell adhesion properties, are extensively utilized in 3D cell culture. Notably, the application of 3D-EVs has shown promise in the treatment of IS. Han et al. conducted a study where they cultured MSCs in GelMA hydrogels in a 3D manner, and subsequently isolated 3D exosomes (3D-Exos) to investigate their therapeutic potential for IS in comparison to 2D exosomes (2D-Exos) [307]. The findings revealed that the 3D culture environment led to increased MSC survival rates and exosome production compared to traditional 2D cultures. Importantly, 3D-Exos, being smaller in size than 2D-Exos, are more readily phagocytosed by target cells and exhibit enhanced neuroprotective effects. *In vivo* experimentation demonstrated that 3D-Exos could selectively accumulate in ischemic cerebral regions, dampen neuroinflammation, and stimulate angiogenesis, possibly due to the improved ability of 3D-Exos to traverse the BBB. This study primarily

utilized hydrogels as a platform for cells to secrete EVs. It is suggested that the direct secretion of EVs by cells into hydrogels to form *in-situ* EViH holds promise for future investigations. The EViH developed through this innovative approach offers multiple advantages: firstly, enabling synergistic interactions between cells and EVs; secondly, incorporating other bioactive substances secreted by cells that enhance their regulatory functions; and thirdly, facilitating the bypassing of the BBB, thereby enhancing the localization efficiency of EVs at the injury site.

In conclusion, we summarized that EViH provides a new perspective for solving the puzzle of neuronal regeneration (Table 4).

8. Liver and kidney regeneration

The liver is the only solid organ that utilizes regenerative mechanisms to maintain the liver-to-body weight ratio necessary for homeostasis in the body. This unique regenerative capacity of the liver is crucial for the functioning of most organisms, as it ensures the appropriate liver function. Hepatocytes in the normal liver tissue environment have practically unlimited regenerative capacity. However, chronic loss of hepatocytes and activation of hematopoietic stem cells (HSC) can lead to an abnormal tissue environment, resulting in chronic compensatory regeneration of hepatocytes that can lead to negative outcomes, including the development of tumors. Chronic liver injury (CLI) is a significant health concern characterized by abnormal liver structure, inflammation, portal hypertension, fibrosis, inflammatory damage, loss of metabolic capacity, and cell death [311]. Orthotopic liver transplantation (OLT) is currently the most effective treatment for CLI, but it is limited by factors such as organ donor availability, immune rejection, and post-operative complications. To overcome these limitations, researchers are exploring the use of MSCs and EVs for treating acute and chronic liver injuries [312,313]. Maintaining an effective concentration of EVs at the site of injury is crucial for achieving therapeutic results. Since the liver has a propensity to accumulate EVs following systemic administration [22,314,315], creating a reservoir for sustained release of EVs *in vivo* is feasible. Researchers have developed an *in-situ* gel-forming, biodegradable hydrogel for the sustained release of EVs [310]. By injecting this hydrogel into the peritoneal cavity, a continuous release of EVs over a month was achieved. This approach resulted in a 50 % increase in liver regeneration in an animal model compared to the injection of free EVs. Controlled-release systemic delivery of EVs *via* hydrogel presents a viable option for sites where topical delivery is not practical, leveraging the propensity for drug accumulation in the body. EViH offers a promising avenue for treating liver injuries and has the potential to overcome the limitations associated with traditional therapies like OLT.

The kidney, in comparison to the liver, is an organ with relatively low cellular regeneration potential. Despite the kidneys' baseline limited regenerative capacity, renal tubular epithelial cells display a remarkable ability to proliferate following injury. There is still no consensus on which specific cells are accountable for the regeneration of the tubular epithelium post-injury. Several hypotheses have been suggested concerning the regenerative potential within renal tissue, with most studies attributing this potential to either the dedifferentiation of mature renal tubular epithelium or the presence of a resident pool of progenitor cells in the renal tissue. EVs show great promise in kidney tissue repair. Self-assembled peptides (SAPs), nanostructures created from the self-assembly of natural amino acids to form hydrogels [316], present distinct advantages in drug delivery due to their exceptional accessibility and biocompatibility [317]. In efforts to address kidney injury induced by I/R, an MMP-2-sensitive SAP hydrogel (KMP2) was employed to locally deliver EVs [309]. This approach was grounded on the observation of significantly increased MMP-2 levels in the affected region [318]. The EVs were enclosed within nanofibers of the KMP2 hydrogel without compromising the structure. Release kinetics revealed that the KMP2 hydrogel displayed reduced efficacy in releasing EVs when in a PBS solution (less than 18 % of EVs released in 168 h), while it exhibited faster EVs release in the presence of MMP-2 in a dose-dependent manner (approximately 28 % and 60 % of EVs released in 168 h by the 0.4 and 4 mg/ml MMP-2 groups, respectively). Experimental findings showcased the successful fabrication of the KMP2-EVs hydrogel, demonstrating excellent responsiveness to MMP-2 and resulting in a notable decrease in apoptosis, anti-inflammatory effects, and enhanced functionality of microvascular endothelial cells [309].

Table 4
The application of EViH for neuronal regeneration, and liver and kidney regeneration.

Disease	EVs species	Composite hydrogel type	Hydrogel loading	Crosslinking mode	Release Kinetics	Biological effects	References
SCI	Exosomes of hPMSCs	pGel	Pre-gel loading	Schiff base reaction	90 % in 11 days	Regulation of the pathological microenvironment of CNS diseases and tissue traumas	[287]
	Exosomes of USCs	Not mentioned	Not mentioned	Not mentioned	Not mentioned	Promotes angiogenesis by activating the PI3K/AKT signaling pathway and enhances neurological recovery	[291]
	Exosomes of MSCs	HAMA	Pre-gel loading	Photo-crosslinking	Not mentioned	Soft water gel is better for nerve repair	[295]
	Exosomes of BMSCs	GM/PPy	Post-gel loading	Photo-crosslinking	90 % in 14 days	Promotes nerve regeneration and functional repair through the activation of PTEN/PI3K/AKT/mTOR pathway	[297]
	Exosomes of Flos Sophorae Immaturus	pDA-Gel	Not mentioned	Schiff base reaction	Not mentioned	Promotes nerve regeneration by ameliorating the inflammatory and oxidative condition	[308]
IS	Exosomes of NSCs	HAD hydrogel	Pre-gel loading	Photo-crosslinking	Over 28 days	Improves cerebral infarction *via* promoting cell proliferation angiogenesis and reducing inflammatory effects	[117]
I/R injury	EVs of MSCs	KMP2	Pre-gel loading	Self-assembling	60 % in 168 h	Improved renal function *via* reduction of apoptosis, anti-inflammation, and improvement of microvascular endothelial cells	[309]
CLI	EVs of MSCs	PEG	Pre-gel loading	Michael addition between thiol and maleimide groups	Over 1 month	Continuous systemic drug delivery for liver injury	[310]

Abbreviations: EVs: extracellular vesicles; SCI: Spinal cord injury; hPMSCs: human placental mesenchymal stem cells; pGel: peptide-modified HA (hyaluronic acid) hydrogel; CNS: central nervous system; USCs: urine stem cells; MSCs: mesenchymal stem cells; HAMA: hyaluronic acid methacrylate; BMSCs: bone marrow derived mesenchymal stem cells; GM/PPy: gelatin methacrylate hydrogels and polypyrrole hydrogels; pDA-Gel: polydopamine modified hydrogel; NSCs: neural stem cells; HAD: hyaluronic acid; I/R: ischemia-reperfusion; KMP2: matrix metalloproteinases-2 (MMP-2)-sensitive self-assembled peptides (SAP) hydrogel; CLI: chronic liver injury; PEG: polyethylene glycol.

In contrast, EViH has been relatively understudied in the realms of liver and kidney regeneration despite demonstrating efficacy similar to that of other systems. Looking ahead, substantial progress is anticipated for EViH in these domains. An overview of the use of EViH systems for liver and kidney regeneration is also presented in Table 4.

9. Conclusions and perspectives

The pursuit of tissue regeneration is a prevalent and crucial goal among researchers and medical professionals. The EViH therapy serves as a potent tool for achieving this goal by leveraging the unique properties of EVs and hydrogels. EVs, small membrane-bound particles, play a pivotal role in ECM interaction, immunoregulation, regulation of sentence and proliferation, and angiogenesis. Hydrogels, 3D polymers closely resembling the natural ECM, exhibit high biocompatibility and act as a scaffold for encapsulating and delivering EVs. The EViH system optimally combines the strengths of EVs and hydrogels, mitigating the individual weaknesses of EVs or hydrogels alone. This article elaborates on the benefits and characteristics of EViH, enhancing the understanding and recognition of its potential.

This comprehensive review presents a detailed overview of recent advancements in utilizing EViH for tissue regeneration across various domains, including wound healing, bone and cartilage repair, vascularization, cardiac restoration, neuronal regeneration, as well as liver and kidney revitalization. Each section outlines the pathological processes of tissue damage, the physiological mechanisms of tissue regeneration and repair, and discusses the distinct properties of EViH in the context of specific tissue regeneration processes. The meticulous design and development of these strategies are anticipated to yield significant clinical outcomes and propel the field of tissue regeneration forward.

Despite the initial progress in regenerative medicine achieved through the utilization of EViH, several unresolved issues necessitate further investigation and discussion. Primarily, the methods employed for isolating EVs significantly influence their quantity, quality, purity, specificity, and the integrity of their outer membrane components. While ultracentrifugation and density gradient flotation are recommended techniques for EVs extraction in analytical and experimental settings, they fail to meet the requirements for large-scale production and clinical isolation of EVs. Therefore, there is an urgent need for robust and scalable bioproduction programs and suitable EVs isolation methods.

Secondly, the selection of properties and types of hydrogels is critical for different pathological contexts. The characteristics of hydrogels, such as their mechanical properties, degradability, and stress distribution, play a crucial role in optimizing the retention and release patterns of EVs. Furthermore, emphasis should be placed on preserving the viability and biological functions of EVs during hydrogel synthesis and EViH application. Nevertheless, the impact of various types of hydrogels on the activity of EVs has not been systematically studied, impeding the further advancement of EViH.

Additionally, although numerous studies have showcased the regenerative potential of non-cellular therapies utilizing EVs as an alternative to cellular therapies, a direct comparison regarding the therapeutic effectiveness of EViH and stem cell-loaded hydrogels in facilitating tissue repair remains elusive. Further investigations are required to assess and compare the outcomes of these two approaches. Furthermore, enhanced with various advantages and disadvantages, different sources of EVs require thorough consideration in determining the most suitable option to employ.

Lastly, regulatory-approved healthcare applications for EViH are currently non-existent. The potential increase in bio-productivity associated with the complexity of the EViH system poses a significant challenge for the clinical translation of EViH. It remains uncertain whether this complexity will lead to enhanced bio-productivity.

In conclusion, the incorporation of hydrogels and EVs presents significant potential in the realm of regenerative medicine. Despite existing challenges, it is plausible to anticipate the surmounting of these obstacles in due course, facilitating the clinical application of this strategy. The enduring impact of this review on the advancement of EViH as a pivotal modality for tissue restoration and rejuvenation is assured.

CRediT authorship contribution statement

Lubin Liu: Writing – original draft. **Wei Liu:** Writing – original draft. **Zeyu Han:** Writing – original draft. **Yansheng Shan:** Writing – original draft. **Yutong Xie:** Writing – original draft. **Jialu Wang:** Writing – original draft. **Hongzhao Qi:** Writing – review & editing, Funding acquisition, Conceptualization. **Quanchen Xu:** Writing – review & editing, Funding acquisition, Conceptualization.

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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